Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

carboxin (ISO); 2-methyl-N-phenyl-5,6-dihydro -1,4-oxathiine-3-carboxamide; 5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide

EC Number: 226-031-1
CAS Number: 5234-68-4

CLH-O-0000001412-86-180/F

Adopted

5 December 2017

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5 December 2017

CLH-O-0000001412-86-180/F

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

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

Chemical name: **carboxin (ISO); 2-methyl-N-phenyl-5,6-dihydro-1,4-oxathiine-3-carboxamide; 5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide**

EC Number: **226-031-1**

CAS Number: **5234-68-4**

The proposal was submitted by **United Kingdom** and received by RAC on **18 October 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom 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 **20 December 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 February 2017**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Bogusław Barański**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2017** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	616-RST-VW-Y	carboxin (ISO); 2-methyl-*N*-phenyl-5,6-dihydro-1,4-oxathiine-3-carboxamide; 5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide	226-031-1	5234-68-4	STOT RE 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 2	H373 (kidneys) H317 H400 H411	GHS07 GHS08 GHS09 Wng	H373 (kidneys) H317 H410		M=1	
RAC opinion	616-RST-VW-Y		226-031-1	5234-68-4	STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H373 (kidneys) H317 H400 H410	GHS07 GHS08 GHS09 Wng	H373 (kidneys) H317 H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	616-RST-VW-Y		226-031-1	5234-68-4	STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H373 (kidneys) H317 H400 H410	GHS07 GHS08 GHS09 Wng	H373 (kidneys) H317 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification is proposed by the Dossier Submitter (DS) for physical hazards based on the following observations:

- carboxin does not meet the criteria for flammable solids based on the results of testing according to EEC A.10 method (Tremain, 2001b).
- carboxin does not exhibit explosive properties based on results of testing according to EEC A.14 method (Tremain, 2001b).
- carboxin does not meet the criteria for oxidising solids based on results of testing according to EEC A.17 method (Tremain, 2001b).

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

As Carboxin does not meet the criteria for classification for physico-chemical properties, RAC supports the proposal of the DS **not to classify** the substance for these properties.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No classification was proposed by the DS for acute toxicity by the oral, inhalation and dermal routes based on the following data.

Acute toxicity: oral route

Carboxin was tested for acute oral toxicity in Sprague-Dawley rats (5 male and 5 female per dose), according to US EPA OPP 81-1 guideline in a GLP-compliant study (Goldenthal, 1992a). Carboxin was administered orally at doses 2430, 3500 and 5040 mg/kg bw as a suspension in 0.5% water solution of carboxymethylcellulose.

The oral calculated LD_{50} was found to be 2588 and 3080 mg/kg bw for male and female rats, respectively and were above the top range values for classification in the acute toxicity category 4 ($300 < LD_{50} \leq 2000$). Therefore, no classification for acute toxicity via the oral route was proposed by the DS.

Acute toxicity: dermal route

Carboxin was tested for acute dermal toxicity in New Zealand White rabbits (5 male and 5 female per dose), according to US EPA OPP 81-2 guideline in GLP-compliant study (Goldenthal, 1992b).

The LD_{50} for both male and female rabbits was > 4000 mg/kg bw, which is above the top range for classification in acute dermal toxicity category 4 (1000 < LD_{50} ≤ 2000). Therefore, no classification for acute toxicity via the dermal route was proposed by the DS.

Acute toxicity: Inhalation

In an acute inhalation study (Ulrich, 1993), the LC_{50} of carboxin was > 4.7 mg/L for rats. The mean mass aerodynamic diameter (MMAD) was 6.8 ± 2.2 µm (inhalable fraction ≤ 4 µm), meaning that certain proportion of the dose might have been deposited in the upper part of respiratory tract and not deposited in the alveolar zone. The particles trapped in the upper part of the respiratory tract might have been translocated to the GI tract.

The DS did not propose classification for acute inhalation for carboxin.

Comments received during public consultation

No specific comments were received during the public consultation.

Assessment and comparison with the classification criteria

Oral

Taking into account that the oral LD_{50} value in male and female rats is above the threshold value for classification (2000 mg/kg bw), carboxin should not be classified for acute oral toxicity according to the CLP criteria.

Dermal

Taking into account that the dermal LD_{50} value in male and female rats and rabbits is above the threshold value for classification (2000 mg/kg bw), carboxin should not be classified for acute dermal toxicity according to the CLP criteria.

Inhalation

Taking into account that the inhalation LC_{50} value in male and female rats is above 4.7 mg/L air/4h (the maximum achievable concentration) and no significant clinical signs of toxicity were exhibited during the fourteen day post-exposure period the exceedance of the threshold value for classification (5 mg/L air/4 h) is not expected. It is also noted that very low acute oral toxicity supports a low inherent toxicity of carboxin, also by the inhalation route.

Therefore, carboxin should not be classified for acute inhalation toxicity according to the CLP criteria.

Overall, RAC agrees with the DS that **no classification for Acute Toxicity by the oral, inhalation and dermal routes** is warranted.

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

Summary of the Dossier Submitter's proposal

The DS did not propose classification of carboxin for the STOT SE hazard class based on the following observations.

In the available acute oral toxicity study (Goldenthal, 1992a), signs of reduced locomotor activity, ptosis, coldness to the touch and decreased defecation were observed at doses well above 2000 mg/kg bw and were considered to be indicative of general toxicity. As there was no clear

evidence of specific toxic effects on a target organ or tissue, the criteria for classification for STOT SE 1 or 2 are not met.

In a study of acute toxicity via the dermal route in the rabbit (Goldenthal, 1992b), no overt signs of toxicity were observed in any of the animals at dose 4000 mg/kg bw.

In an acute inhalation toxicity study (Ulrich, 1993), laboured breathing was observed in 1/5 females at 4.7 mg/L/4h [aerosol, whole body (MMAD 6.8 ± 2.2 µm)] only. No clinical effects on the respiratory tract were observed in males. Carboxin was not irritating in the available skin and eye irritation studies (Goldenthal, 1992c,d). The observations from the acute inhalation toxicity study are not considered to provide sufficient justification for classification of carboxin as a respiratory tract irritant (STOT SE 3).

Since there was no evidence of sedation in single and repeated dose studies in rats or other species, classification for STOT SE 3 for central nervous system (CNS) effects including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination and vertigo is not justified.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

There was no specific, non-lethal target organ toxicity arising during or after single oral, dermal or inhalation exposure to carboxin. The observed effects were indicative of non-specific, general acute toxicity, so RAC agrees with the DS that there is no clear evidence of specific effects on target organs or tissues independent from general systemic toxicity and mortalities, as well as no definitive signs of respiratory tract irritation or narcotic effects. Therefore, RAC is of the opinion that **classification for specific target organ toxicity single exposure (STOT SE) is not required**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation.

The skin irritation potential of carboxin was assessed in a standard skin irritation GLP study (US EPA OPP 81-5) in three females and three males of New Zealand White rabbit (Goldenthal, 1992c).

No dermal irritation was observed in any rabbit during the study period. No other signs of dermal irritation were recorded.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

In the available study, no erythema/eschar or oedema were observed in any of the tested animals. According to the CLP criteria for skin irritation a mean score of ≥ 2.3 for both erythema/eschar or oedema are needed for classification in Cat. 2.

Taking into account that the classification criteria were not met, **RAC considers that carboxin does not warrant classification for skin corrosion/irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS did not propose classification for eye effects based on the results of a reliable study.

The eye irritation potential of carboxin was assessed in a standard eye irritation GLP study (guidance US EPA OPP 81-4 equivalent to OECD TG 405) in New Zealand White rabbits (Goldenthal, 1992d).

