Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CARBETAMIDE (ISO)

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: carbetamide (ISO); (2*R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate
CAS number: 16118-49-3
EC number: 240-286-6
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Germany**		**MSCA**	**1**
Comment received				
The German CA supports the proposed classification and labelling for Carbetamide				
Dossier Submitter's Response				
Thanks for your support				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
09/07/2014	**Germany**	**ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel)**	**Company-Manufacturer**	**2**
Comment received				
see previous comment: attached are the two additional reports mentioned in the comment submitted before - two submissions due to file size ECHA note: see attachement 1 and 2: *2b Carbetamide_PWG report on liver and adrenal tumors and 2a Carbetamide_PWG report on brain tumors*				
Dossier Submitter's Response				
See response to comment 5				
RAC's response				
See RAC's response to comment 5.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CARBETAMIDE (ISO)

Date	Country	Organisation	Type of Organisation	Comment number
09/07/2014	**Germany**	**ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel)**	**Company-Manufacturer**	**3**

Comment received

Classification with Carc. Cat. 2 / H351 / R40 is not warranted for Carbetamide. New information has been generated and is submitted herewith. A pathology working group consisting of six internationally recognized senior pathology experts has re-evaluated all relevant tissues from the existing carcinogenicity studies on Carbetamide according to state-of-the-art diagnostic criteria. The working group followed the rigorous standards of a PWG and concluded by consensus that the rare tumours highlighted in the CLH report do not warrant classification for carcinogenicity.

Three documents were produced by the PWG and are submitted herewith: one overall discussion, and two detailed reports (brain tumours and liver/adrenal tumours).

ECHA note see attachment 3: 1 Carbetamide. PWG overall discussion and conclusion on human carcinogenic potential.

Dossier Submitter's Response

New documents including a re-examination of liver, adrenal gland and brain slides by an independent pathology working group (PWG) have been provided by the applicant and have been considered by the DS.

After evaluation of these documents, DS is of opinion that the single incidence of pheochromocytoma observed in female mice exposed to the higher dose, should be considered to represent an incidental finding. Similarly in mice exposed to the higher dose of carbetamide, the reclassification of cholangiocellular carcinomas as hepatoblastoma leads to a consideration in conjunction with the other hepatocellular tumours. These types of tumours can be related to the MOA for phenobarbital (PB)-like inducers which should not be considered relevant for humans.

However, the higher incidence of astrocytomas was confirmed by the PWG in female rats fed the 9000ppm diet. Moreover, the PWG diagnosed an additional astrocytoma in this same group. The incidence of this rare brain tumor (3/60; 5%) is higher than the available HCD range (NTP and Charles River Lab. databases: range 0%-2%). Due to the rarity of this tumour type and the absence of a mechanistic explanation explaining why this tumour would not be applicable to humans, DS is of opinion that the finding in female rats remains a concern and is considered as limited evidence of carcinogenicity. The same conclusion has been proposed by the Committee for risk assessment (RAC) in 2011 for aclonifen (higher incidence of astrocytoma observed in female rat exposed to the higher dose).

In conclusion, DS still support the initial proposal for classification of carbetamide as Carc. 2 H351

For more details see response to comment 5

RAC's response

RAC agrees with the DS's response.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CARBETAMIDE (ISO)

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Germany**		**MSCA**	**4**

Comment received

Considering the increased incidences of several tumour types in mice and rats, the proposed classification for carcinogenicity is supported.

For rats and mice, historical control data from NTP and Charles Rivers was presented in the CLH report. Their relevance for the present evaluation is unclear: It seems they originate from different laboratories and/or different time frames that the studies with Carbetamide.

The incidence of astrocytomas was increased in female rats treated with Carbetamide. This diagnosis was also seen in female rats treated with Aclonifen and was used by RAC to classify this latter substance as a carcinogen (cat 2).
In addition to astrocytoma, an increased incidence of malignant hepatocellular carcinoma was reported for top dose females treated with Carbetamide.

The case for the non-relevance of hepatocellular tumours in mice seems not too convincing, considering the presented evidence

Dossier Submitter's Response

No historical control data from the laboratories which performed the cancerogenicity studies were available. By default, HCD from NTP and Charles Rivers (concomitant with the date of completion of the studies for the Charles Rivers HCD) were used for the overall interpretation of the relevance of the increased incidences of tumours observed in studies.

