CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: CARBOXIN (ISO); 2-methyl-N-phenyl-5,6-dihydro-1,4-oxathiine-3-carboxaamide; 5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide

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

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

Index Number: **Not Assigned**

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Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***CARBOXIN***
EC number:	***226-031-1***
CAS number:	***5234-68-4***
Annex VI Index number:	***Not yet assigned***
Degree of purity:	***≥ 98.7 %***
Impurities:	***There are a number of impurities present which have been taken into account, but are not considered relevant to the harmonised classification and labelling proposal.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No current entry
Current proposal for consideration by RAC	Skin Sens. 1B; H317 – May cause an allergic skin reaction STOT RE 2; H373 – May cause damage to the kidneys through prolonged or repeated exposure Aquatic Acute 1; H400 – Very toxic to aquatic life (M = 1) Aquatic Chronic 2; H411 – Toxic to aquatic life with long lasting effects

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens. 1B; H317 – May cause an allergic skin reaction STOT RE 2; H373 – May cause damage to the kidneys through prolonged or repeated exposure Aquatic Acute 1; H400 – Very toxic to aquatic life (M = 1) Aquatic Chronic 2; H411 – Toxic to aquatic life with long lasting effects

Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Conclusive but not sufficient
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	Conclusive but not sufficient
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Conclusive but not sufficient
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient

3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens. 1B; H317 – May cause an allergic skin reaction**	Not applicable	Not classified	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient
3.9.	Specific target organ toxicity – repeated exposure	**STOT-RE 2; H373 – May cause damage to the kidneys through prolonged or repeated exposure**	Not applicable	Not classified	
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Conclusive but not sufficient
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 – Very toxic to aquatic life** **Aquatic Chronic 2; H411 – Toxic to aquatic life with long lasting effects**	**M-= 1**	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Conclusive but not sufficient

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s): GHS07; GHS08; GHS09
Signal word: Warning
Hazard statements: H317 - May cause an allergic skin reaction;
H373 - May cause damage to the kidneys through prolonged or repeated exposure;
H410 – Very toxic to aquatic life with long lasting effects

Precautionary statements: Precautionary statements are not listed on Annex VI of CLP

Proposed notes assigned to an entry: Not applicable

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Carboxin is a pesticidal active substance and has been reviewed under Directive 91/414/EEC with the UK as the Rapporteur Member State (RMS). There is no existing entry on Annex VI of CLP and there have been no previous harmonised classification and labelling discussions for this substance. In accordance with Article 36(2) of the CLP Regulation, carboxin should now be considered for harmonised classification and labelling.

At the time of submission, the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Carboxin is a systemic fungicide used as a seed treatment to control soil and seed borne diseases in cereals. Following peer-review of the Draft Assessment Report (DAR), EFSA concluded (EFSA Journal 2010;8(10):1857) that carboxin was of low acute oral, dermal and inhalation toxicity. It was not considered a skin or eye irritant. These conclusions are supported in the CLH report and it is not proposed to classify for these hazard classes. The criteria for classification as a skin sensitiser are met, with > 30% of animals responding to challenge with the substance in a standard Guinea pig maximisation test**. Classification with Skin Sens. 1B; H317 – May cause an allergic skin reaction is proposed.**

The target organ for repeated administration in rodents was the kidney, with the presence of lesions of the renal tubules, chronic nephritis and progressive nephropathy observed at, or below, doses relevant for classificaiton. It is therefore proposed to classify Carboxin with **STOT-RE 2; H373 – May cause damage to the kidneys through prolonged or repeated exposure.**

Carboxin is not considered to be mutagenic and therefore no classification is proposed.

An increased incidence of hepatocellular carcinoma (above the historical control data) was noted in male rats, raising concern for classification with Carc 2 in the EFSA conclusion. However, when considering the low incidence observed (8% vs 2% in controls), the sex-specificity of the response, the lack of statistical significance, the absence of a respective response in liver adenomas and more importantly the "excessive toxicity" reported at this dose in males (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%] and the severe nephrotoxicity for which classification with STOT-RE 2 has already been proposed), it is concluded that these liver tumours are of no relevance to human health and therefore it is not proposed to classify for carcinogenicity.

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

Carboxin is considered not-rapidly degradable for the purpose of classification and labelling. Acute toxicity data for fish, invertebrates and algae are available. Algae are the most acutely sensitive trophic level with a carboxin 5-d E_rC_{50} of 0.45 mg a.s./l. Based on the acute ecotoxicity data available, with $L(E)C_{50}$ values < 1 mg/l, classification as **Aquatic Acute 1; H400 – Very toxic to aquatic life** is applicable with an acute **M-factor of 1** based on $0.1 < L(E)C_{50} \leq 1$ mg/l.

The long-term aquatic data suggest chronic toxicity in the range 0.1-1 mg/l. The carboxin algal 5-d NOErC is 0.107 mg a.s./l and the carboxin sulfone algal 72-h NOE_rC is 0.25 mg/l. This results in the classification Aquatic Chronic 2 based on > 0.1 NOEC ≤ 1 mg/l for a non-rapidly degradable substance. A non-standard 17-d NOEC for *Daphnia* supports the Aquatic Chronic 2 classification. Adequate chronic toxicity data for fish are not available.

Given robust chronic endpoints are not available for fish and invertebrates, the surrogate approach to deriving chronic classification should be considered. Using the available acute data for fish and *Daphnia*, the most stringent chronic classification is Aquatic Chronic 2.

Overall classification as **Aquatic Chronic 2; H411 – Toxic to aquatic life with long lasting effects** is applicable.

2.3 Current harmonised classification and labelling

Carboxin is not currently listed in Annex VI to the CLP Regulation.

2.4 Current self-classification and labelling

At the time of submission there are a number of self-classification entries for carboxin in the C&L inventory. These are summarised below:

Table 4: Classification and labelling in the C&L Inventory

Classification		Labelling	
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Acute Tox. 4	H302	H302	GHS07 Wng
Skin Sens. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H373 (Kidney) (Dermal) H400 H410	H317 H373 H410	GHS07 GHS09 GHS08 Wng
Acute Tox. 4 Acute Tox. 4 Acute Tox. 4	H302 H312 H332	H302 H312 H332	GHS07 Wng
Not Classified			
Acute Tox. 4 Acute Tox. 4	H302 H312	H302 H312	GHS07 Wng

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Carboxin is a pesticide active substance that has been reviewed under Directive 91/414/EEC with the UK as the RMS. In accordance with Article 36 (2) of the CLP Regulation carboxin is subject to harmonised classification and labelling and this proposal considers all hazard classes.

Part B

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

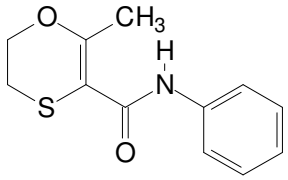
1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	226-031-1
EC name:	Carboxin
CAS number (EC inventory):	5234-68-4
CAS number:	5234-68-4
CAS name:	1,4-Oxathiin-3-carboxamide, 5,6-dihydro-2-methyl-N-phenyl-
IUPAC name:	5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide*
CLP Annex VI Index number:	Not Assigned
Molecular formula:	$C_{12}H_{13}NO_2S$
Molecular weight range:	235.3

***** *As included in the EFSA conclusion*

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Carboxin	98.7 %	≥ 98.7%	

Current Annex VI entry: N/A

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in the substance. These impurities have been taken into consideration and are not considered to further impact on the classification proposed in this report. Further information on the impurities is considered confidential but full details are provided in the technical dossier.

Current Annex VI entry: N/A.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: N/A

1.2.1 Composition of test material

The tested material is considered to be equivalent to that outlined above for the purposes of classification and labelling.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

The physiochemical properties of carboxin are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.2; Physical and Chemical properties – August 2006

All studies were conducted to appropriate quality standards and were considered adequate during the peer review of the active substance.

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White, solid (Pure 99.9 %) Pale yellow solid (Techn. 97 %)	Riggs, 2001a (99.9%) Riggs, 2001b (97%)	OPPTS 830.6302 DAR B.2.1.7
Melting/freezing point	91 – 92 °C	Riggs, 2001a	Purity 99.9 % EEC A1(capillary method) DAR B.2.1.1
Boiling point	Substance decomposes at 210°C	Riggs, 2001a	OECD 103 DAR B.2.1.2
Relative density	1.45	Dunn, 2001	Purity 99.9 % EEC A3 (pycnometer) DAR B.2.1.4
Vapour pressure	2 x 10^{-5} Pa at 25 °C	Tremain, 2001 a	Purity 99.9 % EEC A4 (vapour pressure balance) DAR B.2.1.5
Surface tension	61.2 mN/m at 20 °C	Evans, 2001	Purity 98.2 % EEC A5 DAR B.2.1.24
Water solubility	0.15 g/l at pH 5 and 20 °C 0.13 g/l at pH 7 and 20°C 0.14 g/l at pH 9 and 20 °C	Riggs, 2001d	Purity 99.9 % OECD 105 (flask method) DAR B.2.1.11
Partition coefficient n-octanol/water	Log P_{ow} = 2.3 Range of pHs were not looked at due to the pKa being < 0.5 and the solubility in water not altering with pH.	Riggs, 2001 f	Purity 99.9 % EEC A8 (HPLC) DAR B.2.1.13

Flash point	Not applicable, substance is a solid and melting point > 40 °C		DAR B.2.1.21
Flammability	Not considered highly flammable – experience with handling and use suggests substance is not pyrophoric and does not emit flammable gases on contact with water.	Tremain, 2001 b	Purity 98.2 % EEC A10 DAR B.2.1.20
Explosive properties	Not explosive	Tremain, 2001 b	Purity 98.2 % EEC A14 DAR B.2.1.22
Self-ignition temperature	No self-ignition up to a temperature of 91 °C (melting point)	Tremain, 2001 b	Purity 98.2 % EEC A16 DAR B.2.1.20
Oxidising properties	Non-oxidising	Tremain, 2001 b	Purity 98.2 % EEC A17 DAR B.2.1.23
Dissociation constant	pKa = < 0.5 (no temperature given in DAR)	Thomas and Book, 1998	Purity 99.3 % OECD 112 DAR B.2.1.18

2 MANUFACTURE AND USES

2.1 Manufacture

This substance was manufactured outside of the EU (Canada) for use as a pesticidal active substance.

2.2 Identified uses

Carboxin is used within the EU as a fungicidal active substance, which is applied to the seeds of small grain cereals (wheat, barley, oats, rye and triticale) for control of seed and soil borne fungal diseases.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

3.1 PHYSIO-CHEMICAL PROPERTIES

3.1.1 Summary and discussion of physiochemical properties

3.1.2 Comparison with criteria

In a standard study (EEC Method A10), carboxin was not determined to be flammable. Therefore, it does not meet the criteria for classification as a flammable solid. The self-ignition temperature was found to be > 91 °C. Further, experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.

In a standard study (EEC Method A14), carboxin did not exhibit any explosive properties. Therefore, it does not meet the criteria for classification as an explosive substance.

Finally, in a standard study (EEC Method A17), carboxin did not burn to completion and so it is not classified as an oxidising solid.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4 HUMAN HEALTH HAZARD ASSESSMENT

References are taken from the Draft Assessment Report – DAR – Carboxin – Volume 3, Annex B.6; toxicology and metabolism – August 2006 and the Addendum to the DAR August 2007.

4.1 Toxicokinetics (Absorption, Metabolism, Distribution and Elimination)

4.1.1 Non-human information

ADME of [^{14}C]-carboxin (labelled at the benzene ring)

Following oral administration of a single dose of [^{14}C]-carboxin in rats (5 or 150 mg/kg), extensive absorption of material was observed (approximately 81 %). Elimination of [^{14}C]-carboxin was mainly through urinary excretion, primarily during the first 24 hours post dosing, suggesting rapid absorption from the gastrointestinal tract (77 – 82 %). Biliary metabolism as a route of elimination appeared to be less significant as only 6 – 11 % of the dose was recovered in the faeces. Increasing the dose from 5 to 150 mg/kg resulted in possible saturation of the excretion processes as the rate of urinary excretion in the higher dose group was lower than the 5 mg/kg group. Following oral administration of multiple doses of carboxin in rats (5 mg/kg, qd, 14 days) followed by a single dose of [^{14}C]-carboxin (5 mg/kg), similar recovery to the single dose studies was observed with the rate and extent of recovery in urine being similar to the single dosed 5 mg/kg group.

At 72 h post-dosing the organs were collected and analysed for radioactivity. Tissue levels were low in all studies. The highest levels of radioactivity were found in the liver (0.21 %), red blood cells (0.12 %) and kidneys (0.02 %). [^{14}C]-Carboxin was extensively metabolised (no parent compound detected in the urine samples or faecal sample). In urine, the key pathway involved oxidation to carboxin sulfoxide followed by *p*-hydoxylation of the phenyl ring to yield *p*-hydoxylated carboxin sulfoxide. This metabolite was observed in all urine samples and there was no indication of any quantitative or sex differences. Hydrolysis of the amide bond of *p*-hydoxylated carboxin followed by *N*-acetylation, yielded 4-acetamidophenol and 4-acetamidophenol glucuronide. 4-Acetamidophenol was only identified in the urine of high dose males, suggesting saturation of glucuronide conjugation at high doses. Minor pathways included; further oxidation of carboxin sulfoxide to form carboxin sulfone, *N*-acetylation of liberated aniline to form acetanilide or substitution on the aniline ring resulting in *N*-acetyl cysteinyl conjugate of aniline.

In a follow-up study conducted by McManus *et al*, 1993 enzymatic hydrolysis of the urine samples with β-glucuronidase confirmed the presence of a glucoronide. Due to the low level of faecal excretion, it was not considered feasible to try to identify the faecal metabolites.

ADME of [^{14}C]-carboxin (labelled at the oxathiine ring)

A second guideline oral ADME study (Gupta *et al*, 2006) was conducted in Sprague-Dawley rats to further investigate the fate of the oxathiine ring. This study revealed a similar pattern of absorption, distribution and excretion. The metabolism of ^{14}C-oxathiine-carboxin was extensive and rapid (no parent compound was observed in the excreta) with the main metabolic reactions being ring hydroxylation, oxidation of the sulphur, O-methylation of the ring hydroxyl groups, oxidative cleavage of the oxathiine ring and conjugation (glucuronidation and sulfation).

Refer to DAR B.6.1.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

In rats, carboxin is readily absorbed and rapidly eliminated after oral exposure with no differences between the sexes. The majority of the administered dose was excreted in the urine within 72 h with less being excreted in the faeces. Extensive and rapid metabolism occurred with no parent compound being detected in the excreta. The main metabolic reactions were ring-hydroxylation, oxidation of sulfur and the aromatic ring, *O*-methylation of the ring hydroxyl groups, oxidative cleavage of the oxathiine ring and conjugation of the aniline and phenol moieties (glucuronidation and sulfation).

