# CLH report

# Proposal for Harmonised Classification and Labelling

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

# Substance Name: Carbetamide

**EC Number:** **240-286-6**

**CAS Number:** **16118-49-3**

**Index Number:** **-**

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# Part A.



# Part B.

# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

Table 1: Substance identity

| | |
|---|---|
| **Substance name:** | **Carbetamide** |
| **EC number:** | **240-286-6** |
| **CAS number:** | **16118-49-3** |
| **Annex VI Index number:** | **n.a.** |
| **Degree of purity:** | **Minimum 950 g/kg** |
| **Impurities:** | **None relevant impurities for classification** |

### 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** | None currently in Annex VI, Table 3.1 of the CLP Regulation |
| **Current proposal for consideration by RAC** | Acute Tox. 4 H302: Harmful if swallowed<br>Carc. 2 : H351: suspected of causing cancer<br>Repro 2: H361d: Suspected of damaging the unborn child<br>Aquatic chronic 2 : H411: Toxic to aquatic life with long lasting effects |
| **Resulting harmonised classification (future entry in Annex VI, CLP Regulation)** | Acute Tox. 4 H302;<br>Carc. 2 : H351;<br>Repro 2: H361d;<br>Aquatic chronic 2 : H411 |

## 1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

| 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 | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.2. | Flammable gases | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.3. | Flammable aerosols | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.4. | Oxidizing gases | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.5. | Gases under pressure | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.6. | Flammable liquids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.7. | Flammable solids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.8. | Self-reactive substances and mixtures | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.9. | Pyrophoric liquids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.10. | Pyrophoric solids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.11. | Self-heating substances and mixtures | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.12. | Substances and mixtures which in contact with water emit flammable gases | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.13. | Oxidizing liquids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.14. | Oxidizing solids | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.15. | Organic peroxides | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 2.16. | Substance and mixtures | n.a. | n.a. | Currently not | Conclusive but not sufficient |

| | | | | | |
|---|---|---|---|---|---|
| | corrosive to metals | | | classified | for classification |
| 3.1. | Acute toxicity - oral | **Acute Tox. 4 (H302): Harmful if swallowed** | n.a. | Currently not classified | Conclusive: based on the acute oral LD50 of 1445 mg/kg bw/d in female mouse |
| | Acute toxicity - dermal | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| | Acute toxicity - inhalation | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.2. | Skin corrosion / irritation | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.3. | Serious eye damage / eye irritation | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.4. | Respiratory sensitisation | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.4. | Skin sensitisation | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.5. | Germ cell mutagenicity | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.6. | Carcinogenicity | **Carc. 2 : H351: suspected of causing cancer** | n.a. | Currently not classified | Conclusive: based on several rare tumours occurring in different tissues (brain astrocytoma, liver cholangiocarcinoma and adrenal phaeochromocytoma) in mice and rats at the high dose (exceeding MTD.) |
| 3.7. | Reproductive toxicity | **Repr 2: H361d: Suspected of damaging the unborn child** | n.a. | | |
| 3.8. | Specific target organ toxicity –single exposure | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.9. | Specific target organ toxicity – repeated exposure | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 3.10. | Aspiration hazard | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |
| 4.1. | Hazardous to the aquatic environment | **Aquatic chronic 2 H411: Toxic to aquatic life with long lasting effects** | n.a. | Currently not classified | n.a. |

| | | | | | |
|---|---|---|---|---|---|
| 5.1. | Hazardous to the ozone layer | n.a. | n.a. | Currently not classified | Conclusive but not sufficient for classification |

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

## Labelling:

Signal word: Warning

Hazard pictogram: GHS08: health hazard

Hazard statements:
- H302: Harmful if swallowed
- H351: suspected of causing cancer
- H361d: Suspected of damaging the unborn child
- H411: Toxic to aquatic life with long lasting effects

Precautionary statements: -
- P201: Obtain special instructions before use.
- P202: Do not handle until all safety precautions have been read and understood.
- P281: Use personal protective equipment as required.
- P273: Avoid release to the environment
- P501: Dispose of contents/container in accordance with local/regional/national/international regulations.

**Proposed notes assigned to an entry:**

None

Table 4: Proposed classification according to DSD

| Hazardous property | Proposed classification | Proposed SCLs | Current classification [1)] | Reason for no classification [2)] |
|---|---|---|---|---|
| Classification according to DSD is not anymore necessary in accordance with document CA/58/2012 Rev11 presented at CARACAL 11 & 12. | | | | |

**1)** Including SCLs
**2)** Data lacking, inconclusive, or conclusive but not sufficient for classification

**BACKGROUND TO THE CLH PROPOSAL**

## 1.4 History of the previous classification and labelling

Carbetamide has no existing entry in Annex VI of Regulation 1272/2008 (CLP).

However, the proposal for classification was already discussed in the context of the possible inclusion of Carbetamide in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005, Addendum 1, September 2007, and Additional report, July 2010, RMS France) as well as the conclusion regarding the peer review of the pesticide risk assessment of the active substance Carbetamide (EFSA Journal 2010;8(12):1913). During this process, the manufacturer made some comments on FR proposals on developmental toxicity and carcinogenicity endpoints.

## 1.5 Short summary of the scientific justification for the CLH proposal

Based on the lowest acute toxicity estimate value in the most sensitive appropriate species tested (Oral LD50 for female mouse = 1445 mg/kg bw), classification for acute oral toxicity is proposed.

Several rare tumors including carcinomas were observed in different tissues (brain astrocytoma, liver cholangiosarcoma and adrenal phaeochromocytoma) in mice and rats. The incidence of these tumors is all above the available historical control ranges but they were observed only for the high dose of carbetamide exceeding the maximum tolerated dose (MTD). However, MSCA is of opinion that the possibility of a confounding effect is not justified. Because these lesions are very rare and do not occur in control animals, a substance-related effect cannot be totally excluded and classification for carcinogenicity is proposed (Carc. 2: H351: suspected of causing cancer).

In the developmental study in rats severe abnormalities including complex malformations (associating elongated genital tubercle, imperforate anus, vestigial/absent tail and cardiovascular malformations) were observed at dose levels with no marked maternal toxicity during the dosing period; a slight reduction in maternal body weight gain was observed after dosing (during late gestation) and it was considered unlikely to be the cause of the observed malformations. In the developmental study in rabbit teratogenicity such as skeletal abnormalities, delayed ossifications and post-implantation losses were observed at doses that caused minimal maternal toxicity (i.e. slight reduction in maternal body weight gain). Classification for reproductive toxicity is proposed (Repr 2: H361d: Suspected of damaging the unborn child).

In aquatic toxicity studies, the lowest $EC_{50}$ value was obtained for invertebrates at Carbetamide concentration of 81 mg/L. In aquatic toxicity studies, the lowest NOEC value was obtained for invertebrates at Carbetamide concentration of 1 mg/L. Carbetamide is not considered to have a potential for bioaccumulation (log $K_{OW}$ of 1.78 at pH = 7). Carbetamide cannot be considered as rapidly (less than 60% were consumed after 28 days in two ready biodegradability studies under test conditions). Environmental classification forAquatic Chronic 2 is proposed (H411: Toxic to aquatic life with long lasting effects).

## 1.6 Current harmonised classification and labelling

### 1.6.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No current entry in Annex VI in CLP Regulation.

### 1.6.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No current entry in Annex VI in CLP Regulation.

## 1.7 Current self-classification and labelling

### 1.7.1 Current self-classification and labelling based on the CLP Regulation criteria

**Current self-classification and labelling**

Signal word: warning, danger

Hazard class & statements:

Acute Tox. 4: H302: Harmful if swallowed
Acute Tox. 2: H300: Fatal if swallowed
Repro.2: H361d: Suspected of damaging the unborn child
Aquatic Chronic 2: H411: Toxic to aquatic life with long lasting effects

## 2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no need for justification for pesticides.

# Part B.

## SCIENTIFIC EVALUATION OF THE DATA

## 1 IDENTITY OF THE SUBSTANCE

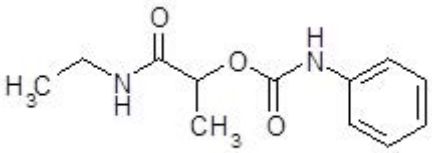
### 1.1 Name and other identifiers of the substance

Table 5: Substance identity

| | |
|---|---|
| EC NUMBER: | 240-286-6 |
| EC NAME: | - |
| CAS NUMBER (EC INVENTORY): | Not attributed |
| CAS NUMBER: | 16118-49-3 |
| CAS NAME: | (*2R*)-*N*-ethyl-2-[[(phenylamino)carbonyl]oxy]propanamide |
| IUPAC NAME: | (*2R*)-1-(ethylamino)-1-oxopropan-2-yl phenylcarbamate |
| CLP ANNEX VI INDEX NUMBER: | Not applicable |
| MOLECULAR FORMULA: | $C_{12}H_{16}N_2O_3$ |
| MOLECULAR WEIGHT RANGE: | 236.27 g/mol |
| SMILES | CCNC(=O)[C@@H](C)OC(=O)Nc1ccccc1 |

| | |
|---|---|
| INCHI | InChI=1/C12H16N2O3/c1-3-13-11(15)9(2)17-12(16)14-10-7-5-4-6-8-10/h4-9H,3H2,1-2H3,(H,13,15)(H,14,16)/t9-/m1/s1 |

**Structural formula:**



## 1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

| CONSTITUENT | MINIMUM PURITY |
|---|---|
| **CARBETAMIDE** | **950 G/KG** |

Current Annex VI entry: no entry

Table 7: Impurities (non-confidential information)

| IMPURITY | TYPICAL CONCENTRATION | CONCENTRATION RANGE | REMARKS |
|---|---|---|---|
| **NONE** | - | - | - |

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

| ADDITIVE | FUNCTION | TYPICAL CONCENTRATION | CONCENTRATION RANGE | REMARKS |
|---|---|---|---|---|
| **NONE** | - | - | - | - |

Current Annex VI entry: -

### 1.2.1 Composition of test material

Confidential data reported in IUCLID

## 1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

| Property | Method/ guideline | Material/ Batch | Results | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|---|---|
| Melting/freezing point | EEC A.1.<br>OECD 102 | Carbetamide<br>99.5%<br>Batch No. : 90422 | 108.7 - 110.6°C | Walter, D. (2002) | Measured |
| Boiling point | EEC A.2.<br>OECD 103<br>OECD 113 | Carbetamide<br>98.8%<br>Batch No. : 030749 | 235 - 240°C<br><br>No decomposition up to 400°C | Smeykal, H. (2005) | Measured |
| Relative density | EEC A.3. | Carbetamide techn.<br>97.2%<br>Batch No. : 20010412 | $\rho^{20\,2}{}_{4\,0}$=1.175 kg/L | Walter, D. (2002) | Measured |
| | EEC A.3. | Carbetamide<br>98.8%<br>Batch No. : 030749 | $\rho^{20\,0}{}_{4\,0}$=1.177 kg/L | Walter, D. (2005) | Measured |
| Vapour pressure | OECD 104 | Carbetamide<br>99.7%<br>Batch No. : EA635P3 | $3 \times 10^{-7}$ Pa at 20°C | Chabassol, Y., Giraud J.P. (1989) | Measured |
| | EEC A.4.<br>OECD 104 | Carbetamide -COOH<br>99.1%<br>Batch No. : RP 10810 | $6.2 \times 10^{-7}$ Pa at 20°C<br>$1.6 \times 10^{-6}$ Pa at 25°C<br>$1.2 \times 10^{-4}$ Pa at 50°C | Smeykal, H. (2008) | Measured |
| Surface tension | EEC A.5.<br>OECD 115 | Carbetamide techn. 97.2%<br>Batch No. : 20010412 | 68.6 mN/m at 20°C | Walter, D. (2001) | Measured |
| | EEC A.5.<br>OECD 115 | Carbetamide<br>98.8%<br>Batch No. : 030749 | 68.1 mN/m at 20°C | Walter, D. (2005) | Measured |
| Water solubility | EEC A.6. | Carbetamide<br>97.8%<br>Batch No. : 020134 | 3.67 g/L at 25°C (pH 6)<br>pH 5: 3.05 g/L at 23°C<br>pH 7: 3.27 g/L at 23°C<br>pH 9: 3.15 g/L at 23°C | Schneider, V. (2002) | Measured |

| | | | | | |
|---|---|---|---|---|---|
| | EEC A.6. OECD 105 | Carbetamide -COOH<br>99.0%<br>Batch No. : FC2407 | pH 2.56: 3.67 g/L at 20°C<br>pH 4.01: 21.1 g/L at 20°C<br>pH 7.58: 412 g/L at 20°C<br>pH 9.02: 409 g/L at 20°C<br><br>Solubility in water at pH 2.58<br>9.0°C: 2.02 g/L<br>19.6°C: 3.67 g/L<br>30.7°C: 5.25 g/L<br><br>Solubility in water at pH 9<br>9.0°C: 360 g/L<br>19.6°C: 409 g/L<br>30.7°C: 455 g/L | Bär, C. (2008) | Measured |
| Solubility in organic solvents | CIPAC MT 181 | Carbetamide techn. 97.2%<br>Batch No. : 20010412 | Solubility at 20°C:<br>Acetone: >250 g/L<br>Dichloroethane: > 250 g/L<br>Ethyl acetate: >250 g/L<br>n- Heptane: <10 g/L<br>Methanol: >250 g/L<br>p-Xylene: <10 g/L | Walter, D. (2001) | Measured |
| | EEC A.6. OECD 105 | Carbetamide techn. 97.0%<br>Batch No. : 020958 | n-heptane: 0.026 g/L at 20°C<br>p-xylene: 2.4 g/L at 20°C | Messerschmidt, S. (2006) | Measured |
| Partition coefficient n-octanol/water | HPLC method | Batch No. : 4488 | $P_{OW}$: 47.5<br>log $P_{OW}$: 1.67 | Courtier, M. & Caude M.-C. (1984) | Measured |
| | EEC A.8. OECD 107 | Carbetamide techn. 98.8%<br>Batch No. : 030749 | Water at 20°C:<br>pH 7.32: log $P_{OW}$: 1.75<br><br>Buffered at 20°C:<br>pH 4.19: log $P_{OW}$: 1.76<br>pH 7.13: log $P_{OW}$: 1.78<br>pH 9.98: log $P_{OW}$: 1.76 | Wilfinger, W. (2005) | Measured |
| Flash point | | - | - | - | Not relevant since Carbetamide is a solid. |
| Flammability | EEC A.10 | Carbetamide techn. 97.2%<br>Batch No. : 20010412 | Carbetamide is not highly flammable | Walter, D. (2001) | Measured |
| Explosive properties | Calculation method/ ASTM (2002) | - | Based on structural analysis no explosive properties can be assumed | Anonymous, (2000) | Calculated statement |

| | | | | | |
|---|---|---|---|---|---|
| | Calculation method/ ASTM (2002) | - | Based on structural analysis no explosive properties can be assumed | Tiemann, J. (2005) | Calculated statement |
| Self-ignition temperature | EEC A.16. | Carbetamide techn.<br>97.2%<br>Batch No. : 20010412 | No auto ignition occurred up to a temperature of 400°C | Smeykal, H. (2001) | Measured |
| Oxidizing properties | Calculation method/ ASTM (2002) | - | Based on structural analysis no oxidizing properties can be assumed | Anonymous, (2000) | Calculated statement |
| | Calculation method/ ASTM (2002) | - | Based on structural analysis no oxidising properties can be assumed | Tiemann, J. (2005) | Calculated statement |
| Dissociation constant | Potentio-metric titration | Carbetamide techn.<br>99.5%<br>Batch No.: EA635SD4 | No determination of a dissociation constant possible. | Cousin, J. (1993) | Measured |
| | Potentio-metric titration and spectropho-tometric method | Carbetamide techn.<br>99.5%<br>Batch No.: EA635SD4 | No dissociation observed. An approximate $pK_a$ was 11.3 at 20°C. | Cousin, J., & Valcarce, M. H. (1993) | Measured |
| | OECD 112 | Carbetamide -COOH<br>99.0%<br>Batch No.:<br>FC2407 | $pK_a$ 3.14 ± 0.03 at 20$^{o}$C | Bär, C. (2008) | Measured |
| Viscosity | - | - | - | - | Not relevant since Carbetamide is a solid. |

# 2 MANUFACTURE AND USES

## 2.1 Manufacture

Not relevant for Classification and Labelling

## 2.2 Identified uses

Carbetamide is a systemic herbicide used for the control of annual grasses and broad weeds.

# 3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

| **Property** | **Method** | **Results** | **Reference** | **Remarks** |
|---|---|---|---|---|
| Thermal stability | OECD 113/<br>DSC measurement | No decomposition up to 400°C | Smeykal, H. (2005) | Study acceptable |
| Flammability | EEC A.10 | Carbetamide is not highly flammable | Walter, D. (2001) | Study acceptable |
| Explosive properties | Calculation method/ ASTM (2002) | Based on structural analysis no explosive properties can be assumed | Tiemann, J. (2005) | Statement acceptable |
| Oxidizing properties | Calculation method/ ASTM (2002) | Based on structural analysis no oxidizing properties can be assumed | Tiemann, J. (2005) | Statement acceptable |

3.1. Summary and discussion of physico-chemical properties

See Section 1.3 (Table 8).

3.2. Comparison with classification criteria

The data presented in Section 1.3 (Table 8) and Table 10 show that Carbetamide does not meet the criteria for classification for physico-chemical hazards under CLP and this is not considered further in this proposal.

3.3. Conclusions on classification and labelling

Carbetamide has no properties with respect to flammability, explosive and oxidising properties that lead to a classification under CLP.

# 4 HUMAN HEALTH HAZARD ASSESSMENT

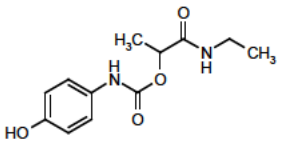
## 4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

### 4.1.1 Non-human information

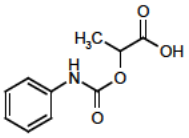
The absorption, distribution, metabolism and elimination of Carbetamide have been studied in male and female rats after single oral dosing with $^{14}C$ labelled Carbetamide at both, a low dose level of 100 mg/kg and a high dose level of 1000 mg/kg as well as after repeated dosing at 100 mg/kg for 14 days. The results obtained suggest a rapid absorption and quantitative elimination of Carbetamide (Laurent & Buys, 1973; Buys et al.; 1972, Buys et al., 1974; Leuschner, 2002)

When given orally to rats the herbicide Carbetamide is intensively metabolised mainly by ring- hydroxylation (in para position) and degradation of the side chain. Five major metabolites were identified. Besides these major metabolites some minor metabolites occurred, which could not be definitely identified. The identified metabolites are:

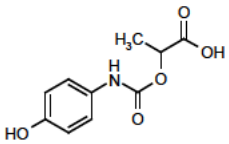
1) N-(4-hydroxyphenyl)-1-(ethylcarbamoyl-1)-ethyl-carbamate (29 835 R.P.)



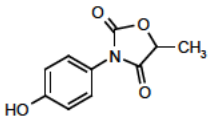
2) N-phenyl-1-(carboxy-1)-ethyl-carbamate (10 810 R.P.)



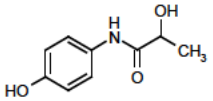
3) 2-(4-Hydroxy-phenylcarbamoyloxy)-propionic acid (29 896 R.P.)



4) 3-(4-hydroxy-phenyl)-5-methyl-oxazolidine-2,4-dione (12 913 R.P.)



5) 2-hydroxy-N-(4-hydroxy-phenyl)-propionamide (29 428 R.P.)



Rate and pattern of absorption, distribution, metabolism and excretion did not differ between high and low dose, nor did it after repeated dosing at 100 mg/kg. Carbetamide and its metabolite were completely excreted predominantly in the urine within 168 h after single high and low dose and after repeated dosing at 100 mg/kg for 14 days. Thus, no accumulation of Carbetamide in tissue occurs. The only significant radioactivity found in the organs was in the liver (Laurent & Buys, 1973; Buys et al., 1972; Buys et al., 1974; Leuschner, 2002).

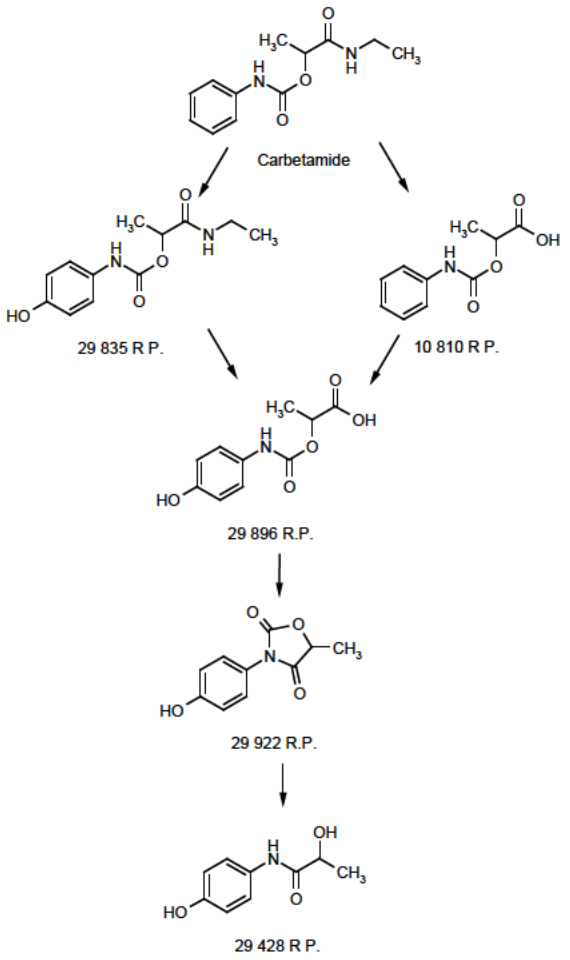



**Fig 1. Proposed scheme for Carbetamide metabolism in rats**

### 4.1.2 Human information

No data

### 4.1.3 Summary and discussion on toxicokinetics

The absorption, distribution, metabolism and elimination of Carbetamide have been studied in male and female rats after single oral and repeated oral dosing with $^{14}C$ labelled Carbetamide. Five major metabolites were identified in rats.

Carbetamide and its metabolite were completely excreted (> 99%) predominantly in the urine within 168 h after single high and low dose and after repeated dosing at 100 mg/kg for 14 days. Rate and pattern of absorption, distribution, metabolism and excretion did not differ between high and low dose, nor did after repeated dosing at 100 mg/kg. The only significant radioactivity found in the organs was in the liver.