The test item (0.055 g) was instilled into one eye of 4 male and 2 female rabbits. Individual scores for each animal, calculated as mean scores at 24, 48 and 72h were:

- cornea: 0, 0, 0, 0, 0, 0
- iris: 0, 0, 0, 0, 0, 0
- conjunctival redness: 0.7, 0.7, 0.3, 1.0, 0.3, 0.3
- conjunctival chemosis: 0, 0, 0, 0.3, 0, 0

No signs of corneal opacity or iris were observed. Slight conjunctival redness (6 rabbits) and chemosis (1 rabbit) were observed. Additionally, all animals exhibited low-grade clear discharge, which cleared within 24 h.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Carboxin caused reversible eye irritation in an *in vivo* study in the rabbit.

Mean scores for specific ocular effects did not exceed the CLP criteria for classification in Category 2. Only slight conjunctival redness and chemosis were observed, but the average scores were < 2 (i.e., the relevant average score for conjunctival redness and oedema).

Therefore, RAC agrees with the DS that **classification for eye damage/irritation is not required**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The potential of carboxin to cause respiratory sensitisation was not investigated directly. However, in an acute inhalation toxicity study (Ulrich, 1993) only laboured breathing was observed in 1/5 females at 4.7 mg/L/4h [aerosol, whole body (MMAD 6.8 ± 2.2 µm)]. No clinical effects on the respiratory tract were observed in males.

Carboxin was not irritating in the available skin and eye irritation studies (Goldenthal, 1992c,d).

There is no indication from the available data that classification for respiratory sensitisation is required. Therefore, no classification was proposed by the DS.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

As there are no data suggesting that carboxin may cause respiratory sensitisation, RAC proposes **no classification for respiratory sensitisation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The potential of carboxin to cause skin sensitisation was investigated in a GLP Magnusson and Kligman Guinea Pig Maximisation test (Hall, 2002), according to the EEC B.6 method (OECD TG 406). Concentrations used for induction and challenge exposures were based on results from a preliminary study.

Intradermal induction was performed at a concentration of 10% of carboxin in corn oil. Challenge was performed at a concentration of 75% in corn oil. Dermal reactions were graded at 24 and 48h following challenge.

In the main challenge phase, the 75% concentration of carboxin induced skin sensitisation reactions in 37% and 56% of animals at 24 and 48h after removal of the dressing, respectively.

No dermal reactions were observed in the vehicle control group. No signs of erythema were observed in the negative control group at challenge. Appropriate results were obtained with the positive control material. Body weight changes were normal in both test and control animals.

According to the DS, it can be concluded that carboxin is a skin sensitiser and meets the criteria for classification for skin sensitisation, and there is sufficient data for sub-categorisation, thus it should be classified as Skin Sens. 1B; H317.

Comments received during public consultation

Three MSCAs supported the DS's proposal to classify carboxin as Skin Sens. 1B; H317.

Assessment and comparison with the classification criteria

The criteria for classification in the Guinea Pig Maximisation test (positive response in ≥ 30% of animals) were fulfilled and, therefore, carboxin should be classified as a skin sensitiser, Category 1; H317.

It is unlikely that carboxin meets the classification criteria for Skin Sens. 1A:

1) sensitise at least 60% of guinea pigs at intradermal induction concentration being in the range > 0.1% to ≤ 1%

   Carboxin sensitisation rate did not reach the required 60% after intradermal induction at a concentration of 10%, so fulfilling this criterion is rather not probable;

2) sensitise at least 30% of guinea pigs at intradermal induction concentration ≤ 0.1%

   Such concentration is 100 times lower than the 10% used in the test producing sensitisation rate of 37% and 56%, hence it is unlikely carboxin can fulfil this criterion.

However, since there are no data on sensitising properties of carboxin used in the Guinea Pig Maximisation test at these lower induction concentrations below ≤ 0.1% or ≤ 1%, the classification for subcategory 1A cannot be excluded and therefore the substance should be classified as a Category 1 skin sensitiser without subcategorisation.

Taking into account all available data and these considerations, RAC is of the opinion that carboxin warrants classification as **Skin Sens. 1; H317**.

Specific concentration limit

Carboxin has, based on existing data, a moderate potency for skin sensitisation and therefore, the generic concentration limit of 1% should be applied.

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

Summary of the Dossier Submitter's proposal

The CLH dossier contains several repeated dose toxicity studies of carboxin in rats (28-day by gavage and 90-day in diet), chronic oral studies (carcinogenicity and reproductive toxicity) in rats and mice and repeated dose toxicity studies in dogs (28-day, 90-day and 1-year) and a dermal repeated dose toxicity in rats.

Based on results from these studies the DS proposed classification of carboxin for STOT RE 2; H373 with kidneys as target organ, via the oral and inhalation routes of exposure.

Oral route

Rats

28-day (Ullmann, 1983):

In a 28-day study (Ullmann, 1983), Wistar rats (10/sex/dose) were administered 0, 30, 90 or 270 mg/kg bw/day carboxin orally via gavage. After treatment, 5/10 animals were sacrificed whilst the other 5/10 animals were retained and observed for a 21-day recovery period. General signs of toxicity (small reductions in body weight, ruffled fur and sedation) were observed from 30 mg/kg bw/day. The critical target organs identified in this study were the kidney and liver. An increased incidence of kidney lesions was observed from the lowest dose administered.

At 30 mg/kg bw/day, there was an increased incidence of vacuolar swelling of epithelial cells in the proximal tubules in males and females and tubular atrophy in males only. At the mid to high doses (90 and 270 mg/kg bw/day), there was an increased incidence of inflammatory foci and tubular casts in males and females and tubular dilation in males only. An increased incidence of glomerular sclerosis and thickening of the tubular basal membrane/Bowman's capsule of the glomeruli was also observed in males of the top dose group. Many of the lesions were still observed in rats sacrificed after the 21-day recovery period (week 7), indicating that they are persistent. However, the high incidence of tubular casts and vacuolar swelling of the proximal tubule epithelial cells observed in the untreated control recovery group suggest that these particular lesions may not be compound-related and/or are an exacerbation of an age-related effect.

Changes in some clinical chemistry and urinalysis parameters indicative of possible reduced kidney function were observed at doses ≥ 90 mg/kg bw/day. These included a statistically significant increase in serum creatinine in females (22.6% at 270 mg/kg bw/day), an increase in urine volume (males at ≥ 90 mg/kg bw/day and females at 270 mg/kg bw/day), an increase in urine specific gravity (males and females at ≥ 90 mg/kg bw/day) and reduced urine pH (males at ≥ 90 mg/kg bw/day and females at 270 mg/kg bw/day). Increased urine volume was still evident in mid and high dose males after the 21-day recovery period. A statistically significant increase in serum creatinine (35.4%) was observed in high dose males of the recovery group, but not in males sacrificed immediately after treatment.

In the liver, a low incidence of hepatocyte necrosis was observed at week 4 in females of all treatment groups. Slight to moderate centrilobular liver hypertrophy was reported in 5/5 males and 2/5 females of the top dose and was still evident in 1/5 males and 1/5 females at the end of the recovery period. No incidence of hypertrophy was reported in the control, low and mid dose groups. Mean liver weight relative to body weight was statistically significantly increased in top dose males at week 4 (20.9%) and week 7 (9.0%). In females, absolute and relative mean liver weights were increased in the mid dose groups at week 4 (absolute = 12.5%; relative to body weight = 11.2%; relative to brain weight = 16.2%). Absolute and mean liver weights were also increased in females in the high dose group (absolute = 16.5%; relative to body weight = 22.7%; relative to brain weight = 21.6%) and relative liver weight was still increased in top dose females at the end of the recovery period (8.0%). Serum alkaline phosphatase, a non-specific marker for liver toxicity, was increased in high dose females (40.8%) at week 3.

All doses given in this study were within guidance values for classification as STOT RE 2 which, for a 28-day study in the rat, are 30 < C ≤ 300 mg/kg bw/day.