4.2 Acute toxicity

Three acute toxicity studies are available for carboxin and are summarised below:

Table 11: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
Rat, Sprague-Dawley, 5/sex/dose 2430, 3500 and 5040 mg/kg bw 0.5 % Carboxymethylcellulose (CMC) (aq.) US-EPA F, 81-1 GLP Purity 102.2 % Goldenthal, EI (1992a) DAR B.6.2.1	M - 2588 mg/kg F - 3080 mg/kg	*Mortalities:* 2430 mg/kg/bw: M 2/5, F 1/5 3500 mg/kg/bw: M 4/5, F 4/5 5040 mg/kg/bw: M 4/5, F 4/5 *Clinical signs 1 – 4 h post dosing:* Decreased activity, ptosis, ataxia *Clinical signs up to 13 days post dosing:* Coldness to the touch, decreased defecation, prostration, chromodacryorrhea and impaired limb function & ataxia (1 animal only) *Necropsy:* Yellow fluid in the lumen of the ileum/duodenum/jejunum (≥ 2430 mg/kg) and focal discolouration of the stomach glandular mucosa (≥ 3500 mg/kg bw)
Acute Inhalation		
Method	**LC50**	**Observations and remarks**
Rat, Sprague-Dawley, 5/sex/dose 4.7.mg/l/4 hr (aerosol; whole body-maximum achievable concentration) MMAD 6.8 ± 2.2 µm US EPA F 81-3 GLP Purity: 102.2% Ulrich (1993) DAR B.6.2.3	M > 4.7 mg/l F > 4.7 mg/l	No mortalities occurred. On removal from the exposure chamber, all animals were covered with the test material. *Clinical signs 1 – 4 h post dosing:* Increased salivation, corneal opacity and labored breathing (F: 1/5). *Clinical signs up to 13 days post dosing:* Red/brown material around the nose and corneal opacity (F:1/5). *Necropsy*: No abnormalities observed.

Acute Dermal		
Method	**LD50**	**Observations and remarks**
Rabbit, New Zealand White, 5/sex 4000 mg/kg bw (powder moistened in deionised water) Exposure time: 24 hrs US EPA F81-2 GLP Purity: 102.2% Goldenthal (1992b) DAR B.6.2.2	M > 4000 mg/kg F > 4000 mg/kg	No mortalities or overt signs of toxicity were observed. *Necropsy:* Gross necropsy revealed a cyst on the right kidney of one male. No other abnormalities were observed.

4.2.1 Non-human information

See Table 11.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

No information on the acute toxicity of carboxin in humans is available. Based on animal data (presented in Table 11), carboxin should not be classified for acute toxicity.

4.2.4 Comparison with criteria

The oral LD_{50} values of 2588 and 3080 mg/kg bw for male and female rats, respectively, 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.

In an acute inhalation study, the LC_{50} was > 4.7 mg/L for rats. The mean mass aerodynamic diameter (MMAD) was 6.8 ± 2.2 µm (inhalable fraction ≤ 4 µm), meaning a large proportion of the dose might not have deposited in the respiratory tract and may well have translocated to the GI tract. The criteria for classification of dusts and mists as acute toxicity category 4 is $1.0 < LC_{50} \leq 5.0$. In the absence of any conclusive data to show that carboxin causes acute toxicity by the inhalation route no classification is proposed.

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

4.2.5 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Please refer to Table 11 and section 4.4.3.

4.3.2 Comparison with criteria

Three guideline studies investigating the effects of carboxin after a single dose by oral, dermal and inhalation routes were reported. The signs that were apparent after a single exposure to carboxin were indicative of non-specific, general acute 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.

Whilst there was no evidence of CNS depression during the acute studies, there was evidence of sedation in a four-week oral gavage toxicity study in rats. At doses of 30 mg/kg bw/day animals displayed symptoms of sedation shortly after treatment, lasting up until the afternoon of the same day. These effects were noted to be more pronounced and lasting for a longer duration in the higher dose groups indicating a dose-related cause. According to the regulation, the criteria for classification as STOT-SE 3 are central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. Since there was no evidence of sedation in other repeated dose studies in rats or with other species and coupled with the transient nature and lack of severity it is considered unnecessary to classify for STOT-SE 3 for CNS effects.

In an acute inhalation toxicity study (data presented in Table 11), laboured breathing was observed in 1/5 females at 4.7.mg/l/4 hr [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 (see Sections 4.4.2 and 4.4.1). 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).

4.3.3 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results
Rabbit, New Zealand White, 3/sex 500 mg Vehicle: deionised water (4 ml) Semi-occlusive US EPA F81-5 GLP Purity: 102.2% Goldenthal (1992c) DAR B.6.2.4	Mean scores over 24 - 72 hours for six rabbits: Erythmea: 0 – 0 – 0 – 0 – 0 – 0 Oedema: 0 – 0 – 0 – 0 – 0 – 0

4.4.1.1 Non-human information

See Table 12

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

No signs of dermal irritation were observed in any rabbit during the study period. There were no deaths or overt signs of toxicity observed during the study.

4.4.1.4 Comparison with criteria

No signs of erythema or oedema were reported in a guideline skin irritation study. Therefore, carboxin does not meet the criteria for classification as a skin irritant.

4.4.1.5 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results
Rabbit, New Zealand White, 4 males and 2 females 55 mg US EPA F81-4 GLP Purity: 102.2% Goldenthal (1992d) DAR B.6.2.5	Mean scores over 24 - 72 hours for 6 rabbits: Cornea: 0 – 0 – 0 – 0 – 0 – 0 Iris: 0 – 0 – 0 – 0 – 0 – 0 Conjunctivae (redness): 0.7 - 0.7 - 0.3 - 1.0 - 0.3 - 0.3 Conjunctivae (chemosis): 0 – 0 – 0 - 0.3 – 0 – 0

4.4.2.1 Non-human information

See Table 13

4.4.2.2 Human information

No data available

4.4.2.3 Summary and discussion of eye irritation

No effects on the cornea or iris were observed. Slight conjunctival redness (6 rabbits) and chemosis (1 rabbit) was observed in an eye irritation study conducted with rabbits. Additionally, all animals exhibited low-grade clear discharge, which cleared within 24 hrs.

4.4.2.4 Comparison with criteria

Since this study was conducted with six animals, the criteria laid out in the Regulation are not directly applicable. However, in accordance with the "Guidance on the Application of the CLP Criteria" classification is only required if the individual average is greater than the values specified in Annex I of CLP in 4 of the 6 animals. No effects on the cornea or iris were observed. 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, the criteria for classification are not met.

4.4.2.5 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In an acute inhalation toxicity study (data presented in Table 11), labored breathing was observed in 1/5 females at 4.7.mg/l/4 hr [aerosol, whole body (MMAD 6.8 ± 2.2 µm)]. No clinical effects on the respiratory tract were observed in males.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

The observations from the acute inhalation toxicity study are not sufficient to justify classification of carboxin as a respiratory tract irritant. In addition, carboxin was not irritating in the available skin and eye irritation studies (see Sections 4.4.2 and 4.4.1).

4.4.3.4 Comparison with criteria

See Section 4.4.3.3.

4.4.3.5 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.5 Corrosivity

Please refer to Table 12.

4.5.1 Non-human information

Please refer to section 4.4.1

4.5.2 Human information

No data available

4.5.3 Summary and discussion of corrosivity

No signs of corrosivity were observed in an *in vitro* skin irritation study, therefore, no classification is proposed.

4.5.4 Comparison with criteria

Carboxin does not fulfil the criteria for classification as corrosive.

4.5.5 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Although two sensitisation studies are included in the DAR, only one of these is presented below (Hall, 2002). In a study by Thompson *et al* (1982) no conclusion on the skin sensitising potential could be drawn due to major study deficiencies (including no positive control, different vehicle used in induction and challenge phases, responses not scored using recommended scale and poor reporting).

Method	Doses	Results
Guinea pig, Hartley, 10/sex for test group; 5/sex for vehicle control (corn oil) Magnusson and Kligman maximisation test Occluded Purity: 98.8% OECD 406 GLP Hall (2002) DAR B.6.2.6	*Induction:* Intradermal = 10% w/v in corn oil Epidermal = 75% w/v in corn oil *Challenge:* Left flank = 75% w/v in corn oil Right flank = vehicle control (corn oil)	**Positive** 37 % and 56 % response at 24 and 48 hours, respectively.

4.6.1.1 Non-human information

See Table 14.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

In a guinea pig maximisation study, carboxin induced skin sensitisation reactions in 37% and 56% of animals challenged with 75% carboxin 24 and 48 hours after removal of the dressing, respectively. The intradermal induction dose was 10 %.

Clinical signs: One male and one female guinea pig in the test group died during the study on day 9 and 11, respectively. Clinical signs noted during the observation period included: diarrhoea, red staining of the anogenital area, tachypnea, tremors, and lethargy. Gross necropsy in the premature decedents revealed red intestinal areas, intestines distended with gas, peritoneal cavity distended

with food/excess fluid, soiling of the anogenital area, a red raised area on the kidney and the caecum displayed opening into the cavity. Body weight changes were normal in test and control animals.

4.6.1.4 Comparison with criteria

A positive response was observed after assessment of carboxin for skin sensitising potential using the Magnusson and Kligman guinea-pig maximisation test. The criteria for classification (positive response in ≥ 30 % of animals) was fulfilled and, therefore, carboxin should be classified as a skin sensitiser, category 1 (H317). In order to be classified in sub-category 1A, a response of ≥ 30 % must be observed at an intradermal induction dose of ≤ 0.1 % or a response of ≥ 60 % at an intradermal induction dose of > 0.1 but ≤ 1 % is required. To be classified in sub-category 1B, a response of ≥ 30% must be observed at an intradermal induction dose of > 1% or a response of ≥ 30% but < 60% at an intradermal induction dose of >0.1% but ≤ 1% is required. Carboxin fulfils the criteria for classification in sub-category 1 B as a response of > 30 % was observed at an intradermal induction dose > 1 %. Whilst there was no evaluation carried out after an intradermal induction at lower concentrations (i.e., ≤ 1 %), category 1A is not considered appropriate as the response after intradermal induction with 10% was < 60%.

4.6.1.5 Conclusion on classification and labelling

Skin Sensitisation Category 1B (H317) – May cause an allergic skin reaction

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data available

4.6.2.2 Human information

No data available

4.6.2.3 Summary and discussion of respiratory sensitisation

Not applicable

4.6.2.4 Comparison with criteria

Not applicable

4.6.2.5 Conclusions on classification and labelling

No classification, data lacking.

4.7 Repeated dose toxicity

No information on the repeated dose toxicity of carboxin in humans is available. 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. These studies are summarised in Table 15.

In addition, two lifetime carcinogenicity studies are available in the rat and mouse respectively. Details of these studies are provided in Table 17 in section 4.10. A 2-generation study in rats is also available with details provided in Table 18 of section 4.11. Summaries of these studies are included in section 4.7.1 as relevant.

Table 15: Summary table of relevant repeated dose toxicity studies

The following table summarises the most significant toxicological effects observed in the repeated dose toxicity studies. Further information can be found in the carcinogenicity and reproductive toxicity sections (4.10 and 4.11 respectively).

Oral Studies:

Dose schedule	Dose levels	Results (effects of major toxicological significance)
Studies in the rat		
28-day study Oral (gavage) **Rat**/Wistar 10/sex/dose (of which 5/sex/dose were sacrificed after a 21 day recovery period – week 7) c.f. OECD 407 Non-GLP Purity: 99.1% (Blood samples taken at week 3 rather than the end of the 4 week treatment period, all other analyses were carried out week 4) Ullmann (1983) DAR B.6.3.1a Guidance value for 28-day rat study) ≤ 300 mg/kg bw/d	0, 30, 90 or 270 mg/kg bw/day Vehicle: 2% CMC (aq.) Vol.: 10 ml/kg	**<u>270 mg/kg bw/day:</u>** *Clinical chemistry*: ↓Lactate dehydrogenase (wk 3- M: 45.0%, F: 26.5% and wk 7- F: 41.4%) ↑Creatinine (wk 3-F: 22.6% and wk 7- M: 35.4%) ↑Alkaline phosphatase (wk 3-F: 40.8%) *Urine:* ↑ Volume (wk 3- M: 117 %, F: 68 % and wk 7- M: 268 %, F: 45 %) *Liver*: ↑ Liver weight (wk 4 - abs F: 16.5%; rel. M: 20.9%, F: 22.7) and (wk 7 – rel M: 9.0%, F: 8.0%) Liver centrilobular hypertrophy (wk 4 - M:5/5, F:2/5 and wk 7- M:1/5, F:1/5) Hepatocyte necrosis (wk 4-F:2/5) *Kidney*: ↑ Kidney weight (wk 4 – abs. M: 18.3%; rel M: 31.9%, F: 8.1%) and (wk 7- abs. M: 28.2%; rel M: 27.3%) Tubular casts: 5/5 M and 5/5 F Inflammatory foci: 5/5M and 2/5 F Tubular dilation: 5/5M Vacuolar swelling of epithelial cells in the proximal tubules: 5/5M and 4/5F Irregular thickening of tubular basal membrane of Bowman's capsule of the glomeruli: 5/5M Tubular atrophy: 5/5M and 1/5 F Glomerular sclerosis: 2/5M and 0/5 F

		<u>90 mg/kg bw/day:</u> *Clinical chemistry*: ↓ Lactate dehydrogenase (wk 3- M: 42.9%, F: 28.7%) *Urine:* ↑ Volume (wk 3- M: 50%, F: 25 % and wk 7 – M: 81 %) *Liver*: ↑ Liver weight (wk 4- rel. to bw F: 11.2%) Hepatocyte necrosis (wk 4-F:1/5) *Kidney:* Tubular casts: 2/5 M and 3/5 F Inflammatory foci: 2/5M Tubular dilation: 2/5M and 1/5F Vacuolar swelling of epithelial cells in the proximal tubules: 4/5M and 2/5F Irregular thickening of tubular basal membrane of Bowman's capsule of the glomeruli: 1/5F Tubular atrophy: 1/5M **<u>30 mg/kg bw/day:</u>** *Clinical chemistry*: ↓ Lactate dehydrogenase (wk 3 - M: 30.1%, F: 22.7%) *Liver:* Hepatocyte necrosis (F:1/5) *Kidney:* Tubular casts: 1/5 F Vacuolar swelling of epithelial cells in the proximal tubules: 3/5M and 3/5F Tubular atrophy: 1/5M *NOAEL not determined.*
90-day study Oral (diet) **Rat/**Crl:CD(SD)B 10/sex/dose US EPA F 82-1, GLP Purity:97.7% MacKenzie (1987) DAR B.6.3.1b Guidance value for 90-day rat study) ≤ 100 mg/kg bw/d	0, 200, 800 and 2000 ppm (Equivalent to 0, 10, 40 and 100 mg/kg bw/day for both males and females)	**<u>2000ppm (100 mg/kg bw/day m/f)</u>** *Clinical signs:* ↓ bw (M: 26.4%, F: 14.1%) ↓Food consumption (M: 21.4%) *Clinical chemistry:* ↑ Urea nitrogen (M: 12.8%, F: 37.9%) ↑ Creatinine (M: 28.6%) *Brain:* ↑brain weight (rel. M: 33.8%, F: 14.2%) *Liver:* ↑ Liver weight (rel F: 9.5%) *Testes/epididymis:* ↑Testes weight (rel. left 26.7%, right 33.4 %)

		Kidney ↑Kidney weight (rel. left M: 13.3% F: 11.3%, right M: 10.5% F: 11.6%) Chronic nephritis (interstitial mononuclear cell infiltrate, thickening of tubular walls and hypertrophy/regeneration of tubular epithelium): 10/10M and 10/10F Poteinaceous casts: 8/10 M and 10/10F Tubular cell degeneration of the outer medulla connecting tubules: 8/10M and 5/10F Tubular mineralization of the renal papilla: 7/10M and 6/10F **<u>800ppm (40 mg/kg bw/day in M/F):</u>** *Clinical signs:* ↓ bw (M: 13.1%) ↓Food consumption (M: 13.1%) *Clinical chemistry:* ↑ Urea nitrogen (M: 24%, F: 12.8%) ↑ Creatinine (M: 14.3 %) *Brain:* ↑ Brain weight (rel. M: 15.3%) *Testes/epididymis:* ↑ Right testes weight (rel. 12.3%) *Kidney:* ↑ Kidney weight (rel. to bw right M: 9.5%). Chronic nephritis (interstitial mononuclear cell infiltrate, thickening of tubular walls and hypertrophy/regeneration of tubular epithelium): 10/10M and 4/10F Poteinaceous casts: 6/10 M and 1/10F Tubular cell degeneration of the outer medulla connecting tubules: 7/10M and 3/10F Tubular mineralization of the renal papilla: 8/10M **<u>200 ppm (10 mg/kg bw/day M/F):</u>** ↑ Urea nitrogen (M: 26.3%) ↑ Creatinine (M: 28.6%) *Kidney* Chronic nephritis (interstitial mononuclear cell infiltrate, thickening of tubular walls and hypertrophy/regeneration of tubular epithelium): 9/10M Poteinaceous casts: 4/10 M and 1/10F Tubular cell degeneration of the outer medulla connecting tubules: 3/10M Tubular mineralization of the renal papilla: 1/10M *NOAEL of 200 ppm for females. NOAEL for males not determined.*