## 4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

| Species | Sex | Route | $LD_{50}$ (mg/kg bw) | References |
|---|---|---|---|---|
| Rat | Male | Oral | > 2000 | Cummins, 1988a |
| Rat | Female | Oral | > 2000 | Cummins, 1988a |
| Mouse | Male | Oral | 2033 | Cummins and Gardner (1984) |
| Mouse | Female | Oral | 1445 | Cummins and Gardner (1984) |
| Rat | Male | Dermal | >2000 | Cummins, 1988b |
| Rat | Female | Dermal | >2000 | Cummins, 1988b |

### 4.2.1 Non-human information

No data

#### 4.2.1.1 Acute toxicity: oral

When rats received a single oral dose of 2000 mg Carbetamide /kg bw via stomach tube (highest achievable dose) two mortalities occurred on Day 1. Ante-mortem signs were confined to unconsciousness and breathing irregularities. In surviving animals, signs of reaction to treatment comprised lethargy, decreased motor activity, ataxia, piloerection and hunched posture. In some animals breathing irregularities, prone position and unconsciousness were observed. From day 2 until end of the observation period all surviving animals were overtly normal. Necropsy of the surviving animals on Day 15 revealed no significant macroscopic lesion (Cummins, 1988a).

Mice treated with a single oral doses of 1000, 1414, 2000 and 4000 mg/kg by gavage showed treatment-related signs of toxicity, such as bradypnoea, hyperpnoea, proneness and/or unconsciousness within 15-30 min after administration of Carbetamide. Deaths occurred at dosages of 1414 mg/kg or above up to Day 3 of the study. Ante-mortem signs were the above mentioned signs of toxicity and less frequent observations of lethargy, decreased motor activity, muscular tremor, apnoea and cyanosis. All surviving mice were overtly normal on Day 3 and throughout the remainder of the observation period. Necropsy of surviving animals at study termination revealed slight pulmonary congestion in several animals and less frequent observations of aerated serous fluid in the trachea and congestion of the submaxillary salivary lymph nodes (Cummins and Gardner, 1984).

The $LD_{50}$ of Carbetamide in mice after oral administration was found to be 2033 mg/kg b.w for males and 1445 mg/kg for females. Thus a sex difference is apparent.

#### 4.2.1.2 Acute toxicity: inhalation

No acute inhalation toxicity study has been submitted with the dossier. The vapour pressure of Carbetamide at 20°C is $3 \times 10^{-7}$ Pa and is, thus, far below the value of $1 \times 10^{-2}$ Pa, for which an inhalation study is required.

#### 4.2.1.3 Acute toxicity: dermal

All rabbits dermally exposed to 2000 mg Carbetamide/kg bw survived without signs of toxicity and gross necropsy revealed no treatment-related abnormalities. The percutaneous $LD_{50}$ of Carbetamide in rats was found to be greater than

2000 mg/kg bw under the condition of the study. Carbetamide does not warrant classification as being harmful or toxic on the basis of its acute percutaneous toxicity (Cummins, 1988b).

#### 4.2.1.4 Acute toxicity: other routes

No data

### 4.2.2 Human information

No data.

### 4.2.3 Summary and discussion of acute toxicity

Carbetamide is of low toxicity by the dermal route in rabbit.

Carbetamide exhibits low-to-moderate toxicity by the oral route as a single dose, depending on the species. Results of acute oral studies showed $LD_{50}$ higher than 2000 mg/kg bw in rats (males and females) and of 2,033 and 1,445 mg/kg bw in mice (males and females respectively).

According to the manufacturer, who submitted a CLH report proposal to the French authorities in december 2012, the data obtained for mice in the acute oral toxicity study should be considered as irrelevant and his arguments are described below:

« *In an experimental study on acute oral toxicity, mice were more sensitive than rats (Cummins and Gardner 1984, Cummins 1988a). Female mice showed an oral $LD_{50}$ of 1445 mg/kg, whereas the oral $LD_{50}$ for males was about 2000 mg/kg. However, these results are based on 5 animals per sex only. In a micronucleus test (August, 2006d) mice received oral doses of 2000 mg/kg bw with no mortality but only moderate signs of toxicity (reduced motility and ataxia). Further evidence for the lack of oral toxicity in mice was observed in chronic feeding studies mice were not more sensitive as compared to rats rather the rat proved to be the most sensitive species. In fact at the highest dose level of 9000 ppm daily intake exceeded 2000 mg/kg bw daily for two years for 50 mice per sex. There was no increase in mortality; mortality was even less as compared to the control group (Amyes et al., 1988b). Also in a recent mechanistic study groups of female mice received Carbetamide at dose levels of up to 9000 mg/kg bw/d for 14 days without any signs of toxicity (Bogaards & Grossouw, 2006).*

*Thus, the acute oral toxicity observed in the study by Cummins & Gardner (1984) is considered to be induced by the vehicle (Tween 80) which was administered at about 100 mg/kg bw and has been shown in the past to be notorious for these kind of effects. The overall database indicates that the data obtained for mice in the acute oral toxicity study are considered irrelevant and labelling of Carbetamide as harmful by the oral route is not warranted.* »

However, MSCA does not agree with the manufacturer's position to consider irrelevant the data obtained for mice in the acute oral toxicity study.

Indeed, this GLP study from Cummins and Gardner (1984) performed in accordance with the OECD Guideline 401, is considered as acceptable. Moreover, the batch of technical carbetamide (Batch DA 565, purity 96.3%) used in the mouse study was the same than the batch used for the rat study (Cummins 1988). Both studies were performed in the same laboratory at similar periods. In addition, both studies were reliable without restriction and considered as key studies. Taking into account that both studies were considered suitable in term of study quality and reliability and relevant to the substance in terms of technical specification, the classification must be based on the lowest acute toxicity estimate value in the most sensitive appropriate species tested.

According to the manufacturer, the acute oral toxicity observed in the mouse study was considered to be induced by the vehicle (Tween 80). MCSA do not support this argument. Tween 80 is a current well tolerated vehicle used for animal studies toxicology drug development (Shane Cox Gad, 2009). Mice were exposed to only 0.1g/kg bw of Tween 80 and the oral $LD_{50}$ of Tween 80 is equal to 25g/kg (Lewis, R.J. 2004). In some studies it has been shown that Tween 80 could increase the bioavailability of some poorly soluble compounds, but there is no evidence that it may increase in the same way the carbetamide bioavailability. Anyway, the carbetamide is already well absorbed after oral administration (> 80%). Thus, a possible artificial increase of oral bioavailability by the presence of Tween 80 (as well as the toxicity increased associated) will be limited.

In conclusion, the lowest acute toxicity estimate value in the most sensitive appropriate species tested is:

Oral $LD_{50}$ for female mouse = 1445 mg/kg bw (Cummings and Gardner 1984)

### 4.2.4 Comparison with criteria

The lowest acute toxicity estimate value identified in the most sensitive appropriate species tested is 1445 mg/kg bw (oral $LD_{50}$ for female mouse). This value lies within the range (300-2000 mg/kg) for classification as Acute Oral Tox. 4 (H302: Harmful if swallowed) under regulation (EC) 1272/2008.

The dermal $LD_{50}$ lies lies above the classification cut-off of 2000 mg/kg bw under regulation (EC) 1272/2008; therefore no classification is required.

### 4.2.5 Conclusions on classification and labelling

Based on the results of the acute oral toxicity studies, Carbetamide should be classified as : Acute Tox. 4 (H302) (Regulation (EC) 1272/2008).

No classification is proposed by dermal route.

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

None

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

Not required

### 4.3.2 Comparison with criteria

Not applicable

### 4.3.3 Conclusions on classification and labelling

No classification required.

## 4.4 Irritation

### 4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

| Species | Sex | Route | Effect | References |
|---|---|---|---|---|
| Rabbit | Male and female | Dermal | No skin irritation | Smith & Cummins, 1988a |

#### 4.4.1.1 Non-human information

The potential of Carbetamide to irritate skin was tested in New Zealand White albino rabbits. (Smith and Cummins, 1988a ).

- **Reference:**

**Smith KD, Cummins HA (1988):** Carbetamide: acute dermal irritation/corrosion test in the rabbit. Life Science Research, Ltd., Suffolk, UK - Unpublished report N° 88/0561 - Dates of work: 13/08/1988 to 05/10/1988.

- **Guidelines:** OECD N° 404 (1981); EPA pesticide Assessment Guidelines (1982).
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch DA 565, purity of 96.3%), was applied at the dose-level of 0.5g moistened with 0.2 mL of distilled water to the closely clipped dorsal region of 3 male and 3 female New Zealand White rabbits (approximately 3 months old, bw range 2.90-3.44 kg) under a gauze square. The test article was kept in contact with the skin for 4 hours.

Cutaneous observations were performed for erythema and oedema, at 1, 24, 48 and 72 hours after gauze removal.

- **Results**

No cutaneous irritation was observed at any test site during the observation period (see Table 13).

Table 13: Individual and mean skin irritation scores according to the Draize scheme

| Animal | 1. Erythema score | | | | | | Oedema score | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | 1 | 2. | 3 | 4 | 3. | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Sex | M | M | M | F | 4. | F | M | M | M | F | F | F |
| 1h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 72 h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Irritation Index | 0 | | | | | | 0 | | | | | |

- **Conclusion**

Under the conditions of the study, no local inflammatory reaction was observed after topical cutaneous application of carbetamide to the skin of rabbits for 4 hours under occlusion. Carbetamide was designated "non-irritating to the skin".

#### 4.4.1.2 Human information

No data

#### 4.4.1.3 Summary and discussion of skin irritation

A test on skin irritation with Carbetamide was negative. Carbetamide does not require any hazard classification.

#### 4.4.1.4 Comparison with criteria

A test on skin irritation with Carbetamide was negative.

#### 4.4.1.5 Conclusions on classification and labelling

Carbetamide does not require any hazard classification.

### 4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

| Species | Sex | Route | Effect | References |
|---|---|---|---|---|
| Rabbit | Male and female | Eye | No ocular irritation | Smith & Cummins, 1988b |

#### 4.4.2.1 Non-human information

An eye irritation study with Carbetamide was performed in New Zealand White albino rabbits. (Smith & Cummins, 1988b).

- **Reference:**

**Smith KD, Cummins HA (1988):** Carbetamide: acute eye irritation/corrosion test in the rabbit. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 88/0562 - Dates of work: 13/09/1988 to 05/10/1988.

- **Guidelines:** OECD N° 405 (1987); EPA pesticide Assessment Guidelines (1982).
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch DA 565, purity of 96.3%) was instilled at the dose-level of 0.1 g into the conjunctival sac of the right eye of six female New Zealand White rabbits (three months old, bw range 3.02-3.48 kg). The upper and lower lids were gently held together for 1 second to prevent loss of material. The other eye served as an untreated control.

After instillation of the test article the animals were observed for signs of clinical toxicity at least once daily. The treated eyes were observed and scored for ocular irritation at 1, 24, 48, and 72 hours after instillation.

- **Results**

All observations are reported in Table 15.

In all rabbits, congestion of the conjunctival blood vessels was observed during the first 48 hours following instillation of the test material. In one animal a transient redness of the conjunctiva was noted at the 24-hour examination. Slight chemosis was also observed in two rabbits at the one hour observation only.

The eyes of all rabbits were overtly normal at the 72-hour examination.

Table 15: Eye irritation score

| | Animal | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| **Conjunctival redness (max=3)** | | | | | | |
| After 1 hr | 1 | 1 | 1 | 1 | 1 | 1 |
| After 24 hr | 1 | 1 | 2 | 1 | 1 | 0 |
| After 48 hr | 1 | 0 | 1 | 1 | 1 | 0 |
| After 72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean score 24-72 hr | 0.66 | 0.33 | 1 | 0.66 | 0.66 | 0 |
| **Group mean score 24-72 hr** | **0.55** | | | | | |
| **Conjunctival chemosis (max=4)** | | | | | | |
| After 1 hr | 1 | 0 | 0 | 1 | 0 | 0 |
| After 24 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 48 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean score 24-72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| **Group mean score 24-72 hr** | **0** | | | | | |
| **Cornea opacity (max=4)** | | | | | | |
| After 1 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 24 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 48 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean score 24-72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| **Group mean score 24-72 hr** | **0** | | | | | |
| **Iridial inflammation (max=2)** | | | | | | |
| After 1 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 24 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 48 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| After 72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean score 24-72 hr | 0 | 0 | 0 | 0 | 0 | 0 |
| **Group mean score 24-72 hr** | **0** | | | | | |

- **Conclusion**

Under the conditions of the study, after application to the eye of New Zealand White rabbits at the dose-level of 0.1 g, only minimal conjunctival redness was observed during the first 48 hours after application, and slight chemosis in 2 animals one hour after dosing. Carbetamide was designated "non-irritating to the eye".

### 4.4.2.2 Human information

No data

### 4.4.2.3 Summary and discussion of eye irritation

A test on eye irritation with Carbetamide was negative. Carbetamide does not require any hazard classification.

### 4.4.2.4 Comparison with criteria

A test on eye irritation with Carbetamide was negative.

### 4.4.2.5 Conclusions on classification and labelling

Carbetamide does not require any hazard classification.

### 4.4.3 Respiratory tract irritation

No data and no indications for concern.

## 4.5 Corrosivity

A skin irritation test conducted in rabbits was negative. Carbetamide does not require classification for corrosivity.

## 4.6 Sensitisation

### 4.6.1 Skin sensitisation

Table 16: Summary table of relevant skin sensitisation studies

| Species | Sex | Route | Effect | References |
|---|---|---|---|---|
| Guinea-pig | Males and females | Intra- and epidermal | No skin sensitization | Cummins & Gardner, 1985 |
| Guinea-pig | Males | Intra- and epidermal | No skin sensitization | Haferkorn, 2008 |

#### 4.6.1.1 Non-human information

Two Maximisation test according to Magnusson and Kligman with guinea pigs was performed to study the sensitizing properties of Carbetamide Cummins & Gardner 1985; Haferkorn 2008).

- **Reference: Cummins HA, Gardner JR (1985):** carbetamide: delayed contact hypersensitivity study in guinea-pigs. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 85/RHA059/319 - Dates of work: 22/11/1984 to 04/07/1985.
- **Guidelines:** OECD N° 406 (1981).
- **GLP standards:** Yes.
- **Deviations:** No positive control.
- **Study acceptable:** Yes.
- **Test system:**

A sensitization study was conducted with technical carbetamide (batch 84 040 01, purity of 96.4%) on 20 treated (10/sex) and 20 controls (10/sex) Dunkin-Hartley guinea pigs (age unknown, bw range 314-446 g), using the Magnusson-Kligman maximisation method. The vehicles were aqueous methylcellulose (1% w/v) for intracutaneous injections and paraffin oil for topical applications.

The concentrations of carbetamide for intradermal induction (2% and 4% w/v in 1% w/v aqueous methylcellulose) were selected as inducing slight to moderate erythema at the injection sites in 2/4 animals 24 hours after injection in a preliminary primary irritation screening test. The second induction was performed on day 8 by application of the active substance at a concentration of 50% w/v in paraffin oil. As the active substance did not induce any primary irritation by topical application in the preliminary screening test, this induction application was preceded by a topical application of sodium lauryl sulfate on Day 7.

The challenge application was conducted at the concentration of 50% w/v of carbetamide in paraffin oil.

- **Results**

All observations are reported in Table 17.

The challenge test resulted in slight to moderate erythema of the vehicle application site and slight erythema of the test article application site in one control group male, and in slight erythema of the test article application site in one control group female. These responses were only apparent 24 hours after removal of the challenge test dressings. No dermal reactions were noted in the 48 hour scoring of the challenge period.

Table 17: Dermal responses to challenge test for each group

| Group | | 1 (control) | | 2 (test) | |
|---|---|---|---|---|---|
| Topical treatment | | Vehicle | Vehicle + test material | Vehicle | Vehicle + test material |
| 24 h examination | Incidence | 0.05 | 0.10 | 0 | 0 |
| | Severity | 0.10 | 0.05 | 0 | 0 |
| 48 h examination | Incidence | 0 | 0 | 0 | 0 |
| | Severity | 0 | 0 | 0 | 0 |

- **Conclusion**

Under the conditions of this maximisation test according to Magnusson and Kligman protocol, carbetamide does not exhibit any potential to induce delayed hypersensitivity in guinea-pigs.

- **Reference: Haferkorn, J. (2008):** Examination of carbetamide in the skin sensitisation test in guinea pigs according to Magnusson and Kligman (Maximisation test). LPT Lab. Of Pharm. And Tox. GmbH & CoKG, Hamburg, Germany, published: no, report N° 22838 (Dates of work: 14/05/2008 to 19/07/2008).
- **Guidelines:** OECD Guideline for the Testing of Chemicals N° 406: "Skin Sensitisation" (07/17/1992) / EC method B.6. Skin Senisitisation (96/54/EC)
- **GLP standards:** Yes.
- **Deviations:** none
- **Batch:** 020946
- **Purity:** 97.03%
- **Study acceptable:** Yes.

**Test system**
Male guinea pigs (strain Dunkin Hartley) with a body weight in the range of 377 - 436 g were used in this study. The guinea pigs were grouped into a test compound group (10 animals) and a control group (5 animals).

The test article (carbetamide, batch # 020946 with a purity of 97.03%) was administered intracutaneously to the shoulder region in stage 1 and 7 days later on Day 8 topically to the same shoulder region (stage 2) for 48 hours and, finally, in a challenge test on study day 21 to the flank region (stage 3) for 24 hours. Sesame oil served as the vehicle for the intradermal injection.

Based on the outcome of a preliminary study the following concentrations were chosen (table 18).

**Table 18**

| Stage | application | Concentration |
|---|---|---|
| I | Intracutaneous | 10% suspension of Carbetamide in vehicle |
| II | Topical | 50% suspension of Carbetamide in vehicle |
| III | Topical | 25% suspension of Carbetamide in vehicle |

Stage 1 (induction): The skin was shorn 24 hours before administration. For induction the test article was mixed in the vehicle with and without Freund´s complete adjuvants. Each animal received two intracutaneous injections each of

1. 0.1 mL Freund´s complete adjuvants
   (diluted 1:1 with physiological saline)
2. 0.1 mL Carbetamide in vehicle (10%)
3. 0.1 mL Carbetamide in vehicle (10%) in a 1+1 mixture (v/v) Freund´s complete adjuvants /physiological saline

The skin reaction was evaluated at 24 and 48 h after begin of the exposure

Stage 2 (induction): As a 50% suspension of Carbetamide in sesame oil was non-irritating to the non-depilated skin of the test animals in the preliminary experiment, the fur was shaved from the application area and the exposed skin was coated with 0.5 mL sodium laurylsulfate 10% in vaseline in order to induce a local irritation.
7 days after the intracutaneous injection, the shoulder region of the same animals was shaved again and treated topically using the patch-test technique (exposure time: 48 hours)

The skin reaction was evaluated at 48 and 72 h after begin of the exposure.

Stage 3 (challenge): Two weeks after the topical application (corresponds to a monitoring period of 21 days) the flanks of the same animals were shaved and depilated for a further topical application using the patch-test technique. The filter paper containing the test item was applied to the left flank, the filter paper with the vehicle to the right flank of the animal (exposure time: 24 hours). 21 hours after the filter paper had been removed, the treated skin was cleaned.

At 24h and 48h from the removal of the occlusive dressing (48h and 72h after the start of challenge application)the skin reaction was observed and recorded using a scoring from 0 to 3 according to Magnusson and Kligman.

- **Results**

A 10% suspension of Carbetamide in sesame oil chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema in all 10 animals 24 and 48 hours after administration. A 50% suspension of Carbetamide in sesame oil/animal chosen for the 2nd (topical) induction stage was non-irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with sodium laurylsulfate on the day before stage 2 induction in order to induce a local irritation. The challenge with 2 mL of a 25% suspension of Carbetamide in sesame oil/animal

revealed no skin irritation in any animal and, thus, the test item had no sensitising properties. The vehicle control revealed no skin reactions (Table 19).

The body weight gain of the animals treated with Carbetamide was within the range of the vehicle control during the experiment. Behaviour remained unchanged.

Table 19: Dermal responses to challenge test for each group

| Animal N | 1st stage | | 2nd stage | | 3rd stage | | | |
|---|---|---|---|---|---|---|---|---|
| | Hours after start of treatment | | | | | | | |
| | Shoulder | | Shoulder | | flank | | | |
| | 24 | 48 | 24 | 48 | 48 | | 72 | |
| | | | | | 1 | r | l | r |
| Group 1: Vehicle control | | | | | | | | |
| 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Group 2: carbetamide | | | | | | | | |
| 6 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 7 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 8 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 9 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 10 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 11 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 12 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 13 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 14 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 15 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Group3: Positive control (Benzocaine) | | | | | | | | |
| 1 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 2 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 3 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 4 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 5 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 6 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 7 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 8 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 9 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 10 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 11 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 12 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 13 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 14 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 15 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 0 |
| 16 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 17 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 18 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 19 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |
| 20 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 0 |

0 no visible change
1 discrete or patchy erythema
2 Moderate and confluent erythema
3 Intense erythema and swelling

l: left
r: right

- **Conclusion**

Under the conditions of this maximisation test according to Magnusson and Kligman, Carbetamide does not exhibit skin sensitisation properties in guinea-pigs

### 4.6.1.2 Human information

No incidences of allergic reaction reported.

#### 4.6.1.3 Summary and discussion of skin sensitisation

In two sensitisation tests using the Magnusson-Kligman maximisation method, carbetamide aqueous methylcellulose 1% w/v for intracutaneous injections or paraffin oil for topical applications does not exhibit any potential to induce delayed hypersensitivity in guinea-pigs.

Carbetamide does not require any hazard classification.

#### 4.6.1.4 Comparison with criteria

Tests on skin sensitization with Carbetamide did not lead to (more than) 30% positive responders and were thus negative.

#### 4.6.1.5 Conclusions on classification and labelling

Carbetamide does not require any hazard classification.

### 4.6.2 Respiratory sensitisation

No data and no indications for concern.

## 4.7 Repeated dose toxicity

The potential toxicity of Carbetmide by oral route has been investigated in rats and dogs up to 13 weeks. A single batch (number 84 040 01) with a purity of 96.4% was used for all these studies. No study was performed by inhalation. No study was conducted by dermal repeated application.

