Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate

EC number: 240-286-6
CAS number: 16118-49-3

CLH-O-0000001412-86-50/F

Adopted

12 March 2015

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12 March 2015

CLH-O-0000001412-86-15/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 (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 harmonized classification and labelling (CLH) of:

Chemical name: **Carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate**

EC number: **240-286-6**

CAS number: **16118-49-3**

The proposal was submitted **France** and received by the RAC on **1 July 2014.** All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS).

PROCESS FOR ADOPTION OF THE OPINION

France 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 **8 July 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 August 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-rapporteur, appointed by RAC: **José Luis Tadeo**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation. The RAC opinion on the proposed harmonized classification and labelling was reached on **12 March 2015** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on Carbetamide (ISO) that should be classified and labelled as follows:

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
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	**No current Annex VI entry**									
Dossier submitters proposal	TBD	carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate	240-286-6	16118-49-3	Carc. 2 Repr. 2 Acute Tox. 4 Aquatic Chronic 2	H351 H361d H302 H411	GHS08 Wng	H351 H361d H302 H411		
RAC opinion	TBD	carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate	240-286-6	16118-49-3	Carc. 2 Repr. 1B Acute Tox. 4 Aquatic Chronic 2	H351 H360D H302 H411	GHS08 GHS07 GHS09 Dgr	H351 H360D H302 H411		
Resulting Annex VI entry if agreed by COM	TBD	carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate	240-286-6	16118-49-3	Carc. 2 Repr. 1B Acute Tox. 4 Aquatic Chronic 2	H351 H360D H302 H411	GHS08 GHS07 GHS09 Dgr	H351 H360D H302 H411		

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

As separate annexes to the CLH report, the Dossier Submitter (DS) included two evaluation documents (EFSA, 2010; EC, 2010) on carbetamide as the active substance in plant protection products (PPP); these were considered relevant for the harmonised classification proposal by RAC.

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

The physico-chemical studies showed no thermal decomposition of carbetamide up to 400°C and no flammability. Structural analysis showed no evidence for explosive or oxidising properties. Therefore, the DS did not propose to classify carbetamide for physical hazards.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The substance did not reveal any properties relevant for classification for physical hazards. RAC agrees with the view of the DS that based on the data presented no classification is warranted for physical hazards.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Three acute toxicity studies by oral and dermal routes were summarised by the DS in the CLH report. No acute inhalation toxicity study is available since the vapour pressure of carbetamide at 20°C is 3 x 10^{-7} Pa whichis far below the limit of 1 x 10^{-2} Pa for which an inhalation study is required, according to the DS.

Oral toxicity
The DS briefly summarised the available acute oral toxicity studies conducted in rats and mice. In Sprague-Dawley rats, carbetamide is of low acute toxicity after oral administration. Two male rats died on Day 1 at 3 hours post-dosing to 2000 mg/kg bw. Other male and female rats survived until the end of the observation period (14 days) although they presented clinical signs for about two days after carbetamide administration (lethargy, decreased motor activity, ataxia and piloerection). Three female rats showed breathing difficulties, prone position and unconsciuousness. The acute oral LD_{50} of carbetamide in rats is > 2000 mg/kg bw.

In mice, administration of carbetamide produced bradypnoea, hyperpnoea, proneness and/or unconsciousness directly after dosing and less frequently lethargy, decreased motor activity, muscular tremor, apnoea and cyanosis. Deaths occurred at dose levels of 1414 mg/kg bw and above within the first three days after dosing. The LD50 of Carbetamide in mice after oral administration was found to be 2033 mg/kg b.w for males and 1445 mg/kg for females. Thus a

sex difference is apparent. The DS reported that the manufacturer considered the study as being irrelevant on the basis of the following arguments: i) results are based on 5 animals/sex only; ii) a micronucleus test showed no mortality and moderate signs of toxicity on mice exposed to 2000 mg carbetamide/kg bw; iii) chronic feeding studies showed similar sensitivities between mice and rats; iv) the mortality of mice exposed to carbetamide at 2000 mg/kg bw/d for 2 years was similar to mortality of controls; v) a mechanistic study showed no toxicity in mice receiving 9000 mg/kg bw/d of carbetamide for 14 days. These data support, according to the manufacturer's opinion cited in the CLH report, the hypothesis that the lethality observed in mice female was due to the vehicle (Tween 80), that was administered at a dose of 100 mg/kg bw.

The DS was of the opinion that since both oral toxicity studies for rats and mice were performed in the same laboratory in similar periods of time, following OECD test guideline (TG) 401 and with the same carbetamide batch, both studies must be considered reliable without restrictions. In addition, the LD_{50} of Tween 80 is equal to 25 000 mg/kg bw while the dose in this study was 100 mg/kg suggesting no influence of Tween 80 on mortality. An increase of the bioavailability of carbetamide due to Tween 80 is also not supported because a toxicokinetic study showed an oral absorption which is quick and higher than 80%.

Based on the oral LD_{50} for female mice of 1445 mg/kg bw which is within the range of ATE = 300-2000 mg/kg bw, the DS proposed to classify carbetamide as Acute Tox. 4; H302 (Harmful if swallowed).

Dermal toxicity
Rabbits dermally exposed to 2000 mg carbetamide/kg bw survived without signs of toxicity and gross necropsy revealed no treatment-related abnormalities.

Inhalation toxicity
No acute inhalation toxicity study is available since the vapour pressure of carbetamide at 20 °C is 3×10^{-7} Pa and is far below of 1×10^{-2} Pa for which an inhalation study is required.

Comments received during public consultation

Three Member States Competent Authorities (MSCAs) supported the proposed classification. One MSCA asked the DS to provide additional information for the mouse study. All the additional information supplied by the DS in the Annex 2 (Comments and response to comments on CLH proposal on carbetamide) has already been included in this opinion.

Assessment and comparison with the classification criteria

A table summarising the key information of the available information from the CLH report and the draft assessment report (DAR, Vol. 3, Annex B, B.6, part 1, July 2006) is presented in the background document (Supplemental information - In depth analyses by RAC).

RAC agrees with the DS that no classification is warranted for the dermal route in the absence of effects in rabbits exposed to 2000 mg carbetamide/kg bw. Furthermore, in the absence of data, RAC has not assessed the acute toxicity via inhalation route.

RAC supports the DS opinion and considers that female mice lethality cannot be supported on the basis of toxicity of the vehicle. Thus, in absence of other reasons to diminish reliability of the study conducted in mice, and following the Guidance on the Application of the CLP Criteria (CLP guidance; version 4.0, November 2013) the lowest available LD_{50} must be used for setting classification.

In conclusion, RAC agrees with the DS that the critical LD_{50} value of 1445 mg/kg bw is within the range of 300-2000 mg/kg bw and therefore carbetamide warrants classification as **Acute Oral Toxicity Category 4 (H302: Harmful if swallowed).**

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

Summary of the Dossier submitter's proposal

In the absence of evidence for target organ toxicity after single exposure (for STOT SE 1 or 2) or signs of respiratory irritation or narcotic effects (for STOT SE 3) in the available studies, the DS proposed 'no classification' for this hazard class.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

According to the CLP Regulation and Section 3.8 of the CLP guidance, specific organ toxicity following single exposure should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. Regarding STOT SE 1 or 2, standard acute toxicity studies did not indicate that there was specific organ toxicity following a single exposure. The effects observed in these studies were general and systemic, occurred at high doses of carbetamide, were transitory in nature without significant functional change in any organ system and were not considered to support STOT SE 1 or 2 classification. Necropsy of rats on day 15 revealed no significant macroscopic lesions. In mice, there were slight and infrequent respiratory effects (pulmonary congestion, aerated serous fluid in the trachea) as well as congestion of the submaxillary salivary lymph nodes. The DAR (2006) reported pulmonary congestion and abnormal (mucoid) gastro-intestinal contents. RAC noted that histopathology data were not available. Relevant human information with respect to, e.g. epidemiological studies, medical surveillance and reporting schemes and national poisons centres was also not available. Overall, it is concluded that classification of carbetamide for STOT SE 1 or 2 is not warranted.