<table>
<tr>
<td>90-day
Oral (diet)

Rat/CD (Crl:CD)
10/sex/dose

OECD 408
GLP

Purity: 98.8%

Goldenthal (2002a)
DAR B.6.3.1c

Guidance value for 90-day rat study) ≤ 100 mg/kg bw/d</td>
<td>0, 80, 160 and 240 (males)/480 (females) ppm

(Equivalent to 0, 5.5, 10.5 and 16.1 mg/kg bw/day in males and 0, 6.0, 12.1 and 37.0 mg/kg bw/day in females)</td>
<td>Microscopic findings in the kidneys:
<table>
<tr><th>Finding</th><th colspan="4">Males (mg/kg bw/day)</th><th colspan="4">Females (mg/kg bw/day)</th></tr>
<tr><th></th><th>0</th><th>5.5</th><th>10.5</th><th>16.1</th><th>0</th><th>6</th><th>12.1</th><th>37</th></tr>
<tr><td>Chronic progressive nephropathy</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Total</td><td>3/10</td><td>3/10</td><td>7/9</td><td>10/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>1/10</td></tr>
<tr><td>Trace</td><td>3/10</td><td>3/10</td><td>6/9</td><td>8/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>1/10</td></tr>
<tr><td>Mild</td><td>0/10</td><td>0/10</td><td>1/9</td><td>2/10</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Tubular mineralisation</td><td>0/10</td><td>1/10</td><td>0/9</td><td>2/10</td><td>2/10</td><td>0/10</td><td>0/10</td><td>4/10</td></tr>
<tr><td>Hyperplasia of urothelial epithelium</td><td>0/10</td><td>0/10</td><td>0/9</td><td>1/10</td><td></td><td></td><td></td><td></td></tr>
</table>
NOAEL of 80 ppm for males and 160 ppm for females</td>
</tr>
<tr><td colspan="3">Studies in the dog</td></tr>
<tr>
<td>28-day study
Oral (diet)

Dog/Beagle
3/sex/dose

Guideline not specified
GLP

Purity: 97%

Atkinson (1989)
DAR B.6.3.3a

Guidance value of ≤ 300 mg/kg bw/d calculated from guidance value for a 90-day rat study.</td>
<td>0, 600, 1200 and 2400 ppm

(Equivalent 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)</td>
<td><u>2400ppm, 1200ppm and 600 ppm (69.3/ 65.7, 32.8/30.8 and 19.3/19.3 mg/kg bw/day in M/F):</u>

No adverse effects noted.

NOAEL of 2400 ppm</td>
</tr>
</table>

90-day study Oral (diet) Dog/Beagle 4/sex/dose OECD 409 GLP Purity: 98.8% Goldenthal (2002b) DAR B6.3.3b Guidance value of ≤ 100 mg/kg bw/d calculated from guidance value for a 90-day rat study	M: 0, 160, 240 and 960 ppm (Equivalent to 0, 5.3, 7.9 and 34.4 mg/kg bw/day) F: 0, 160, 240 and 480 ppm (Equivalent to 0, 5.9, 9.0 and 17.7 mg/kg bw/day)	**960 ppm (34.4 mg/kg bw/day - M only):** No adverse effects noted. **480 ppm (17.7 mg/kg bw/day – F only):** *Uterus and Ovaries:* ↑ Uterus/cervix weight (abs. 235.4%; rel. to bw 216.3%); ↑ Ovary weight (abs. 219.4%; rel. to bw 203.1) 240 ppm and 160 ppm (7.9/9.0 and 5.3/5.9 mg/kg bw/day M/F): No adverse effects noted. *NOAEL of 34.4 mg/kg bw/day and 17.7 mg/kg bw/day M/F respectively.*
1 year study Oral (diet) Dog/Beagle 6/sex/dose US EPA F 83-1, GLP Purity: 98.8% Goldenthal (1991) DAR B.6.3.3c Guidance value of ≤ 25 mg/kg bw/d calculated from guidance value for a 90-day rat study	0, 40, 500 and 3000/5000/ 7500 ppm (Equivalent to 0, 1.13, 16.07 and 158.40 mg/kg bw/day in males and 0, 1.28, 15.00 and 169.70 mg/kg bw.day in females) Initial top dose of 3000 ppm was increased to 5000 ppm after 7 weeks and to 7500 ppm after 13 weeks	**3000/5000/7500 ppm (158.4 /169.7 mg/kg bw/day M/F:** *Clinical signs:* ↓ bw gain (M: 47.6%, F: 60.9%) *Haematology*: ↓ Haemocrit (M: 13.9%) ↑ Mean Cell Volume (M: 5.2%) ↑ Mean Cell Haemoglobin (M: 8.2%) *Clinical chemistry:* ↑ Alkaline phosphatase (M: 111.9%, F: 111.3%) ↑ Cholesterol (12 months - M: 48.1%) ↑ Creatinine (12 months - F: 40%) *Liver:* ↑ Rel. weight (M: 27.5%, F: 27.4%) *Heart:* ↑ Rel. weight (F: 20.8%) *Pituitary:* ↑ Rel. weight (F: 30.8) **500 ppm (16.07/15.00 mg/kg bw/day M/F:** *Clinical signs* ↓ bw gain (M: 19.1%, F: 65.2%) *Heart*: ↑ Rel. weight (F: 16.9%). **40 ppm (1.13/1.28 mg/kg bw/day M/F):** No adverse effects. *NOAEL of 500 ppm for M and 40 ppm for F.*

Dermal Study:

<table>
<tr><th>Dose schedule</th><th>Dose levels</th><th>Results</th></tr>
<tr>
<td>28 day study

Rat/CD (Crl:CD (SD) IGS BR) 10/sex/dose

US EPA OPPTS 870.3200
GLP

Purity: 98.8%

Goldenthal (2002c)
DAR B.6.3.4

Guidance value of ≤ 600 mg/kg bw/d for a 28-day dermal study in the rat.</td>
<td>0, 30, 400 and 1000 mg/kg/6 hr/day
[Moistened with distilled water and applied to clipped dorsal skin (10% of body surface) under gauze dressing and tape].</td>
<td><u>1000, 400 and 30 mg/kg bw/day</u>
Microscopic findings in the kidneys:
<table>
<tr><th rowspan="2">Finding</th><th colspan="4">Males (mg/kg bw/day)</th><th colspan="4">Females (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>30</th><th>400</th><th>1000</th><th>0</th><th>30</th><th>400</th><th>1000</th></tr>
<tr><td>Tubular degeneration:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Trace</td><td>0/10</td><td>0/10</td><td>1/10</td><td>9/10</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Tubular regeneration:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Total</td><td>4/10</td><td>3/10</td><td>9/10</td><td>10/10</td><td>3/10</td><td>0/10</td><td>0/10</td><td>3/10</td></tr>
<tr><td>Trace</td><td>4/10</td><td>3/10</td><td>9/10</td><td>3/10</td><td>3/10</td><td>0/10</td><td>0/10</td><td>3/10</td></tr>
<tr><td>Mild</td><td>0/10</td><td>0/10</td><td>0/10</td><td>7/10</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
</table>
NOAEL of 30 mg/kg bw/day for males and 1000 mg/kg bw/day was determined for females.</td>
</tr>
</table>

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

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. As well as these studies, additional repeated dose toxicology can be found in the carcinogenicity and reproductive toxicity sections for rats and mice. The key points from all these studies are summarised below.

Rat Studies

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 increase 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 of 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. An increase in kidney weight was also observed in high dose males/females sacrificed after treatment (absolute M: 18.3 %; relative M: 31.9 % and F: 8.1 %) and high dose males sacrificed after the recovery period (absolute M: 28.2 %, relative M: 27.3 %).

Finding	**Males (mg/kg bw/day)**				**Females (mg/kg bw/day)**			
wk 4 (wk 7)	**0**	**30**	**90**	**270**	**0**	**30**	**90**	**270**
Tubular casts	2[a]/5[b] (3/5)	0/5 (5/5)	2/5 (4/5)	5/5 (5/5)	0/5 (3/5)	1/5 (2/5)	3/5 (0/5)	5/5 (4/5)
Inflammatory foci	0/5 (1/5)	0/5 (0/5)	2/5 (5/5)	5/5 (5/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	2/5 (4/5)
Tubular dilation	0/5 (0/5)	0/5 (3/5)	2/5 (4/5)	5/5 (5/5)	0/5 (1/5)	0/5 (0/5)	1/5 (1/5)	0 /5 (1/5)
Vacuolar swelling of epithelial cells in the proximal tubules	1/5 (2/5)	3/5 (0/5)	4/5 (5/5)	5/5 (5/5)	0/5 (5/5)	3/5 (1/5)	2/5 (5/5)	4/5 (4/5)
Irregular thickening of the tubular basal membrane and the Bowman's capsule of the glomeruli	0/5 (0/5)	0/5 (1/5)	0/5 (5/5)	5/5 (5/5)	0/5 (1/5)	0/5 (0/5)	1/5 (0/5)	0/5 (2/5)
Tubular atrophy	0/5 (0/5)	1/5 (0/5)	1/5 (5/5)	5/5 (5/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	1/5 (4/5)
Glomerular sclerosis	0/5 (0/5)	0/5 (0/5)	0/5 (2/5)	2/5 (5/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	0/5 (1/5)

[a] number of affected animals. [b] total number of animals examined

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 statically 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 in this study are 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.

Ninety-day studies:

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 mg/kg bw/day in males and at 100 mg/kg bw/day in females. However, the critical target organ was identified as the kidney.

Histopathology of the kidneys in males treated with ≥ 10 mg/kg bw/day and females treated with ≥ 40 mg/kg bw/day revealed an increase in the incidence of chronic nephritis. This 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 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 mg/kg bw/day and mineralisation of the tubes of the renal papilla in males ≥ 40 mg/kg bw/day and females at 100 mg/kg bw/day. These findings were more prevalent in males and severity increased with dose.

Finding	**Males (mg/kg bw/day)**				**Females (mg/kg bw/day)**			
	0	**10**	**40**	**100**	**0**	**10**	**40**	**100**
Chronic nephritis (interstitial mononuclear cell infiltrate, thickening of tubular walls and hypertrophy/regeneration of tubular epithelium)	0[a]/10[b]	9/10	10/10	10/10	0/10	0/10	4/10	10/10
Proteinaceous casts	0/10	4/10	6/10	8/10	0/10	1/10	1/10	10/10
Tubular cell degeneration of the outer medulla connecting tubules	4/10	3/10	7/10	8/10	0/10	0/10	3/10	5/10
Tubular mineralization of the renal papilla	0/10	1/10	8/10	7/10	0/10	0/10	0/10	6/10

[a] number of animals effected. [b] number of animals examined

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 100 mg/kg bw/day and increased urea nitrogen (males: ≥ 10 mg/kg bw/day, females: ≥ 40 mg/kg bw/day). 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.

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, however, one male in the 10.5 mg/kg bw/day dose group was sacrificed in extremis due to a nasal tissue fracture (not considered treatment-related). 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. 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.

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

A statistically significant increase in serum total protein (3.7 %) and albumin (5.7 %) was observed in top dose males. These changes in clinical chemistry parameters could be associated with the adverse effects on the kidneys. In females only, an increase in cholesterol was observed in the high dose group (26.2 %).

All doses in these studies are within guidance values for classification as STOT RE 2, which for a 90-day study are $10 < C \leq 100$.

Chronic studies (carcinogenicity and reproductive toxicity studies):

Refer to tables 17 and 18 in sections 4.10 and 4.11.

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

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) (See table 17 for summary). 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 400 ppm (weeks 65 - 102) and was lower in females at 600 ppm (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 in males and from 300 ppm in females. Water consumption was statistically significantly increased in males from 200 ppm and in females at 600 ppm.

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 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 increase incidence of kidney cysts at 200 ppm and above in males and at 600 ppm in females.

In males that died or were sacrificed prematurely, there was an apparent treatment-related increase in fibrous osteodystropathy 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 there was no dose-response relationship.

The top dose for males and females and the mid dose for females 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 (1991):

In a two-generation reproduction study (Kehoe (1991), 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) (See table 18 for summary).

From a dose of 200 ppm (10 mg/kg bw/day), there was an increased incidence of kidney lesions consistent with those observed in the shorter-term and carcinogenicity studies including chronic nephritis.

Mouse Studies

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

Additional information for repeated dose toxicity is available from a 19-month lifetime carcinogenicity study in CD-1 mice (50/sex/dose) (See table 17 for summary). 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, 480 (females only) and 960 (males only) ppm carboxin (corresponding to 0, 5.3, 7.9 and 34.4 mg/kg bw/day in males and 0, 5.9, 9.0 and 17.7 mg/kg bw/day in females). 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. 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, 16.07 and 158.40 mg/kg bw/day in males and 0, 1.28, 15.00 and 169.70 mg/kg bw/day in females). The initial high dose of 3000 ppm 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, 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 (15.9% relative to body weight at 15.00 mg/kg bw/day), (20.8% relative to body weight at 169.7 mg/kg bw/day). 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 158.40/169.70 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).

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

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 multi-foci were observed these were more numerous in the mid and high dose 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 $60 < C \leq 600$ mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

In the available repeated-dose toxicity studies, the kidney and liver were identified as the critical target organs. These findings and their relevance for classification and labelling are discussed below.

Kidney:

In the three short-term repeated dose studies in the rat, 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 were shown to be more sensitive to these effects than females. In the three studies, effects occurred below the top range value for classification. Additional information on renal toxicity was presented in the carcinogenicity and reproduction studies in rats and mice. In the studies in rats, there was an increased incidence of treatment-related chronic nephritis, tubular cell degeneration and tubular mineralisation in the kidneys of both sexes at doses of 8.65/15.08 mg/kg bw/day (males/females) and above and in the reproductive study at doses ≥ 10 mg/kg bw/day. The carcinogenicity study report noted that it was difficult to distinguish spontaneous chronic progressive nephropathy from treatment-related chronic nephritis, because both have common characteristics such as mononuclear infiltrates, interstitial fibrosis, tubular changes (dilation, proteinaceous casts, thickened walls and regeneration of epithelium) and glomerular changes (dilation and thickened membranes). It was

noted that treatment-related chronic nephritis tends to occur in the area of the interlobular vasculature whereas spontaneous nephropathy tends to be more generalised and involves the outer cortex. The mineralisation in the tubules of the papillae, the tubular cell degeneration in the medullar and the tubular hyperplasia of the tubules of the medulla and papillae are not commonly associated with chronic nephropathy and therefore the effects were considered treatment-related. The lesions appeared to occur more frequently and to be more severe with time, which may indicate that the test material had accentuated the chronic nephropathy.

In mice, there was an increase in the incidence of renal tubular nephrosis in both sexes at the top dose of 752/951 mg/kg bw/day (males/females) in a lifetime carcinogenicity study.

There were no effects in the kidneys of the dog.