90-day (MacKenzie, 1987):

In the first 90-day study (MacKenzie, 1987), Crl:CD rats (10/sex/dose) were fed diets containing 0, 200, 800 or 2000 ppm carboxin (equating to 0, 10, 40 and 100 mg/kg bw/day, respectively).

Signs of general toxicity (reduced body weight and food consumption) were observed at 40 and 100 mg/kg bw/day in males and at 100 mg/kg bw/day in females. The critical target organ was identified as the kidney. Histopathology of the kidneys in males treated with 10, 40 and 100 mg/kg bw/day revealed an increase in the incidence of chronic nephritis and proteinacous casts and an increase in the incidence of chronic nephritis in females treated with 40 and 100 mg/kg bw/day (see table below).

Dose (mg/kg bw/day)	0	10	40	100
Males (n=10)				
Kidneys: Chronic nephritis	0	9	10	10
Proteinaceous casts	0	4	6	8
Tubular cell degeneration in outer medulla collecting tubules	4	3	7	8
Tubular mineralisation in renal papilla	0	1	8	7
Females (n=10)				
Kidneys: Chronic nephritis	0	0	4	10
Proteinaceous casts	0	1	1	10
Tubular cell degeneration in outer medulla collecting tubules	0	0	3	5
Tubular mineralisation in renal papilla	0	0	0	6

The chronic nephritis was characterised by interstitial mononuclear cell infiltrate, thickening of the tubular walls and hypertrophy/regeneration of the tubular epithelium, primarily affecting the inner cortex near the interlobular vascular system. Additional renal findings included, proteinaceous casts in males at doses 10, 40 and 100 mg/kg bw/day and females at 100 mg/kg bw/day, degeneration of tubular epithelium in the collecting tubule of the outer medulla in males and females at doses 40 and 100 mg/kg bw/day and mineralisation of the tubes of the renal papilla in males at doses 40 and 100 mg/kg bw/day and females at 100 mg/kg bw/day. These findings were more prevalent in males and severity increased with dose.

Changes in clinical chemistry, possibly indicative of reduced kidney function were observed from 10 mg/kg bw/day. These included increased serum creatinine in males at all doses, statistically significant at 10 and 40 mg/kg bw/day and increased urea nitrogen (males: ≥ 10 mg/kg bw/day, females: 100 mg/kg bw/day).

Dose (mg/kg bw/day)	0	10	40	100
Males (n=10)				
Glucose (mg/dl)	105.0	101.9	92.6*	83.5*
Total protein (g/dl)	6.8	6.7	6.6	6.2*
Globulin (g/dl)	2.8	3.0	2.8	2.4*
Urea nitrogen (mg/dl)	13.3	16.8*	16.5*	15.0
Creatinine (mg/dl)	0.7	0.9*	0.8	0.9*
Females (n=10)				
Glucose (mg/dl)	87.1	93.6	89.5	91.5
Total protein (g/dl)	6.7	7.0	6.8	6.6
Globulin (g/dl)	2.4	2.6	2.6	2.5
Urea nitrogen (mg/dl)	14.5	14.0	14.9	20.0*
Creatinine (mg/dl)	0.8	0.8	0.8	1.0

* statistical significant

A statistically significant increase in kidney weight relative to body weight was observed in males of the top and females of the top dose [males: 13.3%/10.5% (left/right); females: 11.3/11.6% (left/right)] and mid dose males only [males: 9.5% (right)].

Increases in brain (males: 33.8% and females: 14.2%), liver (females: 9.5%) and testes/epididymis (26.7%/33.4%, left/right) weight relative to body weight were also observed at 100 mg/kg bw/day. However, there were no histopathological changes associated with these findings and they are likely to be a consequence of generalised body weight loss.

A NOAEL was not determined for males based on increased chronic nephritis and associated kidney lesions and clinical chemistry changes at 200 ppm (estimated to be equivalent to 10 mg/kg bw/day), the lowest dose used. Based on chronic nephritis and associated kidney effects at 40 mg/kg bw/day, a NOAEL of 10 mg/kg bw/day was determined for females.

All doses in this study are within guidance values for classification as STOT RE 2, which for a 90-day study are 10 < C ≤ 100 mg/kg bw/day, although the lower one (10 mg/kg bw/day) is an upper bound of guidance value for STOT RE 1.

90-day (Goldenthal, 2002a):

In a second 90-day study (Goldenthal, 2002a), conducted at lower doses to the first, CD rats 10/sex/dose) were fed diets containing 0, 80, 160, 240 (males only) or 480 (females only) ppm carboxin (equivalent to 0, 5.5, 10.5 and 16.1 mg/kg bw/day in males and 0, 6.0, 12.1 and 37 mg/kg bw/day in females).

No signs of general toxicity were observed. Similar to the previous two studies, the critical target organ was identified as the kidney.

A dose-related increase in the incidence of chronic progressive nephropathy of a trace to mild severity was observed in males treated with ≥ 10.5 mg/kg bw/day and a single trace incidence was observed in one female of the top dose group (see table below). An increase in the incidence of tubular mineralisation was also noted in the top dose males and females. However, the incidence in top dose males was the same as that reported in the female control group. A single incidence of hyperplasia for the urothelial epithelium was observed in one male of the top dose group.

Dose (mg/kg bw/day)	**0**	**5.5**	**10.5**	**16.1**
Males				
Chronic progressive nephropathy: Total Trace Mild	 3/10 3/10 0/10	 3/10 3/10 0/10	 7/9 6/9 1/9	 10/10 8/10 2/10
Tubular mineralisation	0/10	1/10	0/9	2/10
Hyperplasia of urothelial epithelium	0/10	0/10	0/9	1/10
Females				
Dose (mg/kg bw/day)	**0**	**6.0**	**12.1**	**37**
Chronic progressive nephropathy: Total Trace	 0/10 0/10	 0/10 0/10	 0/10 0/10	 1/10 1/10
Tubular mineralisation	2/10	0/10	0/10	4/10

A statistically significant increase in serum total protein (3.7%) and albumin (5.7%) was observed in the top dose males. These changes in clinical chemistry parameters could be

associated with the adverse effects on the kidneys. An increase in cholesterol was observed only in females of the high dose group (26.2%).

A NOAEL of 5.5 mg/kg bw/day was determined for males based on the chronic progressive nephropathy at the next highest dose (10.5 mg/kg bw/day). A NOAEL of 12.1 mg/kg bw/day was determined for females based on the single incidence of chronic progressive nephropathy at the next highest dose (37 mg/kg bw/day) and increased cholesterol.

Two higher doses in this study are within guidance values for classification as STOT RE 2, which for a 90-day study are 10 < C ≤ 100 mg/kg bw/day, although the medium one for males (10.5 mg/kg bw/day) is very close to upper bound of guidance value for STOT RE 1.

Repeated long-term toxicity studies (carcinogenicity and reproductive toxicity) in rats and mice

102 week study in the rat (Kehoe, 1991a);

In a 102 week guideline carcinogenicity study, Sprague-Dawley rats (60/sex/dose) were fed diets containing either 0, 20, 200, 400 ppm (males only) or 0, 20, 300, 600 ppm (females only) carboxin (corresponding to 0, 0.82, 8.65 and 16.86 in males and 0, 1.05, 15.08 and 33.48 mg/kg bw/day in females). Ten animals/sex/dose were sacrificed after 52 weeks. The study was terminated two weeks early (at week 102 instead of week 104) due to the 27% survival rate in males dosed with 400 ppm.

Survival was significantly lower in males at the highest dose of 16.86 mg/kg bw/day (weeks 65 - 102) and was lower in females at a dose of 33.48 mg/kg bw/day (weeks 85 - 102).