Table 20: Summary of repeated dose toxicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Rats<br>CD<br>(5/sex)<br>Carbetamide<br>5-week oral (dietary),<br><br>0, 2000, 4000, 8000, 16000, 32000 ppm<br>Correspond to 193, 378, 783, 1642, 3210 mg/kg bw/d in males and 201, 403, 851, 1646, 2886 mg/kg bw/d in females | <u>At 32000 ppm:</u><br>- ↓ MCV ↓ Hb and ↓ PCV<br>- ↑ AP ; ↑ Bilirubin<br><br><u>From 16000 ppm:</u><br>- ↓ plasmatic ACh-esterase : significant only in females<br>- ↑ cellular Ach-E : significant only in males<br><br><u>From 8000 ppm:</u><br>- ↓ BWG or transient BW loss correlating with ↓ food intake<br>- Centrilobular hepatocyte hypertrophy (♀)<br><br><u>From 4000 ppm:</u><br>- ↑ absolute and/or relative liver weight<br><br><u>From 2000 ppm:</u><br>- ↑ Platelet counts<br>- ↓ brain ACh-esterase : in all groups (Dose related)<br>- Centrilobular hepatocyte hypertrophy (♂)<br><br>**Targets: liver at all doses; inhibition of brain ACh-esterase**<br>**No NOAEL determined**<br>**LOAEL = 193 mg/kg/day** | **Guidelines:** Not stated; the study was roughly in accordance with OECD No. 407 (1995).<br><br>**GLP**<br><br>**Deviation**: Only 5 animals per sex per group; treatment duration of five weeks instead of four; haematological parameters measured at three weeks; not all organs were histologically examined.<br><br>**Purity**: 96.4% (batch 8404001) | West, 1984 |
| Rats<br>CD<br>(15/sex)<br>Carbetamide<br>13-week oral (dietary)<br><br>0, 160, 1600, 16000 ppm<br><br>Correspoding to mean dose-levels of 0, 12, 119 and 1365 mg/kg bw/d in males and 13, 137, 1480 mg/kg bw/d in females | <u>At 16000 ppm:</u><br>- ↓ BWG, BW and food intakes (both sex)<br>- ↓ RBC counts, PVC, Hb; ↑ platelet counts<br>- ↑ AP, ↓ AST, ALT<br>- Inhibition of ACh-esterase<br>↓ plasma Ach-E in females<br>↑ cellular Ach-E in females<br>- Hydrometra and uterus dilatations<br><br><u>From 1600 ppm:</u><br>- Centrilobular hepatocyte hypertrophy<br><br>**NOAEL = 12 mg/kg/day**<br>**LOAEL = 119 mg/kg bw/day [target organ = liver]** | **Guidelines:** Not stated, but the study was in accordance with OECD No. 408 (1981).<br><br>**GLP**<br><br>**Deviation**: none<br><br>**Purity**: 96.4% (batch 8404001) | West, 1985 |
| Dogs<br>Beagle<br>(1/sex)<br>Carbetamide<br>4-week oral (capsules)<br><br>0, 15, 30, 75, 150, and 300 mg/kg/day | <u>At 300 mg/kg bw/d:</u><br>- ↑ liver and thyroid weights<br><br><u>From 150 mg/kg bw/d:</u><br>- Transient unsteadiness of hind limbs and non-specific neurovegetative reactions<br>- ↓ AP, ALT, AST (both sex)<br>- ↓ PCV, Hb and RBC, and ↑ platelet counts (♀ only)<br>- ↓ urine volumes (♂)<br>- ↓ BWG correlated with ↓ food intakes<br>- Enlargement of the follicular epithelium in thyroids.<br>- Blood Ach-E : transient variations without dose-relationship<br><br>**NOAEL = 75 mg/kg/day**<br>**LOAEL= 150 mg/kg bw/d** | **Guidelines:** Not stated but in accordance with OECD N° 409 (1981), intended for 90-day oral studies.<br><br>**GLP**<br><br>**Deviation**: Four week study (no specific guideline). Only one animal per sex per dose-level instead of 4.<br><br>**Purity**: 96.4% (batch 8404001) | Danks, 1985a |
| Dogs<br>Beagle<br>(4/sex)<br>Carbetamide<br>13-week oral (capsules) | <u>At 300 mg/kg bw/day:</u><br>- Transient unsteadiness of hind limbs and non-specific neurovegetative reactions;<br>- ↑ platelet and neutrophil counts;<br>- ↑ RBC Ach-E | **Guidelines:** OECD N° 409 (1981).<br><br>**GLP** | Danks, 1985b |

| | | | |
|---|---|---|---|
| 0, 3, 30, 300 mg/kg/day | - ↑ liver and thyroid weights (no significant histological findings).<br><br>**NOAEL = 30 mg/kg/day**<br>**LOAEL = 300 mg/kg/day** | **Deviation**: none<br><br>**Purity**: 96.4% (batch 8404001) | |
| Dogs<br>Beagle<br>(6/sex)<br>Carbetamide<br>52-week oral (capsules)<br><br>3, 30 and 300 mg/kg/day | <u>At 300 mg/kg bw/day:</u><br>- ↓ BWG and food intake in ♀<br>- ↓ lower PCV, Hb and RBC, and higher platelet counts<br>- ↑ AP, ↓ ALT and AST<br>- ↑ liver and thyroid weights, and brown pigment in hepatocytes<br><br><u>From 30 mg/kg bw/day:</u><br>- Slight neuromuscular clinical signs (drowsiness, unsteadiness of the hind limbs, dry nose, prostration and muscle tremors); lower incidence and frequency observed at 30 mg/kg.<br>- Hemosiderin in Kupfer cells<br><br>**NOAEL = 3 mg/kg/day**<br>**LOAEL = 30 mg/kg/day** | **Guidelines:** Not stated; the study was roughly in accordance with OECD N° 409 (1981).<br><br>**GLP**<br><br>**Deviation**: none<br><br>**Purity**: 95.2% (batch 8404002) and 97.4% (batch 85345) | Danks, 1987 |

### 4.7.1 Non-human information

#### 4.7.1.1 Repeated dose toxicity: oral

- **Five-week oral toxicity study in rats**

- **Reference: West HA (1984):** carbetamide: preliminary toxicity study in dietary administration to CD rats. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 84/RH0034/395 - Dates of work: 02/10/1984 to 15/11/1984.
- **Guidelines:** Not stated; the study was roughly in accordance with OECD No. 407 (1995).
- **GLP standards:** Yes.
- **Deviations:** Only 5 animals per sex per group; treatment duration of five weeks instead of four; haematological parameters measured at three weeks; not all organs were histologically examined.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch 84 040 01, purity of 96.4%) was administered at the dietary dose-levels of 0, 2000, 4000, 8000, 16000 or 32000 ppm to six groups (numbered 1 to 6 respectively) of 5 male and 5 female CD rats (approximately 5 weeks old, bw range 133-163 g for males and 108-137 g for females) for 5 weeks.

Achieved dosages were calculated weekly. On day 28, the test material's plasma levels were measured on blood samples.

The animals were observed twice daily for clinical signs and mortalities. Body weights were recorded twice weekly. Every week, detailed observations involving handling and palpation were conducted and individual food consumption was recorded. Water consumption was assessed visually but not recorded. Ophthalmoscopy was performed on all rats on pre-test and before final necropsy.

Haematological and blood chemistry investigations were conducted after three weeks of treatment. Urinalyses were conducted during week 4. Blood and brain cholinesterase activity was determined at study termination in all rats.

All surviving rats were sacrificed after 5 weeks of treatment by carbon dioxide asphyxiation, weighed, and subjected to necropsy. Necropsy was also conducted on decedents. The weights of adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroids, and uterus were recorded. Designated tissues and organs (adrenals, thyroid and parathyroids, stomach, kidney, testes, brain) of all rats of groups 1, 5 and 6 at study termination and of decedents were histologically examined. Livers of all surviving rats from all groups were also histologically examined.

- **Results**

Homogeneity and stability of carbetamide in the diet under the use conditions were satisfying, as achieved dosages were within -10% and +10% of nominal values. The achieved test material concentrations (groups 2 to 6) were within -10% and +10% (extreme values: 93.1%-105%) of the nominal values of 2000, 4000, 8000, 16000 and 32000 ppm.

Results of achieved test material dose-levels (mg/kg/day) for each group and sex are summarized in Table B.6.3.1.1.-1.

On day 28, plasmatic concentrations of carbetamide were slightly less than linearly correlated with achieved dose-levels in mg/kg (see Table 21).

Table 21: Achieved test material dose-levels (mg/kg/day) and carbetamide plasmatic concentrations on day 28 (mg/kg/day), in rats given carbetamide for 28 days in the diet

| Group | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | 2,000 | | 4,000 | | 8,000 | | 16,000 | | 32,000 | |
| Sex | M | F | M | F | M | F | M | F | M | F |
| Mean for whole study (mg/kg/day) | 193 | 201 | 378 | 403 | 783 | 851 | 1642 | 1646 | 3210 | 2886 |
| Minimal weekly value (mg/kg/day) | 139 | 157 | 262 | 329 | 573 | 669 | 1292 | 1334 | 2540 | 2677 |
| Maximal weekly value (mg/kg/day) | 266 | 255 | 523 | 513 | 1019 | 1116 | 1951 | 2019 | 3940 | 3238 |
| Plasmatic concentration (µg/mL) | 3.8 | 4.1 | 5.0 | 6.6 | 14.2 | 14.7 | 23.0 | 18.9 | 27.4 | 36.4 |

No treatment-related death or premature sacrifice occurred during the study. Mortalities were limited to one male receiving 8000 ppm and one female receiving 32000 ppm, at routine blood sampling on week 4.

The only clinical sign was hair loss from the dorsal area in four males and one female, from the first week of the 32000 ppm diet treatment.

During the first week of treatment, a dose-related reduction in bodyweight gain or a body weight loss was observed in rats receiving 8000, 16000 or 32000 ppm. Thereafter, the body weight gains of all rats given 16000 or 32000 ppm and of females given 8000 ppm remained lower than that of controls.

A dose-related lower food consumption was observed in rats given 16000 or 32000 ppm, accounting for the reduction in body weight gain.

No treatment related change was observed in any rat at ophthalmologic examination at the end of week 3.

Relevant haematological findings are summarized in Table 22 and consisted in lower MCV in rats given 32000 ppm (-5% in males, -10% in females), accounting for lower PCV (-8% in males, -13% in females), and Hb (-6% in males, -11% in females) compared to controls. Platelet counts of each group were higher than that of controls, without any dose-relation. Although this change was attributed in the report to low control platelet counts, it was observed in later 13-week study and therefore should be considered as treatment-related.

Relevant blood biochemical findings are summarized in Table 22 and consisted, mostly in the highest dose group compared to controls, in higher AP (+23% in males, +42% in females, significant in females only), lower urea (-36 % significant in females only) and lower glucose attributable to low food intake, and higher total bilirubin (+200% in males, +100% in females) which together with AP changes suggests a moderate cholestasis.

In the 2 highest dose groups, plasmatic levels of butyrylCh-esterase and ACh-esterase were lower (significant in females only) whereas cellular levels of ACh-esterase were higher (significant in males only) compared to controls. ACh-esterase concentrations were dose-relatedly lower in the brain (significant in males only), for all groups compared to controls (see Table 22). Urine of the three highest dose groups was darkened.

Relevant organ weight changes are summarized in Table 22 and consisted in higher liver weight (absolute in males of group 4 and females of group 5, relative in group 3 to 6). Other changes in absolute and/or relative organ weights were observed, but were related to the differences in the animal body weights, and were not correlated with any histological lesions.

Histological findings were limited to the liver and consisted in centrolobular (periacinar) hypertrophy of the hepatocytes in all surviving males at all doses and in 3/5 females given carbetamide at the concentration of 4000 ppm and all surviving females at higher dose-levels.

Table 22: Haematological, biochemical (blood, brain) changes in rats given carbetamide in the diet for 28 days

| Group | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | 0 | | 2000 | | 4000 | | 8000 | | 16000 | | 32000 | |
| Sex | M | F | M | F | M | F | M | F | M | F | M | F |
| PCV (%) (a) | 48 | 47 | 47 | 47 | 48 | 46 | 46* | 46 | 48 | 46 | 44* | 41* |
| Hb (g/100mL) (a) | 16.2 | 15.7 | 15.9 | 15.8 | 16.4 | 15.6 | 15.4 * | 15.4 | 16.3 | 15.5 | 15.2 * | 14.0 * |
| MCV ($\mu m^3$) (a) | 65 | 67 | 67 | 67 | 67 | 66 | 67 | 66 | 66 | 67 | 62* | 61* |
| Platelet count ($10^3/cm^3$) (a) | 668 | 761 | 818* | 747 | 751* | 802 | 920* | 784 | 805* | 846 | 856* | 844 |
| AP (IU/L)(b) | 160 | 107 | 158 | 116 | 160 | 86 | 153 | 134 | 188 | 112 | 196 | 152* |
| Urea (mg/100mL) (b) | 32 | 39 | 42 | 35 | 39 | 37 | 38 | 29* | 30 | 26* | 21 | 25* |
| Glucose (mg/100mL) (b) | 114 | 113 | 120 | 92 | 120 | 100 | 122 | 91 | 98 | 98 | 60* | 89* |
| Total bilirubin (mg/100mL) (b) | 0.3 | 0.3 | 0.3 | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.9* | 0.6* |
| Plasma butyrylCh-esterase (IU/L) (b) | 413 | 2320 | 325 | 2052 | 311 | 2306 | 405 | 1989 | 295* | 1269 * | 335 | 564* |
| Plasma ACh-esterase (IU/L) (b) | 603 | 2017 | 478 | 1841 | 468* | 2063 | 548 | 1815 | 520 | 1235 * | 563 | 774* |
| RBC ACh-esterase (IU/L) (b) | 913 | 928 | 989 | 844 | 943 | 920 | 1056 * | 966 | 1126 * | 996 | 1111 * | 970 |
| Brain ACh-esterase (IU/kg) (b) | 3200 | 10800 | 12200* | 10900 | 12000* | 9700* | 11600* | 9800 | 10900* | 9500* | 11500* | 9700 |

(a): measured after 3 weeks of treatment; (b): measured after 5 weeks of treatment.
*: significantly different from controls ($p<0.05$).

- **Conclusion**

After oral administration in the diet at the concentrations of 0, 2000, 4000, 8000, 16000, and 32000 ppm (corresponding to mean dose-levels of 0, 197, 390, 817, 1644 and 3048 mg/kg/day) to CD rats for 5 weeks, carbetamide induced:

- a lower body weight gain or transient body weight loss in males and females at the two highest dose-levels and in females at the concentration of 8000 ppm, correlating with a lower food intake,
- a lower MCV accounting for a lower Hb and PCV at the highest dose-level; higher platelet counts in all treated groups,
- a higher AP and total bilirubin in animals at the highest dose-level,
- lower plasmatic levels of butyrylCh-esterase and ACh-esterase and higher blood cellular levels of ACh-esterase at the two highest dose-levels. ACh-esterase concentrations were dose-relatedly lower in the brain, for all test groups compared to controls,
- a higher absolute and/or relative liver weight at the dose-levels of 4000 ppm and higher,
- a centrolobular hepatocyte hypertrophy in males at all dose-levels and in females at the dose levels of 4000 ppm and higher.

The liver was identified as the main target for carbetamide. Hepatocyte enlargement was observed in all dose groups, including the lowest tested dose-level of 2000 ppm. Therefore, a no-effect-level could not be established.

Inhibition of brain cholinesterase activity observed at 16000 and 32000 ppm was considered to be of biological significance.

- **Four-week oral toxicity study in dogs**

- **Reference:Danks A (1985):** carbetamide: 4-week preliminary toxicity study in oral administration to beagle dogs. Life Science Research, Ltd., Suffolk, UK - Unpublished report N° 84/RH0035/670 - Dates of work: 27/12/1984 to 27/02/1985.
- **Guidelines:** Not stated but in accordance with OECD N° 409 (1981), intended for 90-day oral studies.
- **GLP standards:** Yes.
- **Study acceptable:** No.
- **Deviations:** Four week study (no specific guideline). Only one animal per sex per dose-level instead of 4.
- **Test system:**

Technical carbetamide (batch 84 040 01, purity of 96.4%) was administered daily by oral route (capsules) at the dose-levels of 0, 15, 30, 75, 150 and 300 mg/kg/day to six groups (numbered 1 to 6 respectively) of 1 male and 1 female

beagle dogs (approximately 18-20 weeks old, bw range 6.5 to 8.6 kg for males and 6.2 to 8.4 kg for females) for 28 days.

Every day, before and after capsule administration, each animal was observed for signs of toxicity and mortality. Pens were examined daily. Individual food consumption was recorded daily and body weight was recorded twice weekly and before necropsy. Water intake was assessed visually but not recorded. Complete veterinary examinations and ophthalmoscopy were performed before study commencement and after 21 days of treatment.

Before study commencement and after 23 days of treatment, haematology, blood chemistry and urinalysis investigations were conducted. Blood cholinesterase activity was measured before study commencement, on treatment days 9 and 23, and before necropsy (except for the control female before necropsy). Brain cholinesterase activity was determined at study termination.

The test material's plasmatic concentrations were quantified using a validated HPLC/UV method on samples taken from each animal, 30 minutes and 1, 2, 4 and 24 hours after the first dosing.

All surviving dogs were sacrificed on completion of the study and subjected to complete necropsy. At this occasion, any abnormality was recorded, and the corresponding tissue/organ weighed and preserved. Selected organs (adrenals, brain, liver, kidneys, heart, lungs, pituitary, prostate, ovaries, spleen, thyroid with parathyroids, testes, and uterus) were weighed and selected tissue/organ samples (adrenals, stomach, brain, kidneys, liver, testes, urinary bladder, thyroid with parathyroids and any abnormality) were histologically examined.

- **Results**

Plasmatic concentrations of carbetamide are summarized in Table 23.

Carbetamide was rapidly absorbed after administration at all doses, Tmax ranging 0.5-4 hours. Plasma concentrations increased with dose-levels. At the dose-levels of 15, 30 and 75 mg/kg/day, they were similar between sexes, but were lower in females compared to males given 150 and 300 mg/kg/day. carbetamide was detected at low levels (0.1 to 0.41 µg/ml) at 24 hours. Metabolite 29835 RP plasma levels increased with dose and were generally lower than that of carbetamide except at 24 hours.

Table 23: Test material dose-levels (mg/kg/day) and test material plasmatic concentrations on first dosing (µg/ml), for each group and sex

| Group | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Target dose-level (mg/kg/day) | | 0 | | 15 | | 30 | | 75 | | 150 | | 300 | |
| Sex | | M | F | M | F | M | F | M | F | M | F | M | F |
| carbetamide | Cmax (µg/mL) | 0 | 0 | 9.2 | 1.1 | 12.5 | 12 | 21 | 33 | 65 | 25 | 71 | 66 |
| | Tmax (hours) | 0 | 0 | 0.5 | 1 | 0.5 | 0.5 | 2 | 1 | 1 | 2 | 2 | 2 |
| Metabolite* | Cmax (µg/mL) | 0 | 0 | 1.2 | 1.2 | 2.3 | 7.4 | 6.8 | 4.3 | 5.4 | 4.6 | 5.4 | 7.3 |
| | Tmax (hours) | 0 | 0 | 0.5 | 2 | 1 | 1 | 4 | 2 | 2 | 4 | 4 | 4 |

* carbetamide metabolite: 29835 RP

No death occurred during the treatment.

Clinical signs included dry nose and unsteadiness of the hind limbs in animals receiving 150 or 300 mg/kg/day, and neurovegetative reactions (salivation, vomiting and tremors) on some occasions during the first 2 weeks in animals given 300 mg/kg/day. A yellow staining of the pens was observed.

The female given 300 mg/kg/day lost weight from days 0 to 10, after which bw gain was normalized but did not compensate for initial loss. Compared to controls, mean body weight gain over the 28 day period was inferior in the male given 300 mg/kg/day (-30% bw gain) and in the females given 300 (bw loss) or 150 mg/kg/day (-43% bw gain). Food intake was much lower for the highest dosed females during the first 3 weeks (compared to controls and to pre-test values for the same animals). Total food intake values over the 28 day period in animals dosed at 300 or 150 mg/kg/day were lower than that of controls (females: -48% and -30%; males: -13% and -16%, respectively).

Table 24: Body weight gains of dogs given carbetamide by the oral route daily for 28 days

| Group | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|

| Dose-levels (mg/kg/day) | 0 | | 15 | | 30 | | 75 | | 150 | | 300 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F | M | F | M | F | M | F |
| Body weight gain over 28 days (kg) | 1.0 | 1.4 | 1.8 | 1.3 | 1.3 | 1.4 | 1.5 | 1.2 | 1.0 | 0.8 | 0.7 | -0.1 |

There were no treatment-related sign at ophthalmoscopy.

Haematological examinations showed lower PCV, Hb and RBC, and higher platelet counts in the female given 300 mg/kg/day, compared to control values (-26%, -22%, -27% and +36% respectively) and to pre-test values for the same animal (-11%, -15%, -16% and +71% respectively). The same trend was observed but with lower amplitude for the female treated at 150 mg/kg/day (all variations within $\pm$10% except for platelet counts: +29% of control values) (Table 25).

Biochemical examinations showed lower AP, ALT and AST in the male given 300 mg/kg/day, compared to control values (-40%, -62% and -46% respectively) and to pre-test values for the same animal (-30%, -15% and -12% respectively). The same trend was observed with lower amplitude in males given 150 mg/kg/day (-23, -31 and -38% of pre-test values respectively). Lower AP, ALT and AST were also observed in the female given 300 mg/kg/day, compared to control values (-30%, -47% and -42% respectively) and to pre-test values for the same animal (-33%, -31% and -28% respectively). The same trend was observed for ALT and AST, only relatively to control values on day 23, for the female treated at 150 mg/kg/day (-34% and -35% respectively) (Table 25).

On day 27, males receiving 150 or 300 mg/kg/day produced lower urine volumes, compared to controls (-73% for both dose-levels) and to their pre-test values (-38% and -45% respectively). Higher urine specific gravity was observed in treated animals with a dose-relationship.

Variations in plasmatic or RBC cholinesterase activities were either transient or non dose-related and were therefore not considered to be treatment-related. Differences in brain ACh- and butyrylCh-esterase activities were slight and not dose-related and therefore considered as not treatment related.