The hazard class STOT SE 3 should cover 'transient' respiratory tract irritation and narcotic effects occurring after single exposure. Although classification in Category 3 is primarily based on human data, if available, animal data can be included in the evaluation. Respiratory tract irritation and narcotic effects are generally assessed from standard acute inhalation studies, although it is possible that narcosis could be observed in studies using other routes (see section 3.8 of the CLP Guidance). Since no acute (or sub-chronic) inhalation toxicity studies were available, it is not possible to determine whether carbetamide is irritant to the respiratory tract.

In the CLP guidance (November 2013), it is indicated (Chapter 3.8.2.2.2) that 'narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia'. In the oral single dose studies, some of these symptoms were observed in rats and mice (namely lethargy and ataxia). These symptoms occurred quickly after dosing, appeared to be unspecific and were transient in nature. The short-term toxicity of carbetamide was evaluated by oral route in rats and dogs. No study was performed by inhalation or by dermal repeated application. Carbetamide is considered of low short-term toxicity in mammals but it induced slight signs of neurotoxicity (muscle tremors, hind limbs, drowsiness, unsteadiness and prostration) in dogs from a dose level of 30 mg/kg bw/day. Modifications of plasma or tissular acetylcholine esterase levels were observed in rats and dogs, but with opposite trends across organs/sexes/studies, and often without dose-relationship.

Overall, the very transient and slight reported narcotic/neurotoxic signs do not fulfil the criteria for STOT SE 3. RAC does not consider additional classification for STOT SE 1 or 2 to be necessary either.

RAC therefore proposes, in agreement with the DS proposal, not to classify carbetamide for **STOT SE 3 – H336.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

A skin irritation study performed in accordance with OECD TG 404 under GLP standards showed that 0.5 g carbetamide (96.3% purity) dermally applied during 4 hours caused no local cutaneous inflammatory or irritation reaction during the observation period. The DS did not propose a classification for skin corrosion/irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The scores in the skin irritation study were all zero, and thus, RAC agrees that carbetamide does not meet the CLP criteria to be classified for skin irritation/corrosion.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

A reliable OECD TG 405 eye irritation study performed under GLP showed that 0.1 g carbetamide (96.3% purity) placed into the conjunctival sac of six female rabbits caused slight reversible conjunctival redness (group mean score 24-72 hours = 0.55, range 24-72 hours = 0.0 to 1) and slight reversible chemosis in two animals 1 hour after dosing (group mean score 24-72 hours = 0). The other parameters (cornea opacity and iridial inflammation) were negative in all tested animals.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC agrees that carbetamide does not meet the criteria to be classified for eye irritation/corrosion since: i) no conjunctival chemosis, corneal opacity and iridial inflammation were detected; ii) the mean scores for 24-72 hours for conjunctival redness and conjunctival oedema were lower than 2; and, iii) the mild reported effects were fully reversible 72 hours after exposure.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

No data on respiratory sensitisation nor any indication of possible concern were included in the CLH report.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the absence of data, RAC has not assessed this hazard class.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS reported two available sensitisation studies using the Guinea pig maximisation test (GPMT) of Magnusson and Kligman.

The first test (Cummins and Gardner, 1985) was a reliable study performed following OECD TG 406 under GLP standards and with the only detected deviation being the absence of positive control. The induction was performed with intradermal injection of carbetamide 2% and 4% w/v, while the challenge was performed with topical application of 50% w/v carbetamide on day 8 preceded by sodium lauryl sulfate exposure on day 7. Challenge test caused slight to moderate erythema in controls. No dermal reactions were noted in the 48 hours following the challenge exposure in treated animals.

The second test (Haferkorn, 2008) was a reliable study performed following OECD TG 406 under GLP and with no detected deviations. Benzocaine was used as a positive control. A first induction was performed with intracutaneous injection of 10% suspension of carbetamide in sesame oil and caused a discrete patchy erythema 24-48 hours after administration in vehicle control and carbetamide groups and intense erythema and swelling in positive control group. A second process of induction was performed 7 days after the first one. In this case, a suspension of 50% carbetamide in sesame oil was used preceded by 0.5 mL sodium lauryl sulfate. It caused discrete or patchy erythema in all animal groups (vehicle control, carbetamide and positive control). The challenge was applied 2 weeks after the second induction with a topical application (24 hours) of a suspension of 25% carbetamide in sesame oil. No skin irritation in any vehicle control or carbetamide animals was detected. On the other hand, 14 animals exposed to benzocaine as a positive control displayed discrete or patchy erythema and 6 displayed moderate and confluent erythema.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS that carbetamide does not meet the CLP criteria to be classified for Skin sensitisation since no positive response was found in the the two available sensitisation studies.

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

Summary of the Dossier submitter's proposal

The potential for repeated dose toxicity of carbetamide by the oral route has been investigated in rats, mice and dogs. No study was performed by inhalation or dermal application. No classification for Specific target organ toxicity after repeated exposure (STOT RE) was proposed by the dossier submitter since carbetamide is considered of low short-term toxicity in mammals.

According to the DS, the toxicity after repeated administration caused the following relevant/adverse effects:

- Slight clinical signs of neurotoxicity (unsteadiness, drowsiness and neurovegetative signs) were found only in dogs but not in rats;
- Slight hematotoxicity (reductions in red blood cell parameters and increases in platelet counts);
- The main target organ is liver (both rats and dogs) and thyroid (dogs).

Hepatocellular effects were slight to moderate increases in relative weight concurrent with hypertrophy in rat and in dogs and with brown pigment deposits in Kupffer cells of dogs.

The main effect on the thyroid was increases in relative weight at the dose-level of 300 mg/kg bw/day in dogs (M/F; 52-week study). This effect might be a secondary effect of the enzymatic induction in liver caused by carbetamide (see mechanistic study in carcinogenicity section). This enzymatic induction might increase the elimination of thyroid hormones in blood through the thyroxine-glucotransferase activity, which would reduce the concentration of thyroxine in blood. The increased thyrotropin production in the pituitary gland would subsequently cause hypertrophy, hyperplasia and neoplasia of the thyroid. This disturbance of the pituitary-thyroid-liver axis and thyroid pathology cannot be extrapolated from rodents and dogs to humans and other primates because the induction of liver enzymes does not cause dramatic decreases in thyroxine circulating levels. Humans and other primates express thyroxine-binding globulin, with an affinity for thyroxin 3-5 orders of magnitude higher than albumin and pre-albumin, the proteins involved in the binding of thyroxin in rats and dogs. Thyroxine that circulates in blood bound to this high affinity protein is not available to be conjugated by thyroxine-glucotransferase for subsequent urine excretion.

A table summarising the key information of the available information from the CLH report and the draft assessment report (DAR, Vol. 3, Annex B, B.6, part 1, July 2006) is presented in the background document (Supplemental information - In depth analyses by RAC).

The DS did not compare relevant/severe repeated dose toxicity effects with the (extrapolated) guidance values and did not assess the data available from the chronic (oncogenicity) toxicity studies in rats and mice. Hepatocellular effects were considered by the DS as adaptative and thyroid effects were considered not relevant for classification as STOT RE 1 or 2.

Comments received during public consultation

One MSCA supported the no classification for STOT RE but requested further discussion of the thyroid effects. Another MSCA proposed classification in Category 2 on the basis of the neurotoxicity detected in dogs. The DS replied that the effects were not of sufficient relevance for warranting classification.

Assessment and comparison with the classification criteria

To get a systematic overview on the carbetamide information critically relevant for the STOT RE classification, the following table with study-specific cut-off levels, the dose range tested and the most critical/severe effects at LOAEL is presented.