The non-relevance of hepatocellular tumours in mice is based on the hypothesis that carbetamide leads to sustained liver activation, likely via interaction with the CAR or PXR-receptor. Indeed, a mechanism study performed on B6C3F1 mice showed carbetamide to be an inducer of a variety of hepatic cytochrome P450 enzymes, which could support a MOA via sustained, receptor-mediated liver toxicity (as activated by Phenobarbital among others). A hepatocarcinogenic response in rodents for compounds that have data to support a MOA for Phenobarbital (PB)-like inducers should not be considered relevant to humans (IPCS, 2006).

RAC's response

RAC agrees with the DS's response.

Date	Country	Organisation	Type of Organisation	Comment number
14/08/2014	**US**		**Individual**	**5**

Comment received

In the draft CLH Report For Carbetamide issued June 2012, the MSCA proposition was the a classification of carbetamide in category 2 (H351) be proposed based on several rare tumours occurring in different tissues (brain astrocytoma, liver cholangiocarcinoma and adrenal pheochromocytoma) in mice and rats at the high dose (exceeding MTD.) The applicant's proposition was that no hazard classification required since these neoplasms were unrelated to exposure.
I was the chairperson of Pathology Working Groups (PWG) which were convened to assess the brain neoplasms reported in rats and liver and adrenal neoplasms reported in the mice. The PWGs were composed of independent consulting pathologists with expertise in the evaluation and interpretation of rodent toxicity and carcinogenicity studies.
Although, a slightly higher incidence relative to controls of astrocytomas of the brain was reported in female rats fed the 9,000 ppm (Group 4, the high concentration) diet, examination of all brain sections did not reveal any morphological evidence of non-neoplastic glial cell proliferation in the brain of male or female rats. There was no increase in tumour latency with the brain tumours in the Group 4 female rats as compared to the lower dose groups. All astrocytomas were observed in rats between test days 721 and 749, as would be expected with age-related neoplasms. In male rats, no astrocytomas were observed in the treated or control groups. Carbetamide was not mutagenic in a

battery of in-vitro assays. The totality of the weight of evidence indicates that the slight increase in the number of astrocytomas in the Group 4 females, as compared to lower dose groups and the control group is an incidental finding and unrelated to the test article. The PWG regarded this difference to represent normal biologic variation in the incidence of brain tumours in F344 rats and did not consider it to be indicative of evidence of a carcinogenic response.
The results of the PWG review of the adrenal gland confirmed a previously diagnosed 'benign pheochromocytoma' in a control male, and one of two reported malignant pheochromocytomas in females at 9000 ppm. In a second case in the 9000 ppm female group that was previously diagnosed as a malignant pheochromocytoma was considered by the peer review pathologist as a benign pheochromocytoma. The incidence of hyperplasia in the adrenal medulla was low and randomly distributed throughout the groups in both sexes. The totality of the weight of evidence indicates that the minimal increase in the number of pheochromocytomas in the Group 4 females, as compared to lower dose groups and the control group, is a spurious finding and unrelated to the test article. The PWG regarded the difference in the incidence of proliferative changes in the adrenal medulla to represent normal biologic variation and did not consider it to be indicative of evidence of a carcinogenic response.
The PWG was in agreement with the applicant and the CLH Report For Carbetamide issued June 2012 that the proposed mode of action (MoA) for the generation of livers tumours in mice is plausible. The proposed MoA involves an epigenetic mechanism of hepatocarcinogenesis by Carbetamide in the mouse. Carbetamide leads to sustained liver activation, likely via interaction with the CAR or PXR-receptor. Indeed, a mechanism study performed on B6C3F1 mice showed carbetamide to be an inducer of a variety of hepatic cytochrome P450 enzymes, which could support a PB-like MoA. A hepatocarcinogenic response in rodents for compounds that have data to support a PB-like MoA is not considered relevant to humans (Holsapple et al. 2006). However, the PWG did not confirm the original diagnosis of cholangiocarcinoma in 3 of the high dose male mice. The PWG confirmed the reviewing pathologist's observation that these tumors were all considered to be malignant hepatocellular neoplasms (hepatoblastoma) and are related to the MoA for the induction of hepatocellular neoplasms and do not represent the induction of a "rare" tumour type in the liver.