Table 25: Haematological and biochemical changes in dogs given carbetamide by the oral route daily for 23 days

| Group | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 15 | | 30 | | 75 | | 150 | | 300 | |
| Sex | M | F | M | F | M | F | M | F | M | F | M | F |
| PCV (%) | 39 | 43 | 37 | 38 | 38 | 43 | 44 | 37 | 36 | 39 | 36 | 32 |
| Hb (g/100mL) | 13.5 | 14.4 | 13.1 | 13.5 | 13.0 | 14.6 | 15.2 | 13.1 | 12.4 | 12.9 | 12.3 | 11.2 |
| RBC ($10^6$/cm$^3$) | 5.62 | 6.21 | 5.31 | 6.04 | 5.74 | 6.26 | 6.29 | 5.29 | 5.17 | 5.52 | 5.37 | 4.51 |
| Platelet count ($10^3$/cm$^3$) | 276 | 192 | 241 | 212 | 294 | 263 | 147 | 185 | 272 | 198 | 287 | 262 |
| AP (IU/L) | 102 | 111 | 126 | 86 | 98 | 112 | 127 | 109 | 74 | 115 | 61 | 78 |
| ALT (IU/L) | 58 | 38 | 52 | 29 | 37 | 50 | 20 | 22 | 36 | 25 | 22 | 20 |
| AST (IU/L) | 28 | 31 | 23 | 24 | 18 | 23 | 17 | 26 | 13 | 20 | 15 | 18 |

Liver and thyroid relative weights were higher in males (+16% and +9%) and females (+28% and +66%) given 300 mg/kg/day (Table 26).

At the two highest dose-levels, all the thyroids (bilateral in each sex) presented an enlargement of the follicular epithelium, when examined microscopically. Testicular lesions were observed at every dose-level from 30 to 300 mg/kg/day (presence of sperm giant cells, dilated tubules, vacuolated germinal epithelia and protein accumulation). Their relation with treatment was not certain because the animals were pre- or pubertals, and the absence of these findings at the lowest dose-level and in the control male could be linked to the fact that these two males were less mature than the other ones.

Table 26: Organ weight changes in dogs given carbetamide by the oral route daily for 28 days

| Group | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 15 | | 30 | | 75 | | 150 | | 300 | |
| Sex | M | F | M | F | M | F | M | F | M | F | M | F |
| Liver | | | | | | | | | | | | |
| Absolute (g) | 239 | 323 | 303 | 298 | 309 | 294 | 325 | 343 | 325 | 281 | 345 | 302 |
| Relative (%) | 3.2 | 3.2 | 3.4 | 3.1 | 3.2 | 3.4 | 3.2 | 3.6 | 3.5 | 4.0 | 3.7 | 4.1 |
| Thyroid with parathyroids | | | | | | | | | | | | |
| Absolute (g) | 0.86 | 0.75 | 1.04 | 0.63 | 0.69 | 0.62 | 1.03 | 0.89 | 1.10 | 0.89 | 1.18 | 0.91 |
| Relative (%) x1000 | 11.6 | 7.4 | 11.6 | 6.6 | 7.0 | 7.2 | 10.1 | 9.5 | 11.8 | 12.5 | 12.7 | 12.3 |

- **Conclusion**

After oral administration by gavage at the dose-levels of 0, 15, 30, 75, 150 and 300 mg/kg/day to beagle dogs for 4 weeks, carbetamide induced:

- clinical signs limited to dry nose, unsteadiness of the hind limbs and transient, non-specific neurovegetative reactions in animals receiving 150 or 300 mg/kg/day,
- a weight loss in the female given 300 mg/kg/day, and lower bw gains over 4 weeks in dogs treated at dose-levels of 150 or 300 mg/kg/day, these observations being correlated with lower food intakes,
- lower PCV, Hb and RBC, and higher platelet counts in females given 150 or 300 mg/kg/day,
- lower AP, ALT and AST in both sexes treated at 150 or 300 mg/kg/day,
- lower urine volumes in males given 150 or 300 mg/kg/day,
- higher relative liver and thyroid weights in both sexes and especially in females, at the dose-level of 300 mg/kg/day,
- an enlargement of the follicular epithelium in all the thyroids of dogs treated at 150 or 300 mg/kg/day.

All these observations draw to a NOAEL of 75 mg/kg/day, considering the microscopic findings in testes to be due to differences in pubertal stages between dog groups.

- **Thirteen-week oral toxicity study in rats**

- **Reference: West HA (1985):** carbetamide: 13-week toxicity study in dietary administration to CD rats. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 85/RH0031/139 -Dates of work: 12/06/1985 to 25/09/1985.
- **Guidelines:** Not stated, but the study was in accordance with OECD No. 408 (1981).
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch 84 040 01, purity of 96.4%) was administered at the dietary dose-levels of 0, 160, 1600 or 16000 ppm to four groups (numbered 1 to 4 respectively) of 15 male and 15 female CD rats (approximately 5 weeks old, bw range 114-145 g for males and 106-122 g for females) for at least 13 weeks.

Achieved dosages were calculated weekly. The animals were observed twice daily for clinical signs and mortalities. Body weights were recorded on day 1 (first day of treatment), weekly thereafter and before terminal necropsy. Every week, detailed observations involving handling and palpation were conducted and individual food consumption was recorded. Water consumption was assessed visually but not recorded.

Ophthalmoscopy was performed on all rats on pre-test, and on all rats of groups 1 (controls) and 4 (highest dose) after 12 weeks of treatment.

At treatment weeks 6 and 12, haematological investigations, blood chemistry investigations including cholinesterase activity, and urinalyses were conducted. Brain cholinesterase activity was determined at study termination in all rats.

All surviving rats were sacrificed after 13 weeks of treatment by carbon dioxide asphyxiation, weighed, and subjected to necropsy. Necropsy was also conducted on decedents. The weights of adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroids, and uterus were recorded. Designated tissues and organs (adrenals, aorta, epididymides, femoral bone and marrow, heart, skin, eye, lung, caudal mammary

glands, trachea, oesophagus, thyroid and parathyroids, stomach, duodenum, thymus, jejunum, cervical and mesenteric lymph nodes, ileum, caecum, colon, salivary gland, liver, spleen, kidneys, urinary bladder, prostate, rectum, testes, uterus with cervix, ovaries, pancreas, pituitary, brain, optic and sciatic nerve) of all rats from all groups were histologically examined.

- **Results**

Homogeneity and stability of carbetamide in the diet under the use conditions were satisfying, as achieved dosages were within -10% and +10% of nominal values. The achieved test material concentrations (groups 2 to 4) were within -10% and +10% (extreme values: 92.5%-107.5% on week 13) of the nominal values of 160, 1600 and 16000 ppm.

Results of achieved test material dose-levels (mg/kg/day) for each group and sex are summarized in Table 27. They were well correlated with dietary concentrations.

Table 27: Achieved test material dose-levels (mg/kg/day) in rats given carbetamide in the diet for 13 weeks

| Group | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|
| Concentration (ppm) | 160 | | 1600 | | 16000 | |
| Sex | M | F | M | F | M | F |
| Mean for whole study (mg/kg/day) | 12 | 13 | 119 | 137 | 1365 | 1480 |
| Minimal weekly value (mg/kg/day) | 7 | 9 | 75 | 90 | 946 | 981 |
| Maximal weekly value (mg/kg/day) | 22 | 21 | 231 | 223 | 2026 | 2092 |

No treatment-related death or premature sacrifice occurred during the study. Mortalities were limited to two females receiving 160 ppm and one male receiving 1600 ppm, at routine blood sampling on week 6. One male receiving the intermediate concentration of 1600 ppm was prematurely sacrificed on week 9, without specific clinical sign and lesion which could be attributed to treatment.

There was no treatment-related clinical sign in any group.

On almost every week during the study, bodyweight and bw gain were significantly lower in males and females receiving 16000 ppm, compared to controls (weight change values from weeks 0 to 13 in group 4: -39% and -34% of control values respectively in males and females).

On almost every week during the study, food consumption was moderately lower in males and females receiving 16000 ppm, compared to controls (mean values for weeks 1 to 13 in group 4: -9% and -6% of control values respectively in males and females).

No treatment related change was observed in any of the rats examined at ophthalmologic examination at the end of week 12 (groups 1 and 4).

Relevant haematological findings are summarized in Table 28 and consisted in lower RBC counts in rats given 16000 ppm (-8% in males, -12% in females), accounting for lower PCV (-8% in males, -10% in females) and Hb (-5% in males, -8% in females) compared to controls on week 12. Platelet counts of rats given 16000 ppm were higher than that of controls on week 12 (+25% in males, +27% in females), contrary to groups 2 and 3.

Relevant blood biochemical findings on week 12 are summarized in Table 28 and consisted, in males of the highest dose group compared to controls, in higher AP (+27%), and lower AST (-21%), ALT (-17%) and glucose levels (-17%). Females of this group only had lower blood glucose levels (-15%). These observations suggest lower protein synthesis and glucose balance linked with lower food intake. Higher concentrations of β-globulins (and, thus, total proteins), bilirubin, calcium and potassium, and lower urea were noted, but they were biologically not significant and often observed at a single sampling and/or in only one sex, and therefore considered to be not treatment related.

In the highest dose group females, plasmatic levels of butyrylCh-esterase and ACh-esterase on week 12 were lower (-41% and -35% respectively), whereas cellular levels of ACh-esterase were higher (+14%) compared to controls. Brain ACh-esterase concentrations were lower in both sexes (males -7%, females -8%) in all 3 treated groups than in controls, without any dose-relation (see Table 28).

In the highest dose group, urine concentration showed changes of opposite and moderate trends between sexes when compared to controls. These trends were correlated with inverse variations in urine volume and were not considered biologically significant nor treatment related.

Table 28: Haematological, and biochemical (blood and brain) changes in rats given carbetamide for 13 weeks in the diet

| Group | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | | 0 | | 160 | | 1600 | | 16000 | |
| Sex | | M | F | M | F | M | F | M | F |
| Haematology (a) | PCV (%) | 51 | 51 | 51 | 50 | 50 | 50 | 47* | 46* |
| | Hb (g/100mL) | 16.4 | 16.1 | 16.3 | 16.1 | 16.4 | 16.0 | 15.5* | 14.8* |
| | RBC ($10^6$/$cm^3$) | 8.49 | 8.06 | 8.5 | 7.97 | 8.47 | 7.89 | 7.83* | 7.06* |
| | Platelet count ($10^3$/$cm^3$) | 794 | 768 | 739 | 763 | 769 | 712 | 993* | 975* |
| Blood biochemistry (a) | AP (IU/L) | 75 | 54 | 73 | 46 | 81 | 48 | 95* | 52 |
| | AST (IU/L) | 80 | 70 | 88 | 70 | 82 | 74 | 63* | 68 |
| | ALT (IU/L) | 35 | 26 | 39 | 29 | 36 | 30 | 29* | 27 |
| | Glucose (mg/100mL) | 126 | 125 | 126 | 122 | 117* | 118 | 104* | 106* |
| | Plasma butyrylCh-esterase (IU/L) | 235 | 4143 | 301 | 4991* | 351* | 3684 | 240 | 2439* |
| | Plasma ACh-esterase (IU/L) | 324 | 2019 | 348 | 2455* | 348 | 1963 | 294 | 1307* |
| | RBC ACh-esterase (IU/L) | 1502 | 1518 | 1537 | 1450 | 1347 | 1442 | 1815* | 1731* |
| Brain biochemistry (b) | ACh-esterase (IU/kg) | 8700 | 8800 | 8200 | 8300 | 8300 | 8100 | 8100 | 8100 |

(a): measured after 12 weeks of treatment; (b): measured after 13 weeks of treatment
*: significantly different from controls ($p<0.05$).

Relevant organ weight changes in comparison to controls are summarized in Table 29 and consisted in higher liver absolute and/or relative weights (+30% and +35% relative weights in males and females respectively) and uterus absolute and relative weights (+30% and +60% respectively) in group 4 compared to controls. Other changes in absolute and/or relative organ weights were observed, but were related to the differences in the animal body weights, were not correlated with any histological lesions, and therefore considered to be not treatment related.

Table 29: Changes in organ weights of rats given carbetamide for 13 weeks in the diet

| Group | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | | 0 | | 160 | | 1600 | | 16000 | |
| Sex | | M | F | M | F | M | F | M | F |
| Liver | Absolute weight (g) | 18.8 | 10.3 | 19.0 | 10.3 | 19.6 | 10.5 | 17.9 | 11.3* |
| | Relative weight (%) | 3.71 | 3.56 | 3.59 | 3.58 | 3.86 | 3.60 | 4.82* | 4.82* |
| Uterus | Absolute weight (g) | - | 0.61 | - | 0.68 | - | 0.56 | - | 0.79 |
| | Relative weight (%) | - | 0.21 | - | 0.24 | - | 0.20 | - | 0.34* |

*: significantly different from controls ($p<0.05$)

Histological findings were essentially observed in group 4 and were systematic periacinar (centrolobular) hepatocytic enlargement (100% of both sexes in group 4), hydrometra (47% of group 4 females) and other luminal uterus dilatations (total: 60% of group 4 females). Other non dose-related lesions were observed, similar to those commonly found in animals of this strain and age.

Table 30: Histological lesions observed in rats given carbetamide for 13 weeks in the diet

| Group | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | | 0 | | 160 | | 1600 | | 16000 | |
| Sex | | M | F | M | F | M | F | M | F |
| Liver | Periacinar hepatocytic enlargement | 0/15 | 0/15 | 0/13 | 0/15 | 2/15 | 0/13 | 15/15 | 15/15 |
| Uterus | Hydrometra | - | 2/15 | - | 2/13 | - | 2/15 | - | 7/15 |
| | Other luminal dilatation | - | 0/15 | - | 0/15 | - | 0/15 | - | 2/15 |

- **Conclusion**

After oral administration in the diet at the concentrations of 0, 160, 1600 and 16000 ppm (corresponding to mean dose-levels of 0, 12.5, 128 and 1423 mg/kg/day) to CD rats for 13 weeks, carbetamide induced:

- a lower body weight gain, body weight and food intake in both sexes at the highest dose-level of 16000 ppm, compared to controls,

- a lower RBC count in rats given 16000 ppm, accounting for lower PCV and Hb compared to controls; a 25% higher platelet count in rats given 16000 ppm,
- in rats given 16000 ppm: a higher AP; lower AST, ALT and glucose levels probably linked to insufficient dietary protein and energy supplies due to inappettence,
- lower plasmatic levels of butyrylCh-esterase and ACh-esterase and higher blood cellular levels of ACh-esterase in the highest dose group females,
- a higher absolute and/or relative liver weight, and uterus weight, at the dose-level of 16000 ppm,
- a centrolobular hepatocyte hypertrophy in all rats given 16000 ppm and in two males given 1600 ppm,
- a higher frequency of hydrometra and other uterus dilatations (accounting for the higher but scattered uterus weights) in group 4, compared to groups 1 to 3.

The liver was identified as the main target for carbetamide. Hepatocyte enlargement was observed at 1600 and 16000 ppm. Under the conditions of the study, the no-effect-level is 160 ppm, which corresponds to a mean dose-level of 12.5 mg/kg/day (males: 11.58, females: 13.37 mg/kg/day).

Inhibition of cholinesterase activity observed at 16000 ppm was considered to be of biological significance.

- **13-week oral toxicity study in dogs**

❑ **Reference:Danks A (1985):** carbetamide: 13-week toxicity study in oral administration to beagle dogs. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 85/RHA048/425 - Dates of work: 27/08/1985 to 25/11/1985.
❑ **Guidelines:** OECD N° 409 (1981).
❑ **GLP standards:** Yes.
❑ **Deviations:** None.
❑ **Study acceptable:** Yes.
❑ **Test system:**

Technical carbetamide (batch 84 040 01, purity of 96.4%) was administered by oral route (capsules) at the dose-levels of 0, 3, 30 and 300 mg/kg/day to four groups (numbered 1 to 4 respectively) of 4 male and 4 female beagle dogs (approximately 20-23 weeks old, bw range 7.0 to 9.1 kg for males and 6.6 to 8.2 kg for females on treatment start) for 13 weeks.

Every day, before and after capsule administration, each animal was observed for signs of toxicity and mortality. Pens were examined daily. Individual food consumption was recorded daily and body weight was recorded weekly and before necropsy. Water intake was assessed visually but not recorded. Complete veterinary examinations were conducted before study commencement and after 4, 8 and 12 weeks of treatment. Ophthalmoscopic examinations were performed before study commencement and after 12 weeks of treatment.

Before study commencement and after 6 and 12 weeks of treatment, haematology and standard blood chemistry were performed and blood cholinesterase activity was evaluated. Brain cholinesterase activity was determined at study termination. Urinalysis was performed before study commencement and after 5 and 11 weeks of treatment.

All surviving dogs were sacrificed on completion of the study and subjected to complete necropsy. At this occasion, any abnormality was recorded, and the corresponding tissue/organ weighed and preserved. Selected organs (adrenals, brain, liver, kidneys, heart, lugs, pituitary, prostate, ovaries, spleen, thyroid with parathyroids, testes and uterus) were weighed and a complete microscopic examination of all designated organs was performed (including bone marrow smears).

❑ **Results**

No death occurred during the treatment.

Treatment-related clinical signs were observed only at the dose-level of 300 mg/kg/day and limited to (in order of decreasing incidence) unsteadiness of the hind limbs (all study long in males, mostly till week 9 in females), drowsiness (from week 3 till study termination), prostration (till week 9 in males and 6 in females), and an increase in the incidence of dry nose (a sign observed in all groups mostly till week 7). Sporadic cases of emesis or retching were observed in all groups but controls.

There was no significant difference in body weight, body weight gain or food intake between any of the four groups for each sex.

Ophthalmoscopic examinations did not show any treatment-related finding.

Haematological examinations on week 12 showed higher platelet counts compared to pre-test values for the same group (males +52%, females +50%) and to control values (males +70%, females +44%) in group 4. Higher neutrophil counts were observed in group 4 but with low biological significance (+123% and +49% in males and females compared to pre-test, less than +50% compared to controls). All the other differences between groups were already present in pre-test (Table B.6.3.2.2.-1).

The only relevant biochemical finding was a higher RBC ACh-esterase activity in animals given 300 mg/kg/day (+36% in both sexes compared to pre-test values). All other differences were not treatment-related. There was no treatment-related variation of brain ACh-esterase activity (Table 31).

Table 31: Blood haematological and biochemical parameters in dogs given carbetamide for 12 weeks

| Group | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 3 | | 30 | | 300 | |
| Sex | M | F | M | F | M | F | M | F |
| Neutrophils ($10^3$/cm$^3$) | 7.7 | 5.9 | 6.7 | 8.0* | 9.1 | 6.9 | 11.6* | 7.7 |
| Platelet count ($10^3$/cm$^3$) | 258 | 261 | 288 | 284 | 321 | 281 | 427* | 376* |
| RBC Ach esterase (iu/L) | 2400 | 3152 | 2624 | 2576 | 3536* | 3280 | 3712* | 3648 |

*: significantly different from controls ($p<0.05$).

Urinalysis did not reveal any treatment-related finding at any sampling.

Liver and thyroid absolute and relative weights were higher in both sexes treated at 300 mg/kg/day compared to controls (relative liver weight: males +24% and females +34%; relative thyroid weight: males +70% and females +91%) (Table 32).

None of the macroscopic or microscopic pathology findings were considered to be treatment-related. Notably, the cases of thyroid epithelial volume increase were sporadic and non dose-related.

Table 32: Absolute and relative organ weights of dogs given carbetamide for 13 weeks

| Group | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | | 0 | | 3 | | 30 | | 300 | |
| Sex | | M | F | M | F | M | F | M | F |
| Liver | Absolute (g) | 366 | 307 | 342 | 282 | 353 | 332 | 420 | 410* |
| | Relative (%) | 2.9 | 2.9 | 2.9 | 3.1 | 2.9 | 3.0 | 3.6* | 3.9* |
| Thyroid with parathyroids | Absolute (g) | 0.76 | 0.62 | 0.82 | 0.56 | 0.79 | 0.58 | 1.20* | 1.17* |
| | Relative (%) x1000 | 6.0 | 5.8 | 6.8 | 6.0 | 6.4 | 5.2 | 10.2* | 11.1* |

*: significantly different from controls ($p<0.05$).

- **Conclusion**

After gavage at the dose-levels of 0, 3, 30 and 300 mg/kg/day to beagles for 13 weeks, carbetamide induced:

- clinical signs limited to dry nose, unsteadiness of the hind limbs, drowsiness, prostration and transient, non-specific neurovegetative reactions in animals receiving 300 mg/kg/day,
- higher platelet and neutrophil counts in animals given 300 mg/kg/day,
- higher absolute and relative liver and thyroid weights in both sexes at the dose-level of 300 mg/kg/day, without any significant histological finding.

The NOAEL of carbetamide administered orally to Beagle dogs is 30 mg/kg/day under the conditions of the study.

- **52-week oral toxicity study in dogs**

- **Reference: Danks A (1987):** Carbetamide: toxicity study by oral (capsule) administration to beagle dogs for 52 weeks. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 86/RHA062/486 - Dates of work: 24/06/1985 to 02/07/1987.
- **Guidelines:** Not stated; the study was roughly in accordance with OECD N° 409 (1981).
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test method:**

Technical carbetamide (batches 84 040 02 and 85345, purity of 95.2% and 97.4%) was administered daily by oral route (capsules) at the dose-levels of 0, 3, 30 and 300 mg/kg/day to four groups (numbered 1 to 4 respectively) of 6 male and 6 female beagle dogs (approximately 20-23 weeks old, bw range 7.1 to 10.0 kg for males and 6.6 to 8.7 kg for females on treatment start) for 52 weeks.

Every day, before and after capsule administration, each animal was observed for signs of toxicity and mortality. Pens were examined daily. Additional, detailed examinations were performed weekly. Individual food consumption was recorded daily and body weight was recorded weekly and before necropsy. Water intake was assessed visually but not recorded. Complete veterinary examinations were conducted before study commencement, and after 11, 23, 37 and 51 weeks of treatment. Ophthalmoscopic examinations were performed before study commencement and after 31 and 51 weeks of treatment.

Before study commencement and after 12, 24 and 50 weeks of treatment, haematology and standard blood chemistry were performed and blood cholinesterase activity was evaluated. Urinalysis was performed before study commencement and after 11, 23 and 49 weeks of treatment.

All surviving dogs were sacrificed on completion of the study and subjected to complete necropsy. At this occasion, any abnormality was recorded, and the corresponding tissue/organ weighed and preserved. Selected organs (adrenals, brain, liver, kidneys, heart, lungs, pituitary, prostate, ovaries, spleen, thyroid with parathyroids, testes) were weighed and a complete microscopic examination of all organs was performed (including bone marrow smears).

- **Results**

No death occurred during the treatment.

At the dose-level of 300 mg/kg/day, treatment-related clinical signs were limited to (in order of decreasing incidence):
- drowsiness (during the whole treatment and more frequent in females),
- unsteadiness of the hind limbs (less frequent after week 30),
- an increase in the incidence of dry nose (a sign observed in all groups),
- prostration (males: till week 9; females: during the whole treatment with decreasing frequency)
- and muscle tremors (from week 7 till study termination in females, with low incidence),.