Table 1. Summary of repeated dose toxicity studies with carbetamide and comparison with STOT RE criteria

	STOT RE 1	**STOT RE 2**	**NOAEL and LOAEL**	**Significant/severe effects at LOAEL**
Rat 5 w	26	**260**	No NOAEL LOAEL = 193 mg/kg/day	Liver centrolobular hypertrophy (all males, no severity grade provided)
	STOT RE 1	**STOT RE 2**	**NOAEL and LOAEL**	**Significant/severe effects at LOAEL**
Rat 13 w	10	100	NOAEL = 12 mg/kg/day LOAEL = 119 mg/kg bw/day	None (centrolobular hepatocytic enlargement in 2/15 males, no severity grade provided)
Rat 2 y	1.25	12.5	NOAEL = 6-8 mg/kg/day LOAEL = 50-60 mg/kg bw/day	None at LOAEL
Mouse 2 y	1.25	12.5	NOAEL = 20.1-22.7 mg/kg/day LOAEL = 150.3-172.1 mg/kg bw/day	None at LOAEL
Dog 4 w	30	**300**	NOAEL = 75 mg/kg/day LOAEL= 150 mg/kg bw/day	- ↓ 43% bodyweight gain (female) - ↓ 16-30% food intake (male and female, respectively)
Dog 13 w	10	100	NOAEL = 30 mg/kg/day LOAEL = 300 mg/kg/day	Clinical signs, hematology, increased liver and thyroid relative weights
Dog 52 w	2.5	25	NOAEL = 3 mg/kg/day LOAEL = 30 mg/kg/day	Clinical signs, haemosiderin in Kupffer cells (no incidence or severity grade provided)

The only studies with LOAEL values below the cut-off values for triggering classification were the 5-week study in rats (liver effect) and the 4-week study in dogs (neurotoxicity, hepatotoxicity and toxicity to thyroid).

Liver effects were detected in rats at doses well above the limit for warranting classification in the 2-year study and slightly above of the limit in the 13-week study. The only study with hepatotoxicity below the limit for classification was the 5-week study, but in this case the severity grade was not indicated. The liver impairments in dogs were also well above the respective guidance values for classification. These facts all together suggest that the liver effects were probably adaptive responses and do not warrant classification.

The neurotoxicity in the dog studies was seen either above the guidance value for classification or, when around or below the guidance value, was of minimal and transient nature. Hence, this does not seem to fulfill the classification criteria.

Thyroid impairments were consistently found in all studies in dog. However, only in the 4-week study the LOAEL was found below the guidance values for classification. In this study, enlargement of the follicular epithelium was seen at 150 and 300 mg/kg bw/day and increase of the relative weight at 300 mg/kg bw/day (upper guidance value for justifying classification). Taking into consideration that the severity of the follicular epithelium enlargement was not

reported in the 4-week study and that this effect was not found in the 13- and 52-week studies; and that this 4-week study was performed with only 1 animal/dose, RAC is of the opinion that thyroid effects do not warrant classification.

In summary, in the available short- and longer term studies with Carbetamide, the liver (rat, mouse, dog), the thyroid and the nervous system (dog), the kidneys (variable and sex-dependent changes), the testes (rat) and the blood system (rat, dog) were identified as target organs. Considering the low incidence and severity of the non-neoplastic effects at the LOAEL and the fact that most LOAELs or severe effects were at doses well above the (extrapolated) guidance values for classification as STOT RE 2 in sub-chronic and chronic studies, RAC is the opinion that no classification is warranted for kidney, testes and blood system effects.

In conclusion, for all the above stated reasons, RAC agrees with the DS that **no classification for STOT RE** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In the CLH report, an array of *in vitro* and *in vivo* test for assessing the germ cell mutagenicity of carbetamide were reported by the DS. The available *in vitro* information includes two Ames Test, three chromosome aberration tests (two with mouse cells and one with human cells), one sister chromatid exchange test and one unscheduled DNA synthesis assay. The available *in vivo* information includes two micronucleus tests in mouse bone marrow and one unscheduled DNA synthesis assay.

The main conclusions drawn by the DS from studies acceptable for regulatory purposes were:

1. A positive result in two of the five strains of *Salmonella typhimurium* was obtained in a single assay in absence of metabolic activation. This result could not be reproduced in independent experiments. Thus, carbetamide had no mutagenic potential in *Salmonella typhimurium*;

2. Carbetamide caused significant increase in the frequency of mutations in mouse lymphoma cells in absence (but not in presence) of metabolic activation at cytotoxic concentrations but not at non-cytotoxic concentrations;

3. Carbetamide in absence and presence of metabolic activation did not significantly increase the frequency of mutations in human lymphoma cells;

4. Carbetamide did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after 1 or 2 oral administrations (with a 24-hour interval) at the dose-levels of 325, 650 and 975 mg/kg bw;

5. Carbetamide tested up to 975 mg/kg bw showed, in absence of systemic toxicity, no genotoxic properties in the mouse bone marrow micronucleus study at the two tested sampling times of 24 and 48 hours;

6. Carbetamide tested up to the maximum tolerated dose level of 2000 mg/kg bw in male CD rats showed no ability to induce unscheduled DNA synthesis at the two tested sampling times of 2 hours and 16 hours.

The results from studies considered not acceptable for regulatory purposes but supporting the above conclusions were:

1. Carbetamide showed no mutagenic effect against 4 different Salmonella typhimurium strains in a test conducted without metabolic activation.

2. Carbetamide did not increase the number of sister chromatid exchanges when tested in the presence or absence of metabolic activation in CHO cells.

3. Carbetamide did not induce significant changes in the nuclear labelling of primary rat hepatocytes when compared to solvent controls.

Thus, the overall conclusion of the DS is that carbetamide is not genotoxic either *in vivo* or *in vitro* and that it therefore does not fulfil the CLP criteria for classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

According to the criteria in the CLP Regulation, a substance should be classified in Category 1 if known to induce heritable mutations or to be regarded as if it induces heritable mutations in the germ cells of humans; while Category 2 is reserved for those substances causing concern for humans.

Studies with carbetamide have not provided positive evidences neither *in vivo* nor *in vitro* of inducing heritable mutations in germ cells.

Thus, RAC agrees with the DS's proposal of **no classification for mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The CLH report contains information for assessing carcinogenicity through a combined chronic toxicity/oncogenicity study in rats and one oncogenicity study in mice both by dietary administration during 2 years. A complementary study assessing the mechanisms of liver tumour induction in mice is also included.

Carbetamide at 9000 ppm induced astrocytoma in female rats and was carcinogenic in mice at 1200 ppm (hepatocellular tumour in both sexes) and 9000 ppm (hepatocellular tumours in both sexes, cholangiocarcinoma in males, and thyroid adenomas and pheochromocytomas in females.

In the view of the DS, the increased liver weights in rats, the hyperplasia of thyroid epithelial cells in rats, and the carcinogenic effects in mice may be related to an induction of hepatic CYP450, and to a secondary hyper-stimulation of the thyroid gland correlated with an increased elimination of thyroid hormones by glucurono-conjugation in the liver. In a supporting mechanistic study, carbetamide led to a liver induction pattern similar to that induced by phenobarbital, CYP2A and CYP2B activities being most strongly induced. The mode of action (MoA) for phenobarbital-like P450 inducers was determined to be unlikely by ICPS in humans after kinetic and dynamic factors were considered. The DS considered that the proposed MoA for the generation of liver and thyroid tumours is plausible considering the mechanistic data submitted. Thus, hepatocellular carcinoma/adenoma as well as thyroidal follicular adenomas should be disregarded for carcinogenicity classification.

However, at the highest dose of carbetamide (exceeding MTD) several rare tumours, including carcinomas, occurred in different tissues (brain astrocytoma, liver cholangiosarcoma and adrenal phaeochromocytoma) in mice and rats. The tumour incidences were all above the available historical control ranges.

The DS proposed that carbetamide fulfils the criteria for classification as Carc. 2 (H351).