JERRY F. HARDISTY, D.V.M., DACVP, FIATP
PWG Chairperson

Dossier Submitter's Response

The new documents (re-examination of liver, adrenal gland and brain slides by an independent pathology working group) provided by the applicant have been considered by the DS. The principal results of this histological slides re-examination were:

- **Mouse adrenal gland tumours:** a case of malignant pheochromocytomas diagnosed previously in the female high dose group in the original study was reconsidered by the review pathologist as a begnin pheochromocytoma. A comparison for adrenal gland tumours of the original Pathologist's finding and the findings reported by the PWG is presented in Table 1.

Table 1: Mouse adrenal - original study pathologist interpretation and PWG interpretation:

	Original diagnosis females (incidence/number animal)				PWG conclusion females (incidence/number animal)			
Doses (ppm)	0	160	1200	9000	0	160	1200	9000
Pheochromocytoma begnin	0/52	0/52	0/52	0/52	0/52	0/52	0/52	1/52
Pheochromacytoma malignant	0/52	0/52	0/52	2/52	0/52	0/52	0/52	1/52

Based on the PWG re-interpretation of histological slides, the incidence of malignant pheochromocytoma (1/52; incidence:1.9%) in the 9000 ppm dose group of female mice should be considered to represent an incidental finding as this incidence is within the expected range of spontaneous occurrence for this type of tumour in B6C3F1 mice.(NTP database: 0-4%)

- **Mouse liver cholangiosarcoma**: the PWG did not confirm the original diagnosis of cholangiocarcinoma in 3 of the high dose males but considered these tumours to be malignant hepatocellular neoplasms (hepatoblastoma) and related to the MoA for the induction of hepatocellular neoplasms. Another neoplasm diagnosed as "carcinoma from miscellaneous" in

male high dose group was also diagnosed as hepatoblastomas by the reviewing pathologist. A comparison for Liver tumours (cholangiocarcinoma and hepatoblastome only) of the original Pathologist's finding and the findings reported by the PWG is presented in Table 2.

Table 2: Mouse liver – original study pathologist interpretation and PWG interpretation:

	Original diagnosis males (incidence/number animal)				PWG conclusion males (incidence/number animal)			
Doses (ppm)	0	160	1200	9000	0	160	1200	9000
Cholangiocellular carcinoma	0/52	0/52	0/52	3/52	0/52	0/52	0/52	0/52
hepatoblastoma	0/52	0/52	0/52	0/52	0/52	0/52	0/52	4/52

Based on the PWG re-interpretation of histological slides, the 4 new diagnosed hepatoblastoma can be related to the MOA for the induction of hepatocellular neoplasms and do not represent the induction of a "rare" tumour type in the liver

- **Rat Brain tumours**: Astrocytomas diagnosed by the original study pathologist were confirmed by the reviewing pathologist in the high dose group in 2 females. Moreover, the ganglioneuroma diagnosed in the original study pathologist was reclassified by the reviewing pathologist as an additional astrocytoma in the female high dose. A comparison for brain tumours of the original Pathologist's finding and the findings reported by the PWG is presented in Table 3..

Table 3: Rat brain - Original study pathologist interpretation and PWG interpretation:

	Original diagnosis females (incidence/number animal)				PWG conclusion females (incidence/number animal)			
	0	160	1200	9000	0	160	1200	9000
Astrocytoma	0/60	0/60	0/60	2/60	0/60	0/60	0/60	3/60
Ganglioneuroma	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60
Mixed glioma Malignant	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
Oligodendrioglioma	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60

DS conclusions

On the basis of the new document provided by the applicant (PWG re-evaluation of the histological slides), DS is of opinion that the single incidence of pheochromocytoma observed in female mice exposed to the higher dose, should be considered to represent an incidental finding. Similarly in mice exposed to the higher dose of carbetamide, the reclassification of cholangiocellular carcinomas as hepatoblastoma leads to a consideration in conjunction with the other hepatocellular tumours. These types of tumours can be related to the MOA for phenobarbital (PB)-like inducers which is not considered relevant for humans.

However, the higher incidence of astrocytomas was confirmed by the PWG in female rats fed the 9000ppm diet. Moreover, the PWG diagnosed an additional astrocytoma in this same group. The incidence of this rare brain tumour (3/60; 5%) is higher than the available HCD range (NTP and Charles River Lab. Databases: range 0%-2%). Due to the rarity of this tumour type and the absence of a mechanistic explanation provided to justify that this finding is not relevant for human, DS is of opinion that the finding in female rats remains a concern and is considered as limited evidence of carcinogenicity. The same conclusion has been proposed by the Committee for risk assessment (RAC) in 2011 for aclonifen (slightly higher incidence of astrocytoma observed in female rat exposed to the higher dose).