Liver:

Adverse effects were seen in the livers of rats following repeated dosing for 28-days. These included a slight to moderate centrilobular liver hypertrophy accompanied by increased liver weight in males and females of the top dose group and increased serum alkaline phosphatase in top dose females only. At the end of the seven week recovery period, slight hypertrophy was still evident in one male and female. In addition to this, there was a low incidence of hepatocyte necrosis in females in all dose groups. These effects were not observed in any of the other studies in rats nor were they observed in the studies with other species at doses relevant for classification. These findings are therefore not considered further for classification.

4.8 Specific target organ toxicity – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

Carboxin has been tested for repeated dose toxicity by the oral route in rats, mice and dogs and in a dermal study in rats. Following dosing, changes were seen in the kidneys and liver of rats. The key findings in rats, mice and dogs at doses relevant for classification are summarised in the table below:

Study Design	Doses (mg/kg bw/day)	Severe effects at doses relevant for classification (STOT-RE 1)	Severe effects at doses relevant for classification (STOT-RE 2)	Other significant adverse effects at doses relevant for classification
Studies involving oral exposure				
Rat, 28-day, gavage	0, 30, 90 and 270	None	• Increased incidence of chronic nephritis	• Increased kidney weight • Clinical chemistry and urinalysis changes associated with reduced kidney function - increased urine volume, specific gravity and pH and increased serum creatinine • Increased liver weight associated with clinical chemistry (increased serum alkaline phosphatase) • Slight-moderate centrilobular liver hypertrophy • Low incidence of hepatic necrosis in females of all dose groups
Rat, 90-day, diet	0, 10, 40 and 100	None	• Increased incidence of chronic nephritis	• Clinical chemistry changes associated with reduced kidney function – increased serum creatinine, urea nitrogen • Increased kidney weight in top dose males and females and mid dose males
Rat, 90-day, diet	0, 5.5, 10.5 and 16.1 (males) 0, 6.0, 12.1 and 37.0 (females)	None	None	• Increase in trace – mild severity chronic progressive nephropathy in males ≥ 10.5 mg/kg bw/day and one female (37 mg/kg bw/day). • Increase in serum total protein and albumin in top dose males
Rat, carcinogenicity study, diet	0, 0.82, 8.65 and 16.86 (males) 0, 1.05, 15.08 and 33.48 (females)	None	• Renal lesions were a major factor in premature deaths in both sexes • Treatment-related nephritis in males at doses ≥ 8.65 mg/kg bw/day • Increased incidence of kidney cysts in males (≥ 8.65 mg/kg bw/day)	• Decreased body weight and weight gain in males (≥ 8.65 mg/kg bw/day) and females 33.48 mg/kg/ bw/day • Increased water consumption in males (≥ 8.65 mg/kg bw/day) and females 33.48 mg/kg/ bw/day
Rat, reproduction study, diet	0, 1, 10 and 20 (males) 0, 1, 15 and 30 (females)	None	None	•Kidney lesions ≥ 10 mg/kg bw/day •Increased incidence of chronic nephritis ≥ 10 mg/kg bw/day •Decreased body weight gain in F0 and F1 males at 20 mg/kg bw/day

Mouse, carcinogenicity study, diet	0, 8, 385 and 752 (males) 0, 9, 451 and 912 (females)	None	None	None
Dog, 28-day, diet	0, 19.3, 32.8 and 69.3 (males) 0, 19.3, 30.8 and 65.7 (females)	None	None	None
Dog, 90-day, diet	0, 5.3, 7.9 and 34.4 (males) 0, 5.9, 9.0, 17.7 (females)	None	None	None
Dog, 1-year, diet	0, 1.13, 16.07 and 158.4 (males) 0, 1.28, 15.0, 169.70 (females)		None	• Reduced body weight gain and increase in relative heart weight in females only 16.07/15.00 mg/kg bw/day • No associated clinical chemistry
Studies involving dermal exposure				
Rat, 28-day	0, 30, 400 and 1000	None	None	• Increased incidence of tubular regeneration in the kidneys of males only at 400 mg/kg bw/day • No associated clinical chemistry

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

In a number of short-term and chronic oral and dermal studies involving repeated doing of carboxin, there was clear evidence of significant organ (kidney) toxicity at doses relevant for classification as STOT-RE 2 (i.e. based on guidance values of $10 \leq C \leq 100$ mg/kg bw/day from a 90-day study in the rat). Effects in rats included chronic nephritis with associated lesions and chronic progressive nephropathy increasing in severity with dose. Kidney weight (absolute and relative) was increased and there were clinical chemistry and urinalysis parameter changes related to reduced kidney function. These effects were consistently observed across all rat studies (oral and dermal) and occurred in both male and females rats, with males appearing to be more sensitive.

No significant effects were noted at doses relevant for classification in STOT-RE 1 (i.e. ≤ 10 mg/kg bw/day based on a 90-day study in the rat). As such it is proposed to classify carboxin in category 2.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

STOT RE 2; H373 – May cause damage to the kidneys through prolonged or repeated exposure

4.9 Germ cell mutagenicity (Mutagenicity)

Table 16: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro			
Method	**Organism/Strain**	**Concentrations Tested**	**Result**
Bacterial Mutation Assay (Ames) Guideline (Ames *et* al, 1975) GLP Purity: 98% Brusick (1982) DAR B.6.4.1a	*Salmonella typhimurium* (TA1535, TA 1537, TA1538, TA98 and TA100)	0, 1, 10, 50, 100, 500, 1000, 2500 and 5000 µg/plate carboxin	**Negative** ± S9 metabolic action Dose selection was based on a preliminary study using strain TA100 in which toxicity was exhibited at ≥ 2500µg/ml (reduced number of revertants on minimal media plates).
Mammalian cell gene mutation test OECD 476 (1997), GLP Purity: 98.2% San & Clarke (2001) DAR B.6.4.1b	Chinese Hamster Ovary cells	0, 150, 250, 500, 750 and 1000 µg/ml carboxin in DMSO	**Negative** ± S9 metabolic activation Precipitation observed at 1000 µg/ml.
Mammalian chromosome aberration test Non-guideline (in house protocol designed to chromosome aberration frequencies in CHO cells) GLP Purity: 98% Galloway (1982) DAR b.6.4.1c	Chinese Hamster Ovary Cells	-S9 metabolic activation: 0, 17, 50, 167, 400 and 500 µg/ml +S9 metabolic activation: *1st trial:* 17-500 µg/ml *2nd trial:*400-1400 µg/ml *3rd trial:* 500-900 µg/ml	**Negative** – S9 metabolic activation Precipitation observed at ≥ 400 µg/ml Toxicity observed at 600 µg/ml. **Positive** + S9 metabolic activation *1st trial:* Negative *2nd trial:* Slight increases in aberrations at 600 and 1200 µg/ml and a statistically significant increase at 800 µg/ml. Precipitation observed at all dose levels. 1200 µg/ml was the highest that could be scored *3rd trial:* statistically significant increase in chromosome aberrations at ≥ 700 µg/ml. Chromatid exchanges were also seen at 700 and 7500 µg/ml Cytotoxicity observed at ≥ 700 µg/ml – severe in nature ≥ 850 µg/ml Precipitation observed at ≥ 850 µg/ml. Negative and positive controls behaved appropriately.

<table>
<tr><td>Unscheduled DNA Synthesis Assay

Non-guideline (in house protocol designed to investigate unscheduled DNA synthesis in rat hepatocytes)
GLP

Purity: 98%

Myhr (1982)
DAR B.6.4.1d</td><td>Rat hepatocytes</td><td>0, 0.513, 1.03, 2.56, 5.13, 10.3, 25.6, 51.3, 103 and 256 µg/ml</td><td>Positive

Dose-related positive response at 5.13-103 µg/ml

Cytotoxicity was observed at ≥ 25.6 µg/ml

Nuclear grains were not counted at 256 µg/ml due to only 8.2 % cell survival

Negative and positive controls behaved appropriately.</td></tr>
<tr><td colspan="4">In Vivo</td></tr>
<tr><td>Method</td><td>Organism/Strain</td><td>Concentrations Tested</td><td>Result</td></tr>
<tr><td>Bone marrow chromosome aberration study in the rat

Oral (gavage)

Non-guideline
GLP

Purity: 98%

Cortina (1983)
DAR B.6.4.2a</td><td>Sprague-Dawley rats (20/sex/dose)</td><td>0, 200, 660 and 2000 mg/kg bw

Vehicle: 0.5% CMC

Volume: 10 ml/kg

Sacrificed at 6, 12, 24, 48 h</td><td>Negative

Increased number of aberrations at 660 mg/kg bw at 12 h only (males and females combined):
<table>
<tr><th>Treatment (mg/kg bw)</th><th>Percentage aberrant cells/group</th><th>Mean mitotic index</th></tr>
<tr><td>Vehicle</td><td>0.3</td><td>1.8 ± 1.3</td></tr>
<tr><td>200</td><td>0</td><td>3.4 ± 2.5</td></tr>
<tr><td>660</td><td>3.6</td><td>2.1 ± 1.9</td></tr>
<tr><td>2000</td><td>0.9</td><td>1.9 ± 1.6</td></tr>
<tr><td>Positive Control (CP)</td><td>17.3</td><td>0.3 ± 0.6</td></tr>
</table>
Toxicology: No animals died. Reductions in mean body weight and clinical signs of toxicity (slightly depressed, rough coat and urine stains) at ≥ 660 mg/kg bw.

Negative and positive controls behaved appropriately.</td></tr>
</table>

Bone marrow chromosome aberration study in the rat Oral (gavage) Non-guideline GLP Purity: 98% Cortina (1985) DAR b.6.4.2b	Sprague-Dawley rats 20/sex/dose in the acute study 5/sex/dose in the sub-acute study	Acute: single dose of 0, 750, 2000 and 4000 mg/kg bw Sub-acute: 5 doses of 0, 100, 400 and 800 mg/kg bw/day Volume: 10 ml/kg	Acute: **Negative** Toxicology: soft faeces, rough coat, eyes squinted, red stains on eyes/nose, slightly depressed, cold, prostrate and laboured respiration at ≥ 750 mg/kg bw. Gross necropsy revealed darkened adrenals, spleens and lungs, pale kidneys and gas-filled distended stomachs. Sub-acute: **Negative** Toxicology: One female was sacrificed moribund on day 3 (800 mg/kg bw). Clinical signs were noted at ≥ 400 mg/kg bw. Negative and positive controls behaved appropriately.
Unscheduled DNA synthesis assay Oral (gavage) OECD 486 GLP Purity: 98.13% Pant and Sly (2006) DAR Addendum 1 (Aug 2007) B 6.4	Rat/Sprague-Dawley 5/sex/dose	0, 500, 1000, 2000 mg/kg bw	**Negative** No animals died and there were no clinical signs during the study

4.9.1 Non-human information

4.9.1.1 In vitro data

A battery of four *in vitro* studies is available to assess the mutagenic potential of carboxin. The gene mutation assays in bacterial (Brusick, 1982) and mammalian cells (San & Clarke, 2001) were both negative. In the chromosome aberration test (Galloway, 1982), carboxin was considered to be clastogenic in the presence of metabolic activation and it was also considered to be positive in two out of three replicates of the unscheduled DNA repair test in rat hepatocytes (Myhr, 1982).

4.9.1.2 In vivo data

Two *in vivo* bone marrow chromosome studies were performed in rats. There was an increase in the number of chromosomal aberrations at one time-point at 660 mg/kg bw (Cortina, 1983), but this finding lacked statistical significance and/or a dose response relationship. Furthermore, a comparable increase was not observed under similar conditions at three other time-points (Cortina, 1985). In this second study, there was no evidence of any carboxin-induced clastogenic activity after acute and sub-acute dosing. Although the mitotic indices were not affected by carboxin administration, severe toxicity was noted in the sub-acute study at the top dose tested. An in vivo unscheduled DNA synthesis test was performed in order to provide reassurance on the genotoxicity of carboxin. 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).

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Four in vitro studies and two in vivo studies were presented in the draft assessment report. In an in vitro unscheduled DNA repair test and a mammalian chromosome aberration test, positive results were obtained. Therefore, it was considered appropriate for the applicants to provide further reassurance on the genotoxicity of carboxin in the form of an in vivo unscheduled DNA repair test. The results of this test proved negative up to a dose of 2000 mg/kg.

4.9.5 Comparison with criteria

No positive results were observed in the available *in vivo* studies. Therefore, the available data is not considered to support classification for mutagenicity

4.9.6 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.10 Carcinogenicity

No information on the carcinogenicity of carboxin in humans is available. One carcinogenicity study in the rat (oral route (Kehoe, 1991)) and one study in the mouse (oral route (Gunderson, 1982)) are summarised in Table 17.

Table 17: Summary table of relevant carcinogenicity studies

<table>
<tr><th>Dose schedule</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>2-year study
Oral (diet)

Terminated at 102 weeks due to low survival rates in males at 400 ppm

Rat/Crl:CD (SD) BR 60/sex/dose (of which 10/sex/dose were sacrificed after 52 weeks)

Purity= 97.7%

OECD 453, GLP

(Kehoe, 1991)
DAR B.6.5.1</td><td>M: 0, 20, 200 and 400 ppm

(Equivalent to 0, 0.82, 8.65 and 16.86 mg/kg bw/day)

F: 0, 20, 300 and 600 ppm

(Equivalent to 0, 1.05, 15.08, 33.48 mg/kg bw/day)</td><td><u>Neoplastic findings (decedents and terminal)</u>
<table>
<tr><th rowspan="3">Finding</th><th colspan="4">Males ppm (mg/kg bw/day)</th><th colspan="4">Females ppm (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>20</th><th>200</th><th>400</th><th>0</th><th>20</th><th>300</th><th>600</th></tr>
<tr><th>0</th><th>(0.82)</th><th>(8.65)</th><th>(16.86)</th><th>0</th><th>(1.05)</th><th>(15.08)</th><th>(33.48)</th></tr>
<tr><td>Liver-hepatocellular adenoma (B)</td><td>3/50 (6%)</td><td>6/50 (12%)</td><td>6/50 (12%)</td><td>3/50 (6%)</td><td>4/50 (8%)</td><td>3/50 (6%)</td><td>4/50 (8%)</td><td>3/50 (6%)</td></tr>
<tr><td>Liver-hepatocellular carcinoma (M)</td><td>1/50 (2%)</td><td>0/50 (0%)</td><td>1/50 (2%)</td><td>4/50 (8%)</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td></td><td colspan="8">Laboratoy historical control data for hepatocellular carcinoma in male rats:
0-1.7% (6 studies) 1988-1990
0-3.3% (9 studies) 1991-1996</td></tr>
<tr><td>Parathyroids-adenoma (B)</td><td>0/47 (0%)</td><td>1/27 (3.7%)</td><td>1/27 (3.7%)</td><td>1/50 (2%)</td><td>0/47 (0%)</td><td>0/29 (0%)</td><td>0/21 (0%)</td><td>1/48 (2.1 %)</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control data for parathyroid adenoma in male rats:
mean 3.3% 1986-1991</td></tr>
</table>
<u>Non-neoplastic findings:</u>

<u>600 ppm:</u>
Clinical:
60%F died by wk 102 vs 44%F in controls (majority of deaths occurred between weeks 52-102)
↓ bw (21.15%)
↓ bw gain (28.7%)
↑ water consumption (78.0%)
Increased incidences of: anorexia, thin, few/no faeces, piloerection, low body temperature

* see table below for microscopic findings

<u>400 ppm:</u>
Clinical:
73.5%M died by wk 102 vs 44%M in controls (majority of deaths occurred between weeks 52-102)
↓ bw (↓17.3%)
↓ bw gain (23.4%)
↑ water consumption (50.3%)
Increased incidences of: anorexia, thin, few/no faeces, soft faeces, low body temperature, languid