Clinical effects were observed at all dose levels and included: anorexia, thin, few/no faeces, soft faeces, low body temperature, languid and rough hair coat. A statistically significant reduction in body weight was observed from 200 ppm (8.65 mg/kg bw/day) in males and from 300 ppm (15.08 mg/kg bw/day) in females. Water consumption was statistically significantly increased in males from 200 ppm (8.65 mg/kg bw/day) and in females at 600 ppm (33.48 mg/kg bw/day).

At the interim sacrifice, there was an increased incidence of chronic nephritis, tubular cell degeneration and tubular mineralisation in the kidneys of both sexes at the mid dose (8.65 mg/kg bw/day) and above, but these changes were more prominent in males. In the unscheduled deaths, renal lesions were a major factor in premature deaths of male and female rats. There was an increased incidence of kidney cysts at 200 ppm (8.65 mg/kg bw/day) and above in males and at 600 ppm (33.48 mg/kg bw/day) in females.

In males that died or were sacrificed prematurely, there was an apparent treatment-related increase in fibrous osteodystrophy in the femurs at 20 ppm and above which was characterised by parathyroid hyperplasia and reduced renal function.

In females, the incidences of ovarian cysts appeared to be increased at all dose levels compared to the control females but there was no dose-response relationship.

The top dose for males (16.86 mg/kg bw/day) and females (33.48 mg/kg bw/day) and the mid dose for females (15.08 mg/kg bw/day) was above the guidance values for classification of STOT RE 2, which for a 2-year oral study are 1.25 < C ≤ 12.5 mg/kg bw/day.

Two-generation study in rats (Kehoe, 1991b):

In a two-generation reproduction study, Crl:CD rats (25/sex/dose) were administered carboxin through their diet (males: 0, 20, 200 and 400 ppm and females: 0, 20, 300 and 600 ppm, equivalent to 0, 1, 10, and 20 mg/kg bw/day in males and 0, 1, 15 and 30 mg/kg bw/day in females) for 10 weeks prior to mating and then throughout the gestation, lactation and weaning period (up to 33 weeks).

Changes to the kidneys in males at doses ≥ 200 ppm (≥ 10 mg/kg bw/day) and in females at doses ≥ 300 ppm (15 mg/kg bw/day) included pelvic dilation, proteinaceous casts, tubular mineralisation and chronic nephritis. The number of animals examined was limited to those showing gross findings only, however, the lesions were identical to those observed in chronic rat studies and were considered treatment-related.

Lifetime study carcinogenicity (19 month) in the mouse (Gunderson, 1982):

In this study, mice were dosed with 0, 50, 2500 or 5000 ppm (corresponding to 0, 8, 385 and 752 mg/kg bw/day in males and 0, 9, 451 and 912 mg/kg bw/day in females).

From week 78, there was a reduction in female survival at doses ≥ 451 mg/kg bw/day compared to the controls, which was statistically significant at the highest dose of 912 mg/kg bw/day (78% mortality versus 52% mortality in controls).

The incidence of centrilobular hepatocellular hypertrophy of the liver was increased in both sexes at 2500 ppm and above (≥ 385/451 mg/kg bw/day, liver weight not recorded) and there was an increase in hyperplastic liver nodules at 752 mg/kg bw/day in males.

An increase in the incidence of renal tubular nephritis was noted in both sexes at the top dose (752/951 mg/kg bw/day).

Dog Studies

28-day (Atkinson, 1989):

In a 28-day study (Atkinson, 1989), Beagle dogs (3/sex/dose) were fed diets containing 0, 600, 1200 and 2400 ppm carboxin (equating to 0, 19.3, 32.8 and 69.3 mg/kg bw/day in males and 0, 19.3, 30.8 and 65.7 mg/kg bw/day in females).

No signs of general toxicity were observed, except for one male in the high dose group that was reported as being emaciated. A male dog in this group lost 1.2 kg over the treatment period, but a control dog also lost a comparable amount of body weight over the same period. Mean body weights were comparable to controls.

Absolute and relative testes weight appeared to be decreased from control values in top dose males (absolute 24.3%/25% and relative to body weight 35.3%/72.5% (left/right)). However, the lower mean value was due to one animal with very small testes while the other two dogs in this group had comparable testes weights to controls. No treatment-related microscopic findings were reported.

All doses in this study were within guidance values for classification as STOT RE 2 which, for a 28-day oral study are 30 < C ≤ 300 mg/kg bw/day (calculated from the 90-day oral value in the rat).

90-day (Goldenthal, 2002b):

In a 90-day study (Goldenthal, 2002b), Beagle dogs (4/sex/dose) were fed diets containing 0, 160, 240 and 480 ppm (females) and 0, 160, 240 and 960 (males) ppm carboxin (corresponding to 0, 5.9, 9.0 and 17.7 mg/kg bw/day in females and 0, 5.3, 7.9 and 34.4 mg/kg bw/day in males).

No clinical signs or treatment-related effects on haematological, clinical chemistry and urinalysis parameters were reported.

There was a statistically significant increase in uterus/cervix weight (absolute: 235.4% and relative: 216.3%) at the top dose of 17.7 mg/kg bw/day. In addition, ovary weight was also increased in the top dose group (absolute: 219.4% and relative: 203.1%), however, this effect was not found to be statistically significant. The report attributed the increased organ weights to

two females being in oestrus. The increase in ovarian and uterine weight observed in this study was not seen at similar or higher doses in the 12-month dog study. However, it is noted that the dogs were 5 - 6 months of age in this study, whereas the dogs were 7 months of age at initiation of the 12-month study.

There were no findings on microscopic examination of the tissues.

All doses in this study were within guidance values for classification as STOT RE 2 which, for a 90-day oral study are 10 < C ≤ 100 mg/kg bw/day (based on the 90-day oral values in the rat).

One-year (Goldenthal, 1991):

In a 1-year study (Goldenthal, 1991), Beagle dogs (6/sex/dose) were fed diets containing 0, 40, 500 and 3000/5000/7500 ppm carboxin (corresponding to 0, 1.13, 16.1 and 158.4 mg/kg bw/day in males and 0, 1.28, 15.0 and 169.7 mg/kg bw/day in females).

The initial high dose of 3000 ppm (158.4 mg/kg bw/day in males and 169.7 mg/kg bw/day in females) was increased to 5000 ppm after seven weeks and to 7500 ppm after thirteen weeks.

A statistically significant dose-related reduction in body weight gain was observed in females of the mid and high dose groups (65.2% and 60.9%, respectively). Body weight gain was also decreased in mid and high dose males (19.0% and 47.6%, respectively); however, this was not statistically significant.

Slight but statistically significant changes in red blood cell parameters were observed in top dose males. This included a reduction in erythrocyte count (15.4% and 18.2% at 6 and 12 months, respectively), reduced haematocrit (13.9% at 12 months), increased mean cell volume (6.3% and 5.2% at 6 and 12 months, respectively) and increased mean cell haemoglobin (6.9% and 8.2% at 6 and 12 months, respectively). In the absence of any other effects, these are not considered further.

At the top dose level (158.4 mg/kg bw/day in males and 169.7 mg/kg bw/day in females increased after 7 and 13 weeks of exposure), there was an increase in alkaline phosphatase in males and females (6 months - males: 72.9%, females: 85.5%, 12 months - males: 111.9%, females: 111.3%) and an increase in cholesterol levels in males only (6 months - 42.1%, 12 months - 48.1%). In females only, creatinine levels were also increased (12 months - 40.0%). Relative liver weights were increased in both sexes at the top dose level (relative to body weight males: 27.5% and females: 27.4%). However, this was not accompanied by any adverse changes in histopathology. In females, there were small increases in relative heart weight (16.9% and 20.8% relative to body weight at doses of 15.0 and 169.7 mg/kg bw/day, respectively). In addition, an increase in pituitary weight in females (23.5% relative to body weight). These changes were likely to be due to the body weight changes.