Comments received during public consultation

One MSCA supported the proposed classification as Carc. 2 based on the concern for rat astrocytomas and the previous RAC decision on aclonifen. They also raised doubts about the non-relevance of hepatocellular tumours in mice due to the lack of the comparison with historical controls of the same laboratory that performed the carcinogenicity assays and the limited mechanistic evidence. The DS replied that the historical controls of the laboratories where the tests were done were not available and that the mechanistic study (see Annex 2) included in the CLH report showed that carbetamide is an inducer of a variety of hepatic cytochrome P450 enzymes that supports a MoA similar to phenobarbital, which according to IPCS should not be considered relevant to humans (IPCS, 2006).

The manufacturer submitted comments during public consultation. These comments were accompanied by three documents produced by a Pathology Working Group (PWG) which covered one overall discussion and two detailed reports, one dealing with brain tumours and the other liver and adrenal tumours. The manufacturer convened a Pathology Working Group (PWG)to re-evaluate the pathology findings by according to state of the art diagnostic criteria. The PWG was formed by six senior independent consultant pathologists with expertise in the evaluation and interpretation of rodent toxicity and carcinogenicity studies. The chairperson of the PWG also submitted several comments during PC arguing against the relevance of the detected tumours for classification purposes on the basis of these three reports. The DS evaluated these three new documents and their main conclusions are summarised below:

- *Mouse adrenal gland tumours.* Only one of the malignant pheochromocytomas was confirmed, while the second one initially diagnosed was reconsidered as a benign phaeochromocytoma. In this scenario, the DS considered the incidence of this tumour (1/48 = 2.1%) in the 9000 ppm dose group of female mice as an incidental finding as this incidence is within the historical control range reported by other laboratories for this malignant tumour.

- *Mouse cholangiocellular carcinoma.* None of the three initially diagnosed cases was confirmed. These tumours were reclassified as liver hepatoblastomas. A new case, not previously diagnosed, was also assigned to this category. Thus, according to the PGW, male mice exposed to 9000 ppm carbetamide exhibited four cases of hepatoblastoma. The DS considered that these tumours can be related to the phenobarbital-like MoA for the induction of hepatocellular neoplasm and do not represent tumours relevant for humans.

- *Rat brain tumours.* The initially diagnosed ganglioneuroma was reclassified by the PGW as astrocytoma, an extremely rare tumour. Thus, the incidence of malignant astrocytoma in rat females exposed to 9000 ppm carbetamide was 3/60 (5%), which is well above the historical control given as overall incidences and ranges reported by several databases. The DS considered that their concern was additionally supported by a previous RAC opinion on aclonifen, where a slightly higher incidence of astrocytoma observed in female rats led to the classification for Carcinogenicity in Category 2.

The DS, after a complete evaluation of the PWG's information confirmed their proposal for classification of carbetamide as Carc. 2 (H351) (see Annex 2: Comments received on the CLH report, response to comments provided by the Dossier Submitter and Rapporteurs' comments).

Assessment and comparison with the classification criteria

The table below presents a summary of all available information regarding tumours occuring in different organs of mice treated with carbetamide as recorded i.e. from the original study pathologist (SP), from the PWG reports (PWG, 2013a; PWG, 2013c) received during PC and the historical control data from the US NTP.

Table 2. Summary of neoplastic findings in mice treated with carbetamide.

Group	control		160 ppm		1200 ppm		>MTD 9000 ppm		Historical control (NTP database)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	15	13	10	14	8	10	8	4		
Body weight gain (%)			-5	-1	+1	+3	-43	-42		
Neoplastic lesions (no. examined)	52	52	52	52	52	52	52	52		
LIVER										
B-Hepatocellular adenoma										
SP	12	1	7	4	12	7* (13.5%)	27* (51.9%)	22* (42.3%)	18-60%	12-50%
PWG	14	4	12	8	18 (34.6%)	12 (23.1%)	34 **(65.4%)**	24 (46.2%)		
M-Hepatocellular carcinoma										
SP	16	3	10	4	9	2	22 (42.3%)	6 (11.5%)	10-40%	4.1-20%
PWG	16	4	9	4	11	2	19 (36.5%)	6 (11.5%)		
M-Cholangiocellular carcinoma										
SP	0	0	0	0	0	0	3 # (5.8%)	0	0-2%	
PWG	0	0	0	0	0	0	0	0		
M-Hepatoblastoma										
SP	0	0	0	0	0	0	0	0		
PWG	0	0	0	0	0	0	4 **(7.7%)**	0	0-2%	
ADRENAL GLAND										
B-Phaeochromocytoma										
SP	1	0	0	0	0	0	0	0		
PWG	1	0	0	0	0	0	0	1 (1.9%)		0-4%
M-Phaeochromocytoma										
SP	0	0	0	0	0	0	0	2 $ (3.8%)		
PWG	0	0	0	0	0	0	0	1 (1.9%)		0-2%
THYROID										
B-Follicular adenoma	0	1	1	1	0	0	1	5 **(9.6%)**		0-5.9%
PITUITARY										
B Adenoma	0	4	0	6	0	6	0	9 (17.3%)		0-22.9%

* stat. sign.; # re-diagnosed by PWG as hepatoblastomas (as well as one case of a "carcinoma from miscellaneous"); $ 1 out of 2 was re-diagnosed by PWG to be benign; in bold: incidence outside historical control range

The table below shows an integration of all available information regarding tumours occuring in different organs of rats treated with carbetamide as recorded i.e. from the original SP, from the PWG reports (PWG, 2013b; PWG, 2013c) received during PC and the historical control data from the US NTP.

Table 3. Summary of neoplastic findings in rats treated with carbetamide.

Group	**control**		**160 ppm**		**1200 ppm**		**9000 ppm**		**Historical control** (NTP database)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	21	10	28	15	24	15	20	14		
Body weight gain (%)			-3	+1	-4	0	-33	-19		
Neoplastic lesions (no. examined)	60	60	60	60	60	60	60	60		
BRAIN										
M-Astrocytoma SP	0	0	0	0	0	0	0	2 (3.3%)		0-2%
PWG	0	0	0	0	0	0	0	3 **(5%)**		
M-Ganglioneuroma SP	0	0	0	0	0	0	0	1*		
PWG	0	0	0	0	0	0	0	0		

* re-diagnosed by PWG as astrocytoma; in bold: incidence outside historical control range

The analysis of the available information by RAC shows that:

1) The DS interpreted the dose of 9000 ppm in the mouse study and the rat study as above the MTD. However this is unclear. Indeed, the information on the body weight gain alone is not conclusive. As the mortality rates in high dose males and females in the mouse study were lower than those of the control groups and comparable in the rat study to the control values, this and the absence of any severe clinical symptoms indicating significant non-specific toxicity leaves it open as to whether the MTD was exceeded or not.

2) Rat astrocytomas occurred in female mice with an incidence higher than concurrent controls and higher than the reported historical controls of several laboratories at doses causing lower bodyweight gain (19%) compared to the control group.

3) Hepatocellular adenomas occurred in male mice at the top dose only and females at the mid- and top-doses with incidences statistically significantly higher than controls. The mechanism leading to these tumours was ascribed by the DS to a mode of action based on the capability of carbetamide to induce sustained expression of liver cytochrome P450 enzymes likely via interaction with the CAR or PXR-receptor. This has been considered by IPCS framework of the World Health Organization (WHO) IPCS (IPCS, 2006) as not relevant for humans. However, RAC but assesses such proposals on a case-by-case basis. The evidence for this mode of action is weak because at least the following data are missing: i) activation of the CAR nuclear receptor; ii) hepatocyte proliferation, and; iii) evidence that other modes of action are not operative. In addition to hepatocellular adenomas and adenocarcinomas, hepatoblastomas, the latter a rare tumour type of the liver, were observed in 4 (7.7%) of high dose males and raised concern that the observed liver tumours were not related to a species-specific modes of action.