* see table below for microscopic findings</td></tr>
</table>

300 ppm:
Clinical:
46.9% F died by wk 102 vs 44% F in controls (majority of deaths occurred between weeks 52-102)
↓ bw (8.7%)
Increased incidence of anorexia and piloerection

* see table below for microscopic findings

200 ppm:
Clinical:
49% M died by wk 102 vs 44% M in controls (majority of deaths occurred between weeks 52-102)
↓ bw (7.5%)
↑ water consumption (78%)

Clinical chemistry and urinalysis:
↑ Creatinine (75%)
↑ Urea nitrogen (110.5%)
↑ Urine volume (91.7%)

* see table below for microscopic findings

20 ppm:
Clinical:
58% (M) and 54% (F) died by wk 102 vs 44% M/F in controls
Increased incidence of anorexia and piloerection in females only

* see table below for microscopic findings

*** Non-neoplastic microscopic findings (decedents and terminal)**

Finding	Males (ppm)				Females (ppm)			
	0	20	200	400	0	20	300	600
Kidney								
chronic progressive nephropathy	35/50 (70%)	43/50 (86%)	47/50 (94%)	40/50 (80%)	14/50 (28%)	10/50 (20%)	24/50 (48%)	21/50 (42%)
cysts	6/50 (12%)	10/50 (20%)	38/50 (76%)	32/50 (64%)	2/50 (4%)	2/50 (4%)	1/50 (2%)	10/50 (20%)
chronic nephritis	0/50 (0%)	0/50 (0%)	3/50 (6%)	9/50 (18%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	25/50 (50%)
undifferentiated nephritis	0/50 (0%)	1/50 (2%)	44/50 (88%)	38/50 (76%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	19/50 (38%)
tubular mineralization (renal papilla)	0/50 (0%)	1/50 (2%)	48/50 (96%)	47/50 (94%)	0/50 (0%)	0/50 (0%)	6/50 (12%)	46/50 (92%)
tubular cell degeneration (medulla)	3/50 (6%)	3/50 (6%)	23/50 (46%)	23/50 (46%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	20/50 (40%)
tubular epithelium hyperplasia (papillary-medullary)	2/50 (4%)	1/50 (2%)	39/50 (78%)	38/50 (76%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	12/50 (24%)
pelvic dilation	16/50 (32%)	10/50 (20%)	37/50 (74%)	32/50 (64%)	13/50 (26%)	12/50 (24%)	7/50 (14%)	14/50 (28%)
Parathyroids-hyperplasia	4/47 (8.5%)	7/27 (26%)	14/27 (52%)	19/48 (40%)	0/47 (0%)	0/29 (0%)	2/21 (10%)	4/48 (8%)
Femur- fibrous osteodystrophy	1/50 (2%)	4/29 (14%)	9/26 (35%)	9/50 (18%)	0/50 (0%)	0/27 (0%)	0/23 (0%)	1/50 (2%)
Ovaries- cysts					4/50 (8%)	6/31 (19%)	7/27 (26%)	8/50 (16%)

NOAEL of 20 ppm for females. NOAEL for males not be determined

<table>
<tr>
<td>Lifetime study (19 months)
Oral (diet)

Mouse/CD-1 50/sex/dose in treatment groups
75/sex/dose in control group

Purity= not specified

Not guideline or GLP (organ weights were not recorded)

(Gunderson, 1982)
DAR B.6.5.2</td>
<td>0, 50, 2500 and 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.</td>
<td>
<u>Neoplastic findings (decedents and terminal)</u>
<table>
<tr><th rowspan="2">Finding</th><th colspan="4">Males ppm (mg/kg bw/day)</th><th colspan="4">Females ppm (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>50</th><th>2500</th><th>5000</th><th>0</th><th>50</th><th>2500</th><th>5000</th></tr>
<tr><td></td><td>(0)</td><td>(8)</td><td>(385)</td><td>(752)</td><td>(0)</td><td>(9)</td><td>(451)</td><td>(912)</td></tr>
<tr><td>Lung - adenoma</td><td>13/75 (17%)</td><td>7/49 (14%)</td><td>7/50 (14%)</td><td>17/50 (34%)</td><td>10/75 (13%)</td><td>-</td><td>-</td><td>6/50 (12%)</td></tr>
<tr><td>Lung - Alveolar-bronchiolar carcinoma</td><td>0/75 (0%)</td><td>2/49 (4%)</td><td>3/50 (6%)</td><td>0/50 (0%)</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control data for lung adenoma in males:
6.3-16.7% (6, 18mth studies) from 1971-1976
4-31.1% (7, 20-22mth studies) from 1973-1978
24% (1, 18mth study) from 1979-1980

Laboratory historical control data for lung carcioma in males:
6% (1, 18mth study) from 1979-1980</td></tr>
<tr><td>Liver - carcinoma</td><td>2/75 (3%)</td><td>-</td><td>-</td><td>3/50 (6%)</td><td>1/75 (1%)</td><td>-</td><td>-</td><td>0/50 (0%)</td></tr>
</table>
<u>Non-neoplastic findings</u>
<u>5000 ppm</u>:
Reduced female survival compared to controls at weeks 78 and 84 (72 %* died week 78 and 78 %* died week 84)
Liver;
Hyperplastic nodules 13/50M and 3/50 F (compared to 10/75M and 2/75F in controls)
Centrilobular hypertrophy: 37/50M and 8/50F (compared to 0/75 in M/F controls)
Kidney:
Tubular nephrosis: 41/50M and 40/50 F (compared to 37/75M and 38/75F in controls)

<u>2500 ppm:</u>
Reduced female survival compared to controls at weeks 78 and 84 (58% died week 78 and 66% died week 84)
Liver;
Hyperplastic nodules 5/50M and 5/49 F (compared to 10/75M and 2/75F in controls)
Centrilobular hypertrophy: 17/50M and 1/49F (compared to 0/75 in M/F controls)

NOAEL of 50 ppm for M and F.
</td>
</tr>
</table>

4.10.1 Non-human information

As shown in Table 17 there is one study in the rat and one study in the mouse that are considered of sufficient quality to assess the carcinogenic potential of carboxin. Both studies were conducted via the oral route. No information is available via the inhalation or dermal routes. The key effects observed in these studies are described below.

4.10.1.1 Carcinogenicity: Oral

Two carcinogenicity studies have been reported, one guideline study in rats and one non-guideline study in mice. The non-neoplastic findings presented in Table 17 have been discussed and summarised in the repeated dose toxicity section (4.7.1.1). The neoplastic findings are presented below.

Rat

In a 102 week carcinogenicity study (OECD 453), Sprague-Dawley rats (60/sex/dose) were fed diets containing carboxin [0, 20, 200 or 400 ppm (males) and 0, 20, 300 and 600 ppm (females), corresponding to 0, 0.82, 8.65 and 16.86 mg/kg bw/day (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 vastly reduced survival rate in males dosed with 16.86 mg/kg bw/day (27% survival). This increased mortality was considered to be due to carboxin-induced nephrotoxicity as the anatomical and clinical pathology findings at the interim sacrifice of one year clearly showed dose-related increase of chronic nephritis, tubular cell degeneration and mineralisation.

Neoplastic findings:

Finding	**Males ppm (mg/kg bw/day)**				**Females ppm (mg/kg bw/day)**			
	0	**20**	**200**	**400**	**0**	**20**	**300**	**600**
	0	**(0.82)**	**(8.65)**	**(16.86)**	**0**	**(1.05)**	**(15.08)**	**(33.48)**
Liver-hepatocellular adenoma (B)	3/50 (6%)	6/50 (12%)	6/50 (12%)	3/50 (6%)	4/50 (8%)	3/50 (6%)	4/50 (8%)	3/50 (6%)
Liver-hepatocellular carcinoma (M)	1/50 (2%)	0/50 (0%)	1/50 (2%)	**4/50 (8%)**	-	-	-	-
	Laboratoy historical control data for hepatocellular carcinoma in male rats: 0-1.7% (6 studies) 1988-1990 0-3.3% (9 studies) 1991-1996							
Parathyroids-adenoma (B)	0/47 (0%)	1/27 (3.7%)	1/27 (3.7%)	1/50 (2%)	0/47 (0%)	0/29 (0%)	0/21 (0%)	1/48 (2.1 %)
	Laboratoy historical control data for parathyroids- adenoma in male rats: mean 3.3% 1986-1991							

At the top dose of 400 ppm, there was an increase in the incidence of hepatic carcinoma in males (8% versus 2 % in the concurrent control group). In addition to the carcinoma, the incidence of liver adenoma in males increased to 12 % at 20 and 200 ppm however, at 400 ppm incidences were the same as the control group indicating a lack of dose-response. The liver adenomas in males are therefore considered unrelated to treatment.

The incidence of hepatic carcinoma in this study was above the range of the laboratory historical control data (HCD). HCD for hepatocellular adenomas and carcinomas in male rats for the period of 1988 – 1990 was provided by the applicants. The initial data range was 0 – 1.7% for hepatocellular carcinoma incidence in male rats (six studies). Further data was provided from the period immediately following the carboxin study (1991 – 1996). This showed a slight increase in incidence in control males compared to previous HCD, with a range of 0 – 3.3% (nine studies). However, when considering the low incidence observed (8% vs 2% in controls), the sex-specificity of the response, the lack of statistical significance, the absence of a respective response in liver

adenomas and more importantly the excessive toxicity observed at this dose in males (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%] and the severe nephrotoxicity for which classification with STOT-RE 2 has already been proposed), it can be concluded that these liver tumours are of no relevance to human health.

In addition to the liver findings, there was an increase in adenoma of the parathyroids in males (3.7% [1/27] at 20 and 200 ppm and 2 % [1/50] at 400 ppm versus 0 % in control group) and females (2.1 % at 600 ppm versus 0 % in control group). Although the incidence of these tumours in males at 20 and 200 ppm was slightly outside the HCD for males in the period of 1986 – 1991 (mean incidence of 3.3 %), it is noted that there was a lack of dose-response. In addition, the incidences at 20 and 200 ppm were confounded by the number of animals examined (only 27). Therefore these benign tumours in males are considered unrelated to treatment. In females, the very low incidence (1/48) of this tumour at the top dose, in the absence of any pre-neoplastic findings and toxic effects in this organ is considered a chance finding unrelated to treatment.

Mouse

In a 19-month non-guideline lifetime study, CD-1 mice (treatment groups: 50/sex/dose, control group: 75/sex/dose) were fed diets containing 0, 50, 2500 or 5000 ppm carboxin (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).

Neoplastic findings

There was an increased incidence of lung adenoma in males at 5000 ppm (34% vs 17% in controls) and examination of the Kaplan Meier curve indicated that the tumours in the high dose group appeared earlier than in all other groups. There were no alveolar-bronchiolar carcinomas in males and no alveolar-bronchiolar adenomas or carcinomas in females. The male historical control data for six 18-month studies (1971 - 1976) had a mean incidence of 12.1 % (range 6.3 - 16.7 %) and seven 20 - 22 month studies (1973 - 1978) had a mean incidence of 18.8 % (range 4.0 - 31.1 %). Further data provided for male CD-1 mice from this test lab showed that in nine 2-year studies bronchiolar adenoma and carcinoma combined were in the range of 14 – 37 %. The male incidence for this 19-month (34 %) study exceeds slightly the upper limit of the historical control data sets (31.1%), but the combined incidence of adenoma and carcinoma at 5000 ppm (34%) is within the laboratory HCD upper limit for combined adenoma and carcinoma (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, it is concluded that the slight increase (compared to controls) in lung adenomas observed in males at 5000 ppm is unrelated to treatment with carboxin.

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available

4.10.2 Human information

No data available

4.10.3 Other relevant information

Two additional chronic toxicity studies (one conducted with carboxin and one conducted with carboxin sulfone (a urinary metabolite of carboxin)) are available. However, these studies are not presented in the CLH report as no conclusion on the carcinogenic potential could be drawn due to major study deficiencies (pre-GLP, protocol limitations and poor health status of the animals).

In the study conducted with carboxin (July 1966 - July 1968), the following points were taken into consideration when deciding on the acceptability of the study for classification and labelling purposes:

i) the purity of the test material was not reported (assumed to be 100% purity by the contract laboratory)
ii) the number of test animals (30/sex) was lower than the minimum number required by current international guidelines
iii) the investigations in test animals were limited to 5/sex/dose (e.g. clinical chemistry, haematology and histopathology of selected organs at the mid and high dose levels) or 10/sex/dose (e.g. histopathology in control and top dose groups)
iv) reduced survival was evident in males at the top dose (attributed to incidental deaths with 50% mortalities at 18 months and terminated in the 89th week)
v) prophylactic injections of Duracillin were administered to control and test animals in the latter part of the study
vi) mortalities which occurred in all groups were considered by the authors of the report to be due to spontaneous disease, predominantly lung disease (deaths from lung disease were reported prior to the 12 month sacrifice).

In the study conducted with carboxin sulfone (July 1966 – July 1968) the following points were taken into consideration when deciding on the acceptability of the study for classification and labelling purposes:

i) the purity of the test material was not reported (the report states that the test material was considered to be free from impurities by the contract laboratory)
ii) the number of test animals (30/sex) was lower than the minimum number required by current guidelines
iii) the investigations in test animals were limited to 5/sex/dose (e.g. clinical chemistry, haematology and histopathology of selected organs at the mid and low dose levels) or 10/sex/dose (e.g. histopathology in the control and top dose groups)
iv) prophylactic injections of Duracillin were administered to control and test animals in the latter part of the study
v) a high mortality rate occurred in all groups during the last 6 months of the study and the authors of the report considered these mortalities to be due to spontaneous disease, predominantly lung disease.

4.10.4 Summary and discussion of carcinogenicity

Carcinogenicity has been investigated in one guideline study in rats and one non-guideline study in mice.

In rats 4/50 (8 %) males, receiving 400 ppm of carboxin, presented with hepatocellular carcinoma (compared to 1/50 (2%) in the control and mid-dose groups). The value of 8 % was outside of all laboratory HCD provided and therefore the increased incidence of hepatic carcinoma in male rats is

considered to be treatment-related. However, when considering the low incidence observed (8% vs 2% in controls), the sex-specificity of the response, the lack of statistical significance, the absence of a respective response in liver adenomas and more importantly the "excessive toxicity" reported at this dose in males (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%] and the severe nephrotoxicity for which classification with STOT-RE 2 has already been proposed), it can be concluded that these liver tumours are of no relevance to human health.

In mice, 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%). However the combined incidence of adenoma and carcinoma at 5000 ppm (34%) was within the laboratory HCD upper limit for combined adenoma and carcinoma 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, it is concluded that the slight increase (compared to controls) in lung adenomas observed in males at 5000 ppm is unrelated to treatment with carboxin.

4.10.5 Comparison with criteria

Category 1A (known to have carcinogenic potential for humans) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to carboxin and the development of cancer.

Category 1B (presumed to have carcinogenic potential for humans) is also not appropriate as *there is not sufficient evidence of carcinogenicity in experimental animals*. Tumours of relevance to human health or tumours related to treatment with carboxin were not observed either in rats or mice.

Category 2 (suspected to have carcinogenic potential for humans) is also not appropriate as *there is no evidence of carcinogenicity in experimental animals.* Tumours of relevance to human health or tumours related to treatment with carboxin were not observed either in rats or mice.