There were no treatment-related macroscopic or microscopic changes observed at necropsy.

The top dose of 158.4/169.7 mg/kg bw/day in this study was significantly outside the guidance values for classification as STOT RE 2 which, for a 1-year study, are considered to be 2.5 < C ≤ 25 mg/kg bw/day (calculated from the value for a 90-day oral study in the rat).

Repeated dose toxicity: dermal (Goldenthal, 2002c)

In a 28-day study (Goldenthal, 2002c), CD rats (10/sex/dose) were treated with dermal applications of 0, 30, 400 or 1000 mg/kg bw/day carboxin for at least 28 consecutive days (6 hours/day). The test material was moistened with distilled water and applied to clipped dorsal skin (10% surface area) under a gauze dressing and tape (plus Elizabethan-like collar).

No substance-related signs of general toxicity or changes in clinical chemistry, haematology or urinalysis parameters were observed. There were no treatment-related macroscopic findings on post mortem examination or organ weight changes.

Microscopic changes were observed in the kidneys. There was an increase in the incidence and severity of tubular degeneration and tubular regeneration in males from 400 mg/kg bw/day. Regenerative foci were usually singular, however, when multifoci were observed these were more numerous in the mid and high dose (400 and 1000 mg/kg bw/day) males and were generally located in the inner cortex and outer stripe of the medulla. No kidney lesions exceeding the control data were reported in females.

The top dose of 1000 mg/kg bw/day applied in this study was outside the guidance values for classification as STOT RE 2 which, for a 28-day dermal study in rats, is 60 < C ≤ 600 mg/kg bw/day.

Comments received during public consultation

One MSCA supported DS's proposal to classify carboxin for STOT RE 2 and one MSCA considered that carboxin warrant classification as STOT RE 1.

Assessment and comparison with the classification criteria

Classification for target organ toxicity (repeated exposure) identifies the substance as being a specific target organ toxicant and, as such, it may present a potential for adverse health effects in people who are exposed to it. These adverse health effects include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. According to the CLP Regulation, substances are classified for target organ toxicity STOT RE 1 if they have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Classification with STOT RE 1 is triggered by the occurrence of significant toxic effects in experimental animals after exposure at or below the guidance values, which for oral route amounts ≤ 10 mg/kg bw/day for a 90-day repeated-dose study and ≤ 30 mg/kg bw/day for a 28-day repeated-dose study.

For STOT RE 2, the relevant guidance values for oral exposure are ≤ 100 mg/kg bw/day (rat 90-day study) and ≤ 300 mg/kg bw/day (rat 28-day study) according to tables 3.9.2 and 3.9.2 and paragraph 3.9.2.9.6 in Annex I to the CLP Regulation. The Regulation clearly specifies that these guidance values "are intended only for guidance purposes, i.e., to be used as part of a weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values" (Annex I, 3.9.2.9.8.).

There is no information on the repeated dose toxicity of carboxin in humans. However, there are two 90-day studies and one 28-day study available in the rat and a 28-day, 90-day and 12-month study in the dog. In the evaluation of repeated dose toxicity also the non-oncogenic effects observed on carcinogenicity and reproductive toxicity studies in rats and mice were included.

In the three repeated dose studies in the rat (one 28-day study and two 90-day studies), the critical target organ was identified as the kidneys, with increased weight, lesions of the renal tubules, chronic nephritis and progressive nephropathy accompanied by changes in clinical chemistry associated with kidney toxicity. Males are shown to be more sensitive to these effects than females. In the 28-day study the effects occurred at dose levels 90 and 270 mg/kg bw/day, but not at a dose of 30 mg/kg bw/day (Ullmann, 1983), and in the 90-day studies they were

observed at doses of 10/10.5 mg/kg bw/day - 100 mg/kg bw/day in male rats and at doses of 37/40 mg/kg bw/day - 100 mg/kg bw/day in females rates (MacKenzie, 1987; Goldenthal, 2002a), thus classification criteria were met in females rats for STOT RE 2, while in male rats the effects, which were seen at the upper limit for classification as STOT RE 1 were not sufficiently severe, and could be related to chronic progressive nephropathy, which spontaneously occurs with high frequency in male rats.

In a 102-week guideline carcinogenicity study in rats (Kehoe, 1991) there was an increased incidence of chronic nephritis; tubular cell degeneration and tubular mineralisation in the kidneys of both sexes at the mid dose (8.65 mg/kg bw/day) and above, but these changes were more prominent in males. Renal lesions were a major factor in premature deaths of male and female rats. There was an increased incidence of kidney cysts at 200 ppm (8.65 mg/kg bw/day) and above in males and at 600 ppm (33.48 mg/kg bw/day) in females. In this study, was also observed an increased incidence of fibrous osteodystrophy of femur which was most probably related to kidney injury causing disturbance of minerals turnover starting at the dose level of 0.82 mg/kg bw/day but the difference in incidence of fibrous osteodystrophy of femur in comparison with concurrent control group reached statistical significance in the Fisher Exact Probability Test only at a doses of 8.65 mg/kg bw/day and higher. At the dose level of 0.82 mg/kg bw/day no adverse effects were seen in kidney and other organs, therefore a NOAEL of 0.82 mg/kg bw/day (20 ppm in a diet) was proposed based on this study (Kehoe, 1991).

Thus, the significant adverse effects seen in both sexes at the dose of 8.65 mg/kg bw/day meet the criteria for classification as STOT RE 2 using the range of guidance values > 1.25 mg/kg bw/day and ≤ 12.5 mg/kg bw/day.

The dogs and mice seem to be less sensitive to nephrotoxicity of carboxin. No adverse effects were observed in histopathological examination of kidneys of dogs fed with diet for 28 days at doses of 19.3 – 69.3 mg/kg bw/day, or fed with diet for 90 days at dose 5.3 – 34.4 mg/kg bw/day or for one year at doses of 1.13 to 169.7 mg/kg bw/day, what suggests considerable interspecies variation of carboxin nephrotoxicity.

There are no data on repeated toxicity by inhalation, however, nephrotoxic effects were observed in male rats, although not in female rats, exposed in a 28-day study by dermal route 6 h/day (Goldenthal, 2002c) to carboxin at daily dose of 400 and 1000 mg/kg bw/day, what meets classification criteria for STOT RE 2 with the guidance values for dermal exposure being 60 < C ≤ 600 mg/kg bw/day.

Taking into account evidence from all three tested animal species, indicating a considerable interspecies variation in sensitivity to nephrotoxicity of carboxin, RAC is of the opinion that carboxin warrants **classification as STOT RE 2; H373: May cause damage to organs (kidney) through prolonged or repeated exposure**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS provided the results of a battery of four *in vitro* studies and two *in vivo* studies to assess the mutagenic potential of carboxin. Based on the results of these studies, the DS concluded that classification of carboxin for mutagenicity is not required.

In vitro assays

The *in vitro* gene mutation assay (Ames test) in *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) (Brusick, 1982) and the *in vitro* mammalian gene mutation test using Chinese hamster ovary cells (San and Clarke, 2001) were both negative.