In conclusion, DS still support the initial proposal for classification of carbetamide as Carc. 2 H351.

RAC's response

RAC agrees with the DS's response.

Date	Country	Organisation	Type of Organisation	Comment number
09/07/2014	**Germany**	**ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel)**	**Company-Manufacturer**	**6**

Comment received

p. 52 of CLH report, chapter 4.10.4:

Brain astrocytoma: The Pathology Working Group regarded this difference to represent normal biologic variation in the incidence of brain tumors in F344 rats and did not consider it to be indicative of evidence of a carcinogenic potential of carbetamide.

Liver tumours: The PWG did not confirm the original diagnosis of cholangiocarcinoma in 3 of the high dose male mice. These tumours were all diagnosed as malignant hepatocellular neoplasms. (hepatoblastoma) and are related to the MOA for the induction of hepatocellular neoplasms and do not represent the induction of a "rare" tumour type in the liver.

Adrenal tumours: The PWG regarded the incidence of proliferative changes in the adrenal medulla in this study to represent normal biologic variation, unrelated to treatment with carbetamide, and not indicative of a carcinogenic response.

Dossier Submitter's Response

See response to comment 5

RAC's response

See RAC's response to comment 5.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Germany**		**MSCA**	**7**

Comment received

Considering the reported findings in offspring, the proposed classification for developmental toxicity is supported. It would have been helpful, if the litter incidences for foetal findings had been reported too.

Dossier Submitter's Response

Please find below the tables for foetal findings completed with the litter incidence for the two teratogenicity studies in rat and rabbit. For more clarity and in response to other comments, the historical control data of the laboratories which performed both studies have been added.

1- RAT study (Tesh et al. 1985)

Table 77: Main parameters of pregnant rats treated with carbetamide during the organogenesis period, and their foetuses.

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

Text highlighted in yellow: information about litter incidences that have been added.
Bold text : incidences above the historical control data of the study

2-RABBIT study (Tesh et al. 1986)

Table 81: Main parameters of pregnant rabbits treated with carbetamide during the organogenesis period, and their foetuses

Group	**1**	**2**	**3**	**4**	**Historical control data– Study range**
Dose-level (mg/kg/day)	**-**	**5**	**40**	**320**	

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CARBETAMIDE (ISO)

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

Text highlighted in yellow: information about litter incidences that have been added.
Bold text : incidences above the historical control data of the study

RAC's response

Thank you. Opinion will include the information as is showed in this Table.

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Belgium**		**MSCA**	**8**

Comment received

Regarding the reproductive toxicity endpoint, we question the important consideration established by the DS for the maternal toxicity:

• The studies are not guideline compliant.

• The study in rabbit (Tesh et al., 1986) reveals a NOAEL for maternal toxicity at 40 mg/kg bw/d while the foetal toxicity is observed at 5 mg/kg bw/d (NOAEL). The foetotoxic evidences (signs of immaturity - incomplete ossification of vertebrae and long bones- and the skeletal abnormalities (higher number of ribs and of presacral vertebrae)) appear before the maternal toxicity (slight decrease bodyweight).

• The study in rats (Tesh et al., 1985) indicates a slight lower bodyweight gain in adults during late gestation with a generalized foetal immaturity (soft tissue variations and ossification retardation) and several foetal abnormalities (imperforate anus, cardiovascular malformations,…) at 1000 mg/kg. The table 77 page 94 presents some inconsistencies: some effects (skeletal or other abnormalities) observed at the highest dose are very high in comparison with the control and the lower doses but are not considered significant: for example the incidence of the foetuses with higher number of ribs was 0, 2.9, 3.5 and 93.6 respectively at 0, 150, 450 and 1000 mg/kg, the incidence of imperforate anus was 0, 0.8, 0 and 7.0, …

Regarding those effects, we consider that Cat.1B cannot be disregarded in comparison with the

criteria of the Regulation: data that provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered no to be a secondary non-specific consequence of other toxic effects.