4.10.6 Conclusions on classification and labelling

No classification, conclusive but not sufficient for classification

4.11 Toxicity for reproduction

Table 18: Summary table of relevant reproductive toxicity studies

<table>
<tr><th colspan="3">Fertility studies</th></tr>
<tr><th>Method</th><th>Dose Levels</th><th>Observations and Remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Two-generation reproduction study

Rat/Crl:CD (SD) BR 25/sex/dose

Oral (diet)

US EPA SF 83-4, GLP

Purity= 97.5%

Kehoe (1991)

F0 animals were mated twice to produce F1a and F1b litters and the F1b animals were mated twice to produce F2a and F2b litters.</td><td>M: 0, 20, 200 and 400 ppm

(Corresponding to ~ 0, 1, 10 and 20 mg/kg bw/day)

F: 0, 20, 300 and 600 ppm

(Corresponding to ~ 0, 1, 15 and 30 mg/kg bw/day)

Administration was 10 weeks prior to mating and then throughout gestation, lactation and weaning.</td><td><u>Parental toxicity</u>

<u>600 (females only):</u>
↓ Mean food consumption (F1b)
↓ bw gain during second mating period (F1b:26.1 %)**

*see table below for microscopic findings

<u>400 ppm (males only):</u>
↓ Food consumption (F0 and F1b)
↓ bw gain during first and second mating periods (F0: 10.8% - 28.5, F1b: 16.5 – 49.1 %)**
↓ bw (F0: range 4.7 – 9.1 %, F1: 10.4 – 14.4 %)**

*see table below for microscopic findings

<u>300 ppm (females only):</u>

*see table below for microscopic findings

<u>200 ppm (males only):</u>

↓ bw gain during first and second mating periods (F1b – 7.8 – 46.5 %)*

*see table below for microscopic findings

<u>20 ppm (males and females):</u>

No adverse effects.

* Microscopic findings in the kidney:
<table>
<tr><th rowspan="3">Finding</th><th rowspan="3"></th><th colspan="4">Males ppm (mg/kg bw/day)</th><th colspan="4">Females ppm (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>20</th><th>200</th><th>400</th><th>0</th><th>20</th><th>300</th><th>600</th></tr>
<tr><th>0</th><th>1</th><th>10</th><th>20</th><th>0</th><th>1</th><th>15</th><th>30</th></tr>
<tr><td rowspan="2">Proteinaceous cast</td><td>F0</td><td>1/2
50%</td><td>-</td><td>0/2
0%</td><td>1/1
100</td><td>-</td><td>1/2
50%</td><td>0/3
0%</td><td>1/3
33.3</td></tr>
<tr><td>F1b</td><td>2/2
100</td><td>1/3
33.</td><td>5/9
55.6</td><td>6/8
75</td><td>1/4
25</td><td>1/1
100</td><td>3/3
100</td><td>3/6
50%</td></tr>
<tr><td rowspan="2">Pelvic dilation</td><td>F0</td><td>1/2
50%</td><td>-</td><td>2/2
100</td><td>1/1
100</td><td>-</td><td>2/2
100</td><td>3/3
100</td><td>3/3
100%</td></tr>
<tr><td>F1b</td><td>0/2
0%</td><td>2/3
66.</td><td>7/9
77.8</td><td>7/8
87.</td><td>3/4
75</td><td>1/1
100</td><td>2/3
66.7</td><td>5/6
83.3</td></tr>
<tr><td rowspan="2">Chronic nephritis</td><td>F0</td><td>1/2
50%</td><td>-</td><td>2/2
100</td><td>1/1
100</td><td>-</td><td>0/2
0%</td><td>0/3
0%</td><td>2/3
66.7</td></tr>
<tr><td>F1b</td><td>2/2
100</td><td>0/3
0%</td><td>9/9
100</td><td>8/8
100</td><td>0/4
0%</td><td>0/1
0%</td><td>2/3
66.7</td><td>6/6
100%</td></tr>
<tr><td rowspan="2">Tubular mineralization</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0/2
0%</td><td>0/3
0%</td><td>3/3
100%</td></tr>
<tr><td>F1b</td><td>0/2
0%</td><td>0/3
0%</td><td>9/9
100</td><td>8/8
100</td><td>0/4
0%</td><td>1/1
100</td><td>0/3
0%</td><td>6/6
100%</td></tr>
</table>
</td></tr>
</table>

		Fertility effects: No treatment related effects on pre-coital intervals, gestation duration or fertility were observed **Offspring toxicity:** **400 ppm (males F2b pups only):** ↓ bw gain Lactation day 4 (post cull): 7.6 %*, lactation days 7 – 21: range 9.4 - 10.6%** Reproductive parameters: NOAEL of 400 and 600 ppm male and female rats, respectively. Parental toxicity: NOAEL of 20 ppm
Developmental Toxicity		
Method	**Dose Levels**	**Observations and Remarks (effects of major toxicological significance)**
Prenatal developmental study Rat/Charles River 25 females/dose Oral (gavage) OECD 414 GLP Purity=97.0% Schardein (1989)	0, 10, 90 and 175 mg/kg bw/day (suspended in 0.5% CMC and administered on days 6 to 15 of gestation) Volume= 10 ml/kg	**Maternal toxicity:** 175 mg/kg bw/day - ↓bw (7.7%, day 16), hair loss (8/25) 90 mg/kg bw/day: - ↓bw (4.6%, day 16); hair loss (4/25) 10 mg/kg bw/day – no adverse effects **Developmental findings:** 175 mg/kg bw/day: - ↓ mean fetal bw 6% 90 and 10 mg/kg bw/day: - No adverse effects Maternal NOAEL of 10 mg/kg bw/day Developmental NOEL of 90 mg/kg bw/day
Prenatal developmental study Rabbit/Dutch Belted 16 females/dose Oral (gavage) Non-guideline Non-GLP Purity= 99.0% Laughlin (1981)	0, 75, 375 and 750 mg/kg bw/day (suspended in 0.5% carboxymethylcellulose and administered on days 6 to 27 of gestation) Volume= 8 ml/kg	**Maternal toxicity:** 750 mg/kg bw/day- found dead/sacrificed (3/16), 375 mg/kg bw/day- found dead/sacrificed (1/16), 75 mg/kg bw/day- no adverse effects **Developmental findings:** 750 mg/kg bw/day- 3 animals aborted 375 mg/kg bw/day- 1 animals aborted 75 mg/kg bw/day- no adverse effects Maternal NOAEL of 75 mg/kg bw/day Developmental NOAEL of 75 mg/kg bw/day (based on abortions)

* p = < 0.05; ** p = < 0.01

4.11.1 Effects on fertility

4.11.1.1 Non-human information

One guideline multi-generation study is available in rats. In this study, groups of 25 male and 25 female Crl:CD rats (F0 and F1b) were fed diets containing carboxin at concentrations of 0, 20, 200 and 400 ppm (males) and 0, 20, 300 and 600 ppm (females) for a period of 10 weeks prior to mating and then continuing throughout gestation, lactation and weaning of the F1b and F2b pups until sacrifice. The F0 animals were mated twice to produce F1a and F1b litters; the F1b animals were mated twice to product F2a and F2B litters.

Parental toxicity was evidenced as changes in body weight associated with reduced food consumption and an increased incidence of kidney lesions (comparable to those observed short-term and chronic toxicity studies). Reductions in body weight gain were more marked in F0 and F1 males at 200 and 400 ppm. These changes occurred during both first and second matings. Changes to the kidneys in males at doses ≥ 200 ppm and females at doses ≥ 300 ppm 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. There were no specific reproductive effects during this study and the only developmental effect was reduced weight gain in male pups from the second litter of the second generation. This reduction in weight gain was measured on lactation days 4 (post cull), 7, 14 and 21 and the ranged between 7.6 – 10.6 %.

4.11.1.2 Human information

No data available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Two studies are available to investigate developmental toxicity. The first prenatal development study in rats was carried out to GLP standards following the relevant guideline, and the second was carried out in rabbits to neither guideline nor GLP standard.

In the rat developmental study, groups of 25 pregnant female Charles River COBS CD rats were orally administered carboxin technical by gavage (0, 10, 90 and 175 mg/kg bw/day) on days 6 – 15 of gestation.

During the study, there was a slight reduction in body weight gain associated with reduced food consumption at doses ≥ 90 mg/kg bw/day and a dose-related increase in hair loss in the dams. Gross necropsy of the dams did not reveal any treatment-related findings. A slight reduction in fetal body weight (↓ 6% compared to controls) was observed in the top dose.

In the rabbit developmental study, groups of 16 artificially inseminated Dutch Belted rabbits were orally administered carboxin technical by gavage (0. 75, 375 and 750 mg/kg bw/day) on days 6 – 27 of gestation.

Dam body weights and body weight gain were reduced and there was an increased incidence of clinical signs of abortion at doses ≥ 375 mg/kg bw/day. There was no evidence of treatment-related malformations or variations.

4.11.2.2 Human information

No data available

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

See sections 4.11.1 and 4.11.2

4.11.5 Comparison with criteria

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

4.11.6 Conclusions on classification and labelling

Not classified, conclusive but not sufficient for classification

4.12 Other effects

Not relevant.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Carboxin is a fungicide used as an agricultural seed treatment to prevent fungal pathogens. Available environmental fate and hazard studies have been reviewed under Directive 91/414/EEC. The studies are summarised in the Pesticide Draft Assessment Report (DAR), Volume 3, B.8 and B.9 – August 2006. The key information pertinent to determining a classification is presented below.

The carboxin purity profiles were considered as part of the DAR peer review process. The original purity of 97% technical carboxin was under review and a revised value of 98.7% was determined. Ecotoxicity studies used 97 to 97.5% carboxin, which were above the original purity profile minimum but slightly less than the proposed revision. This is not considered to affect the classification and labelling proposal.

Carboxin degrades to a number of degradants in the environment – where available data on the metabolites have also been included.

5.1 Degradation

Table 19 presents a summary of key degradation information for carboxin.

Table 19: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD Guideline 111	Carboxin is hydrolytically stable at environmentally relevant pH and temperature		Clayton and Lowrie, 2003 DAR B.8.4.1a
Aquatic photolysis EPA Guideline subdivision N 161-2	Carboxin DT_{50} at 25 °C 1.54 hours (linear regression) 2.64 hours (non-linear regression)		Horree, 1992 DAR B.8.4.2
Ready biodegradation OECD Guideline 301B	Carboxin is not readily biodegradable		Van Dijk, 1989 DAR B.8.4.3
Water/sediment simulation Dutch Regulation for Biocides Section G.2.1	Carboxin mean DT_{50} total system 17.3 days at 20 °C Carboxin mean DT_{50} total system 32.8 days at 12 °C Carboxin sulfoxide DT_{50} total system 27.7 days at 20 °C Carboxin sulfoxide DT_{50} total system 52.5 days at 12 °C	Carboxin undergoes primary degradation to carboxin sulfoxide in the aquatic phase with partitioning to the sediment phase and limited mineralisation	Muttzall, 1994 DAR B.8.4.4

5.1.1 Stability

Hydrolysis

Carboxin

Two aqueous hydrolysis studies using radiolabelled carboxin were carried out showing the substance was hydrolytically stable at environmentally relevant pH values.

Study 1 (Clayton and Lowrie, 2003)

Using ^{14}C-phenyl]-carboxin and following GLP and OECD Guideline 111, hydrolysis was assessed over 7 days at pH 4, 7 and 9 at 50 °C. Carboxin was analysed by High Performance Liquid Chromatography (HPLC) and Think Layer Chromatography (TLC) while radioactivity was analysed by Liquid Scintillation Counting (LSC). Minimal hydrolysis was observed and half-lives were not calculated.

Study 2 (Dzialo and Lengen, 1983)

A second study using [^{14}C]-carboxin is available. However, the guideline and duration are not specified and the study is not GLP compliant. On this basis it is considered supporting evidence. The study results support that carboxin is hydrolytically stable at environmentally relevant pH values.

Degradants

Two aqueous hydrolysis studies using carboxin degradants carboxin sulfoxide and carboxin sulfone were carried out:

Carboxin sulfoxide (Jewell, 1990)

A GLP compliant study following BBA guidelines assessed hydrolysis of carboxin sulfoxide over 30 days at pH 4, 7 and 9. Analysis by HPLC showed no significant hydrolysis at pH 4 or 7. Hydrolysis was observed at pH 10 with a calculated half-life of 4.9 days at 22 °C.

Carboxin sulfone (Dzialo, 1995)

A GLP compliant study following US EPA guidelines assessed hydrolysis of [^{14}C-phenyl]-carboxin sulfone over 30 days at pH 5, 7 and 9. Radioactivity was analysed by LSC and the test substance by HPLC with TLC. No significant hydrolysis was observed at pH 5 but increasing hydrolysis of carboxin sulfone was observed with increasing pH. The calculated half-lives at the study temperature 25 °C at pH 7 and 9 are 9.8 days and 3.9 hours.

Aqueous photolysis

Carboxin

Study 1 (Horree, 1992 and DAR, 2006)

A GLP compliant study assessed the aqueous photolysis of [^{14}C-phenyl]carboxin following US EPA guidelines at 25 °C. Radioactivity was analysed by LSC and the test substance by HPLC-MS. Rapid photolysis was observed. The original study reported a first-order DT_{50} of 1.54 hours based on linear regression analysis.

For the purpose of the DAR, the rapporteur a calculated DT_{50} of 2.64 hours based on non-linear regression. Two major metabolites were observed: oxo(phenyl amino)acetic acid (max. 54.9% AR) and carboxin sulfoxide (max. 20.4% AR).

Study 2 and Study 3 (Harned, 2003a and 2003b)

The quantum yield of carboxin was determined considering the UV/visible absorption spectrum, the first order aqueous photodegradation rate, including wavelength distribution and distribution, (Horree, 1992) and the Swanson *et al* method (details in DAR, 2006).

The calculated quantum yield of carboxin was 0.685%.

For the purpose of the DAR, the recalculated quantum yield based on non-linear aqueous degradation rate was 0.4%.

Using the study calculated quantum yield, a pseudo first-order rate constant for direct phototransformation of carboxin in the surface layer (0.5 m depth) of natural water at 40-50^{o}N was calculated to be DT_{50} 1.01 hours. This considered yearly averaged mid-day sunlight values and reflection from water surface.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Study 1 (Van Dijk, 1989)

A GLP ready biodegradation study following OECD Guideline 301B was carried out. The dissolved organic carbon concentration did not change over the 28-day study period indicating no significant biodegradation and carboxin was not considered readily biodegradable.

5.1.2.3 Simulation tests

***Aquatic/Sediment system* (Muttzall, 1994 and Wanner, 2004a)**

A GLP water/sediment study using [^{14}C-phenyl]-carboxin, following Dutch Regulation of Biocides guidelines has been carried out. Two laboratory water/sediment systems (TNO ditch system and Kromme Rijn river system) were used to assess the fate of carboxin over 13 weeks. The TNO system used a clay loam sediment and water pH 8.7. The Kromme Rijn system used a sandy loam sediment and water pH 7.9. The study was run in the dark at 20 ^{o}C ± 2 ^{o}C with the water phase being considered as aerobic and the sediment phase considered as anaerobic. The Applied Radioactivity (AR) distribution can be found in Table 20. The initial decrease in parent carboxin was considered due to degradation during storage of extracts prior to addition to the test system.