They were also observed, at lower frequencies, at 30 mg/kg/day:
- low incidence of transient drowsiness within the hours following dosing up to week 7 (males) or 9 (females),
- muscle tremors from week 20 in females only (tables 33 and 34)

Table 33: Number of animals presenting with muscle tremors, drowsiness, unsteadiness and prostration during the study:

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | 3 | 30 | 300 |
| Male | | | | |
| Muscle tremors | 2/6 | 0/6 | 0/6 | 2/6 |
| Drowsiness | 2/6 | 1/6 | 4/6 | 5/6 |
| Unsteadiness | 1/6 | 1/6 | 3/6 | 6/6 |
| Prostration | 1/6 | 0/6 | 1/6 | 5/6 |
| Female | | | | |
| Muscle tremors | 1/6 | 3/6 | 3/6 | 6/6 |
| Drowsiness | 1/6 | 3/6 | 5/6 | 6/6 |
| Unsteadiness | 2/6 | 0/6 | 3/6 | 6/6 |
| Prostration | 2/6 | 0/6 | 4/6 | 6/6 |

Table 34: Muscle tremors, drowsiness, unsteadiness and prostration incidences observed during the study (52 weeks):

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | 3 | 30 | 300 |
| Male | | | | |
| Muscle tremors | 3[(a)] | 0 | 0 | 3 |
| Drowsiness | 4[(b)] | 0 | 10 | 167 |
| Unsteadiness | 2[(c)] | 1 | 6 | 65 |
| Prostration | 1[(d)] | 0 | 1 | 22 |
| Female | | | | |
| Muscle tremors | 1[(e)] | 12 | 12 | 63 |
| Drowsiness | 13[(f)] | 6 | 28 | 723 |
| Unsteadiness | 8 [(g)] | 0 | 4 | 82 |
| Prostration | 4[(h)] | 0 | 8 | 90 |

(a) incidences observed at week 38, week 49 and week 52
(b) incidences observed at week 1, week 42 and week 52
(c) incidences observed at week 21
(d) incidences observed at week 38
(e) incidences observed at week40
(f) incidences observed at week 37 to week 39
(g) incidences observed at week 3, week 16 and week 38
(h) incidences observed at week 1, week 16 and week 37

There was no significant difference in body weight gain over 52 weeks (Table B.35), but bw gain was lower in females given 300 mg/kg/day during the first 6 months (-33% when compared to controls). In these females, food intake was moderately lower than control values during most of the treatment period (-6% for 52 weeks). Water intake was unaffected by treatment.

Table 35: Mean body weight gains in non-treated control and carbetamide treated groups

| Dose-level (mg/kg/d) | Not treated | | 3 | | 30 | | 300 | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | Females | Males | Females | Males | Females | Males | Females |
| Weeks 0-26 (kg) | 4.7 | 4.2 | 4.3 | 4.5 | 5.0 | 4.1 | 4.9 | 2.8* |
| Weeks 26-52 (kg) | 0.4 | 0.5 | 0.4 | 0.8 | 0.5 | 0.8 | 0.6 | 0.6 |

*: significantly different from controls ($p<0.05$)

Ophthalmoscopic examinations did not show any treatment-related finding.

Haematological examinations of group 4 on week 50, compared to control values, showed lower PCV, Hb (males -14%, females -12% for both parameters) and RBC (males -15%, females -14%), and higher platelet counts (males +42%, females +24%). The same trends were observed when compared to pre-test values for the same group. Blood reticulocyte, and to a lesser extent normoblast counts were marginally higher in group 4 (Table 36).

Biochemistry investigations showed higher AP (males +103%, females +39%) and lower ALT (males -26%, females -10%) in group 4 compared to controls. Urinalysis only showed a transient presence of reducing substances in group 4.

Table 36: Summary of haematological and biochemical parameters and relative organ weights in dogs treated with carbetamide for 50 weeks

| Group | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 3 | | 30 | | 300 | |
| Sex | M | F | M | F | M | F | M | F |
| PCV (%) | 51 | 50 | 49 | 50 | 48 | 49 | 44* | 44* |
| Hb (g/100mL) | 17.0 | 16.5 | 16.6 | 16.5 | 16.2 | 16.4 | 14.7* | 14.6* |
| RBC ($10^6$/cm$^3$) | 7.40 | 7.11 | 7.27 | 7.23 | 6.94 | 7.08 | 6.26* | 6.08* |
| Platelet count ($10^3$/cm$^3$) | 177 | 241 | 167 | 201 | 204 | 223 | 252* | 298 |
| Dogs with reticulocyte count $\geq$ 2% | 1 | 0 | 0 | 0 | 1 | 0 | 6 | 2 |
| AP (IU/L) | 35 | 44 | 35 | 42 | 35 | 54 | 71* | 61 |
| ALT (IU/L) | 50 | 41 | 56 | 39 | 41 | 35 | 37* | 37 |

*: significantly different from controls ($p<0.05$)

Liver and thyroid absolute and relative weights were higher in both sexes treated at 300 mg/kg/day compared to controls (relative liver weight: males +24% and females +34%; relative thyroid weight: males +70% and females +91%) (Table 38).

There was no treatment-related macroscopic finding.

Microscopic examinations showed liver lesions: higher amounts of haemosiderin in Kupffer cells in males treated at 30 or 300 mg/kg/day, and presence of another, brown pigment in hepatocytes and sinusoids in both sexes treated at 300 mg/kg/day.

Table 37: Number of animals presenting with haemosiderin in Kupfer cells during the study:

| Group | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 3 | | 30 | | 300 | |
| Sex | M | F | M | F | M | F | M | F |
| Haemosiderin in Kupfer cells | 0 | 4 | 0 | 5 | 3 | 4 | 6 | 6 |

Table 38: Organ weights of dogs treated with carbetamide for 52 weeks

| Group | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Dose-levels (mg/kg/day) | 0 | | 3 | | 30 | | 300 | |
| Sex | M | F | M | F | M | F | M | F |
| Liver | | | | | | | | |
| Absolute (g) | 388 | 375 | 388 | 390 | 431 | 413 | 529* | 437 |
| Relative (%) | 2.8 | 3.0 | 2.8 | 2.9 | 2.9 | 3.2 | 3.6* | 3.9* |
| Thyroid with parathyroids | | | | | | | | |
| Absolute (g) | 0.88 | 0.71 | 0.86 | 0.76 | 0.90 | 0.77 | 1.63* | 1.29 |
| Relative (%) x1000 | 6 | 6 | 6 | 6 | 6 | 6 | 11* | 11* |

❑ **Conclusion**

After oral administration (capsules) at the dose-levels of 0, 3, 30 and 300 mg/kg/day to beagles for 52 weeks, carbetamide induced:

- clinical signs including drowsiness, unsteadiness of the hind limbs, dry nose, prostration and muscle tremors in animals receiving 300 mg/kg/day. With lower incidence and lower frequency, they were also observed at 30 mg/kg/day, i.e., transient drowsiness within the hours following dosing up to week 7 (males) or 9 (females) and muscle tremors from week 20 in females.
- a lower body weight gain and food intake in females given 300 mg/kg/day, only for the first 6 months;
- lower PCV, Hb and RBC, and higher platelet counts in animals given 300 mg/kg/day;
- higher AP and lower ALT and AST in both sexes treated at 300 mg/kg/day.
- higher absolute and relative liver and thyroid weights in both sexes at the dose-level of 300 mg/kg/day;
- higher amounts of haemosiderin in Kupffer cells in males treated at 30 or 300 mg/kg/day, and presence of another, brown pigment in hepatocytes and sinusoids in both sexes treated at 300 mg/kg/day.

The NOAEL is proposed at 3 mg/kg bw.day. However, the adverse effects observed at the LOAEL of 30 mg/kg/day were minimal, i.e. low incidence of transient drowsiness within the hours following dosing up to week 7 (males) or 9 (females) and muscle tremors from week 20 in females only, and higher haemosiderin content in hepatic Kuppfer cells without modifications of red blood cell parameters.

### 4.7.1.2 Repeated dose toxicity: inhalation

Carbetamide is not a volatile compound. The vapour pressure is $3 \times 10^{-7}$ Pa (20°C), and is, therefore, well below the $10^{-2}$ Pa value. Due to its low vapour pressure a 28-day or a 90-day inhalation toxicity study was not conducted.

### 4.7.1.3 Repeated dose toxicity: dermal

Exposure to Carbetamide by the percutaneous route is not expected to be significant. Therefore, no such study over 28 or 90 days has been conducted.

#### 4.7.1.4 Repeated dose toxicity: other routes

No data

#### 4.7.1.5 Human information

No data

#### 4.7.1.6 Other relevant information

No data

#### 4.7.1.7 Summary and discussion of repeated dose toxicity

The short-term toxicity of carbetamide was evaluated by oral route in rats and dogs. No study was performed by inhalation or by dermal repeated application.

Clinical signs of neurotoxicity were observed in dogs but not in rats. Modifications of plasma or tissular acetylcholine esterase levels were observed in rats and dogs, but with opposite trends across organs/sexes/studies, and often without dose-relationship. Slight changes in haematological parameters were noted in rats and dogs. The liver was identified as the main target organ in rats and dogs, and the thyroids were also identified as a target organ in dogs (Table 15).

Based on the results of the available 90-day studies and of the 52-week dog study, carbetamide is considered of low short-term toxicity in mammals.

Similar effects and target organs were identified in rats and dogs after oral administration respectively by dietary mix and capsules, and included:

- Slight signs of neurotoxicity (unsteadiness, drawsiness and non specific neurovegetative reactions) in dogs and in rats increased or decreased in plasma and erythrocyte cholinesterase activities, respectively.
- Slight hematotoxicity with lower red blood cell parameters, and higher platelet counts,
- The liver was identified as a target organ in rats and dogs (increases in liver weight associated in rats with hepatocellular hypertrophy).

In addition, dogs in 13- and 52-week studies showed higher thyroid weights, associated with thyroid epithelium enlargement. Dogs in the 52-week study also showed:

- A lower body weight gain and food intake in females,
- Higher AP and lower ALT and AST in both sexes,
- Minor pigment accumulations: haemosiderin in Kupffer cells (males) and another, brown pigment in hepatocytes and sinusoids (both sexes).

***Liver effects***

Repeated dose oral toxicity studies with Carbetamide in rodent and in dogs revealed the liver and the thyroid as target organs. Increased liver weight –at high dose levels associated with hepatocellular hypertrophy- was the most common finding. The effect on the liver may be caused by adaptation to the xenobiotic burden, which should be regarded as a physiological response rather than an adverse effect.

***Thyroid effects***

The effect on the thyroid observed with rats, mice (cf section 4.10) and dogs should be regarded as a secondary effect to the induction of liver enzymes by Carbetamide. The effects on the thyroid resulted from increased metabolic turnover of thyroid hormones in the liver presumably by an induced thyroxine-glucotransferase activity. In response to the decrease in thyroid hormones thyrotropin (TSH) production is stimulated in the pituitary gland and this causes subsequently overstimulation of the thyroid gland apparent as hypertrophy, hyperplasia, and finally neoplasia of the thyroid gland (US EPA Risk Assessment Forum, 1998; IARC, 1999).

The Carbetamide treatment-related effect on the thyroid has been noted in rodents and also in dogs. This is not uncommon for effects on the thyroid, which are frequently not species-specific and observable in mouse, rats, and dog

studies with the same chemical (Spielmann & Gerbracht, 2001) . However, major species differences exist for humans. Thyroxine-binding globulin is the predominant plasma protein in humans and non-human primates that binds and transports thyroid hormone in the blood. This protein has binding affinities 3 and 5 orders of magnitude greater than the other two thyroxine-binding proteins, albumin and pre-albumin, respectively. The lack of thyroxine-binding globulin in the adult rat is an important difference. Other differences are present in the half-life of thyroxine, 12 h in the rat versus 5–9 days in humans, and in the serum level of TSH which is 25 or more times higher in the rodent than in humans. The rat also exhibits enhanced thyroid hormone elimination with less efficient enterohepatic recirculation than humans. The histology of the resting rodent thyroid is similar to that of the stimulated human gland, with small follicles lined by tall follicular cells. Thus, both the physiological parameters and the histological appearance indicate that the rodent thyroid gland is more active and operates at a higher level with respect to thyroid hormone turnover as compared to the human gland (IARC, 1999).

Thus, the treatment-related effect of Carbetamide on the thyroid of mouse, rat, and dog is mediated by an unspecific impairment of hepatic thyroid hormone metabolism and subsequently disturbance of the pituitary-thyroid-liver axis. This disturbance is frequently observed with liver enzyme inducers in rodents and the dog and can not be extrapolated to primates including man. This unspecific alteration in the thyroid gland (focal hyperplasia and increased organ weight) is therefore not considered as a relevant substance-specific effect (Spielmann & Gerbracht, 2001).

In conclusion, the main target organ identified in repeated dose toxicity studies with Carbetamide is the liver and subsequently the thyroid. Inductions of adverse effects in both organs were related only to high and unrealistic dose levels of Carbetamide administered. Moreover, the effect on the thyroid observed in rodents and dogs is not regarded as relevant for humans.

#### 4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Not applicable

#### 4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Not applicable

#### 4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Not applicable

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

#### 4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Repeated dose oral toxicity studies with Carbetamide in rodent and in dogs revealed the liver and the thyroid as target organs.

Hepatocellular effects of Carbetamide on the liver (i.e. increased liver weight at high dose levels associated with hepatocellular hypertrophy) were the most common findings. These effects are most probably caused by an adaptative response to the xenobiotic burden, which should be regarded as a physiological response rather than an adverse effect.Effects of Carbetamide on the thyroid of mouse, rat, and dog are mediated by an unspecific impairment of hepatic thyroid hormone metabolism and subsequently disturbance of the pituitary-thyroid-liver axis. This disturbance is frequently observed with liver enzyme inducers in rodents and the dog and can not be extrapolated to primates including man. This unspecific alteration in the thyroid gland (focal hyperplasia and increased organ weight) is therefore not considered as a relevant substance-specific effect (Spielmann & Gerbracht, 2001).

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

The main target organs identified in repeated dose toxicity studies with Carbetamide are the liver and the thyroid. Inductions of adverse effects in both organs were related only to high and unrealistic dose levels of Carbetamide administered. Hepatocellular effects are considered as adaptative and thyroid effects are considered not relevant for classification.

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

Not applicable

## 4.9 Germ cell mutagenicity (Mutagenicity)

The genotoxic potential of carbetamide has been investigated in a battery of *in vitro* and in vivo tests including reverse mutation tests on bacterial S. typhymurium strains, forward mutation tests on mouse lymphoma *tk +/-* cells, *in vitro* chromosome aberration tests, one UDS test on rat hepatocytes, one SCE test, one *in vivo* micronucleus test in bone marrow and one *in vivo* UDS test with rat liver cells.

Table 39: Summary table of relevant in vitro and in vivo mutagenicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **In vitro** | | | |
| **Ames Test**<br>*Salmonella typhimurium*<br>TA 1535, TA 1537, TA 98 and TA 100.<br>Carbetamide:<br>- Main test: 125-1000 µg/plate, +/- S9<br>- Spot test: 200 µg/ 10µL per spot, +/- S9<br>Negative controls: solvent (DMSO)<br>Reference mutagens: S propiolactone, Hycanthone and Niridazole | Negative | **Guidelines:** None. Plate incorporation according to standard procedure published by Ames et al., 1975, on which the current Guideline is based. In addition a "spot test" was performed.<br>**GLP:** Not in force at the time of the study.<br>**Deviation**: Only four concentrations were tested. The assay with metabolic activation wasnot repeated.<br>**Purity**: unknown ((batch RAB 3 161)<br>**Study acceptable:** as supportive | Benazet & Cartier, 1977 |
| **Ames Test**<br>*Salmonella typhimurium*<br>TA 1535, TA 1537, TA 1538, TA 98 and TA 100<br>Carbetamide:<br>Main test: 312-5000 µg/plate, +/- S9<br>Negative controls: solvent (DMSO)<br>Reference mutagens: S propiolactone, Hycanthone and Niridazole | Negative | **Guidelines:** None. Plate incorporation according to standard procedure published by Ames et al., 1975, on which the current Guideline is based.<br>**GLP:** Yes<br>**Deviation**: none<br>**Purity**: 96.0% ((batch MAG 1 326)<br>**Study acceptable:** Yes | Cordier & Bonneau, 1984 |
| **Mouse Lymphoma Assay (Tk locus)**<br>L5178Y cells<br>Carbetamide:<br>156.3 to 2500 µg/ml (-S9)<br>156.3 to 2500 µg/ml (+S9)<br>Negative controls: solvent (DMSO)<br>Positive controls: methylmethanesulfonate (-S9 mix) and 3-methylcholanthrene (with S9 mix). | Negative | **Guidelines:** OECD guideline for testing chemicals (No.476, 1997)<br>**GLP:** Yes<br>**Deviation:** experiment without metabolic activation not made in duplicate. Only 4 concentrations tested instead of 8. Statistical evaluation not performed<br>**Purity**: 97.6% ((batch 020946)<br>**Study acceptable:** Yes | August, 2006b |
| **Chromosome aberration Test**<br>Human lymphocytes<br>Carbetamide:<br>78.1-1250 µg/mL (-S9, 24h)<br>78.1-1250 µg/mL (-S9, 4h)<br>312.5-5000 µg/mL(+S9, 24h)<br>Negative controls: solvent (DMSO)<br>Positive controls: Mitomycin C (-S9) or cyclophosphamide (+ S9) | Negative | **Guidelines:** OECD guideline for testing chemicals No.473 (07/21/1997): *In vitro* mammalian Chromosome Aberration Test<br>**GLP:** Yes<br>**Deviation:** No control of the treatment solutions concentrations has been performed : homogeneity, stability of the test item applied in the vehicle<br>**Purity**: 97.6% ((batch 020946)<br>**Study acceptable:** Yes | August, 2006a |
| **Chromosome aberration Test**<br>CHO cells<br>Carbetamide:<br>0.6-1.0 mg/mL (-S9, 23h)<br>1.5-2.2 mg/mL (+S9, 2h)<br>2.0-2.4 mg/mL (+S9, 2h)<br>Negative controls: solvent (DMSO)<br>Positive controls: mitomycin C (-S9) or cyclophosphamide (+S9) | Negative | **Guidelines:** none<br>**GLP:** Yes<br>**Deviation:** One single assay without metabolic activation.<br>**Purity**: unknown ((batch 04040.02)<br>**Study acceptable:** as supportive | Ivett, 1984a |

| | | | |
|---|---|---|---|
| **Sister Chromatid Exchange**<br>CHO cells<br>Carbetamide:<br>7.5- 750 µg/mL (-S9, 25h),<br>75-2250 µg/mL (+S9, 2h)<br>Negative controls: solvent (DMSO)<br>Positive controls: mitomycin C (-S9) or cyclophosphamide (+S9) | Negative | **Guidelines:** not mentionned<br>**GLP:** Yes<br>**Deviation:** One single assay without metabolic activation.<br>**Purity**: unknown ((batch 04040.02)<br>**Study acceptable:** as supportive | Ivett, 1984b |
| **In vitro Unschedules DNA Synthesis assay (UDS)**<br>Primary hepatocyte culture<br>Carbetamide<br>19.4 – 826 µg/ml<br>Negative controls: solvent (1% DMSO)<br>Positive controls: 2-acetyl aminofluorene 2-AAF, 0.10 µg/mL | Negative | **Guidelines:** Procedures described by Williams G.M. Cancer Res. 37:1845-1851 (1977) and Chemical Mutagens, Plenum Press, NY, 1980, pp. 61-79. OECD Guideline 482 is based on comparable protocol.<br>**GLP:** Yes<br>**Deviation:** Only one assay was conducted without repetition.<br>**Purity**: unknown (batch 04040.02)<br>**Study acceptable:** as supportive | Cifone, 1984 |
| **In vivo** | | | |
| **In vivo Micronucleus test (bone marrow)**<br>Male and female CD1 mice<br>Carbetamide oral route:<br>325, 650, 975 mg/kg bw (one or two administrations)<br>Negative control: vehicle (10% Arabic gum)<br>Positive control: mitomycin C | Negative<br>Lack of clinical toxicity, hematotoxicity, no proof of exposure | **Guidelines:** Not stated. Design of the study is roughly in accordance with current OECD guideline 474 "Mammalian Erythrocyte Micronucleus Test" (1983).<br>**GLP:** Yes<br>**Deviation:** Per animal, 500 polychromatic erythrocytes were examined in bone marrow smears, instead of 200 in bone marrow and 1000 in blood.<br>**Purity**: 96.0% (batch MAG 1326)<br>**Study acceptable:** Yes | Cordier & Fournier, 1984 |
| **In vivo Micronucleus test (bone marrow)**<br>Female NMRI mice<br>Pharmacokinetic evaluation<br>Carbetamide oral route:<br>500,1000, 2000 mg/kg<br>Negative control: vehicle (0.8% hydroxypropylmethyl cellulose gel)<br>Positive control: cyclophosphamide | Negative<br>Proof of systemic availability of Carbetamide | **Guidelines:** OECD guideline for testing of chemicals No. 474 (07/21/1997)<br>**GLP:** Yes<br>**Deviation:** minor.<br>**Purity**: 97.6% (batch 020946)<br>**Study acceptable:** Yes | August, 2006d |
| **In vivo UDS test with rat liver cells**<br>Male CD rats<br>Carbetamide<br>500, 1000 mg/kg bw<br>Negative control: vehicle (0.8% hydroxypropylmethyl cellulose gel)<br>Positive control: Dimethylnitrosamine and 2-Acetaminofluorine | Negative | **Guidelines:** OECD guideline for testing of chemicals No. 486 (07/21/1997):<br>**GLP:** Yes<br>**Deviation:** minor.<br>**Purity**: 97.03% (batch 020946)<br>**Study acceptable:** Yes | Flügge, 2008 |

### 4.9.1 Non-human information

#### 4.9.1.1 In vitro data

The genotoxic potential of carbetamide has been investigated on a battery of *in vitro* and *in vivo* tests.

**Bacterial studies**

Two Ames tests were submitted by the manufacturer. The first one was conducted before GLP was in force and is reported for completeness below.

- **Reference: Benazet, Cartier (1977):** carbetamide (11561 RP): Etude de l´activité mutagène vis-à-vis de *Salmonella typhymurium.* Rhone-Poulenc / CNG, Vitry sur Seine, France - Unpublished report No. RP/RD/CNG 19231 - Dates of work: ? to 27/07/1977.
- **Guidelines:** None. Plate incorporation according to standard procedure published by Ames et al., 1975, on which the current Guideline is based. In addition a "spot test" was performed.
- **GLP standards:** Not in force at the time of the study.
- **Deviations:** Only four concentrations were tested. The assay with metabolic activation was not repeated.
- **Study acceptable:** As supportive
- **Test method:**

Carbetamide (batch RAB 3 161, unknown purity) was tested in a plate incorporation assay using bacterial strains of *Salmonella typhymurium* TA 1535, TA 1537, TA 98 and TA 100.