4) Thyroid follicular adenomas in female mice occurred with incidences higher than concurrent control and historical controls of several laboratories. It occurred at doses causing lower body weight gain (42% compared to the control group) and might be attributable to the disturbance of the pituitary-thyroid-liver axis. ECHA CLP guidance (November 2013) states that "Certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999; EU Specialised Experts, 1999)" are not relevant to humans. It was not demonstrated that the MoA proposed here is UGT mediated. In addition, the specialised experts excluded "liver enzyme inducing agents such as phenobarbital" from their recommendation. Although there is a plausible MoA suggested for induction of thyroid tumours via CAR mediation, the evidence of such tumours is scarce. Based on the limited data provided, RAC can not conclude whether a CAR mediated MoA is contributing to the formation of thyroid tumours or not. Indeed, other MoAs have not been excluded (e.g. thyroid peroxidase). Nor has direct cytotoxicity and thyroid hormone disturbances been investigated. RAC does not support the conclusion

of the DS that the benign thyroid follicular adenomas would not pose a cancer hazard to humans. The human relevance of thyroid tumours can not be excluded due to the lack of data on the underlying MoA and lack of data to assess the MTD, and therefore the thyroid tumours were taken into account for classification.

5) Adrenal phaeochromocytoma in females mice with incidences higher than control, and in the border line of the historical control records of other laboratories, but at doses causing lower body weight gain of 42% compared to the controls. Incidence of benign and malignant phaechromocytomas were very low (1 case each) leading to uncertainties whether these tumour are treatment-related.

RAC based its conclusions on carcinogenicity on two available studies, a 2-year study in rats and a 2-year study in mice. The neoplastic lesions observed in the liver at the mid and high dose groups in the mouse study may be considered relevant for classification due to the fact that the evidence for the the postulated CAR or PXR-receptor mediated MoA is absent. The thyroid tumours occurring in female mice was also ascribed to CAR or PXR-receptor mediation, although RAC notes that some of the evidence necessary to draw such a conclusion is missing. Therefore, these tumours should be taken into consideration for classification purposes. As they occur at doses where body weight gain was lower than in the respective controls, the DS concluded that they appeared at doses clearly over the MTD. However clear evidence that the MTD was exceeded is missing for both studies.

RAC considers that the evidence of carcinogenicity for carbetamide is limited, on the basis of the following arguments:

- The lack of data demonstrating with confidence that the liver and thyroid tumours in mice are consistent with CAR or PXR-receptor mediated MoA. The occurrence of hepatoblastomas as a tumour rarely observed in mice has to be taken into account.
- The abnormal incidence of brain astrocytomas might be of concern for humans due to:
  - the rarity of this tumour type
  - the absence of mechanistic information
  - a lower body weight gain of 19% alone without any other significant sign of toxicity such as loss of body weight or severe clinical symptoms is unlikely to cause brain tumours
  - it is on-line with former RAC decision for this type of tumours with similar records
  - EFSA has considered carbetamide as a non-genotoxic carcinogen (EFSA, 2010).

On the other hand, when comparing the neoplastic effects of carbetamide with the CLP criteria for classification in category 1B (Carc. 1B), RAC also notes that:

- Carbetamide is not genotoxic.
- Rat astrocytomas were observed in conjunction with:
  - No dose-effect relationship was observed.
  - Astrocytomas were observed in one species only.
  - No benign astrocytomas (a precursor of malign tumour) were observed.
  - The incidence observed in females treated with 9000 ppm carbetamide is only slightly above the historical controls for females in Charles River and NTP laboratories.
  - Tumours appeared only in females, while toxicokinetics studies have not revealed sex-related differences in metabolism of carbetamide.

In conclusion, RAC supports the DS opinion that carbetamide warrants classification for Carc. 2 - H351 (Suspected of causing cancer)

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The reproductive toxicity was assessed by the DS on the basis of an oral rat multigenerational study and two oral teratogenicity studies (one in rat and one in rabbit).

Sexual function and fertility

A two-generation reproductive study in rats (Tesh *et al.*, 1987) was performed under GLP but no OECD test guideline was stated. CD rats received 1000, 3000 or 7500 ppm carbetamide in the diet, resulting in the following mean doses:

	Male			Female		
Concentration (ppm)	1000	3000	7500	1000	3000	7500
F0 (mg/kg/ bw/day)	65	194	506	74	221	570
F1 (mg/kg/ bw/day)	82	253	676	91	284	749

Mortalities seen were not treatment-related and there were no clinical signs of toxicity. Bodyweights were lower (20-23% in F0 and 12-11% in F1) in both sexes given 7500 ppm than in controls, and correlated with a minimally lower (-6%) food intake.

Relative liver weights were higher (17-22%) than in controls in males of the F0 and F1 generations treated at the highest dose-level and in females (7-27%) treated at all dose-levels. These changes in organ weights were associated with centrilobular hepatocyte hypertrophy in males and females treated at 3000 and 7500 ppm but no other treatment-related macroscopic abnormalities could be detected.

Oestrous cycles, precoital intervals, mating, conception rate, fertility index and gestation index were not affected by treatment and there were no cases of dystocia. Gestation length was normal in all F0 groups, but was minimally longer in F1 (3000 and 7500 ppm) for the first pregnancy and F1 (3000 ppm) for the second, but this was not associated with changes in litter size, foetal viability or birth weight.

Sex ratio, litter size, live birth index and pup weight were not altered by carbetamide neither in F1 nor in F2. Litter size and birth viability were not altered in any litter by day 4 after birth. Until weaning, offspring body weight gains were lower in all litters of the highest dose-level group (up to -16%) when compared to controls. At necropsy of all litters and at skeletal examination, there were no treatment-related abnormalities.

Higher relative liver weights were observed in male and female pups of the F2B generation treated at dose-levels of 3000 and 7500 ppm, which could be associated with incidences of peroacinar hepatocytic hypertrophy in males and females exposed to 7500 ppm.

In conclusion, no NOAEL could be determined for parental toxicity. The LOAEL for parental toxicity was 1000 ppm because effects observed at the lowest dose level were minimal and not associated with lesions at histopathological examination of the liver.

The NOAEL for toxicity to reproduction was 3000 ppm (194 mg/kg bw/day for males and 221 mg/kg bw/day for females), while the LOAEL of 7500 ppm (506 mg/kg bw/day for males and 570 mg/kg bw/day for females) was associated with lower body weight gain up to weaning in all litters treated at the highest dose-level, and liver enlargement associated with centrilobular hepatocyte hypertrophy in F2B pups treated at the highest dose-level.

The DS concluded that the reproductive parameters were not affected by carbetamide exposure and that carbetamide should not be classified for effects on sexual function and fertility.

Developmental toxicity

A teratology study of carbetamide in rats (Tesh *et al.*, 1985) was performed under GLP but no OECD test guideline was stated. CD rats received 150, 450 or 1000 mg/kg bw/day. No deaths occurred during the study and the only observed clinical sign was occasionally increased salivation in females treated with 1000 mg/kg bw/day. This group also showed a lower bodyweight gain. There were no significant differences in food or water intake. Necropsy of adults showed no treatment-related findings.

Litters presented no treatment-related finding in terms of pregnancy rate (100% of all females), implantations, live and dead foetuses, pre- and post-implantation losses, and mean placental weight. Mean foetal bodyweight was markedly lower when females were treated at 1000 mg/kg bw/day (-18% compared to controls).
Foetuses from the 1000 mg/kg bw/day group showed higher incidences of signs of immaturity (dilatation of brain ventricles, space between body wall and organs, subcutaneous haemorrhages and reduced ossification of cranial bones, sternebrae, vertebrae, metacarpals/metatarsals and pubic bones), which can be interpreted as a sign of toxicity. They also presented higher incidences of darkened thyroid glands, several arterial or cardiac defects (no historical control data available), elongated genital tubercle, imperforated anus and vestigial/absent tail. The skeletal lesions also included fused sternebrae, higher number of ribs and presacral vertebrae and asymmetric pelvis.