Table 20 – Distribution of Applied Radioactivity (as mean percentage) in water/sediment systems at 20 °C ±2 °C

'TNO' ditch system	Incubation time (weeks)				
	0	2	4	8	13
Aqueous phase					
Aqueous phase extract (dichloromethane)					
Carboxin	74	16	6	2	<1
Carboxin sulfoxide	8	11	8	4	4
Total Unknowns *	5	4	3	3	2
Aqueous phase freeze-dried					
Carboxin sulfoxide	1	nd	nd	nd	nd
Total Unknowns **	9	nd	nd	nd	nd
Sediment phase					
Carboxin	1	27	25	16	9
Carboxin sulfoxide	2	2	4	3	3
Total Unknowns ****	3	9	10	7	6
$^{14}CO_2$	0	8.2	10.7	18.0	23.7
Non-extractables	0.8	18.6	25.0	35.4	40.2
Total recovery	102.5	98.1	93.6	90.4	88.1
'Kromme Rijn' river system	**Incubation time (weeks)**				
	0	2	4	8	13
Aqueous phase					
Aqueous phase extract (dichloromethane)					
Carboxin	74	18	4	1	- [1]
Carboxin sulfoxide	6	18	19	12	1 [1]
Total Unknowns *	3	6	6	5	4 [1]
Aqueous phase freeze-dried					
Carboxin sulfoxide	1	1	1 [1]	nd	nd
Total Unknowns ***	10	2	3 [1]	nd	nd
Sediment phase					
Carboxin	2	10	10	4	2
Carboxin sulfoxide	1	6	4	4	1
Total Unknowns ****	1	11	5	5	5
$^{14}CO_2$	0.0	8.4	15.2	25.0	40.1
Non-extractables	0.6	16.0	23.2	27.6	33.4
Total recovery	104.6	98.4	91.4	87.8	83.5

* Total of up to 4 unknowns, each ≤4% AR, ** Total of 2 unknowns, *** Total of up to 3 unknowns, , **** Total of up to 5 unknowns, nd = not determined, - = not detected, [1] = results of one replicate

Carboxin partitioned from the water phase to the sediment phase. The principle degradant in water and sediment was carboxin sulfoxide. Greater partitioning to sediment in the TNO system was considered due to the higher organic matter content.

Mineralisation was observed with 10.7-15.2% AR CO_2 at week 4 and a maximum of 23.7-40.1 % AR CO_2 by week 13.

Whilst noting the infrequent sampling means (particularly for the time between 0 and 2 weeks), the degradation of carboxin and carboxin sulfoxide in the aquatic environment was estimated using ModelMaker 4.0 and a multi-compartment model. For carboxin, the total system DT_{50} values at study temperature (20 °C) were 23.5 days for TNO and 11 days for Kromme Rijn with a mean of 17.3 days. For carboxin sulfoxide, the total system DT_{50} values at study temperature (20 °C) were 33.2 days for TNO and 22.1 days for Kromme Rijn with a mean of 27.7 days.

Converting DT_{50} values to environmentally relevant temperature results in the following DT_{50} values at 12 °C:

- Carboxin mean DT_{50} total system 32.8 days at 12 °C
- Carboxin sulfoxide DT_{50} total system 52.5 days at 12 °C

Overall, carboxin was considered to undergo rapid primary degradation (with carboxin sulfoxide as the principle degradant) but limited mineralisation.

Soil system

Various degradation in soil studies have been carried out:

Study 1 (Mamoumi, 2004 and Wanner, 2004b)

A GLP study following SETAC guidelines assessed the degradation of [^{14}C-oxathiine]-carboxin and [^{14}C-UL-phenyl]-carboxin under aerobic soil conditions at 20 °C ± 1 °C. Three soil types were employed (Soil I - clay loam, Soil II - silty clay loam and Soil III - clay) for the duration of the study, which was carried out in the dark for over 160 days. Sampling was undertaken on days 0, 0.16, 1, 4, 7, 14, 28, 60, 88 and 120. An additional sample was taken at day 160 for soil I and soil II. Extracts were analysed by HPLC and degradants were identified by LC-MS. Carboxin dissipated rapidly with less than 2% AR or not detectable by day 14 in all soil systems. The major degradant was carboxin sulfoxide and the proposed degradation pathway for carboxin in aerobic soil is presented in Figure 1. Mineralisation was observed with a maximum of 53.5% AR on day 160 for Soil II. Mineralisation to CO_2 did not exceed 30% AR in any soil by day 28. For the purpose of the DAR the Rapporteur calculated DT_{50} values using ModelMaker. The carboxin first order DT_{50} ranged from 0.11 to 0.78 days. The carboxin sulfoxide first order DT_{50} ranged from 8.5 to 53.1 days.

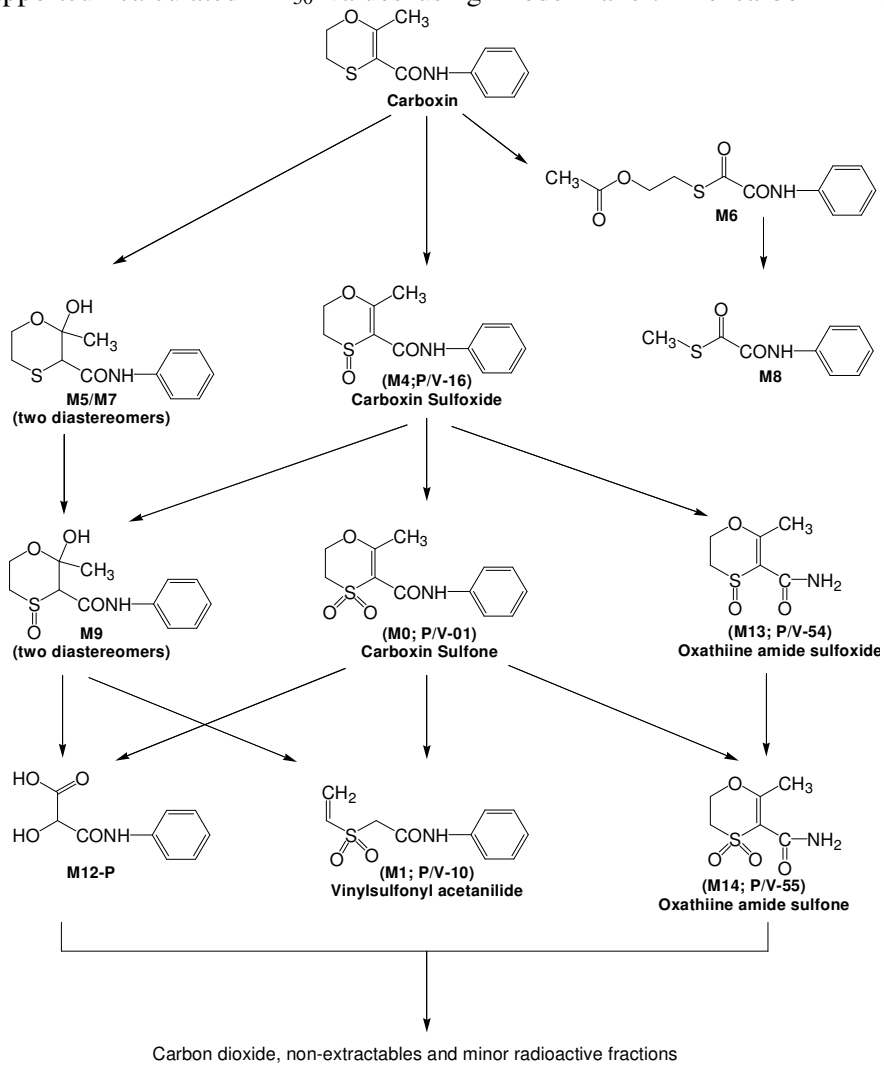


Figure 1 - Proposed degradation pathway for carboxin in aerobic soil

Study 2 (Wanner, 2004c)

A GLP study assessed the degradation of [^{14}C-oxathiine]-carboxin and [^{14}C-UL-phenyl]-carboxin, under aerobic soil conditions in the dark at 20 °C ± 1 °C, for 118 days. A single soil type (sandy loam) was employed. Sampling was undertaken on days 0, 0.16, 1, 3, 7, 14, 28, 61, 90 and 118, with analysis by HPLC. Carboxin dissipated rapidly and was undetectable at day 3. Carboxin sulfoxide was the major degradant with CO_2 mineralisation reaching a maximum of 27% AR by day 118. For the purpose of the DAR, the Rapporteur calculated DT_{50} values at 20 °C using ModelMaker. The carboxin first order DT_{50} ranged from 0.1 to 0.8 days. The carboxin sulfoxide first order DT_{50} ranged from 71.6 to 101.2 days

Study 3 (Gaydosh, 1989 and Beerbaum, 1990)

A GLP study following EPA guideline N-162-2 the degradation of [^{14}C-phenyl]-carboxin under anaerobic soil conditions was assessed for 60 days. A single soil type (sandy loam) was employed. Sampling was undertaken on days 0, 15, 30 and 60, with analysis by HPLC. Carboxin gradually dissipated reaching 27.9% AR by day 60 in soil residues. Carboxin sulfoxide was the major degradant with carbon sulfone a minor degradant in soil residues. Carbon dioxide evolution was not assessed. Overall, the degradation of carboxin under anaerobic conditions was slower compared to aerobic conditions.

Additional studies

Two further 48-hour degradation studies in soil were reported in the DAR. These were not considered for the CLH proposal as they did not provide any further information to the above studies, given their short duration. A second anaerobic soil degradation study was provided which supported the findings of the key study above.

Overview

Carboxin rapidly dissipated under aerobic soil conditions (DT_{50} of <1 day) to form carboxin sulfoxide and carboxin sulfone which had longer residence times. The DAR considered all soil degradation data and presented calculated arithmetic mean first order DT_{50} values at 20 °C for carboxin and its degradants (presented in Figure 1). The values were as follows: carboxin DT_{50} 0.53 days; carboxin sulfoxide DT_{50} 38.6 days; carboxin sulfone DT_{50} 20.2 days; P/V-54 DT_{50} 78.7 days; P/V-55 DT_{50} 18.7 days and M9 DT_{50} 5 days.

5.1.3 Summary and discussion of degradation

In laboratory studies, carboxin underwent minimal hydrolysis, indicating hydrolytical stability at environmentally relevant pH and temperature.

In an aqueous photolysis study at 25 °C, carboxin underwent rapid phototransformation with a half-life of 1.54 to 2.64 hours. The two principal degradants were oxo(phenyl amino) acetic acid and carboxin sulfoxide. Additional data support rapid photodegradation under experimental conditions.

It is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non classifiable substances. Therefore aquatic photolysis is not considered to meet the criteria for rapid degradation. On this basis, a DT_{50} at 12 °C has not been included.

On the basis of a ready biodegradation study, carboxin was not considered readily biodegradable.

In a water/sediment study carboxin dissipated fairly rapidly to the sediment phase. Carboxin sulfoxide was the principle degradant although limited mineralisation was observed. The mean total system DT_{50} at study temperature (20 ^{o}C) for carboxin was 17.3 days and the mean total system DT_{50} at study temperature (20 ^{o}C) for carboxin sulfoxide was 27.7 days. Converting DT_{50} values to environmentally relevant temperature results in the following DT_{50} values at 12 ^{o}C:

- Carboxin mean DT_{50} total system 32.8 days at 12 ^{o}C
- Carboxin sulfoxide DT_{50} total system 52.5 days at 12 ^{o}C

Carboxin rapidly dissipates in soil with a DT_{50} of <1 day to form carboxin sulfoxide and carboxin sulfone which have longer residence times with DT_{50} values 38.6 and 20.2 days respectively.

Taking into account all of the findings summarised above, carboxin is considered to undergo rapid primary degradation but not considered to undergo greater than 70% ultimate degradation in the aquatic environment within 28 days. It is therefore considered not rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Study 1 (Wanner, 2003)

The adsorption of carboxin in five soils (sandy loam – clay, pH 4.5 - 7.7, Organic Carbon 0.9 - 2.2%) was assessed following OECD Guideline 106 and GLP. Adsorption values were in the range 123 - 213 with 1/n=0.79 - 0.82 (mean Kfoc 152, 1/n=0.81). Soil pH appeared to have a small effect on soil adsorption of carboxin, with adsorption increasing with acidity.

5.2.2 Volatilisation

Carboxin has a measured vapour pressure (Tremain, 2001c) of $2x10^{-5}$ Pa at 25 ^{o}C and a calculated Henry's Law Constant (White, 2002) of $3.1x10^{-5}$ Pa m^3 mol^{-1}. On that basis, carboxin is likely to remain in solution and not partition to the atmosphere.

5.3 Aquatic Bioaccumulation

Table 21 presents a summary of key aquatic bioaccumulation information for carboxin.

Table 21: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
EEC Method A8 (HPLC)	Log Pow 2.3		Riggs, 2001g DAR B.2.1.13

5.3.1 Aquatic bioaccumulation

The measured log Pow (Riggs, 2001g) for carboxin was 2.3. This indicates carboxin has low potential for bioaccumulation (less than CLP log Kow trigger of 4). No measured bioaccumulation data are currently available.

5.4 Aquatic toxicity

Tables 22 a-c present a summary of key ecotoxicity information for carboxin and its degradants.

5.4.1 Fish

Table 22a: Summary of relevant information on aquatic toxicity to fish

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Carboxin 97.39 %	*Oncorhynchus mykiss*	US EPA 72-1	96-h LC_{50}	2.3 mg a.s./l	Flow-through Mean measured	Bettencourt, 1994a
Carboxin 97.39 %	*Lepomis macrochirus*	US EPA 72-1	96-h LC_{50}	3.6 mg a.s./l	Flow-through Mean measured	Bettencourt, 1994b
Carboxin sulfoxide 99.6 %	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	>25 mg/l	Static Nominal	Czech, 2002a
Carboxin sulfone unknown	*Lepomis macrochirus* and *Oncorhynchus mykiss*	US EPA-660/3-75-009	96-h LC_{50} 96-h LC_{50}	28.1 mg/l 19.9 mg/l	Static Nominal Losses likely given hydrolysis half-lives at varying pH	Kuc, 1977
P/V-54 100 %	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	>100 mg/l	Static Nominal	Volz, 2004a
Carboxin 97 %	*Cyprinus carpio*	OECD 204	21-d NOEC (based on growth, mortality and symptoms of toxicity)	0.32 mg a.s./l	Semi-static Nominal	Bogers, 1989a

5.4.1.1 Short-term toxicity to fish

Carboxin

Two GLP acute toxicity to fish studies using carboxin are available:

Study 1 (Bettencourt, 1994a)

The acute toxicity to fish was assessed following US EPA guideline 72-1 and Rainbow Trout (*Oncorhynchus mykiss*). The study used carboxin technical with a purity of 97.39%. Under flow-through conditions carboxin concentrations were 80–93% of nominal and the degradant carboxin sulfoxide was not detected. Based on mean measured concentrations, the 96-h LC_{50} was 2.3 mg a.s./l and the 96-h NOEC 0.61 mg a.s./l.

Study 2 (Bettencourt, 1994b)

The acute toxicity to fish was assessed following US EPA guideline 72-1 and Bluegill Sunfish (*Lepomis macrochirus*). Under flow-through conditions carboxin (purity 97.39%) concentrations were 90-120% of nominal and the degradant carboxin sulfoxide was not detected. Based on mean measured concentrations, the 96-h LC_{50} was 3.6 mg a.s./l and the 96-h NOEC 1.8 mg a.s./l.

Additional supporting toxicity to fish data (Bogers, 1989a)

A GLP, semi-static, 21-day sub-lethal fish toxicity study was carried out for carboxin following OECD Guideline 204 and using carp (*Cyprinus carpio*). The study used carboxin technical with a purity of 97%. Exposure solutions were prepared using Tween 80 and a solvent control was included. The nominal 21-d NOEC was 0.32 mg a.s./l based on mortality, toxicity symptoms and growth.

It is noted that the OECD 204 test method is considered a prolonged toxicity to fish test and as such is not considered as a chronic endpoint. In addition, in April 2014, the test guideline was removed by OECD. On this basis the study is not considered to provide a valid chronic NOEC for the purpose of classification and labelling.

Degradants

Three acute toxicity to fish studies are available using three carboxin degradants. These indicate the carboxin parent is more acutely toxic to fish than major degradants.