Dossier Submitter's Response

Table 77 page 94: In the Tesh et al. (1985) study, statistical analysis was performed only on the following parameters: body weight change, water intake, foetal weight and placental weight. No statistical analysis was performed on the incidence of malformation/variation in foetuses. However, these incidences have been compared with the historical control data of the laboratory when available. Only the foetal findings observed above the HCD have been considered significant by DS and have been reported in Table 77 of the CLH report. For more clarity and in response to other comment, Table 77 has been reported here in response to comment 7 and completed with the HCD and the litter incidences.

DS agrees that some evidence of carbetamide teratogenic effects was observed in both species. However, both teratogenicity in rat and rabbit presents some limitations (old study, not guideline compliant) which make the quality of evidence less convincing for a Category 1B. Anyway, we agree that this issue deserves some discussion in RAC meeting.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
12/082014	**The Netherlands**		**MSCA**	**9**

Comment received

Fertility: We agree that carbetamide should not be classified for fertility effects.

Developmental toxicity: We do not agree with the proposed classification for carbetamide as Repro 2 (H361d).
- In the rat developmental toxicity study, foetal toxicity was observed at the highest dose (1000 mg/kg bw/d). These effects included reduced foetal weight, higher incidences of reduced ossification, haemorrhages, dilation of brain ventricles, arterial and cardiac effects, and skeletal lesions (i.e. higher nr of ribs, presacral vertebrae, asymmetric pelvis, fused sternebrae). At the highest dose level also some maternal toxicity was observed and included slightly reduced body weight gain. Fetal effects such as delayed ossification, dilation of brain ventricles and reduced fetal body weight are indications of delayed fetal growth and are most likely related to the reduction of maternal body weight. Further, the reduced bw in the adult rat was mainly observed after the exposure period (day 15-21) and could largely be explained by the reduced body weight of the fetus (12.8 foetuses/litter * (3.21-2.64 g/fetus) = 7.3 g). Is information on corrected body weight gain available?
However, this maternal effect cannot explain all of the observed fetal effects. Especially, the observed fetal malformations are not considered to be secondary to reduced maternal body weight. Also, the observed effects in the repeated dose studies at comparable dose level and duration (5 week study) do not show clear adverse effects that could have caused the observed fetal malformations. Therefore, the observed fetal effects (malformations) in the rat developmental study should be considered for classification for developmental toxicity.
- In the rabbit developmental toxicity study, fetal toxicity was observed at the highest dose (320 mg/kg bw/d). Effects included incomplete ossification, and higher nr of ribs and presacral vertebrae. More importantly, a higher incidence of post-implantation losses was observed in this high dose group. At the highest dose level also some maternal toxicity was observed and included body weight loss during the first 4 days of treatment (body weight gain during whole gestation period was unaffected). However, decreased maternal body weight is not considered responsible for the observed higher incidence of post-implantation losses. Therefore, the observed fetal effects from the rabbit developmental study (post-implantation loss) should be considered for classification for developmental toxicity.

Given that 1) the observed fetal effects can be considered treatment-related, 2) the fetal effects (malformations and post-implantation loss) cannot be related to maternal toxicity and 3) the relevance of the observed fetal effects for human health cannot be excluded, there is clear evidence of an adverse effect on development, which is, though occurring together with other toxic effects, considered not to be a secondary non-specific consequence of other toxic effects. Therefore, carbetamide should be classified as Repro 1B (H360D).

Effects on or via lactation: No data are available.

Dossier Submitter's Response

Concerning the rat developmental study (Tesh et al. 1985), no information on corrected body weight gain is available in the study report. DS agrees that the observed fetal effects should be considered for classification for developmental toxicity as these effects can't be explained by the maternal toxicity. Taking into account others comments received, the tables 77 and 81 of the CLH report have been reported here and completed with additional information (litter incidence, HCD).

DS agrees that some evidence of carbetamide teratogenic effects was observed in both species. However, both teratogenicity in rat and rabbit presents some limitations (old study, not guideline compliant) which make the quality of evidence less convincing for a Category 1B. Anyway, we agree that this issue deserves some discussion in RAC meeting.

RAC's response

Noted.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Germany**		**MSCA**	**10**

Comment received

In Section 1.3 the conclusion regarding the endpoint respiratory sensitisation is „Conclusive but not sufficient for classification". Considering the absence of relevant data as discussed in the CLH report, the conclusion should be rather "data lacking".

Dossier Submitter's Response

Ok. We agree with this proposal. RAC should modify its background document accordingly.