At the mid dose some observations were considered to be indicative of a marginal adverse foetal response to carbetamide. One out of 88 foetuses (1.11%) of the mid dose group showed darkening of the thyroid glands. In the offspring of high dose dams, 15 of 86 foetuses showed this effect (17.4%). According to the DS, the manufacturer argued that this effect is marginal since it does not affect the final development and maturity of the thyroids and was not reproduced in the two-generation study performed in the same lab with the same rat strain.

In conclusion, according to the DS, the NOAEL for both maternal and developmental toxicity was 450 mg/kg bw/day, while the LOAEL for both maternal and developmental toxicity was 1000 mg/kg bw/day based on lower parental body weight gain (maternal) and foetal immaturity (ossification retardation, soft tissue variation, complex malformations associated with elongated genital tubercle, imperforate anus, vestigial/absent tail and cardiovascular malformations). The DS concluded that carbetamide is clearly teratogenic in rats at the maternally toxic dose of 1000 mg/kg bw/day.

A teratology study of carbetamide in rabbit (Tesh *et al.*, 1986) was performed under GLP but no OECD test guideline was stated. New Zealand White (NZW) rabbits received 5, 40 or 320 mg/kg bw/day of carbetamide. No deaths occurred during the study and the only observed clinical signs were unsteadiness, inactivity and increased respiration rate in females treated with 320 mg/kg bw/day. Necropsy of adults showed no treatment-related findings apart from accentuated lobular pattern of the liver.

Two females in the high dose group (320 mg/kg bw/day) aborted, without any specific finding at necropsy. The same group showed a higher incidence of post-implantation loss (almost 4-fold compared to control values. The other litter parameters were unaffected by treatment, except for a lower litter size in the highest dose-level group (-26% when compared to controls), but within historical control values.

There was an increased incidence of skeletal abnormalities in foetuses of treated females at the top dose level: signs of immaturity (incomplete ossification of vertebrae and long bones), higher number of ribs and presacral vertebrae were recorded.

The occurrence of a 13^{th} pair of ribs in the offspring of dams treated at 40 mg/kg bw/day was 40.9% compared to 27.9% in control animals, and 27.7% at 5 mg/kg bw/day (but still in the range of historical control data). It was 86.3% at the top dose level. Thus, the incidence of 13^{th} pair of ribs at 40 mg/kg bw/day is slightly above the mean value for the spontaneous occurrence

(35.9%) but clearly and severely above at 320 mg/kg bw/day. Furthermore there is a clear dose response relationship.

The incidence of incomplete ossification of cervical vertebrae in the high dose group is above the historical range, however this dose level caused slight maternal toxicity. A dose response relationship for incomplete ossification of cervical vertebrae was observed from the dose of 40 mg/kg bw/day. This observation could be considered indicative of an adverse foetal response to carbetamide.

In conclusion, according to the DS, the NOAEL for maternal toxicity was 40 mg/kg/day, and embryotoxic effects were observed at 40 and 320 mg/kg/day, resulting in a NOAEL for foetal toxicity of 5 mg/kg/day. The DS concluded that carbetamide should be classified for developmental toxicity as Repr. 2 (H361d).

Comments received during public consultation

One MSCA commented that classification of carbetamide as toxic to reproduction Category 1B (H360D) cannot be disregarded.

Another MSCA supported the DS's proposal for no classification of carbetamide for fertility but disagreed with the classification of Category 2 and proposed classification of carbetamide as toxic to reproduction Category 1B (H360D).

Assessment and comparison with the classification criteria

Sexual function and fertility

Reproductive parameters were not affected by carbetamide. Thus, RAC agrees and supports the proposal of the DS for no classification of carbetamide for effects on sexual function and fertility.

Developmental toxicity

The table below displays the main parameters of pregnant rats treated with carbetamide during the organogenesis period, and their foetuses. Values in bold shows incidences that were above the historical control data.

Table 2. Summary of developmental findings in rats treated with carbetamide

Group		**1**	**2**	**3**	**4**	**Historical control data– Study range**
Dose-level (mg/kg/day)		**-**	**150**	**450**	**1000**	
MATERNAL OBSERVATIONS						
Body weight gain (g)	Days 6-15	50	51	49	47	
	Days 15-21	73	74	71	**66**	
Pregnant		20/20	26/26	20/20	20/20	
N° with viable young		20/20	26/26	20/20	20/20	
LITTER OBSERVATIONS						
Live young	Males	6.7	7.6	7.4	6.5	
	Females	7.0	6.2	5.5	6.3	
	Total per dam	13.6	13.8	12.9	12.8	
Resorptions	Early	0.6	0.5	0.9	0.7	
	Late	0.2	0.1	0.2	0.2	
	Total	0.8	0.5	1.0	0.9	
Mean pre-implantation loss (%)		7.4	9.5	7.3	8.7	
Mean post-implantation loss (%)		5.2	3.8	7.2	6.6	
Mean foetal weight (g)		3.21	3.24	3.22	**2.64**	
Signs of immaturity						
Observations: % foetal incidence (number of litters)						
Slight dilatation of brain ventricles		0	0.8 (1)	0	**23.3** (6)	0-18
Space between body wall and organs		7.6 (5)	5.9 (2)	8.0 (5)	**65.1** (16)	2.1-47.4
Subcutaneous haemorrhages (nasal, cranial, limbs: extreme %)		2.2-8.7	0-7.6	2.3-6.8	**15.1-20.9**	0-11.5
Incomplete ossification of cranial bones (supraoccipital, interparietal, frontal: extreme %)		0-20.6	0-23.8	0.6-19.4	7.6-**74.1**	0-50.5
Incomplete ossification of 4 or more sternebrae		8.9	5.4	9.4	**44.1**	0-17.5
Incomplete ossification of vertebrae (various locations: extreme %)		0-27.8	0-36.7	0.6-37.1	2.4**-81.8**	0-58.3
Incomplete ossification of metacarpals/metatarsals		1.1 (2)	4.6 (6)	4.1 (4)	**15.9** (13)	0-9.2
Incomplete ossification of pubis		13.3 (10)	8.8 (12)	6.5 (6)	**26.5** (17)	0-18.6
Skeletal abnormalities						
Fused sternebrae		0	0	0	**1.2** (2)	0-0.5
Foetuses with higher number of ribs (14/14 or 14/15)		0	2.9 (5)	3.5 (6)	**93.6** (20)	0-3.5
Foetuses with higher number of presacral vertebrae (27)		0	0	0	**80.9** (20)	0-1.1
Asymmetric pelvis		0.6 (1)	0	0.6 (1)	**3.5** (5)	0-1.8
Vestigial/absent tail		0	0.8 (1)	0	**4.7** (3)	0-3.6
Other abnormalities						
Darkened thyroid glands		0	0	1.1 (1)	17.4 (7)	-
Cardiovascular defects (arteries, aortic arch, cardiac septum: extreme %)		0-0	0-0	0-0	**1.2-5.8**	0-1.2
Elongated genital tubercle		0	0	0	**18.6** (8)	0-3.6
Imperforate anus		0	0.8 (1)	0	**7.0** (5)	0-1.8

The table below displays the main parameters of pregnant rabbits treated with carbetamide during the organogenesis period, and their foetuses. Values in bold shows incidences that were above the historical control data.