In the mammalian chromosome aberration test *in vitro* on Chinese hamster ovary cells (Galloway, 1982), without metabolic activation, negative results were obtained up to concentrations of 17-500 µg/mL in the first trial and the second trial. At higher concentrations, there was suppression of mitotic activity and the highest concentration that could be scored was 500 µg/mL (a precipitate was formed at 400-500 µg/mL with toxicity at 600 µg/mL). With metabolic activation, negative results were obtained at concentrations of 17-500 µg/mL in the first trial. In the second trial, the concentrations tested ranged from 400 µg/mL to 1.4 mg/mL and cells could be scored up to 1.2 mg/mL (precipitate at all dose levels). There were slight increases in aberrations at 600 µg/mL and 1.2 mg/mL and a statistically significant increase in chromosome aberrations at 800 µg/mL. Therefore, a third trial was conducted using doses of 500-900 µg/mL. There was a statistically significant increase in chromosome aberrations at all concentrations from 700-900 µg/mL, however without dose-response relationship, which was accompanied by a dose-related increase in cytotoxicity (at 700 µg/mL there was approximately 50% reduction in cell confluence compared to controls and at 900 µg/mL very few cells survived). When aberrations due to breaks in the X chromosome were not included in analysis, the increase in aberrations was still statistically significant for concentrations between 750 and 850 µg/mL. Chromatid aberrations were also seen at 700 and 750 µg/mL. Cytotoxicity observed at concentrations ≥ 700 µg/mL, and severe cytotoxicity at concentrations ≥ 850 µg/mL. Carboxin was considered to be clastogenic only in the presence of metabolic activation at concentrations ≥ 700 µg/mL. It noted that clastogenicity of carboxin in this study was observed only at cytotoxic concentrations.

In the unscheduled DNA synthesis (UDS) assay *in vitro* (Myhr, 1982) on rat hepatocytes, carboxin (dissolved in DMSO) and ^{3}H-thymidine were added to viable cell cultures for an appropriate length of time (18h). Dose selection was based on the formation of a cloudy suspension of fine precipitate at 256 µg/mL and above. Nine doses were used: 0.513, 1.03, 2.56, 5.13, 10.3, 25.6, 51.3, 103 and 256 µg/mL. Autoradiographic techniques were then used to determine the extent of DNA repair in cells which are not in the S-phase of the cell cycle (i.e. the incorporation of ^{3}H-thymidine into the nuclear DNA). The criteria to conclude that UDS had been induced were a mean nuclear grain count of at least 6 grains/nucleus, or at least 10% of nuclei containing 6 or more grains, or at least 2% of nuclei containing 20 or more grains. One or more of these criteria were achieved or exceeded at concentrations of 5.13 - 103 µg/mL. The response was dose related and all three UDS criteria were exceeded at 51.3 µg/mL with cytotoxicity observed at concentrations ≥ 256 µg/mL.

In vivo assays

In the bone marrow chromosome aberration study (Cortina, 1983), the rats (20/sex/dose) were given carboxin by gavage at 0, 200, 660 and 2000 mg/kg bw/day. They were sacrificed at 6, 12, 24, and 48h after dosing. At 6h, the percentage of aberrant cells and total number of aberrations appeared to be increased at 660 mg/kg bw/day and above. At 12 h, the number of aberrations was noticeably increased at 660 mg/kg bw/day, but there was no dose-response. There were no apparent increases in the number of chromosome aberrations at 24 and 48h after dosing. No significant differences were observed between the negative controls and test groups when comparing modal number and mitotic indices. Appropriate results were obtained with the negative and positive controls. It was concluded that under the conditions of this study, there was no clear evidence that carboxin induced chromosome aberrations *in vivo*.

The second bone marrow chromosome aberration study (Cortina, 1985) included an acute and a subacute dosing regime. In the acute test, the rats (20/sex/dose) were given carboxin by gavage a single dose of 0, 750, 2000 and 4000 mg/kg bw and in the subacute study, the rats (5/sex/dose) were given by gavage for five days doses of 0, 100, 400 and 800 mg/kg bw/day. The animals were sacrificed at 6, 12, 24, and 48h after single dosing and 6h after the last dose in the subacute study. There were no statistically significant increases in the frequencies of chromosome aberrations in any of the treated groups. No statistically significant increases

between the mean mitotic indices of the test groups and vehicle control were seen. Appropriate results were obtained with the negative and positive controls. Under the acute or subacute dosing regimes of this study, carboxin did not induce chromosome aberrations.

In the *in vivo* UDS assay (Pant and Sly, 2006), rats (5/sex/dose) were given carboxin by gavage at doses of 0, 500, 1000, 2000 mg/kg bw. In this study there was no increase reported in mean net nuclear grain counts, nor was there an increase in the percentage of cells in repair, up to the highest dose tested (2000 mg/kg bw). Thus, no increase in unscheduled DNA synthesis was observed in the *in vivo* test in rats.

Comments received during public consultation

One commenting MSCA agreed with DS's proposal while another did not approve nor disapprove of the DS's proposal for no classification of carboxin for mutagenicity.

Assessment and comparison with the classification criteria

Substances, which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans are classified as Category 2, if there is a positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

- somatic cell mutagenicity tests *in vivo*, in mammals; or
- other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays

In case of carboxin, the *in vitro* gene mutation assays in bacteria (Brusick, 1982) and in mammalian cells (San and Clarke, 2001) were negative. The UDS assay *in vitro* (Myhr, 1982) on rat hepatocytes was positive, but carboxin was not genotoxic in the *in vivo* UDS assay in rats (Pant and Sly, 2006).

The *in vitro* mammalian chromosome aberration test on Chinese hamster ovary cells (Galloway, 1982) was positive with metabolic activation, although at concentration causing cytotoxicity. However, carboxin was not clastogenic in two *in vivo* bone marrow chromosome aberration studies (Cortina, 1983; 1985) using high single doses up to 4000 mg/kg bw and 5 repeated doses up to 800 mg/kg bw/day. Therefore, it may be concluded that carboxin does not have the ability to induce chromosome aberrations in somatic cells in the *in vivo* conditions.

Taking into account the results of these studies, RAC is of the opinion that **carboxin does not warrant classification as a germ cell mutagen**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No information on the carcinogenicity of carboxin in humans is available. Two oral carcinogenicity studies, one in the rat (Kehoe, 1991) and the second in the mouse (Gunderson, 1982) were provided by the DS.

In the Kehoe study (1991), 4/50 (8%) male rats receiving 400 ppm of carboxin had hepatocellular carcinoma (compared to 1/50 (2%) in the control and mid-dose groups). There was no increase in incidence of hepatocellular carcinoma in female rats. The value of 8% was outside of the laboratory historical control data (HCD) 0.9% (with a range 0 – 1.7%) and therefore, the increased incidence of hepatic carcinoma in male rats could be considered to be

treatment-related. However, taking into consideration increased incidence of hepatocellular carcinoma observed also in the concurrent control group (2%) and the low incidence of hepatocellular carcinoma observed (8%), the sex-specificity of the response (male only), the lack of statistical significance between the incidence of hepatocellular carcinoma in the concurrent control group and the 400 ppm group, the absence of a respective response in liver adenomas and more importantly the "excessive toxicity" reported in males of the 400 ppm group (75% mortality, clinical signs of toxicity, significant effects on terminal body weights (mean decrease of 17.3%) and on body weight gain (reduction of 23.4%), the severe nephrotoxicity), the DS concluded that these liver tumours are of no relevance to human health.

In mice (Gunderson, 1982), there was an increase in benign lung tumours in males in the 5000 ppm group (34% vs 17% in controls), which marginally exceeded the range of the laboratory HCD (31.1%). There was no treatment-related increase in alveolar-bronchial carcinomas in males and no treatment-related alveolar-bronchial adenomas or carcinomas in females. The combined incidence of adenomas and carcinomas at 5000 ppm (34%) was within the laboratory HCD upper limit for combined adenomas and carcinomas in males (37%). It is well established that CD-1 mice have a high spontaneous incidence of lung tumours, as shown by the concurrent and historical control data. Therefore, the DS concluded that the slight increase (compared to controls) in lung adenomas observed in males at 5000 ppm is unrelated to treatment with carboxin.

Based on results of these studies (Kehoe, 1991; Gunderson, 1982) and the chronic toxicity studies, the DS concluded that the presented data are conclusive, but not sufficient for classification of carboxin for carcinogenicity.

Comments received during public consultation

Two MSCAs supported DS's proposal not to classify carboxin for carcinogenicity and one MSCA considered that carboxin warrants classification Carc. 2; H351: Suspected of causing cancer.