Carboxin sulfoxide (Czech, 2002a)

The acute toxicity to fish was assessed following OECD Guideline 203 and GLP using Rainbow Trout (*Oncorhynchus mykiss*). A static test system with a single replicate of 25 mg/l was used. Analytical measurement at 3, 24, 48, 72 and 96 hours showed measured concentrations were 100% of nominal concentrations. No adverse effects were observed and the 96-h LC_{50} is considered to be > 25mg/l.

Carboxin sulfone (Kuc, 1977)

The acute toxicity to fish was assessed following US EPA-660/3-75-009 guideline using Rainbow Trout (*Oncorhynchus mykiss*) and Bluegill Sunfish (*Lepomis macrochirus*). The study predated GLP. The study temperature was 22 ^{o}C for Bluegill Sunfish and 11 ^{o}C for Rainbow Trout. A static test system was employed and the study did not include analytical support. Based on nominal concentrations the study 96-h LC_{50} for Bluegill Sunfish was 28.1 mg/l. Based on nominal concentrations the study 96-h LC_{50} for Rainbow trout was 19.9 mg/l. Carboxin sulfone was susceptible to hydrolysis with half lives of 9.8 days at pH 7 and 3.9 hours at pH 9. Given the pH ranged from 6.82 to 7.86 for both species over the exposure period of 4-days, some test substance losses are anticipated. However, such losses are not considered to result in a fish acute LC_{50} for carboxin sulfone below the carboxin acute fish LC_{50} or the lowest acute toxicity endpoint for carboxin / degradants (carboxin parent algal 5-d E_rC_{50}).

P/V–54 (Volz, 2004a)

The acute toxicity to fish was assessed following GLP and OECD Guideline 203 guidelines using Rainbow Trout (*Oncorhynchus mykiss*). A static test system with a single 100 mg/l exposure concentration was employed as the substance is considered stable over the study period. Mean measured concentrations at 0 and 48 hours were 97-98% nominal. No adverse effects were observed. Based on nominal concentrations the study 96-h LC_{50} was considered >100 mg/l. On this basis, P/V-54 is considered less acutely toxic to fish than the parent carboxin.

5.4.1.2 Long-term toxicity to fish

No valid studies are available.

5.4.2 Aquatic invertebrates

Table 22b: Summary of relevant information on aquatic toxicity to invertebrates

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Carboxin 97.39 %	*Daphnia magna*	OECD 202	48-h EC_{50}	>57 mg a.s./l	Flow-through Mean measured	Putt, 1994
Carboxin sulfoxide 99.6 %	*Daphnia magna*	OECD 202	48-h EC_{50}	>25 mg a.s./l	Static Nominal	Czech, 2002b
Carboxin sulfone unknown	*Daphnia magna*	US EPA-660/3-75-009	48-h EC_{50}	69.1 mg/l	Static Nominal Losses likely given hydrolysis half-lives at varying pH	Vilkas, 1977
P/V-54 100 %	*Daphnia magna*	OECD 202	48-h EC_{50}	>100 mg/l	Static Nominal	Volz, 2004b
Carboxin 97 %	*Daphnia magna*	OECD 202 17-d Nominal	17-d NOEC 0.32 mg a.s/l based on reproduction and growth	>100 mg/l	Semi-static Nominal	Bogers, 1989b

5.4.2.1 Short-term toxicity to aquatic invertebrates

<u>Carboxin</u>

Study 1 (Putt, 1994)

The acute toxicity to *Daphnia magna* was assessed following US EPA 72-2 guideline and using flow-through conditions. The GLP study used carboxin technical with a purity of 97.39%. Exposure concentrations were dilutions of a saturated stock solution; 13, 22, 36, 60 and 100%. Analysis at 0 and 48 hours were used to present the exposure concentrations as mean measured concentrations; 7, 11, 21, 34, 57 mg a.s./l. In addition the degradant carboxin sulfoxide was not detected at 0 or 48

hours. Based on mean measured concentrations, the 48-h EC_{50} was >57 mg a.s./l reflecting the saturated solution and the 48-h NOEC was 11 mg a.s./l.

Degradants

Three acute toxicity to invertebrate studies are available using three carboxin degradants.

Carboxin sulfoxide (Czech, 2002b)

The acute toxicity to *Daphnia magna* was assessed following GLP, OECD Guideline 202 and using static conditions. A single exposure concentration of 25 mg/l was used reflecting the limit of solubility in test medium. Mean measured concentrations at 0 and 48 hours were 88 - 89% nominal. Based on nominal concentrations, the 48-h EC_{50} was >25 mg/l reflecting the saturated solution and the 48-h NOEC was 25 mg/l

Carboxin sulfone (Vilkas, 1977)

The acute toxicity to *Daphnia magn*a was assessed following US EPA-660/3-75-009 guidelines. The study predated GLP. A static test system was employed and the study did not include analytical support. Based on nominal concentrations, the reported study 48-h EC_{50} was 69.1 mg/l (95 % C.L. 54.2 – 88.2 mg/l) and the 48-h NOEC <16 mg/l reflecting the lowest exposure concentration. Carboxin sulfone was susceptible to hydrolysis with half lives of 9.8 days at pH 7 and 3.9 hours at pH 9. Given the pH ranged from 7.48 to 7.65 over the exposure period of 2 days, some test substance losses were anticipated. However, *Daphnia* are not considered highly acutely sensitive and such losses are not considered to result in an acute LC_{50} for carboxin sulfone below the carboxin acute *Daphnia* LC_{50} or the lowest acute toxicity endpoint for carboxin / degradants (carboxin parent algal 5-d E_rC_{50}).

P/V–54 (Volz, 2004b)

The acute toxicity to *Daphnia magna* was assessed following GLP, OECD Guideline 202 and static conditions. A single exposure concentration of 100 mg/l was used. Mean measured concentrations at 0 and 48 hours were 97-99% nominal. No adverse effects were observed. Based on nominal concentrations, the 48-h EC_{50} was >100 mg/l.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A GLP, semi-static, chronic toxicity study using *Daphnia magna* following OECD Guideline 202 is available (Bogers, 1989b). The study predated the current OECD 211 Guideline and was run over 17 days. The study used carboxin technical with a purity of 97%. The exposure test series was 0.1, 0.32, 1.0, 3.2 and 10 mg a.s./l. Measured concentrations were 91-134% of nominal and results are based on nominal concentrations. Based on inhibition of reproduction and toxicity to juveniles the 17-d NOEC was 0.32 mg a.s./l and the 17-d LOEC was 1.0 mg a.s./l. Given the nature of NOEC values it is not possible to extrapolate a 21-d NOEC.

5.4.3 Algae and aquatic plants

Table 22c: Summary of relevant information on aquatic toxicity to algae

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Carboxin 97.5 %	*Pseudokirchneriella subcapitata*	US EPA FIFRA 123-3	5-d E_rC_{50} 5-d NOE_rC	0.45 mg a.s./l 0.107 mg a.s./l	Static Mean measured	Hughes, 1990
Carboxin sulfoxide 99.6 %	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC50 72-h NOE_rC	>25 mg/l 25 mg/l	Static Nominal	Czech 2002c
Carboxin sulfone unknown	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC50 72-h NOE_rC	2.76 mg/l 0.25 mg./l	Static Mean measured Losses likely given hydrolysis half-lives at varying pH	Czech, 2002d
P/V-54 100 %	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC50 72-h NOE_rC	>100 mg/l 100 mg/l	Static Nominal	Volz, 2004c

Carboxin

Study 1 (Hughes, 1990)

A 5-day, GLP, static algal growth inhibition study was carried out using *Pseudokirchneriella subcapitata* and following US EPA FIFRA 123-2 guideline. The 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. Analysis was undertaken at 0 hours and on day 5, which included analysis for carboxin sulfoxide. At 0 hours, carboxin concentrations were 92-122% of nominal. At day 5, combined mean measured concentrations of carboxin and carboxin sulfoxide were 82-92% of nominal carboxin concentrations. The study reported EC_{50} and NOEC data based on mean measured concentrations at day 0 (carboxin) and day 5 (carboxin + carboxin sulfoxide). Table 23 presents nominal exposure concentrations, analytical data and algal growth inhibition.

Table 23 – Comparison of analytical data and algal growth inhibition from Hughes 1990

Concentration (mg a.s./l)						Algal growth inhibition (%)	
Nominal	**Day 0 Carboxin**	**Day 5 Carboxin**	**Day 5 Carboxin sulfoxide**	**Study reported mean measured carboxin + carboxin sulfoxide**	**Calculated mean measured carboxin on days 0 and 5**	**Day 3**	**Day 5**
Control	Not detected	Not detected	Not detected	Not detected	-	-	-
Solvent control	Not detected	Not detected	Not detected	Not detected	-	-	-
0.125	0.152	0.061	0.046	0.130	0.107	11.3	13.1
0.25	0.276	0.170	0.060	0.253	0.223	28.2	28.2
0.5	0.525	0.375	0.071	0.486	0.450	45.8	49.8
1.0	1.130	0.759	0.056	0.972	0.945	53.4	72.7
2.0	1.830	1.604	0.080	1.757	1.717	82.7	100

The study reported the 5 day E_rC_{50} as 0.48 mg a.s./l and the 5 day NOE_rC was 0.13 mg a.s./l (combined carboxin and degradant concentration) based on analysis of variance and Dunnett's test[1]. 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. Approximately ~50 % inhibition was observed on day 5 at nominal exposure concentration 0.5 mg carboxin a.s./l. This equated to a mean measured concentration of 0.45 mg a.s./l based on carboxin concentrations on days 0 and 5. Similarly, the reconsidered NOE_rC based on mean measured concentration carboxin concentrations on days 0 and 5 is 0.107 mg a.s./l.
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, this was not considered to affect the validity of the 5-day results.

Degradants
Three algal growth inhibition studies are available using three carboxin degradants:

Carboxin sulfoxide (Czech, 2002c)

A 72 hour, GLP, static algal growth inhibition study was carried out using *Pseudokirchneriella subcapitata*, following OECD Guideline 201. A single exposure concentration of 25 mg/l was used reflecting the limit of solubility in test medium. Mean measured concentrations at 0 and 72 hours were 95-96% nominal. Based on nominal concentrations, the E_rC_{50} was >25 mg/l and the NOE_rC was 25 mg/l.

[1] The effect values presented in this document are different than those in the DAR. For the purpose of classification, effects values based on carboxin only have been used to assess ecotoxicity.

Carboxin sulfone (Czech 2002d)

A 72 hour, GLP, static algal growth inhibition study was carried out using *Pseudokirchneriella subcapitata*, following OECD Guideline 201. The study pH ranged from 7.9 to 8.1. Mean measured concentrations at 0 hours were 89-109% of nominal and at 72 hours 43–52% nominal. Losses were assumed to be a result of hydrolysis. Based on mean measured concentrations, the E_rC_{50} was 2.76 mg/l (95% C.L. 1.02 – 6.86 mg/l) and the NOE_rC was 0.25 mg/l.

P/V-54 (Volz (2004c)

A 72 hour, GLP, static algal growth inhibition study was carried out using *Pseudokirchneriella subcapitata*, following OECD Guideline 201. Mean measured concentrations at 0 and 72 hours were 98% nominal. Based on nominal concentrations, the E_rC_{50} was >100 mg/l and the NOE_rC was 100 mg/l.

5.4.4 Other aquatic organisms (including sediment)

No other relevant data.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Carboxin was found to be hydrolytically stable at environmentally relevant pH and temperature.

Carboxin was susceptible to aqueous photolysis with experimental half-lives of 1.54 to 2.64 hours at 25 ^oC. The two principal degradants were oxo(phenyl amino) acetic acid and carboxin sulfoxide. It is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore aquatic photolysis is not considered to meet the criteria for rapid degradation. On this basis, a DT_{50} at 12 ^oC has not been included.

On the basis of a ready biodegradation study, carboxin was not considered readily biodegradable.

In a water/sediment study carboxin dissipated fairly rapidly. Carboxin sulfoxide was the principle degradant although limited mineralisation was observed. At study temperature, the mean total system DT_{50} for carboxin was 17.3 days and the mean total system DT_{50} for carboxin sulfoxide was 27.7 days. Converting DT_{50} values to environmentally relevant temperature results in the following DT_{50} values at 12 ^oC:

- Carboxin mean DT_{50} total system 32.8 days at 12 ^oC
- Carboxin sulfoxide DT_{50} total system 52.5 days at 12 ^oC

Carboxin rapidly dissipated in soil with a DT_{50} <1 day to form carboxin sulfoxide and carboxin sulfone which have longer residence times with DT_{50} values 38.6 and 20.2 days respectively.

Overall, carboxin is considered to undergo rapid primary degradation but is not considered to undergo greater than 70% ultimate degradation in the aquatic environment within 28 days. On this basis it is considered not rapidly degradable for the purpose of classification and labelling.

While a bioaccumulation study is not available, the carboxin log P_{ow} value of 2.3 is lower than the trigger value of 4 for classification and labelling under Regulation EC 1272/2008.

Acute toxicity to fish, invertebrates and algae data are available for carboxin and degradants carboxin sulfoxide, carboxin sulfone and P/V-54. Overall, carboxin parent is considered more acutely toxic than its degradants. Carboxin is considered to exhibit acute aquatic toxicity < 1 mg/l. Algae are the most acutely sensitive trophic level with a carboxin 5-d E_rC_{50} 0.45 mg a.s./l.

Based on the available acute ecotoxicity data, with $L(E)C_{50}$ values < 1 mg/l, classification as Aquatic Acute 1 is applicable with an acute M-factor of 1 based on $0.1 < L(E)C_{50} \leq 1$ mg/l.

The long-term aquatic data suggest chronic toxicity in the range 0.1-1 mg/l. The carboxin algal 5-d NOErC for *Pseudokirchneriella subcapitata* is 0.107 mg a.s./l and the carboxin sulfone algal 72-h NOE_rC for the same species is 0.25 mg/l. This results in the classification Aquatic Chronic 2 based on > 0.1 NOEC ≤ 1 mg/l for a non-rapidly degradable substance.

The carboxin 17-d NOEC for *Daphnia* was 0.32 mg a.s./l. While this was a non-standard duration and was not considered robust for the purpose of deriving a chronic classification, the value supports the Aquatic Chronic 2 classification.

Adequate chronic toxicity data for fish are not available.

Given robust chronic endpoints are not available for fish and invertebrates, the surrogate approach to deriving chronic classification should be considered. Using the available acute data for fish, this would result in classification Aquatic Chronic 2 based on $1 < L(E)C_{50} \leq 10$ mg/l for a non-rapidly degradable substance. Using the available acute data for invertebrates, this would result in classification Aquatic Chronic 3 based on $10 < L(E)C_{50} \leq 100$ mg/l for a non-rapidly degradable substance.

Overall, the most stringent chronic classification should be applied which is Aquatic Chronic 2.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1; H400 - Very toxic to aquatic life

M-factor of 1

Aquatic Chronic 2; H411- Toxic to aquatic life with long lasting effects,

6 OTHER INFORMATION

No other information of relevance to the CLH discussion.

7 REFERECNES

References are taken from the Draft Assessment Report for Carboxin – March 2006 as follows

Volume 3; Annex B1 – Identity

Volume 3; Annex B2 – Physcial and chemical properties

Volume 3; Annex B6 – Toxicology and metabolism

Volume 3: Annex B.8 – Environmental Fate and Behaviour

Volume 3; Annex B.9 – Ecotoxicology

And the Addendum to the DAR dated August 2007

Also refer to
EFSA Journal 2010; 8(10):1857 - Conclusion on the peer review of the pesticide risk assessment of the active substance carboxin

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8 ANNEXES

None