Table 3. Summary of developmental findings in rabbits treated with carbetamide

Group		**1**	**2**	**3**	**4**	**Historical control data– Study range**
Dose-level (mg/kg/day)		**-**	**5**	**40**	**320**	
MATERNAL OBSERVATIONS						
Body weight gain (g)	Days 6-20	0.08	0.04	0.02	-0.09	
	Days 20-28	0.07	0.05	0.12	0.20	
Pregnant		14/15	12/15	15/15	15/15	
N° with viable young		13/15	12/15	15/15	13/15	
LITTER OBSERVATIONS						
Live young	Males	5.1	3.7	4.4	3.2	
	Females	4.8	4.8	4.1	4.2	
	Total per dam	9.9	8.4	8.5	7.3	
Resorptions	Early	0.0	0.4	0.3	0.2	
	Late	0.7	0.4	0.6	2.2	
	Total	0.7	0.8	0.9	2.4	
Mean pre-implantation loss (%)		11.0	23.4	16.1	14.9	
Mean post-implantation loss (%)		6.5	9.0	9.9	**24.6**	
Mean foetal weight (g)		36.9	36.5	38.7	36.2	
Signs of immaturity						
Observations: % foetal incidence (number of litters)						
Incomplete ossification of vertebrae	Cervical	3.9 (4)	1.0 (1)	7.1 (5)	**23.2** (8)	0.0-9.9
	Thoracic	0.8 (1)	0	1.6 (2)	0	0.0-11.5
	Caudal	0.8 (1)	0	0	0	0.0-1.3
	Pooled data	5.5	1.0	8.7	**23.2**	0.0-22.7
Incomplete ossification of long bones		69.0 (13)	73.3 (12)	65.4 (15)	**82.1** (13)	1.9-66.3
Skeletal abnormalities						
Foetuses with higher number of ribs (13/13)		27.9 (11)	27.7 (9)	40.9 (12)	**86.3** (13)	11.9-61.0
Foetuses with higher number of presacral vertebrae (27)		20.2 (12)	21.8 (9)	15.0 (9)	**72.6** (13)	7.2-44.3

In summary, carbetamide altered foetal development in two different species (rabbit and rats). In both species, the developmental effects consisted of generalized foetal immaturity mainly with delayed ossification. Moreover, darkening of the thyroid glands that seems not to affect the final maturity of the thyroid was also observed in the rat stuy while in the rabbit study abortions and post-implantation losses were noted.

RAC considers the cardiovascular defects and the vestigial/absence tail reported in rats to be especially relevant for classification because these defects cannot be explained by developmental delays or inmaturity. RAC made reference to the EFSA peer review report on carbetamide (EFSA, 2010) mentioning "severe and complex malformations" in rats. The developmental impairments appeared at doses with slight maternal toxicity in rats (10% reduction of body weight gain and occasional episodes of salivation) and mild maternal toxicity in rabbits (unsteadiness, inactivity and increased respiratory rate and lobular liver).

RAC is of the opinion that carbetamide is a presumed human reproductive toxicant and proposes classification as Repr. 1B for developmental toxicity (H360D: May damage the unborn child) based on the following arguments:

- Animal studies provided clear evidence of adverse and severe effects on development, as skeletal and visceral abnormalities, or cardiocvascular effects. Teratogenicity appeared in two different animal species.
-

- The maternal toxicity was not high enough to consider a relationship with development impairments, especially in rats where the decrease in bodyweight gain appeared after carbetamide administration and might be partially linked to the decrease in foetal weight. Therefore, if the decrease of bodyweight gain disregarded, the teratogenicity in rats appeared only in concurrence with transient episodes of salivation in mothers.
- The incidence of post-implantation loss was 4 times higher than in controls and cannot be justified only on the basis of mild maternal toxicity in rat/rabbits.
- There is no mechanistic information to conclude that the effects are not relevant for humans.
- The CLP criteria does not set an upper limit value for classification and therefore, the fact that critical effects appeared in rats at doses of 1000 mg/kg bw/day does not preclude the possibility of classification.

Therefore, RAC is of the opinion that classification of carbetamide as **Repr. 1B (H360D: Suspected of damaging fertility or the unborn child)** is justified.

RAC evaluation of aspiration toxicity

Summary of the Dossier submitter's proposal

No information was supplied in the CLH report.

Comments received during public consultation

None.

Assessment and comparison with the classification criteria

The CLH report does not contain information suitable for setting aspiration toxicity classification. In the absence of data, RAC has not assessed this hazard class.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Carbetamide does not currently have a harmonised classification according to CLP. The DS proposed to classify the substance as Aquatic Chronic 2 (H411). The proposal is based on the lowest chronic NOEC (21d) for *Daphnia magna* of 1.0 mg/L (nominal concentration). The DS concluded that carbetamide does not require acute classification given the lowest EC_{50} is higher than 1 mg/L (48 h EC_{50} = 81 mg/L). Furthermore the DS considered carbetamide not rapidly degradable and non-bioaccumulative.

Comments received during public consultation

One MSCA commented on the CLH proposal of carbetamide during public consultation. The CA stated a general agreement with the proposed environmental classification as Aquatic Chronic 2 but requested more information on the type of concentrations (nominal or measured) used for the determination of endpoints and on details of toxicity tests other than the key studies. The DS

replied that the concentrations in the CLH report tables are nominal and further study details on the available ecotoxicity tests are available in relevant sections in the DAR.

Assessment and comparison with the classification criteria

Degradability

In a well-conducted hydrolysis study performed before OECD TG 111 was published, it was determined that the hydrolytic stability of carbetamide in water depends on temperature and pH conditions. At pH 3 and pH 6 (25ºC) carbetamide was hydrolytically stable (< 10% degraded after 1 month; half-life not determined). At pH 9, degradation was faster resulting in a DT_{50} of 21 days (25ºC). The main metabolites were 10810 RP and aniline (16% and 40% of initial applied radioactivity (AR), respectively). In conclusion, hydrolysis is not an expected transformation route for carbetamide. It was not indicated in the CLH report why the hydrolysis metabolites 10810 RP and aniline were not considered further by the DS for classification. RAC understands the reason is that these metabolites were only detected at pH 9 which is not an environmentally relevant condition in European water ecosystems. RAC notes that they were not further considered in the risk assessment performed by EFSA either.

Two studies on ready biodegradability were available. Based on OECD 301D criteria (i.e. > 60% removal of theoretical oxygen demand (ThOD) within 28 days) both studies showed that carbetamide was not readily biodegradable under test conditions (maxiumn ThOD was below 8% in both studies). A simulation test on aerobic degradation in two water/ sediment systems (river and stream) was available. An updated kinetic fitting following FOCUS Kinetics GD estimated DT_{50} of 33.6 and 81 days (river and stream, respectively) for the whole system. Mineralisation accounted for 20 – 29% AR at the end of the study, i.e. after 100 days, leading to the conclusion that carbetamide did not meet the criteria to be ultimately degraded (> 70% degradation within 28 days, i.e. half-life < 16 days). No major metabolites were reported.

Therefore, RAC agrees with the DS's proposal to consider carbetamide as not rapidly (nor readily) degradable.

Bioaccumulation

Based on experimental data, carbetamide has a log K_{ow} value of 1.78 at pH 7. This value is below the CLP criterion (i.e. log Kow ≥ 4) to consider the substance as potentially bioaccumulative. An experimental BCF study was not available.
RAC agrees with the DS's proposal to consider carbetamide a non-bioaccumulative substance for classification purposes.

Aquatic toxicity

Acute aquatic toxicity studies for both the parent substance carbetamide and the metabolite carbetamide-COOH were available for fish, invertebrates, algae and aquatic plants. Chronic aquatic toxicity studies on carbetamide were available for invertebrates, algae and aquatic plants while for the metabolite carbetamide-COOH there were chronic studies on algae, macrophytes and sediment-dwelling organisms. All values were based on nominal concentrations. Information on the analytical concentrations were provided only for the two key studies for classification (invertebrates); the measured concentrations were above 80% of the nominal concentration. Thus, according to the requirement in OECD TGs the nominal concentrations can be used to determine the endpoints. Furthermore carbetamide stability towards abiotic and biotic degradation and the low adsorption coefficient (mean Koc = 88.6 mL/g) would not indicate the need for measured concentrations.

The toxicity to fish was estimated with two acute tests; one on *Oncorhynchus mykiss* and another one on *Cyprinus carpio*. In both tests the reported LC_{50} values were higher than 100 mg/L. In addition, a prolonged acute toxicity test on *O. mykiss* following OECD TG 204 was reported (Douglas, 1989). The study was extended to 21 days instead of the usual 14 days in the guideline.