A preliminary test was performed with concentrations ranging from 1 to 5000 µg/plate, to determine the range to be used in the main test.

Four concentrations of 125, 250, 500 and 1000 µg/plate were used in the main test, with and without metabolic activation (S-9 fraction of liver homogenate prepared from Aroclor 1254 treated Sprague Dawley rats and S-9 cofactor mix). Each test included negative controls (solvent: DMSO) and reference mutagens (S propiolactone, Hycanthone and Niridazole). Two independent assays were conducted without metabolic activation, but the assay was not repeated with metabolic activation.

Another assay was also performed with the test material dropped in the center of the petri plate at 200 µg carbetamide/10 µL per spot, with and without metabolic activation. This qualitative “spot test” consisted in appreciation of revertant colony density in the spot.

- **Results**

Using the standard plate incorporation procedure (Table 40), the number of revertant colonies in the carbetamide treated plates did not differ from control solvent plates, and higher revertant colony counts in treated plates were always inferior to twice that of control plates. The numbers of revertant colonies in plates treated with positive controls were much higher than controls, demonstrating the validity of the assay without and with metabolic activation.

Similar results were qualitatively obtained in the "spot test".

Table 40: Mean number of revertant colonies in bacterial reverse mutation test

| Mean number of revertant colonies | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Product** | **Dose µg/plate** | **TA 1535** | | **TA 1537** | | **TA 98** | | **TA 100** | |
| | | **- S9** | **+ S9** | **- S9** | **+ S9** | **- S9** | **+ S9** | **- S9** | **+ S9** |
| **1st experiment** | | | | | | | | | |
| carbetamide | 1,000 | 11.3 | 11 | 8.3 | 5.6 | 25.6 | 35 | 97.6 | 82.6 |
| | 100 | 9.6 | 9.3 | 8.3 | 6.6 | 23.3 | 31.0 | 97.6 | 92 |
| | 10 | 11.6 | 9.6 | 6.6 | 5.6 | 18.6 | 41.6 | 99.6 | 98.3 |
| | 1 | 10.6 | 12 | 6.3 | 7.6 | 23.3 | 35.6 | 99.3 | 93.3 |
| Solvent: DMSO | - | 10.0 | 9.3 | 7.3 | 8.3 | 26.3 | 41.6 | 117.3 | 104 |
| S propiolactone | 50 | 364 | 728 | | | | | | |
| Hycanthone | 50 | | | 592 | 696 | | | | |
| Niridazole | 0.05 | | | | | 308 | 632 | 1160 | 1384 |
| **2nd experiment** | | | | | | | | | |
| carbetamide | 1,000 | 9.3 | | 7.67 | | 30.7 | | 110.7 | |
| | 500 | 13.67 | | 4 | | 23.3 | | 119 | |
| | 250 | 11.5 | | 5.67 | | 19.7 | | 116.3 | |
| | 125 | 15 | | 6 | | 22.7 | | 115 | |
| Solvent: DMSO | - | 15.5 | | 7.67 | | 31 | | 116.3 | |
| S propiolactone | 50 | 1322 | | | | | | | |
| Hycanthone | 50 | | | 710 | | | | | |
| Niridazole | 0.05 | | | | | 260 | | 1181.3 | |

❑ **Conclusion**

In a study, which cannot be considered as acceptable for regulatory evaluation, because it was conducted prior to GLP enforcement, with major deviations from current OECD guideline, and using a batch of unknown purity, carbetamide had no mutagenic effect on the TA1535, TA1537, TA98, TA100 strains of *Salmonella typhymurium* in a plate incorporation assay conducted without and with metabolic activation according to the Ames test procedure.

❑ **Reference: Cordier A, Bonneau D (1984):** carbetamide (11561 RP) *in vitro* mutagenic activity on five Ames´ tester strains of *Salmonella typhimurium* using the mammalian -microsomes / plate incorporation assay. Rhone-Poulenc, CRV, Vitry sur Seine, France - Unpublished report No. ST/CRV/Tox. 22048-E - Dates of work: 9/12/1983 to 19/03/1984.

❑ **Guidelines:** None. Plate incorporation according to standard procedure published by Ames et al., 1975, on which the current Guideline is based.

❑ **GLP standards:** Yes.

❑ **Study acceptable:** Yes.

❑ **Test method:**

Carbetamide (batch MAG 1 326, purity 96.0%) was tested in a plate incorporation test using bacterial strains of *Salmonella typhymurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100.

A preliminary test was performed with concentrations ranging from 1 to 5000 µg/plate, to determine the range to be used in the main test.

Five concentrations of 312, 625, 1250, 2500 and 5000 µg/plate were used in the main test, with and without metabolic activation (S-9 fraction of liver homogenate prepared from Aroclor 1254 treated Sprague Dawley rats and S-9 cofactor mix), along with negative controls (solvent: DMSO) and reference mutagens (sodium azide, hycanthone and ethidium bromide). Three independent assays were conducted without metabolic activation, and two independent assays with metabolic activation.

❑ **Results**

At the concentration of 5000 µg/plate, carbetamide has no cytotoxic effect and does not precipitate.

In the main test (see Table 41), the frequency of spontaneous revertants and revertants induced by reference mutagens were in accordance with values observed in the same condition and laboratory.

In a first assay, carbetamide caused increases in the frequency of revertants compared to solvent, in strains TA 1535 and 1538 without metabolic activation at the two higher tested concentrations, exceeding the biological significance increase ratio at the highest concentration of 5000 µg/plate. These increases were not reproduced in the two other independent assays.

No increases in the mean number of revertant colonies were observed at any concentration in presence of metabolic activation.

Table 41: Mean number of revertant colonies in bacterial reverse mutation test

| **Mean number of revertant colonies** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Product** | **Dose µg/plate** | **TA 1535** | | **TA 1537** | | **TA 1538** | | **TA 98** | | **TA 100** | |
| | | **- S9** | **+ S9** | **- S9** | **+ S9** | **- S9** | **+ S9** | **- S9** | **+ S9** | **- S9** | **+ S9** |
| **1st assay** | | | | | | | | | | | |
| carbetamide | 5,000 | **47** | 19 | 8 | 12 | **39** | 27 | 18 | 24 | 170 | 107 |
| | 2,500 | 27 | 8 | 6 | 7 | 18 | 17 | 18 | 23 | 130 | 111 |
| | 1,250 | 22 | 13 | 7 | 11 | 12 | 24 | 16 | 23 | 130 | 102 |
| | 625 | 17 | 10 | 9 | 8 | 10 | 18 | 13 | 31 | 136 | 106 |
| | 312 | 18 | 12 | 7 | 8 | 8 | 20 | 16 | 31 | 123 | 102 |
| Solvent: DMSO | - | 14 | 15 | 8 | 9 | 10 | 21 | 14 | 23 | 139 | 114 |
| Sodium azide | 10 | 929 | | | | | | | | 1093 | |
| Hycanthone | 10 | | | 103 | | 137 | | 108 | | | |
| Ethidium bromide | 5 | | | | | | | 22 | 834 | | |
| **2nd assay** | | | | | | | | | | | |
| carbetamide | 5,000 | 24 | 7 | 5 | 6 | 10 | 17 | 11 | 14 | 141 | 85 |
| | 2,500 | 27 | 10 | 6 | 3 | 7 | 13 | 14 | 12 | 134 | 85 |
| | 1,250 | 20 | 8 | 7 | 6 | 5 | 12 | 12 | 16 | 135 | 87 |
| | 625 | 18 | 10 | 5 | 7 | 8 | 16 | 15 | 15 | 133 | 96 |
| | 312 | 20 | 9 | 6 | 5 | 9 | 18 | 14 | 23 | 124 | 88 |
| Solvent: DMSO | - | 18 | 14 | 9 | 7 | 12 | 15 | 14 | 18 | 123 | 92 |
| Sodium azide | 10 | 978 | | | | | | | | 1083 | |
| Hycanthone | 10 | | | 139 | | 406 | | 216 | | | |
| Ethidium bromide | 5 | | | | | | | 18 | 779 | | |
| **3rd assay** | | | | | | | | | | | |
| carbetamide | 5,000 | 24 | | 6 | | 14 | | 17 | | 118 | |
| | 2,500 | 29 | | 5 | | 14 | | 15 | | 117 | |
| | 1,250 | 32 | | 7 | | 15 | | 20 | | 134 | |
| | 625 | 23 | | 9 | | 13 | | 20 | | 132 | |
| | 312 | 21 | | 8 | | 14 | | 19 | | 139 | |
| Solvent: DMSO | - | 19 | | 8 | | 14 | | 15 | | 142 | |
| Sodium azide | 10 | 948 | | | | | | | | 1035 | |
| Hycanthone | 10 | | | 106 | | 187 | | 102 | | | |
| Ethidium bromide | 5 | | | | | | | | | | |

- **Conclusion**

The study report of the manufacturer states that carbetamide had no mutagenic potential in *S. typhymurium*, using the plate incorporation procedure of the Ames test.

However, carbetamide (batch MAG 1 326) induced biologically relevant increases in the number of revertant colonies without metabolic activation in TA1535 and TA1538 strains of *S. typhymurium* in one assay. This effect could not be

reproduced in two other independent assays using the same concentrations. No increases in the number of revertant colonies were observed in presence of metabolic activation, using the plate incorporation procedure.

**Mammalian cell studies**

***Gene mutation assays***

| | |
|---|---|
| ❑ **Reference:** | **Hoorn AJW (1985):** Mutagenicity evaluation of carbetamide technique in the mouse lymphoma forward mutation assay. Litton Bionetics, Inc., Veenendaal, Netherlands - Unpublished report No. E-9265 / E-9361 - Dates of work: 29/04/1984 to 00/01/1985. |
| ❑ **Guidelines:** | Not stated. Design of the study is roughly in accordance with current OECD guideline 476 "*In vitro* Mammalian Gene Mutation Test" (1997). |
| ❑ **GLP standards:** | Yes. |
| ❑ **Deviations:** | Not applicable. |
| ❑ **Study acceptable:** | As supportive |
| ❑ **Test method:** | |

Technical carbetamide (batches 84.040.02 and DA310, unknown purity) in DMSO was tested *in vitro* for its potential to induce mutations at the *tk* locus in mouse lymphoma cells.

The test article's solubility in DMSO and its toxicity were evaluated in preliminary tests with and without metabolic activation (S-9 fraction of liver homogenate prepared from Aroclor 1254 treated Sprague Dawley rats and S-9 cofactor mix).

Based on the results of these preliminary assays, carbetamide concentrations up to 1600 µg/mL without metabolic activation, and 700 µg/mL with metabolic activation were selected for the main assays, which also included negative solvent (DMSO), and positive control cultures treated with ethylmethanesulfonate (EMS) or 3-methylcholanthrene (MCA) respectively without and with metabolic activation. Viability (total relative survival) and 5-triflurothymidine resistance (mutation frequency) were determined on a range of concentrations including toxic ones.

Four independent trials were conducted without metabolic activation (3 using batch 84.040.02 and 1 using batch DA310) and two with metabolic activation (both using batch 84.040.02).

❑ **Results**

In the preliminary test, evidence of cytotoxicity was noted in carbetamide treated cultures, at the concentration of 500µg/mL without metabolic activation, and 1000 µg/mL with metabolic activation.

In all main assays, positive controls elicited the expected positive responses.

With batch 84.040.02A (-S9), c.a. 25% relative growth level was obtained at 600 µg/mL in the first trial (23.3%), 1000 µg/mL in the second one (25.7%), and no toxicity was observed in the third one (97.2% at 1600 µg/mL). With batch DA310 (-S9), the 500 µg/mL test was associated with 17.0% relative growth. With metabolic activation (+S9, batch 84.040.02A), at 400 µg/mL little toxicity was observed in the first trial (51.9% RG) while more toxicity was observed in the second one (25.1% RG).

Without metabolic activation, carbetamide batch 84.040.02 induced a significantly higher frequency of mutation (over the biological relevant threshold defined as 1,5x controls $+10.10^{-6}$, see Table 42) on several occasions: at 600, 700 and 1000 µg/mL in two separate assays. At the concentration of 700 µg/mL, the total relative survival was only 7.4%, and therefore this effect may be considered as toxicity-related, but at 600 and 1000 µg/mL, the relative survival was higher than 20%, and the effect should be considered to be induced directly by carbetamide and not related to excessive cytotoxicity.

Batch DA310 was associated with increased mutation frequency at 600 µg/mL, at a relative survival (4.3%) which was compatible with a cytoxicity-related effect. This batch was too toxic to be tested at higher concentrations.

Differences in toxicity between the different assays conducted with carbetamide batch 84.040.02 suggest that this test material batch was instable along tests. No purity or stability analysis was provided to support this hypothesis. Therefore, carbetamide should be considered genotoxic in these trials without metabolic activation.

With metabolic activation, carbetamide was not associated with higher frequency of mutation on any occasion at any concentration. Carbetamide was considered non genotoxic in these trials.

Table 42: Relative survival and mutation frequencies at the *tk* locus in L5178 mouse lymphoma cells treated with carbetamide

| Treatment | Concentration (µg/ml) | Test conditions | | | | | |
|---|---|---|---|---|---|---|---|
| | | Without S9-mix | | | | With S9-mix | |
| | | Batch 84.040.02 | | Batch DA310 | | Batch 84.040.02 | |
| | | % RS | MF | % RS | MF | % RS | MF |
| Solv. control | 0 | 100 | 58.0 | 100 | 50.9 | 100 | 89.1 |
| | | | 49.5 | | 69.7 | | 89.0 |
| | | | 50.6 | | 48.7 | | 88.8 |
| | | | 24.8 | | | | 82.0 |
| | | | 30.1 | | | | 113.6 |
| | | | 20.2 | | | | 107.3 |
| | | | 19.3 | | | | |
| | | | 16.5 | | | | |
| | | | 18.9 | | | | |
| carbetamide | 10 | 84.0 | 52.3 | 82.8 | 56.8 | 84.6 | 81.0 |
| | 10 | | | | | 72.6 | 95.9 |
| | 50 | 104.0 | 28.4 | | | | |
| | 100 | | | 88.9 | 55.6 | 76.1 | 94.5 |
| | 100 | 72.5 | 60.4 | | | 90.4 | 93.6 |
| | 125 | 106.1 | 18.0 | | | | |
| | 200 | | | 68.7 | 64.5 | 88.9 | 66.0 |
| | 200 | 72.6 | 56.9 | | | 82.0 | 92.0 |
| | 300 | | | 54.1 | 70.7 | 70.5 | 87.1 |
| | 300 | 84.8 | 24.8 | | | 96.7 | 96.1 |
| | 300 | 64.2 | 63.6 | | | | |
| | 400 | | | 34.8 | 84.0 | 51.9 | 102.0 |
| | 400 | 57.5 | 66.3 | | | 25.1 | 93.5 |
| | 500 | 115.4 | 23.3 | 17.0 | 65.1 | | |
| | 600 | 58.6 | 31.6 | 4.3 | 152.1 * | 18.8 | 128.5 |
| | **600** | **23.3** | **117.7 *** | | | | |
| | 700 | 7.4 | 201.4 * | | | 14.3 | 109.9 |
| | 700 | 33.2 | 31.8 | | | | |
| | 800 | 29.6 | 34.6 | | | | |
| | 800 | 89.9 | 16.7 | | | | |
| | **1,000** | **25.7** | **50.0 *** | | | | |
| | 1,000 | 95.3 | 17.1 | | | | |
| | 1,400 | 95.7 | 17.2 | | | | |
| | 1,600 | 97.2 | 28.2 | | | | |
| EMS | 0.25 | 55.1 | 439.4 | | | | |
| | 0.25 | 67.2 | 399.6 | | | | |
| | 0.30 | 50.7 | 549.3 | 47.0 | 538.3 | | |
| | 0.40 | 37.9 | 647.8 | 18.6 | 811.7 | | |
| | 0.40 | 28.8 | 792.8 | | | | |
| | 0.40 | 47.0 | 539.7 | | | | |
| MCA | 2.0 | | | | | 60.9 | 338.3 |
| | 2.0 | | | | | 36.0 | 409.5 |
| | 3.0 | | | | | 52.0 | 473.9 |
| | 3.0 | | | | | 11.3 | 555.7 |

% RS: percentage of relative survival adjusted by post-treatment cell counts
MF: mutant frequency (5-TFT resistant cells /$10^6$ viable cells)
EMS: Ethyl methane sulfonate
MCA: 3-methylcholanthrene
Bold characters: biologically relevant mutagenic activity of the test material

- **Conclusion**

Without metabolic activation, carbetamide batch 84.040.02 significantly increased the frequency of mutations in mouse lymphoma cells at the concentrations of 600 and 1000 µg/mL, with relative survivals higher than 20%. Batch DA310 was associated with increased mutation frequency at the concentration of 600 µg/ml, but at a low relative survival rate (4.3%) compatible with a cytotoxicity-related effect, and was too toxic to be tested at higher concentrations. With metabolic activation, the test conducted with batch 84.040.02 provided negative results.

A shift in cytotoxicity between the assays was observed with batch 84.040.02, suggesting that this batch was instable. However no analytical data were provided to support this hypothesis. This raises doubt on test material quality, and subsequently on the results of the tests performed both with and without metabolic activation.

- **Reference:** **August M. (2006):** Mutagenicity study of carbetamide in the mouse lymphoma forward mutation assay – *in vitro*. LPT; Germany, Unpublished report No. 20080/06.
- **Guidelines**: OECD guideline for testing chemicals (No.476, 1997) / EC method B.14 (2000/32/EC): Methods for determination of toxicity, *in vitro* mammalian cell gene mutation test.
- **GLP standards**: Yes
- **Deviations:**

  *From BPL:* no control of the treatment solutions concentrations has been performed : homogeneity, stability of the test item applied in the vehicle( OECD 1997, paragraph 6.2 point 5).

  *From 476 OECD guideline:* the experiment without metabolic activation was not made in duplicate. The OCDE guideline recommends at least 8 tested concentrations if the experiment were not made in duplicate. In this study only 4 concentrations were tested. However, two exposure times (3 and 24 hours) were tested.

  Final quantity of S9 was non-determined and the acceptability criteria for the activation system wasn't described.

  A statistical evaluation was not performed.

  In the experiment without metabolic activation, one of the positive control (MMS 15µl/ml) did not elicit the expected effect whereas the other (MMS 10µglml) did.

  *From other recommendations (workshops IWGT, Moore et al., 2002[1] and 2006[2]):* the cytotoxicity has been evaluated exclusively on the basis of the clonage efficiency and not on the RTG percentage as recommended by Moore et al. (2002). Negative control spontaneous mutation level should be comprised between 50 and 170 x $10^6$ mutants (Moore *et al.*, 2006). In this study, the spontaneous mutation level of negative control varied from 25.58 to 28.2.

*These deviations did not impair the validity of the study.*

- **Study acceptance**: Yes
- **Batch**: 020946
- **Purity**: 97.6%
- **Test system**

Carbetamide in DMSO was assayed *in vitro* in a gene mutation assay in cultured mammalian cells (L5178 TK+/-) in the presence and absence of metabolic activation by a liver post-mitochondrial fraction (S9 mix) from Arochlor-induced rats. The test was carried out employing 2 exposure times without S9 mix (3 and 24 hours) and one exposure time carried out twice with S9 mix (3 hours).

Cytotoxicity was noted in the preliminary test at concentrations of 2500 and 5000 µg/mL with or without metabolic activation.

In the main study, 6 doses were tested: 156.30, 312.5, 625, 1250 and 2500 µg/mL. It also included negative solvent (DMSO) and positive controls: MMS (methylmethanesulfonate) for experiments without S9 mix and 3-MC (3-methylcholanthrene) for experiments with S9 mix.

Viability (% relative survival), 5-trifluorothymidine resistance (mutation frequency) and clastogenic potential (small/large colonies ratio) were determined on this range of concentration.

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[1] Moore (2002) : Mouse lymphoma thymidine kinase gene mutation assay : Follow-up international worshop on genotoxicity test procedures, New Orleans, Louisiana, April 2000. Environ. Mol. Mutagen., 40: 292-299.

[2] Moore (2006) : Mouse lymphoma thymidine kinase gene mutation assay : Follow-up meeting of the international workshop on genotoxicity testing-Aberdeen, Scotland, 2003-Assay acceptance criteria, positive controls, and data evaluation. Environ. Mol. Mutagen., 47 (1): 1-5.

- **Results**

In the main study, evidence of cytotoxicity (decreased survival) was noted in carbetamide treated cultures, at the concentration of 2500 µg/mL immediately after treatment. No signs of cytotoxicity were noted in the following plating for 5-trifluorothymidine (5-TFT) resistance in the experiments with or without metabolic activation.

The mutation frequency of the solvent controls ranged well within the historical data.

The mutation frequencies of the cultures treated with carbetamide were within the range of solvent controls and no mutagenicity was observed according to the criteria for assay evaluation. Furthermore, no change was observed in the ratio of small to large mutant colonies.

In all main assays, positive controls elicited the expected positive responses except MMS 15 in the 24-hour experiment without S9 mix. The mutation frequency was $78.52/10^6$ cells which is outside the historical range ($135.1\text{-}630.8/10^6$).