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Belgium**		**MSCA**	**11**

Comment received

The Cummins and Gardner study (1984) indicates a LD50 value of 1445 mg/kg bw/d for the female mouse exposed via oral route. This value is within the range of category 4 for acute toxicity and is supportive for a classification Acute Toxicity Cat.4. However, we would recommend the DS to beter substantiate the classification because of a lack of information in the proposal : the number of animals tested, the guidance compliance , vehicle used for the test, number of dead animals per dose. We support the classification due to the LD50 value but the study needs to be beter described in order to constitute sufficient support for the classification. For a confirmation of the validity of studies, we think that more information are needed

Dossier Submitter's Response

More information concerning the Cummins and Gardner study (1984) is presented below:

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CARBETAMIDE (ISO)

- **Reference:**

Cummins HA and Gardner JR (1984): carbetamide: acute oral toxicity in the mouse. Life Science Research, Ltd., Suffolk, UK - Unpublished report N° 84/RH0039/462 - Dates of work: 20/03/1984 to 23/08/1984.

- **Guidelines:** OECD N° 401 (1981).
- **GLP standards:** Yes
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch 840 4001, purity 96.4%) was administered to groups of 5 males and 5 females CD-1 mice (7-week old, bw range 17-25 g) by gavage at the dose-levels of 1000, 1414, 2000 and 4000 mg/kg bw in 20mL/kg of an aqueous solution of methylcellulose (0.5% w/v) and Tween 80 (0.5% w/v).
Mortality and clinical signs were recorded during 15 days after dosing. At the end of the study, animals were sacrificed by carbon dioxide inhalation. Macroscopic examination of organs was performed on decedent animals and on those sacrificed at the end of the observation period, and all abnormalities were recorded.

- **Results:**

Mortality is summarized in Table B.6.2.1.2-1.
Table B.6.2.1.2-1: Mortality following single oral administration of carbetamide to mice on day 1 (d-1)

Dose level (mg/kg bw)	**Males**		**Females**	
	Mortality	Time of death (n° of rats)	Mortality	Time of death (n° of rats)
1000	0/5	-	0/5	-
1414	3/5	d-1 (2), d-3 (1)	2/5	d-1 (1), d-3(1)
2000	2/5	d-1 (2)	5/5	d-1 (3), d-2(1), d-3(1)
4000	4/5	d-1 (4)	5/5	d-1 (5)

Deaths occurred at dose-levels of 1414 mg/kg and above for the first 3 days after dosing. Ante-mortem signs were bradypnea, hyperpnea, proneness and/or unconsciousness within 15-30 min after administration of carbetamide, and less frequently lethargy, decreased motor activity, muscular tremor, apnea and cyanosis.

Bradypnea, hyperpnea, proneness and/or unconsciousness and hunched posture were observed in the surviving animals within 15-30 min after administration of carbetamide. The frequency and nature of signs observed in surviving animals were similar to those in decedents. For surviving mice, recovery was complete by 24 hours after dosing except for one female dosed at 1414 mg/kg and still showing decreased motor activity, proneness and bradypnea on day 2.

All surviving animals achieved a body weight gain stated to be similar to historical controls.

Necropsy of the decedents revealed, externally, yellow urogenital staining and pale staining on the head particularly at high dose-levels, and, internally, abnormal gastro-intestinal contents which were often of mucous aspect, and pulmonary congestion.

No treatment-related macroscopic lesion was observed at necropsy of surviving animals at study termination.

- **Conclusion**

Under the conditions of the study, the acute oral LD_{50} of carbetamide (mg/kg bw) in mice is 2033 (1133-2934) in males, 1445 (1275-1616) in females and 1718 (1328-2109) when sexes are combined. Carbetamide is designated "of low acute oral toxicity". Carbetamide should be classified as Acute Tox. 4 (H302) according to Regulation (EC) 1272/2008.