The DS considered this as long-term test but the CLP guidance does not recognise this study as a chronic test. Hence, although the test duration was extended to 21 days RAC did not accept the acute NOEC being considered for long-term hazard classification.

Regarding toxicity to invertebrates, one acute test (*Daphnia magna*) was available for carbetamide and one for carbetamide-COOH. The most sensitive acute toxicity result was the EC_{50} (48 h) of 81 mg/L for carbetamide. A chronic study was also available (*D. magna*) for carbetamide with a chronic NOEC (21 d) of 1.0 mg/L (based on parental survival) and of 10 mg/L (based on reproduction) indicating that aquatic invertebrates are the most sensitive trophic level for chronic aquatic toxicity.

Carbetamide toxicity toward algae was tested in two species; the 72h E_rC_{50} values were 305 mg/L (*Scenedesmus subspicatus*) and 202 mg/L (*Navicula pelliculosa*), while the NOE_rC values were 125 and 50 mg/L respectively. An additional algae toxicity test was performed on *Desmodesmus subspicatus* using carbetamide-COOH; the 72h EC_{50} and NOE_rC values were > 100 and 100 mg/L respectively.

Studies on aquatic macrophytes were also available for both carbetamide and carbetamide-COOH. For carbetamide the E_rC_{50} (7 d) was 301 mg/L and NOE_rC (7 d) was 100 mg/L, based on change in fronds number produced with *Lemna minor*.

The DS concluded that the most sensitive trophic level was invertebrates for both acute and chronic effects. Therefore the *D. magna* studies for carbetamide were regarded as key studies for classification. Only for these studies details were presented in the CLH report. More details on the other studies should have been added to the CLH report to make data evaluation easier. Furthermore, RAC concludes that the full chronic dataset was not available as it did not consider the available prolonged acute fish toxicity test (according to OECD TG 204) as chronic study.

A summary of the relevant aquatic ecotoxicity results for carbetamide and its metabolite carbetamide-COOH is presented in the Table 4 below (the key studies for classification are highlighted in bold).

Table 4. Summary of relevant aquatic ecotoxicity results for carbetamide and its metabolite

Method	Species	Results	Remarks	Reference
Fish				
OECD 203	*Oncorhynchus mykiss*	LC_{50} (96 h) > 100 mg/L NOEC (96 h) = 50 mg/L (nominal)	Purity:96.5%	Memmert, U. & Knoch, E. (1994a)
OECD 203	*Cyprinus carpio*	LC_{50} (96 h) > 100 mg/L NOEC (96 h) = 46 mg/L (nominal)	Purity:96.5%	Memmert, U. & Knoch, E. (1994b)
OECD 203	*Oncorhynchus mykiss*	LC_{50} (96 h) > 100 mg/L NOEC (96 h) = 100 mg/L (nominal)	Study conducted with Carbetamide-COOH Purity:99.0%	Gonsior, G. (2008)
OECD 204	*Oncorhynchus mykiss* (formerly *Salmo gairdneri)*	LC_{50} (21 d) > 100 mg/L* NOEC (21 d) = 32 mg/L (nominal)	Purity: 95.0%	Douglas, M. T. *et al.* (1989)
Aquatic invertebrates				
OECD 202	*Daphnia magna*	**EC_{50} (48 h) = 81 mg/L** NOEC (48 h) = 13.3	Purity: 96.8%	Knacker, Th. & Hilt, J. (1989)

		mg/L (nominal)		
OECD 202	*Daphnia magna*	EC_{50} (48 h) > 100 mg/L NOEC (48 h) = 10 mg/L (nominal)	Study conducted with Carbetamide-COOH Purity: 99.0%	Bormann, K. (2008)
OECD 202	*Daphnia magna*	**NOEC (21 d) = 1.0 mg/L** (nominal)	Purity: 96.5%	Memmert, U. & Knoch, E. (1994c)
Algae and aquatic plants				
EEC 92/69/EEC C.3	*Scenedesmus subspicatus*	E_bC_{50} (72 h) = 158 mg/L E_rC_{50} (72 h) = 305 mg/L NOE_rC (72 h) = 125 mg/L (nominal)	Purity: 98.8%	Scheerbaum, D. (2003)
EEC 92/69/EEC C.3	*Navicula pelliculosa*	E_bC_{50} (72 h) = 128 mg/L E_rC_{50} (72 h) = 212 mg/L NOE_rC (72 h) = 50 mg/L (nominal)	Purity: 98.8%	Scheerbaum, D. (2003)
OECD 201	*Desmodesmus subspicatus*	E_rC_{50} and E_bC_{50} (72 h) > 100 mg/L* NOE_rC (72 h) = 100 mg/L (nominal)	Study conducted with Carbetamide-COOH Purity: 99.0%	Dengler, D. (2008)
Method	Species	Results	Remarks	Reference
Algae and aquatic plants				
OECD 221	*Lemna minor*	Fronds*, E_bC_{50} (7 d) = 629 mg/L E_rC_{50} (7 d) = 301 mg/L NOE_rC (7 d) = 100 mg/L (nominal)	Purity: 98.8%	Scheerbaum, D. (2003)
OECD 221	*Lemna gibba*	Fronds, E_bC_{50} (14 d*) = 110.8 mg/L E_rC_{50} (14 d*) > 200 mg/L E_yC_{50} (7 d) = 102.7 mg/L NOE_rC (7 d) = 25 mg/L (nominal)	Study conducted with Carbetamide-COOH Purity: 99.0%	Dengler, D. (2008)
Other aquatic organisms				
OECD 219	*Chironomus riparius*	NOEC (28 d) = 640 mg/L (nominal)	Study conducted with Carbetamide-COOH Purity: 99.0%	Gonsior, G. (2008)

*Information taken from the PPP DAR (France, 2006) and/or the EFSA conclusion on the peer review of carbetamide (2010).

The reason why the degradation product carbetamide-COOH was further assessed was not explained by the DS. The EFSA evaluation documents indicated that it is a relevant metabolite for anaerobic degradation in soil. Nevertheless ecotoxicity data provided for carbetamide-COOH proved that the metabolite is less toxic than the parent to fish, invertebrates and algae.

Conclusion on the environmental classification

Acute aquatic hazard

Acute aquatic toxicity data are available for three trophic levels. The most sensitive species is *Daphnia magna*. The lowest and relevant result is EC_{50} (48 h) of 81 mg/L (nominal). This value is above the CLP criteria for acute aquatic hazard classification (i.e. ≤ 1 mg/L). **Therefore no acute aquatic classification is proposed by the DS and RAC agrees with their proposal.**

Long-term aquatic hazard

Chronic aquatic toxicity data are available for two trophic levels. According to the DS the most sensitive aquatic species is *Daphnia magna*. However a chronic fish test was not available and it can thus not be assumed that invertebrates are in fact the most sensitive trophic level. In accordance with the guidance on the application of the CLP criteria both types of information (acute and chronic toxicity) should be assessed and the classification should be based according to the most stringent outcome.

The first step is to classify based on the available chronic toxicity studies. The lowest chronic result is a NOEC (21 d) of 1 mg/L (nominal) on *D. magna*. This value is just at the threshold value for long-term aquatic classification (i.e. NOEC ≤ 1 mg/L), therefore classification as Category Chronic 2 (H411) would be applicable.

The second step is to classify based on the available acute toxicity studies. The lowest acute endpoint is an EC_{50} (48 h) of 81 mg/L (nominal) on *D. magna*. Carbetamide is not considered to be rapidly degradable. The EC_{50} (48 h) is higher than 10 mg/L and lower than 100 mg/L leading to classification as Category Chronic 3 (H412).

The comparison of both outcomes indicates that Category Chronic 2 is the most stringent outcome. Thus carbetamide should be classified as Category Chronic 2 (H411). **In conclusion, RAC agrees with the DS's proposal to classify carbetamide as Aquatic Chronic 2 (H411).**

Additional references

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ANNEXES:

Annex 1 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 Rapporteurs' comments (excl. confidential information).