The results are presented in the tables below:

**Experiments without metabolic activation**

| Concentration | % relative survival (Plating efficiency 1 for survival) | % relative survival (Plating efficiency 2 for mutant frequency) | Mutation frequency $/10^6$ | Ratio small/large colonies |
|---|---|---|---|---|
| 1st experiment (3-hour exposure) | | | | |
| 0 (control)* | 100 | 100 | 25.58 | 1.06 |
| 2500 | 19 | 73 | 23.07 | 1.20 |
| 1250 | 66 | 86 | 25.15 | 1.33 |
| 625 | 68 | 91 | 18.62 | 1.20 |
| 312.5 | 55 | 90 | 22.33 | 1.17 |
| 156.30 | 69 | 78 | 25.77 | 1.17 |
| MMS 15 | 2 | 13 | 406.94 | 3.25 |
| MMS 10 | 7 | 37 | 147.19 | 3.25 |
| 2nd experiment (24-hour exposure) | | | | |
| 0 (control)* | 100 | 100 | 28.20 | 1.00 |
| 2500 | 11 | 99 | 24.43 | 1.40 |
| 1250 | 30 | 77 | 20.71 | 1.00 |
| 625 | 34 | 62 | 25.88 | 1.00 |
| 312.5 | 35 | 65 | 30.93 | 1.00 |
| 156.30 | 74 | 112 | 17.90 | 1.00 |
| MMS 15 | 4 | 68 | 78.52 | 3.33 |
| MMS 10 | 6 | 12 | 497.37 | 3.00 |

* mean value of two parallel cultures

**Experiments with metabolic activation**

| Concentration | % relative survival (Plating efficiency 1 for survival) | % relative survival (Plating efficiency 2 for mutant frequency) | Mutation frequency $/10^6$ | Ratio small/large colonies |
|---|---|---|---|---|
| 1st experiment (3-hour exposure) | | | | |
| 0 (control)* | 100 | 100 | 26.94 | 1.08 |
| 2500 | 13 | 75 | 28.71 | 1.00 |
| 1250 | 63 | 89 | 31.43 | 1.17 |
| 625 | 83 | 87 | 32.34 | 1.17 |
| 312.5 | 77 | 101 | 27.66 | 1.17 |
| 156.30 | 73 | 97 | 26.60 | 1.00 |
| 3-MC 4.0 | 30 | 22 | 346.88 | 1.19 |
| 3-MC 2.5 | 35 | 83 | 113.69 | 1.21 |
| 2nd experiment (3-hour exposure) | | | | |
| 0 (control)* | 100 | 100 | 27.00 | 1.24 |
| 2500 | 9 | 41 | 44.93 | 1.00 |
| 1250 | 76 | 99 | 24.35 | 1.17 |
| 625 | 74 | 98 | 24.69 | 1.17 |
| 312.5 | 95 | 101 | 22.12 | 1.00 |
| 156.30 | 74 | 102 | 20.01 | 1.20 |
| 3-MC 4.0 | 20 | 38 | 174.90 | 1.19 |

| | | | | |
|---|---|---|---|---|
| 3-MC 2.5 | 23 | 40 | 153.49 | 1.29 |

* mean value of two parallel cultures

- **Conclusion**

Carbetamide tested up to cytotoxic concentrations in the absence and presence of metabolic activation did neither induce mutations nor had any chromosomal aberration potential.

***Clastogenicity***

- **Reference:** **August M. (2006):** In vitro assessment of the clastogenic activity of carbetamide in cultured human peripheral lymphocytes. LPT, Germany. Unpublished report No.20079/06
- **Test methods**: OECD guideline for testing chemicals No.473 (07/21/1997): *In vitro* mammalian Chromosome Aberration Test / EC method B.10. Mutagenicity- *in vitro* mammalian Chromosome Aberration Test, 2000/32/EC (05/19/2000).
- **GLP standards**: Yes
- **Deviations**:

  *From BPL:* no control of the treatment solutions concentrations has been performed: homogeneity, stability of the test item applied in the vehicle (OECD 1997, paragraph 6.2 point 5).

  *From 476 OECD guideline:* none
- **Study acceptance**: Yes
- **Batch**: 020946
- **Purity**: 97.6%
- **Test system**

Carbetamide was assayed in an *in vitro* cytogenetic study using human lymphocyte cultures both in presence and in absence of metabolic activation by a rat liver postmitochondrial fraction (S9 mix) from Arochlor 1254 induced animals. The test was carried out employing 2 exposure times without S9 mix: 3 and 24 hours and one exposure time carried out twice with S9 mix: 3 hours. The study was conducted in duplicate.
In a cytotoxicity preliminary test, cytotoxicity, haemolysis and test item precipitation were noted at the top concentration of 5000 µg/mL with or without metabolic activation at any exposure time. Furthermore, the concentration of 2500 µg/mL also induced the test item precipitation. A decrease (>50%) in the mitotic index was observed at 1000 µg/mL and above in the 24-hour exposure experiment without S9 mix and at 2500 and 5000 in the 4-hour exposure experiment with S9 mix

In the main study, 4 doses were tested: 156.3, 312.5, 625 and 1250 µg/mL in experiments without metabolic activation and 625, 1250, 2500 and 5000 in experiments with metabolic activation. It also included negative solvent (DMSO) and positive controls: Mitomycin C for experiments without S9 mix and Cyclophosphamide for experiments with S9 mix.

- **Results**

*Test without metabolic activation (4- and 24-hour exposure)*

The mean incidence of chromosomal aberrations (excluding gaps) of cells treated with carbetamide at concentrations from 156.3 to 1250 µg/mL in the absence of S9 mix is considered to be within the normal range of the solvent control.

The positive control elicited the expected results.

The results are presented in the table below:

| Treatment (µg/mL medium) | 1st experiment (4-h exposure) | | 2nd experiment (24-h exposure) | |
|---|---|---|---|---|
| | Mitotic index | % of cells with aberrations excluding gaps | Mitotic index | % of cells with aberrations excluding gaps |
| 0 (DMSO) | 1.00 | 1.5 | 1.00 | 1.5 |
| 156.3 | 1.07 | 1.5 | 0.77 | 0.5 |
| 312.5 | 0.92 | 1.0 | 0.81 | 1.0 |
| 625 | 0.85 | 1.0 | 0.63 | 3.5 |
| 1250 | 0.32 | 1.0 | 0.28 | 1.5 |
| Mitomycin C 0.1 | 1.02 | 11.0* | 0.58 | 23.0* |

* significantly different from the negative control ($p \leq 0.05$)

*Test with metabolic activation (4-hour exposure)*

The positive control elicited the expected results.

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with carbetamide at concentrations from 625 to 5000 µg/mL in the presence of S9 mix is considered to be within the normal range of the solvent control.

However, a chromatid/chromosome exchange event was observed in two independent assays in presence of S9 at the higher concentration tested (5000 µg/ml). This rare event corresponds to a real clastogenic effect (a break following by a reparation) and was not relied to a eventual interference.

Anyway, an exchange was also observed in the two negative controls in the short term assay without S9.

The results are presented in the table below:

| Treatment (µg/mL medium) | 1st experiment (4-h exposure) | | 2nd experiment (4-h exposure) | |
|---|---|---|---|---|
| | Mitotic index | % of cells with aberrations excluding gaps | Mitotic index | % of cells with aberrations excluding gaps |
| 0 (DMSO) | 1.00 | 3.0 | 1.00 | 2.0 |
| 625 | 1.00 | 2.0 | 0.83 | 0.5 |
| 1250 | 0.97 | 2.0 | 0.85 | 1.0 |
| 2500 | 0.71 | 1.0 | 0.85 | 2.0 |
| 5000 | 0.33 | 3.5 | 0.20 | 4.0 |
| Cyclophosphamide 20 | 0.73 | 16.0* | 0.41 | 17.0* |

* significantly different from the negative control ($p \leq 0.05$)

- **Conclusion**

Carbetamide, tested up to cytotoxic concentrations in the absence and in the presence of metabolic activation, revealed no indications of mutagenic properties with respect to chrosomosal or chromatid damage.

***In vitro sister chromatide exchange test in CHO cells***

- **Reference:** **Ivett JL (1984):** Mutagenicity evaluation of carbetamide technical ref. 04040.024 in an in vitro sister chromatid exchange assay in chinese hamster ovary (CHO) cells. Litton Bionetics, Inc., Kensington, Maryland, USA - Unpublished report N° 20990 E-9265 - Dates of work: 21/07/1984 to 00/12/1984.
- **Guidelines:** Not mentioned.
- **GLP standards:** Yes.
- **Deviations:** One single assay with and without metabolic activation.
- **Study acceptable:** As supportive
- **Test method:**

Chinese Hamster Ovary (CHO) cells were exposed to technical carbetamide (batch 04040.02, unknown purity). The test article's solubility in DMSO and its cytotoxicity towards CHO cells were evaluated in preliminary tests without and with metabolic activation (S-9 fraction of liver homogenate prepared from Aroclor 1254 treated Sprague Dawley rats and S-9 cofactor mix). Precipitates were observed without metabolic activation at doses higher than 2.25 mg/mL.

Based on the results of these assays, the concentrations of 7.5, 22.5, 75, 225 and 750 µg/mL without metabolic activation, and 75, 225, 750 and 2,250 µg/mL with metabolic activation were selected for the main tests. The main tests included negative solvent and positive controls mitomycin C (-S9) or cyclophosphamide (+S9) and evaluated the frequency of sister chromatid exchanges.

- **Results**

The results of the preliminary tests are summarized in Table 43 (see previous study summary under B.6.4.1.2.1.). In the test without metabolic activation, carbetamide was toxic or delayed mitosis at concentrations of 2.25 mg/ml or higher. No toxicity was observed at any tested concentration with metabolic activation at the highest achievable concentration.

There was no difference in frequency of SCEs (neither per chromosome nor per cell) between carbetamide at any tested concentration and negative controls, with or without metabolic activation. Negative control ranges were within normal historical changes and positive controls produced statistically significant increases of aberrations (see Table 43).

Table 43: Sister chromatid exchanges in CHO cells treated with carbetamide

| Treatment | | Cell cycle stages (%)[a] | | | | Time in BrdU (hrs) | SCEs /chromosome | SCEs/cell |
|---|---|---|---|---|---|---|---|---|
| Nature | Conc. µg/ml | M1 | M1+ | M2 | M2+ | | | |
| Without metabolic activation | | | | | | | | |
| None | | 1 | 3 | 95 | 1 | 25.83 | 0.34 | 7.06 |
| DMSO Solvent Control 10 µL/mL | | 1 | 5 | 94 | | 25.83 | 0.44 | 9.12 |
| Mitomycin C | 0.005 | | 5 | 95 | | 25.83 | 0.94 | 19.6 |
| carbetamide | 7.5 | | 6 | 94 | | 25.83 | 0.43 | 9.08 |
| | 22.5 | 1 | 4 | 95 | | 25.83 | 0.35 | 7.24 |
| | 75 | 2 | 11 | 87 | | 25.83 | 0.37 | 7.72 |
| | 225 | 36 | 42 | 22 | | 31.83 | 0.43 | 9.12 |
| With metabolic activation | | | | | | | | |
| None | | 2 | | 96 | 2 | 25.83 | 0.36 | 7.58 |
| DMSO Solvent Control 10 µL/mL | | 1 | | 97 | 2 | 25.83 | 0.45 | 9.44 |
| Cyclophosphamide | 0.0015 | | 4 | 90 | 6 | 25.83 | 1.06 | 22.2 |
| carbetamide | 75 | | 1 | 86 | 13 | 25.83 | 0.42 | 8.9 |
| | 225 | 2 | | 77 | 21 | 25.83 | 0.43 | 9.1 |
| | 750 | | | 94 | 6 | 25.83 | 0.41 | 8.58 |
| | 2,250 | 4 | 1 | 88 | 7 | 25.83 | 0.43 | 9.00 |
| a: % cells that have completed one (M1), between one and two (M1+), two (M2), or more than two (M2+) cycles in BrdU. | | | | | | | | |

- **Conclusion**

In a study which cannot be considered as acceptable for regulatory evaluation, because it was conducted without repeat without metabolic activation and with a batch of unknown purity, carbetamide technical did not increase the number of sister chromatid exchanges when tested in the presence or absence of metabolic activation in CHO cells.

***Unscheduled DNA synthesis assay in rat hepatocytes***

- **Reference:** **Cifone MA (1984):** Evaluation of carbetamide technique in the rat primary hepatocyte unscheduled DNA synthesis assay. Litton Bionetics, Inc., Kensington, Maryland, USA - Unpublished report No. 20991 - Dates of work: 15/05/1984 to 12/08/1984.
- **Guidelines:** Procedures described by Williams G.M. Cancer Res. 37:1845-1851 (1977) and Chemical Mutagens, Plenum Press, NY, 1980, pp. 61-79. OECD Guideline 482 is based on comparable protocol.
- **GLP**: Yes.
- **Deviations**: Only one assay was conducted without repetition.
- **Study acceptable:** As supportive
- **Test method:**

Primary hepatocyte cultures were established by collagenase perfusion of the liver of one male Fischer 344 rat.

Culture dishes (35 mm) were seeded with approximately 0.5 x $10^6$ viable cells and left for 1.5 to 2 hours for cell attachment. The UDS test was then initiated by replacing the culture medium by 10 different treatments containing tritiated thymidine and either carbetamide (batch 04040.02, purity unknown, at 15 concentrations ranging from 0.025 to 1,000 µg/mL), negative control (vehicle: 1% DMSO) or positive control (2-acetyl aminofluorene 2-AAF, 0.10 µg/mL). Treatment was conducted for 18-19 hours on five cultures per treatment dose, from which 3 were used for counting nuclear and cytoplasmic grains, while the 2 others were used for determination of cell viablility.

Cultures from 8 carbetamide dose-levels (ranging from 19.4 to 826 µg/mL) with adequate viability after treatment were selected for UDS analysis. The net nuclear grain count was determined for 50 randomly selected cells from each of the 3 cultures for each treatment and dose-level.

- **Results**

Toxicity was induced by carbetamide over a wide range of concentrations (Table 44) The 826 µg/mL concentration was highly toxic (10.8% survival). The survival improved gradually with decreasing concentrations to reach values greater than 90% at155 µg/mL and below. Some survival rates were higher than 100% due to cell distribution variations and inaccuracies in cell counting.

The positive control elicited the expected nuclear labeling. None of the treatments with carbetamide caused nuclear labeling significantly different from the solvent control, and there was no evidence of dose-relationship in the distribution of netnuclear grain counts in treated hepatocytes (Table 44).

Table 44: Summary results of in vitro UDS with carbetamide on rat hepatocytes

| Test group | Concentration (µg/ml) | UDS grains per nucleus | Average nuclei with ≥ 6 grains | Average nuclei with ≥ 20 grains | % Survival at 20.5 hours |
|---|---|---|---|---|---|
| DMSO 1% | | 1.46 | 2.0 | 0.0 | 100.0 |
| 2-AAF | 0.10 | 15.88 | 89.3 | 24.7 | 84.7 |
| carbetamide | 826 | 0.91 | 0.7 | 0.0 | 10.8 |
| | 620 | 1.03 | 0.0 | 0.0 | 50.2 |
| | 413 | 0.95 | 0.0 | 0.0 | 70.2 |
| | 310 | 0.73 | 0.7 | 0.0 | 84.5 |
| | 155 | 0.99 | 1.3 | 0.0 | 97.2 |
| | 77.4 | 1.16 | 0.7 | 0.0 | 101.1 |
| | 38.7 | 1.07 | 2.0 | 0.0 | 104.6 |
| | 19.4 | 1.11* | 1.0* | 0.0* | Not determined |

*: results corresponding to only two coverslips (100 cells) instead of three (150 cells).

- **Conclusion**

In an unscheduled DNA synthesis test conducted with a batch of unknown purity and without independent repeat, carbetamide did not induce significant changes in the nuclear labeling of primary rat hepatocytes when compared to solvent controls, within a concentration range from 19.4 to 826µg/mL.

### 4.9.1.2 In vivo data

The genotoxic potential of carbetamide has been investigated in two bone marrow micronucleus tests in CD1 and NMRI mice by oral route and on one unscheduled DNA-synthesis (UDS) Test in the liver of orally dosed male rats using an *in vivo/in vitro* procedure

**Micronucleus in mouse bone marrow**

- **Reference:** **Cordier A, Fournier E (1984):** carbetamide (11561 RP) - Micronucleus test in mice by the oral route. Rhône-Poulenc, Centre de Recherche, Vitry sur Seine, France - Unpublished report No. ST/CRV/Tox.N° 22145 - Dates of work: 09/02/1984 to 09/05/1984.
- **Guidelines:** Not stated. Design of the study is roughly in accordance with current OECD guideline 474 "Mammalian Erythrocyte Micronucleus Test" (1983).
- **GLP standards:** Yes.
- **Deviations:** Per animal, 500 polychromatic erythrocytes were examined in bone marrow smears, instead of 200 in bone marrow and 1000 in blood.
- **Study acceptable:** Yes
- **Test method:**

Technical carbetamide (batch MAG 1326, purity 96.0%) was administered orally to CD1 mice (8 week old), by gavage as a suspension in an aqueous solution of 10% arabic gum. The dose-levels were 325, 650 and 975 mg/kg. These dose-levels were chosen to reach up to 75% of a OF1 mouse oral $LD_{50}$ of 1,300 mg/kg determined in a previous assay, but actually reach less than 60% of the CD1 mice $LD_{50}$ (1718 mg/kg). Negative control (vehicle only) and positive controls (intraperitoneal injection of mitomycin C) were included. There were 10 males and 10 females in each of the 5 groups. One half of each group was treated on a single occasion and sacrificed 30h post administration, the other half was treated twice at a 24h interval and sacrificed 24h after the second administration.

For each mouse, bone marrow was collected from one femur and 2 marrow smear slides were prepared. Micronuclei formation was scored for each slide on 500 polychromatic erythrocytes by two different investigators. Polychromatic erythrocytes (PE) and normochromatic erythrocytes (NE) were counted on each slide to determine medullar cytotoxicity: R=100*PE/NE.

- **Results**

For both males and females, none of the values of PE/NE differed from negative controls, whatever the administration scheme (Tables 6.4.2.-1 and 6.4.2.-2). Yet there was no proof of exposition: neither clinical signs nor measure of the test item's plasma level.

The frequency of micronucleated PE was significantly ($p<0.01$) increased in positive controls treated with mitomycin C, indicating the sensitivity of the test system under our experimental conditions.

The frequency of micronucleated PE in carbetamide treated mice was not different at any dose-level from negative controls, whatever the administration scheme (Tables 45 and 46). Carbetamide has no genotoxic effect on bone marrow cells.

Table 45: Medullar toxicity and micronucleus formation after a single administration of carbetamide

| Treatment | Dose (mg/kg bw) | Group mean ratio of PE/NE | | Group mean frequency of micronucleated PE (per 1000) | |
|---|---|---|---|---|---|
| | | Males | Females | Males | Females |
| Vehicle only | - | 2.18 | 1.28 | 0.2 | 0.6 |
| Mitomycin C | 1 | 1.89 | 1.61 | 28.8* | 20.2* |
| carbetamide | 325 | 1.72 | 1.84 | 2.4 | 0.2 |
| | 650 | 1.63 | 1.81 | 0.4 | 0.4 |
| | 975 | 1.70 | 1.97 | 0.3 | 0.2 |
| **PE:** polychromatic erythrocytes; **NE:** normochromatic erythrocytes; *: significantly different when sexes are combined ($p<0.01$) | | | | | |

Table 46: Medullar toxicity and micronucleus formation after two administrations (24h interval) of carbetamide

| Treatment | Dose (mg/kg bw) | Group mean ratio of PE/NE | | Group mean frequency of micronucleated PE (per 1000) | |
|---|---|---|---|---|---|
| | | Males | Females | Males | Females |
| Vehicle only | - | 1.61 | 1.58 | 0.2 | 0.6 |
| Mitomycin C | 1 | 1.11 | 0.95 | 19.8* | 15.8* |
| carbetamide | 325 | 1.48 | 1.82 | 0.4 | 0.4 |
| | 650 | 1.76 | 1.47 | 0.6 | 0.8 |
| | 975 | 1.89 | 1.33 | 0.3 | 0.2 |
| **PE:** polychromatic erythrocytes; **NE:** normochromatic erythrocytes; *: significantly different when sexes are combined ($p<0.01$) | | | | | |

- **Conclusion**

Under the experimental conditions of the study, carbetamide did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after 1 or 2 oral administrations (with a 24-hour interval) at the dose-levels of 325, 650 and 975 mg/kg. Yet, as there were neither mentions of clinical signs nor any measure of plasma levels of the test item, systemic and medullar exposition to the test item remain to be demonstrated. Therefore, the results of the study do not allow the exclusion of a clastogenic potential of the test item.

- **Reference:** **August M. (2006):** Micronucleus test of carbetamide in bone marrow cells of the NMRI mouse by oral administration. LPT, Germany. Unpublished report No.20081/1/06
- **Guidelines**: OECD guideline for testing of chemicals No. 474 (07/21/1997): Mammalian Erythrocyte Micronucleus Test / EC directive 2000/32/EC: Methods for determination of toxicity. Micronucleus Test B.12. (05/19/2000).
- **GLP standards**: Yes
- **Deviations**:

  *From BPL:* no control of the treatment solutions concentrations has been performed : homogeneity, stability of the test item applied in the vehicle( OECD 1997, paragraph 6.2 point 5).

  *From 474 OECD guideline:* none

  *Others:* the test item was suspended to the appropriate concentrations in 0.8% hydroxypropylmethylcellulose gel and not in 0.5% hydroxypropylmethylcellulose gel as stated in the Study plan.

Negative reference item: "injection speed: dose/5 min" was omitted.
*These deviations did not affect the validity of the study.*

- **Study acceptance**: Yes
- **Batch**: 020946
- **Purity**: 97.6%
- **Test system**

Carbetamide was administered orally to NMRI mice by gavage as a suspension in 0.8% hydroxypropylmethylcellulose gel.
The dose levels have been selected based on a preliminary experiment employing one animal per dose and sex. Three doses: 500, 1000 and 2000 mg carbetamide/kg b. w. were tested. Slight to moderate signs of toxicity were noted at the top dose of 2000 mg/kg b.w.: slightly to moderately reduced motility, slight to moderate ataxia and slight dyspnoea.

In the main study, the dose level were 500, 1000 and 2000 mg carbetamide/kg b.w. Further groups received the vehicle and one further group the positive reference item cyclophosphamide (27 mg/kg b.w., ip). Each group consisted in 5 male and 5 female mice. Two sampling times were employed in this study: 24 h after administration (for the negative reference item group, the 3 doses of test item groups and the positive reference item group) and 48 h after administration (for the negative reference item group and the top dose of the test item group).

Moderate signs of toxicity similar to those seen in the preliminary experiment were observed at the dose of 2000 mg/kg b.w. until 6 h after administration.
Immediately after sacrifice, bone marrow smears were prepared. Two thousands erythrocytes were evaluated per animal.

- **Results**

The highest dose of 2000 mg carbetamide/kg b.w. orally administered did not result in an increase in the incidence of micronucleated polychromatic erythrocytes (PCE).
The ratio polychromatic to normochromatic erythrocytes (NCE) was not influenced.
The positive control elicited the expected results.