RAC's response
Noted. All information supplied by the DS is included in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
12/08/2014	**The Netherlands**		**MSCA**	**12**
Comment received				
We agree with the dossier submitter's response to the comments of the manufacturer concerning the results of the mouse study. We agree that carbetamide should be classified based on the lowest available LD50 value, classification for acute toxicity as Acute Tox. 4 (H302) is therefore justified.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Organ Toxicity Repeated

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Germany**		**MSCA**	**13**
Comment received				
The reported signs for neurotoxicity in dogs might trigger classification with STOT-RE. According to the report, these findings were seen in the 4-week and 1-year studies near or below the guidance values for category 2				
Dossier Submitter's Response				
In the 4-week dog study, the neurotoxic effects, i.e. unsteadiness of the hind limbs and transient non-specific neurovegetative reactions (salivation, vomiting and tremors), were observed at 150 or 300 mg/kg bw/d which is above the guidance value for STOT RE 2. In the 1-year dog study, neurotoxic effects observed at 30 mg/kg bw/d were minimal, i.e. low incidence of transient drowsiness within the hours following dosing up to week 7 (males) or 9 (females) and muscle tremors from week 20 in females only. DS is of opinion that these transient and minimal effects alone are not sufficient to justify a classification STOT RE 2.				
RAC's response				
RAC agrees with DS's response. In addition, the lowest LOAEL for liver effects was found in the 13-week oral exposure study in rats (119 mg/kg bw/day) and in the 52-week oral exposure study in dog (30 mg/kg bw/day). When we adjust the exposure duration of the dog study to 90 days using the Haber's law we obtain 120 mg/kg bw/day, very close to the value reported for rats. Both records were above the cut-off point for warranting classification as STOT RE 2 (LOAEL lower than 100 mg/kg bw/day). In conclusion, no classification for STOT RE is warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
12/08/2014	**The Netherlands**		**MSCA**	**14**
Comment received				
STOT RE We agree that the observed liver effects are insufficient for classification. The liver effects are mainly observed at effective dose levels which are above the upper classification criteria for STOT RE. Moreover, the observed effects on liver (increased liver weight, hepatocellular hypertrophy) are most probably an adaptive response. Furthermore, a mechanistic study showed the P450-inducing potential of carbetamide.				

Thyroid effects were observed in mouse, rat and dog. These effects included higher thyroid weights and thyroid epithelial enlargement (hypertrophy/hyperplasia) and are considered in the CLH dossier irrelevant for human health hazard classification based on the hypothesis that "the thyroid effects resulted from increased metabolic turnover of thyroid hormones in the liver by an induced thyroxine-glucotransferase activity". Section 3.9.2.5.3 of the CLP Guidance states that "thyroid effects in rodents caused by an increase in hepatic UDPG-transferase are considered of insufficient concern for classification". However, no experimental data on the contribution of UDPG-transferase to these effects in rodents were presented which justifies an exclusion of these effects. Furthermore, thyroid effects were also observed in dogs, which is not a rodent. These issues deserve some discussion.

Dossier Submitter's Response

In addition to rat and mouse, thyroid effects were also observed in dogs. Changes in thyroid weights were observed in all dog studies (28-day, 90-day and 52-week) but this effect associated with an enlargement of the follicular epithelium was observed only in the 28-day study.
Anyway, the LOAEL (150 mk/kg/d) for thyroid effects lies above the classification cut off for STOT RE 2.

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Aquatic Environmental Hazard

Date	Country	Organisation	Type of Organisation	Comment number
22/08/2014	**Belgium**		**MSCA**	**15**

Comment received

Based on the results of the aquatic toxicity test on the most sensitive species (Daphnia magna with 48hEC50 = 81 mg/l, 21dNOEC=1mg/l), the fact that the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of the regulation 1272/2008, as Aquatic chronic 2, H411.

In conclusion: we agree with the proposed environmental classification by ANSES.

Some editorial or/and minor comments:
Could you please specify whether the aquatic toxicity values reported in the tables are nominal or measured values?
Furthermore we would have appreciated that a concise description (findings) was given for all aquatic toxicity studies reported, besides the more detailed one given for the key studies.

Dossier Submitter's Response

The aquatic toxicity values reported in the tables are nominal values.
For the findings of the toxicity studies, we are of the opinion that a detailed presentation could be considered required only for the key studies. Indeed, for this substance, which is a pesticide, all the summaries are available in public documents (Draft Assessment Report and Addenda).

RAC's response

RAC agrees with the DS's response.

ATTACHMENTS RECEIVED:
Attachment 1: from ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel) [comment 2]:2b Carbetamide_PWG report on liver and adrenal tumors.pdf
Attachment 2: from ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel) [comment 2]:2a Carbetamide_PWG report on brain tumors.pdf
Attachment 3: from ADAMA Agriculture B.V., The Netherlands (on behalf of ADAMA Agan, Israel) [comment 3]: 1 Carbetamide_PWG overall discussion and conclusion on human carcinogenic potential.pdf