Assessment and comparison with the classification criteria

There is no evidence relevant to carcinogenicity from studies in humans. However, two carcinogenicity studies in animals are available (Kehoe, 1991; Gunderson, 1982).

The results of the two animal studies (Kehoe, 1991; Gunderson, 1982) do not provide sufficient evidence of carcinogenicity, because an increased incidence of malignant neoplasms or an appropriate combination of benign and malignant neoplasms was not observed in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. There was also no increase in the incidence of tumours in both sexes of a single species. None of the existing studies (Kehoe, 1991; Gunderson, 1982) meet the criterion according to which a single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur at an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

Furthermore, the existing data do not indicate limited evidence of carcinogenicity, because only at the highest toxic dose, causing increased mortality (75%), kidney damage and significant reduction of terminal body weight, a small, non-statistically significant increase in incidence of hepatocellular carcinoma was observed in male rats, but not in females (Kehoe, 1991). In mice (Gunderson, 1982), a slight increase of benign lung tumours in males (34% versus 17% in controls) at the highest dose level (5000 ppm) is not taken as limited evidence of carcinogenicity

since, as pointed out by the DS, the combined incidence of adenoma and carcinoma was within the laboratory HCD upper limit for combined adenoma and carcinoma in males (37%).

Taking these data into account, RAC considers that **carboxin does not warrant classification as a carcinogen.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The effect of carboxin on fertility and sexual function was assessed based on results of the 2-generation study in rats (Kehoe, 1991b) and developmental toxicity was assessed based on one prenatal toxicity study in rats (Schardein, 1989) and one prenatal toxicity study in rabbits (Laughlin, 1981).

In the opinion of the DS, there was no evidence of any adverse effects on sexual function, fertility or development in rats and rabbits, therefore no classification for reproductive toxicity is proposed.

Comments received during public consultation

One commenting MSCA agreed with the DS's proposal.

Assessment and comparison with the classification criteria

In the two-generation reproduction study (Kehoe, 1991b), male rats were given carboxin in diet at concentrations of 0, 20, 200 and 400 ppm (corresponding to 0, 1, 10 and 20 mg/kg bw/day) and of 0, 20, 300 and 600 ppm (corresponding to 0, 1, 15 and 30 mg/kg bw/day) for female rats. Administration was 10 weeks prior to mating and then throughout gestation, lactation and weaning. F0 animals were mated twice to produce F1a and F1b litters and the F1b animals were mated twice to produce F2a and F2b litters. No treatment related effects on pre-coital intervals, gestation duration or fertility were observed. In histopathological examinations adverse effects in kidneys were observed starting at a dose of 10 mg/kg bw/day in male rats and of 15 mg/kg bw/day in females. In offspring, only the reduction of body weight gain by 7.6% on lactation day 4, and of 9.4 - 10.6% in lactation days 7 – 21 was observed in male F2b pups at a dose of 20 mg/kg bw/day which, most probably, was related to maternal toxicity. No other effects in offspring were reported.

In the prenatal developmental study (Schardein, 1989), Charles River female rats were given by gavage carboxin at doses 0, 10, 90 and 175 mg/kg bw/day (25 females/dose) on days 6 to 15 of gestation. Maternal toxicity was seen at a dose of 175 mg/kg bw/day as reduction of body weight (7.7%, day 16) and hair loss (8/25 females), and at a dose of 90 mg/kg bw/day as reduction of body weight (4.6%, day 16) and hair loss (4/25 females). No adverse maternal effects were seen at the dose of 10 mg/kg bw/day. The mean foetal body weight reduction of 6% was only observed in the 175 mg/kg bw/day group. No treatment-related malformations or developmental variations were observed at necropsy of the foetuses.

In the prenatal developmental study (Laughlin, 1981), Dutch Belted female rabbits were given by gavage carboxin at doses 0, 75, 375 and 750 mg/kg bw/day (16 females/dose) on days 6 to 27 of gestation. A treatment-related increase in the incidence of soft stool was observed at 750 mg/kg bw/day and an increase in the number of animals with reduced amount of stool was observed at 375 mg/kg bw/day and above. Treatment did not affect maternal or foetal body weight. Three out of 16 females at a dose of 750 mg/kg bw/day and 1 out of 16 female at

375 mg/kg bw/day had abortion of entire litter. There was no effect of number of viable foetuses, post implantation loss, total implantations, number of *corpora lutea* and number of dams with resorptions. There was no evidence of treatment-related malformations, variations or teratogenic effects in the pups examined.

Taking into account the results of animals studies in which no adverse effects on fertility, sexual function or development were observed, RAC is of the opinion that **carboxin does not warrant classification as a reproductive toxicant**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Carboxin is a fungicide used as an agricultural seed treatment to prevent fungal pathogens. On the ECHA C&L inventory, 16 out of 89 notifiers submitted a classification as Aquatic Acute 1 and Aquatic Chronic 1.

Degradation

The DS proposed to not consider carboxin as rapidly degradable for classification purposes. The basis for this proposal is that carboxin is hydrolytically stable at environmentally relevant pH and temperature (Clayton and Lowrie, 2003; Dzialo and Lengen, 1983), measured aquatic photolysis of carboxin (Horree, 1992; DAR, 2006; Harned, 2003a and 2003b) was not transferable to photodegradation in the European environment in terms of mineralisation or transformation, carboxin was found to be not readily biodegradable (Van Dijk, 1989). Carboxin is considered to undergo rapid primary degradation in water-sediment system (Muttzall, 1994; Wanner, 2004a) and soil (Mamoumi, 2004; Wanner, 2004b; Wanner, 2004c; Gaydosh, 1989; Beerbaum, 1990) but is not considered to undergo greater than 70% ultimate degradation within 28 days at environmentally relevant pH and temperature (12°C).

Aquatic Bioaccumulation

The DS proposed to not consider carboxin as being bioaccumulative in the aquatic environment for classification purpose. The basis for this proposal is a measured log Pow of 2.3 (Riggs, 2001g) and that no calculated or measured bioaccumulation data are currently available.

Acute Toxicity

The DS included in the CLH report the following aquatic acute toxicity studies for carboxin.

Test guideline and purity	Species	Endpoint	Toxicity value	Conditions	Reference
Fish					
US EPA 72-1 97.39%	*Oncorhynchus mykiss*	96h LC_{50}	2.3 mg/L	Flow-through Mean measured	Bettencourt, 1994a
US EPA 72-1 97.39%	*Lepomis macrochirus*	96h LC_{50}	3.6 mg/L	Flow-through Mean measured	Bettencourt, 1994b
Invertebrates					
OECD TG 202 97.39%	*Daphnia magna*	48h EC_{50}	> 57 mg/L	Flow-through Mean measured	Putt, 1994
Algae & aquatic plants					

US EPA FIFRA 123-3 97.5%	*Pseudokirchneriella subcapitata*	5d E_rC_{50}	0.45 mg/L	Static Mean measured	Hughes, 1990

The DS proposed to classify carboxin as Aquatic Acute 1 with an acute M-factor of 1, based on the results of the acute algae study (Hughes, 1990). The short-term (acute) aquatic ecotoxicity test results showed toxicity of 2.3 mg/L and 3.6 mg/L for fish (96h LC_{50} US EPA 72-1 Bettencourt, 1994a&b), >57 mg/L for invertebrates (48h EC_{50} OECD 202 Putt, 1994), and 0.45 mg/L for algae (5d E_rC_{50} Hughes, 1990). The DS evaluated an OECD 204, GLP, semi-static, 21-day fish (*Cyprinus carpio*) toxicity study with a nominal 21-d NOEC of 0.32 mg/L based on mortality, toxicity symptoms and growth only as supporting data. In addition to the parent carboxin three degradants carboxin sulfoxide, carboxin sulfone and P/V-54 were tested for all three trophic levels: fish, invertebrates and algae. As a result carboxin parent is considered more acutely toxic than its degradants with algae being the most acutely sensitive trophic level and relevant for classification.

Chronic Toxicity

The DS included in the CLH report the following aquatic chronic toxicity studies for carboxin.

Test guideline and purity	**Species**	**Endpoint**	**Toxicity value**	**Conditions**	**Reference**
Fish					
-	-	-	-	-	-
Invertebrates					
OECD TG 202 97%	*Daphnia magna*	17d NOEC reproduction and growth	0.32 mg/L	Semi-static Nominal	Bogers, 1989b
Algae & aquatic plants					
US EPA FIFRA 123-3 97.5%	*Pseudokirchneriella subcapitata*	5d NOE_rC	0.107 mg/L	Static Mean measured	Hughes, 1990

The DS proposed to classify carboxin as Aquatic Chronic 2. The basis for this proposal is that the long-term (chronic) aquatic ecotoxicity test results showed toxicity of 0.107 mg/L for algae (5d NOE_rC Hughes, 1990) for carboxin parent and for carboxin sulfone 0.25 mg/l (72h NOE_rC Czech, 2002c). The 17-d NOEC for *Daphnia* of 0.32 mg/L was not considered robust for the purpose of deriving a chronic classification however the value supports the Aquatic Chronic 2 classification.

Adequate chronic toxicity data for fish and invertebrates are not available. Consequently the surrogate approach to derive a chronic classification was considered, see CLP Regulation Annex I 4.1.2 and the decision scheme in figure 4.1.1, resulting also in Aquatic Chronic 2 based on acute data for fish (2.3 mg/L 96h LC_{50}) and carboxin a non-rapidly degradable substance.

Comments received during public consultation

Four MSCAs commented on the proposals, with three agreeing with the proposed classification. The fourth MSCA supported the classification for acute toxicity but questioned the proposed chronic classification. They considered that carboxin should be classified as Aquatic Chronic 1 (M=1) based on the mean measured values and a significant effect already in the lowest test

concentration of the algae study (Hughes, 1990). The DS replied that the NOEC was calculated on the full study duration, 5 days, and even if close to it is above the limit for Aquatic Chronic 1 classification (NOErC = 0.107 > 0.1 mg/L).

Additional key elements

Acute and chronic algae toxicity test by Hughes, 1990

Following the comment by one MSCA, RAC reevaluated the results of the 5-day, GLP, static algal growth inhibition study (Hughes, 1990) which was carried out using *Pseudokirchneriella subcapitata* and following US EPA FIFRA 123-2 guideline. The original study used carboxin technical with a purity of 97.5%. Exposure solutions were prepared with DMF (dimethylformamide) solvent to aid dispersion and a solvent control was included. Triplicate of all 5 solutions (0.125, 0.25, 0.5, 1.0 and 2.0 mg/L) and of the water and solvent controls were used. Analysis was undertaken at 0 hours and on day 5, which included analysis for carboxin sulfoxide. At 0 hours, carboxin concentrations were 92-122% and, at day 5, 49-80% of nominal carboxin concentrations. The study reported EC_{50} and NOEC data based on mean measured concentrations however using at day 0 carboxin and day 5 carboxin + carboxin sulfoxide. The study used the Dunnett's test as statistical method. The results of this re-evaluation are given below.

The table below presents nominal exposure concentrations, analytical data and algal growth inhibition.

				Algal growth inhibition (%)		
Nominal	**Day 0 measured carboxin**	**Day 5 measured carboxin**	**mean measured carboxin**	**72h**	**96h**	**120h**
Control	Not detected	Not detected	-	-		-
Solvent control	Not detected	Not detected	-	-		-
0.125	0.152	0.061	0.107	11.3	23.4	13.1
0.250	0.276	0.170	0.223	28.2	42.7	28.2
0.500	0.525	0.375	0.450	45.8	59.3	49.8
1.000	1.130	0.759	0.945	74.7	86.5	86.3
2.000	1.830	1.604	1.717	90.6	96.5	97.6

The DS presented different effect and toxicity values than those reported in the DAR. For the purpose of classification, the dossier submitter considered effects based on carboxin only, not carboxin plus carboxin sulfoxide as in the DAR. Carboxin photodegrades and, given the test light conditions at ~4306 lux, losses of the parent carboxin are considered likely. For the purpose of classification, the E_rC_{50} and NOE_rC have been reconsidered to reflect carboxin concentrations only. It was noted that the study was run over 5 days instead of the standard 72 - 96 hour duration. However, after comparing the growth inhibition on days 3 and 5, the dossier submitter did not consider this to affect the validity of the 5 day results.

RAC has reassessed the original data from table 4 on page 21/22 in the study report with a statistical analysis using the software ToxRat Professional Version 3.2 (www.toxrat.com/). See the Background Document for further details.

A summary of Results for all Endpoints is given in the table below, including: Critical effect and threshold concentration as observed at end of experimental time; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration.

Critical Conc.s [mg/L]		**0 - 72h**	**0 - 96h**	**0 - 120h**
Cell number				
	EC_{10}	0.113	***0.063***	0.134
95%-CL	lower	0.072	0.036	0.100
	upper	0.177	0.109	0.178
	EC_{20}	0.183	0.106	0.197
95%-CL	lower	0.122	0.065	0.152
	upper	0.278	0.177	0.258
	EC_{50}	0.465	0.291	0.414
95%-CL	lower	0.286	0.161	0.302
	upper	0.765	0.538	0.572
Cell number	LOEC	≤0.107	≤0.107	≤0.107
	NOEC	<0.107	<0.107	<0.107

n.d.: not determined due to mathematical reasons or inappropriate data

The Williams Test revealed that at 72h, 96h and 120h the lowest test concentration is significantly different from the solvent control. Consequently, RAC concludes that the NOEC is < 0.107 mg/L. RAC further concludes that for the purpose of classification the **96h** EC_{10} of 0.063 mg/L should be used.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal and argumentation of the DS to not consider carboxin as rapidly degradable.

Aquatic bioaccumulation

RAC agrees with the proposal and argumentation of the Ds that carboxin has low potential for bioaccumulation based on the measured log Pow and therefore does not meet the criteria for bioaccumulation.

Acute Toxicity

RAC reassessed the algae toxicity test by Hughes, 1990 and concludes that for the purpose of classification a 96h EC_{50} of 0.291 mg/L should be used, rather than the reported of 0.45 mg/L.

RAC agrees with the proposal of the DS to classify carboxin as **Aquatic Acute 1; H400 with an acute M-factor of 1**.

Chronic Toxicity

RAC agrees with the DS that the 17d NOEC for Daphnia of 0.32 mg/L can not be considered robust enough for the purpose of deriving a chronic classification. Consequently, adequate chronic toxicity data for fish and invertebrates are not available.

RAC agrees with a commenting MSCA that the growth inhibition of 11.3% at 72h and of 23.4% at 96h is significant and that the NOEC is below the lowest test concentration, so below the mean measured concentration of 0.107 mg/L (Hughes, 1990). RAC concludes that for the purpose of classification the 96h EC_{10} of 0.063 mg/L should be used.

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

	fish	**crustacea**	**algae**
included in dossier	-	0.32 mg/L (17d NOEC)	0.107 mg/L (NOEC)
re-assessment by RAC	-	-	0.063 mg/L (96h EC_{10})

The surrogate approach to derive a chronic classification results in Aquatic Chronic 2 based on acute data for fish (2.3 mg/L 96h LC_{50}) and carboxin being a non-rapidly degradable substance.

RAC concludes to classify carboxin as **Aquatic Chronic 1; H410 with M=1 based on the 96h EC_{10} of 0.063 mg/L**.

ANNEXES:

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

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