The results are presented in the table below:

| Test item (mg/kg b.w.) | Sampling time (h) | Ratio PCE/NCE (mean) | Micronucleated polychromatic erythrocytes (mean frequency per 1000 PCE) |
|---|---|---|---|
| 0 (vehicle) | 24 | 0.52 | 2.2 |
| 500 | 24 | 0.36 | 2.5 |
| 1000 | 24 | 0.44 | 1.7 |
| 2000 | 24 | 0.37 | 2.3 |
| 0 (vehicle) | 48 | 0.51 | 1.7 |
| 2000 | 48 | 0.54 | 2.4 |
| Cyclophosphamide 27 | 24 | 0.48 | 11.4* |

* significantly different from the negative control ($p \leq 0.05$)

- **Conclusion**

Carbetamide, tested up to 2000 mg/kg b.w. by oral administration showed no genotoxic properties in the mouse bone marrow micronucleus study at the two tested sampling times of 24 and 48 hours.
But, at any dose, the ratio PCE/NCE was not significantly different from the ratio for the vehicle. That means that carbetamide, even at the highest dose of 2000 mg/kg b.w. was not toxic to bone marrow.
A pharmacokinetic evaluation in this study has been provided and no proofs of bone marrow exposure were given, only blood samples were tested.

**Pharmacokinetic evaluation**

- **Reference:** **August M. (2006):** Micronucleus test of carbetamide in bone marrow cells of the NMRI mouse by oral administration – pharmacokinetic evaluation. LPT, Germany. Unpublished report No.20081/2/06

- **Guidelines**: OECD guideline for testing of chemicals No. 474 (07/21/1997): Mammalian Erythrocyte Micronucleus Test / EC directive 2000/32/EC: Methods for determination of toxicity. Micronucleus Test B.12. (05/19/2000).
- **GLP standards**: Yes
- **Deviations**:

  *From BPL:* no control of the treatment solutions concentrations has been performed : homogeneity, stability of the test item applied in the vehicle (OECD 1997, paragraph 6.2 point 5).

  *From 474 OECD guideline:* none

  *Others:* the test item was suspended to the appropriate concentrations in 0.8% hydroxypropylmethylcellulose gel and not in 0.5% hydroxypropylmethylcellulose gel as stated in the Study plan.

  Negative reference item: "injection speed: dose/5 min" was omitted.

*These deviations did not affect the validity of the study.*

- **Batch**: 020946
- **Purity**: 97.6%
- **Study acceptance**: Yes
- **Test system**

A satellite group of 24 female mice was dedicated to the pharmacokinetic evaluation. They were administered orally a freshly-prepared suspension containing the labelled test item and the unlabelled test item. A dose level of 2000 mg Carbetamide/kg b.w. (nominal radioactive dose of 70.3 MBq/kg b.w.) was tested. Plasma and bone marrow were removed.
Eight sampling times were employed: 0.25, 0.5, 1, 1.5, 2, 3, 4 and 8 hours after administration.
Only plasma samples were analysed. Bone marrow samples will be examined on request.

- **Results**

The mean pharmacokinetic parameters in mouse plasma following single oral administration of 2000 mg/kg of carbetamide are given in the following table:

| Route of administration | Cmax (mg-eq/mL) | Tmax (h) | $t_{1/2}$ elim (h) | AUC (0-8h) (mg-eq x h/mL) | AUC (0-inf) (mg-eq x h/mL) |
|---|---|---|---|---|---|
| p.o. | 1.554 | 1.5 | 2.58 | 4.856 | 5.617 |

- **Conclusion**

Carbetamide is present in mice plasma. Only female mice were studied.
No strong proofs of bone marrow exposure have been given.

**Unscheduled DNA synthesis**

- **Reference:** **Flügge, C. (2008):** Unscheduled DNA synthesis (UDS test of carbetamide by oral administration to CD rats- In vivo/in vitro study. LPT, Germany. Unpublished report No. 22839
- **Guidelines**: OECD guideline for testing of chemicals No. 486 (07/21/1997): Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells In vivo / EC Method L 136 B.39, 'Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*'(2000/32/EC).
- **GLP standards**: Yes
- **Deviations**:

  *From BPL:* no control of the treatment solutions concentrations has been performed: homogeneity, stability of the test item applied in the vehicle( OECD 1997, paragraph 6.2 point 5).

  *From 486 OECD guideline:* no indication was given concerning the sampling time point of the historical controls (2 hours, 16 hours or both times)

  Absence of the criteria for considering study as positive, negative or equivocal.

*These deviations did not affect the validity of the study.*

- **Batch**: 020946
- **Purity**: 97.03%
- **Study acceptance**: Yes
- **Test system**

Carbetamide was tested for its ability to induce unscheduled DNA-synthesis (UDS) in the liver of orally dosed male rats using an *in vivo/in vitro* procedure.

Carbetamide technical was administered orally by gavage at dose levels of 500, and 1000 mg/kg. Hydroxypropylmethylcellulose gel (0.8%) was used as the vehicle. Dimethylnitrosamine and 2-Acetaminofluorine served as positive controls. For the negative control, a further group of 6 male rats per sampling time point selected received the vehicle. Six male CD rats (strain CD / Crl: CD(SD)), 58 - 66 days old, with a body weight of 265 - 336 g were employed per sampling interval and group. Hence, a total of 48 animals were employed. Of these, 5 animals per group were processed for the liver perfusion. The following dose scheme was applied.

Table 47: Test conditions of the unscheduled DNA synthesis in rats

| Group | Dose of Carbetamide (mg/kg b.w., p.o.) | Sampling time (h) after dosing | Number and sex of animals incl. spare animals |
|---|---|---|---|
| 1<br>low dose | 500 | 2 | 6 males |
| 2<br>low dose | 500 | 16 | 6 males |
| 3<br>high dose | 1000 | 2 | 6 males |
| 4<br>high dose | 1000 | 16 | 6 males |
| 5<br>positive control 1 | 10 mg DMN/kg b.w., p.o.<br>dissolved in aqua ad iniectabilia | 2 | 6 males |
| 6<br>positive control 2 | 50 mg 2-AAF/kg b.w., p.o.<br>dissolved in corn oil | 16 | 6 males |
| 7<br>control 1/vehicle | 20 mL 0.8% aqueous hydroxylpropylmethyl-cellulose/kg b.w., p.o. | 2 | 6 males |
| 8<br>control 2/vehicle | 20 mL 0.8% aqueous hydroxylpropylmethyl-cellulose/kg b.w., p.o. | 16 | 6 males |

Two hours (experiment 1) or 16 hours (experiment 2) after dosing, the animals were killed and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were prepared from 5 animals in each dose group and were treated with [3H] thymidine. For each animal 6 slides with fixed hepatocytes were prepared and dipped in photographic emulsion to prepare autoradiograms. Two of the six slides were examined microscopically. The net nuclear grain count (NNG), the number of grains present in the nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm, were determined for each slide, animal and dose group.

- **Result**

An orientating preliminary test was carried out prior to the main test using 2 male animals/dose and revealed 1000 mg/kg to be the maximum tolerated dose.
Negative control animals revealed a mean NNG value of -0.28 (2-h and 16-h sampling time) and <0.01% of cells in repair with a NNG ≥ 5.

Treatment with 500 or 1000 mg Carbetamide/kg b.w. in male rats did not produce a mean NNG value greater than 0.05 and <0.01% of cells in repair with a NNG ≥ 5. It should be noted that 2 of the 3 animals treated with the higher dose of 1000 mg/kg (sampling time 16 hours) showed a NNG > 0, with values comprised between 0.02 and 0.13. However, these values were in the range of the historical control values (between -8.86 and 0.75).

The number of S-phase cells was not given, thus its not possible to conclude on the potential proliferative effect of carbetamide.

The NNG values obtained for Carbetamide are within/below the historical control values. Hence, Carbetamide showed no ability to induce unscheduled DNA synthesis in CD rats at the two tested sampling times of 2 and 16 hours.

- **Conclusions**

Under the present test conditions, Carbetamide tested up to the maximum tolerated dose level of 1000 mg/kg b.w., p.o. in male rats showed no ability to induce unscheduled DNA synthesis in CD rats at the two tested sampling times of 2 hours and 16 hours, respectively.

### 4.9.2 Human information

No data

### 4.9.3 Other relevant information

No data

### 4.9.4 Summary and discussion of mutagenicity

**In vitro data**

Carbetamide was devoid of mutagenic activity in an in vitro mutagenicity assay when tested in Tester strains TA1535, AT1537, TA98 and TA100 with or without the metabolic activation system S9. A negative result was also obtained in the spot test (Benazet & Cartier, 1977). In a second bacterial mutagenicity assay on *Salmonella typhimurium* Carbetamide was tested in strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 with and without liver microsomal S9 homogenate from Aroclor 1254 treated rats at non-toxic concentrations of 312, 625, 1250, 2500, and 5000 µg/plate. The collective results showed that Carbetamide is devoid of mutagenic activity in this *in vitro* mutagenicity assay when tested with or without the metabolic activation system S9. Effects seen in TA1535 and TA1538 without S9 in the first trial were weak and not dose dependent. Moreover, they were not confirmed by two further assays (Cordier & Bonneau, 1984).

Carbetamide has been tested in *in vitro* mammalian cell systems. Carbetamide was assayed for the potential to induce chromosomal aberrations in CHO cells with and without metabolic activation. In the presence of a metabolic activation system Carbetamide was less toxic and could be tested at higher doses than without S9. A positive results observed in the first trial in the presence of S9 was not confirmed in the second trial. In conclusion, Carbetamide technical Ref. 04040.02 did not increase the number of chromosome aberrations when tested in the presence or absence of the metabolic activation system S9 in CHO cells (Ivett, 1984a).

Carbetamide was assayed for Sister Chromatid Exchange (SCE) in CHO cells with and without metabolic activation. Carbetamide technical did not increase the number of Sister Chromatid Exchanges when tested in the presence or absence of the metabolic activation system S9 in CHO cells (Ivett, 1984b).

Carbetamide was assayed for clastogenic activity in human lymphocytes with and without metabolic activation. Carbetamide when tested up to cytotoxic concentrations in the absence (1250 µg/ml) and in the presence of metabolic activation (5000 µg/ml) employing two exposure times (without S9) and one exposure time (with S9) revealed no indications of mutagenic properties with respect to chromosomal or chromatid damage under the present test conditions. (August, 2006a).

In a mammalian cell forward mutation assay in L5178Y mouse lymphoma cells, carbetamide technical did not induce mutation in the TK locus of L5178Y TK +/- cells when tested in the presence of S9. However, in the absence of the metabolic activation system S9 slight increased number of mutants was observed. The increased mutant frequency was, however, associated with marked cytotoxicity. The finding is, therefore, of limited biological significance (Hoorn, 1985). In a second assay in L5178Y mouse lymphoma cells, carbetamide did not induce mutation in the TK locus of L5178Y TK +/- cells when tested up to cytotoxic concentrations in the presence or absence of the metabolic activation system S9. In addition, no change was noted in the ratio of small to large mutant colonies. Therefore, Carbetamide did also not exhibit clastogenic potential at the concentration-range investigated (August, 2006b).

**In vivo data**

The clastogenic activity of Carbetamide was tested in CD1 mice and in NMRI mice in vivo.

In CD-1 mice, significant or dose dependent micronuclei formation was not induced by Carbetamide at any concentration or observation time point (Cordier and Fournier, 1984). Carbetamide treatment did also not increase frequency of micronuclei formation after oral gavage in a recent study in NMRI mice. Indeed, no dose-dependent significant increase in the frequency of polychromatic erythrocytes with micronuclei were noted after oral administration of Carbetamide (August, 2006c). In a supplementary pharmacokinetic study to the recent micronucleus assay plasma levels of Carbetamide were determined and proved exposition of the bone marrow to Carbetamide (August, 2006d).

In a recent additional *in vivo* assay, Carbetamide with well-defined batch specification was assayed for induction of unscheduled DNA synthesis in hepatocytes. Carbetamide tested up to the maximum tolerated dose level of 1000 mg/kg bw, p.o. in male rats showed no ability to induce unscheduled DNA synthesis in CD rats at the two tested sampling times of 2 hours and 16 hours, respectively (Flügge et al., 2008).

Thus, Carbetamide was demonstrated to be not genotoxic *in vitro* or *in vivo*.

### 4.9.5 Comparison with criteria

**Considering the criteria in the CLP classification**, a substance shall be classified in category 2 for germ cell mutagenicity endpoint if the substance causes concern for humans owing to the possibility that they may induce heritable mutation in the germ cells of humans. This classification is based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
    - Somatic cell mutagenicity tests in vivo, in mammals (mammalian bone marrow chromosome aberration test, mouse spot test or mammalian erythrocyte micronucleus test); or
    - Other in vivo somatic cell genotoxicity test (UDS or SCE assay) which are supported by positive results from in vitro mutagenicity assays (in vitro mammalian chromosome aberration test, in vitro mammalian cell gene mutation test or bacterial reverse mutation test).

Available in vitro and in vivo data showed that carbetamide is not genotoxic *in vitro* or *in vivo*.

### 4.9.6 Conclusions on classification and labelling

In this context, the available data did not support a classification for carbetamide for mutagenicity end-points.

## 4.10 Carcinogenicity

A combined chronic toxicity / oncogenicity study was conducted in rats by dietary administration for 2 years, and one oncogenicity study was conducted in mice by dietary administration for 2 years.

Table 48: Summary table of relevant carcinogenicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **104-week oral (dietary) carcinogenicity study**<br><br>**Rat F-334 (60/sex)**<br><br>**Carbetamide: 160, 1,200 and 9,000 ppm** | **NOAEL = 160 ppm**<br>**(6 mg/kg/day in males, 8 mg/kg/day in females)**<br>**LOAEL = 1200 ppm**<br><br><u>At 9000 ppm:</u><br>- Lower BW gain and food intake<br>- Lower PCV, Hb and RBC, and higher platelet counts;<br>- Lower plasmatic Ach-E in females<br>- Higher RBC Ach-E in both sexes<br>- High Brain Ach-E in females<br>- Higher liver and kidney relative weights (males) & lower testes absolute and relative weights<br>- Centrilobular hepatocyte hypertrophy, fatty vacuolation<br>- Thyroid follicular epithelial hypertrophy (2 sexes)<br>- Increased incidence of astrocytoma (female)<br><br><u>From 1200 ppm:</u><br>- Higher plasma urea, plasma phosphorus<br>- Higher RBC Ach-esterase activities (female only) | **Guidelines:** OECD N° 453, 1981.<br><br>**GLP:** Yes.<br><br>**Deviation**: none<br><br>**Study acceptable:** Yes.<br><br>**Purity**: 95.0 – 97.4% (batches 8044001, 8044002 and 85345) | Amyes et al., 1988a |

| | | | |
|---|---|---|---|
| **104-week oral (dietary) carcinogenicity**<br><br>**Mice B6C3F1 (52/sex)**<br><br>**Carbetamide 160, 1,200 and 9,000 ppm** | **NOAEL = 160 ppm**<br>**(20.1 mg/kg/day in males, 22.7 mg/kg/day in females)**<br>**LOAEL = 1200 ppm**<br><br>At 9000 ppm:<br>- Lower BW , higher liver relative weight<br>- Kidney relative weight (lower in males, higher in females)<br>- Thyroid adenomas, phaeochromocytomas (females), cholangiocarcinomas (males)<br><br>From 1200 ppm:<br>- Increases in liver weight,<br>- Hepatic tumors in both sexes at 9000 ppm and only in females at 1200 ppm. | **Guidelines:** OECD N° 453, 1981.<br><br>**GLP:** Yes.<br><br>**Deviation**: none<br><br>**Study acceptable:** Yes.<br><br>**Purity**: 95.0 – 97.4% (batches 8044001, 8044002 and 85345) | Amyes et al., 1988b |

## 4.10.1 Non-human information

## 4.10.1.1 Carcinogenicity: oral

### *4.10.1.1.1* Rats

- **Reference:** **Amyes SJ, Marris CJ, Brown PM, Lee P, Virgo DM, Fowler JSL (1988):** carbetamide: combined oncogenicity and toxicity study in rats. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 87/RHA058/749 - Dates of work: 06/02/1985 to 25/02/1987.
- **Guidelines:** OECD N° 453, 1981.
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

A total of 180 male and 180 female F-344 rats (5-6 weeks old, bw ranging 79-119 g for males and 72-102 g for females) were randomly allocated to 3 groups of 60 males and 60 females given technical carbetamide (batches N° 8044001, 8044002 and 85345, purity: 95.0 to 97.4%) in the diet at the concentrations of 160, 1200 or 9000 ppm for 2 years. Another group of 60 males and 60 females (with same characteristics) received the diet without carbetamide and served as a control group. The first treatment day was noted D0.

The homogeneity and stability of carbetamide in the diet over the range of tested concentrations were validated in previous studies. Achieved concentrations were controlled at regular intervals during the study.

The animals were observed at least once daily for clinical signs and mortality. Detailed observations and palpations were conducted weekly. Each animal was weighed on D0, at weekly intervals for the first 14 weeks and once every two weeks thereafter. Mean food consumption was calculated weekly, and achieved dosages were calculated at the same intervals as body weight for each group and sex. Water consumption was measured on 5 cages for each sex and group over a 5-day period on designated weeks during the study.

Ophthalmology was conducted before the start of treatment and during weeks 51, 76-77 and 103 on all surviving animals from all groups.

Blood was sampled from 10 males and 10 females of each group on weeks 26, 52, 77, 102 and 104 for haematological investigations(packed cell volume, haemoglobin, erythrocyte count, total and differential leucocyte counts, platelet count, reticulocyte count, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration) , blood chemistry determinations (sodium, potassium, chloride, calcium, aspartate aminotransferase, alanine aminotransferase, glucose, urea, total bilirubin, alkaline phosphatase, total cholesterol, creatine phosphokinase, total protein, and electrophoretic protein fraction, at the exception of week 104), and erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was determined at study termination on 10 males and 10 females of each group. Urinalyses (specific gravity, volume, appearance, total reducing substances, microscopic examinations of sediment, protein, pH, glucose, blood, nitrites, urobilin, ketones and bilirubin) were performed after weeks 25, 51, 76, and 101 using overnight urine samples.

All surviving rats were sacrificed after 104 weeks of treatment, weighed, and subjected to necropsy.

The weights of designated organs (adrenals, brain, liver, kidneys, heart, pituitary, ovaries, testes, spleen, thymus, thyroid and uterus cervix) were recorded, selected tissues were preserved and histological examinations were performed on specified tissue samples (adrenals, aorta, epididymides, femoral bone with marrow and joint, harderian glands, heart, eyes with optic nerves, lungs, mammary glands caudal and cranial, trachea, oesophagus, thyroid, stomach, duodenum, thymus, jejunum, lymph node, ileum, caecum, spinal cord, skin, colon, salivary gland, liver, skeletal muscle, spleen, kidneys, seminal vesicles, urinary bladder, prostate, rectum, testes, uterus with cervix, ovary, pancreas, pituitary, sciatic nerve, brain, tumours and gross abnormalities) of all animals.

- **Results**

Concentration of carbetamide in the diet was within ±10% of nominal values except on rare occasions. Mean achieved concentrations were 154, 1160 and 8932 ppm.

Achieved dose-levels are summarized in Table 18. Approximate dose-levels throughout the majority of the treatment were 6, 50 and 400 mg/kg/kg/day in males and 8, 60 and 500 mg/kg/day in females receiving 160, 1200 and 9000 ppm respectively.

Table 49: Mean and extreme values of carbetamide dose-levels in rats given the test article in the diet

| Concentration | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|
| Sex | Males | Females | Males | Females | Males | Females |
| Mean (mg/kg/day) | 6 | 8 | 50 | 60 | 400 | 500 |
| Minimum (mg/kg/day) | 5.20 | 6.36 | 39.6 | 47.7 | 331 | 386 |
| Maximum (mg/kg/day) | 21.08 | 19.99 | 160.4 | 149.4 | 1194 | 1173 |

Mortality was similar in non-treated and carbetamide treated groups, whatever the treatment period (Table 49). Major death causes were various tumours (mainly pituitary tumours and leukaemia) and nephropathy, as usually found in this strain of rats.

Table 50: Summary of mortality in controls and carbetamide treated groups

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | Females | Males | Females | Males | Females | Males | Females |
| Total death/initial | 21/60 | 10/60 | 28/60 | 15/60 | 24/60 | 15/60 | 20/60 | 14/60 |
| Deaths weeks 1-54 | 5 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| Deaths week 55-termination | 16 | 10 | 28 | 14 | 24 | 15 | 19 | 12 |

The only treatment related clinical sign was a significantly higher incidence of a slight and transient staining of the urogenital region of the coat of both sexes at the concentration of 9000 ppm, compared to controls. It was noted from weeks 26 to 86 in most of the cases, with only few cases of staining observed after week 86.

No treatment related palpable swellings were observed, as the incidence of masses was not dose-correlated.

Body weight gain was significantly lower in male and female rats given 9000 ppm, during all the study (Table 50). Food intake values in rats receiving carbetamide at all concentrations were similar or minimally lower (-7% in females given 9000 ppm) to those of controls. Water intake was unaffected by treatment.

Table 51: Mean body weight gains in non-treated control and carbetamide treated groups

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | Females | Males | Females | Males | Females | Males | Females |
| Weeks 0-13 (g) | 249 | 105 | 255 | 108 | 250 | 106 | 224 | 99 |
| % controls | - | - | +2 | +3 | 0 | +1 | -10 | -6 |
| Weeks 0-52 (g) | 381 | 161 | 392 | 161 | 387 | 162 | 342 | 138 |
| % controls | - | - | +3 | 0 | +2 | +1 | -10 | -14 |
| Weeks 0-104 | 343 | 231 | 334 | 234 | 329 | 230 | 231 | 188 |
| % controls | - | - | -3 | +1 | -4 | 0 | -33 | -19 |

Anterior subcapsular lenticular opacities were observed at ophthalmoscopic examinations with higher incidences in rats given 9000 ppm of carbetamide, compared to controls on weeks 51, 76 and 77. On week 102, no difference was observed anymore between groups.

Lower PCV (up to -10%) and Hb (up to -9%) were observed at haematological examinations of rats given 9000 ppm on weeks 26, 52 and 77 compared to controls. In females these effects were associated with significantly lower RBC (up to -7%). Higher platelets counts (up to +43%) were observed on all occasions in both sexes treated with 9000 ppm.

Table 52: Haematological findings in controls and carbetamide treated rats

| **Group** | | **Not treated** | | **160 ppm** | | **1200 ppm** | | **9000 ppm** | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | Sampling time (week) | Males | Females | Males | Females | Males | Females | Males | Females |
| PCV (%) | W 26 | 49 | 51 | 50 | 51 | 50 | 50 | 47* | 49* |
| | W 52 | 50 | 51 | 50 | 51 | 50 | 50 | 46* | 47* |
| | W 77 | 50 | 51 | 49 | 51 | 50 | 51 | 45* | 47* |
| Hb (g/100mL) | W 26 | 15.2 | 15.7 | 15.5* | 15.6 | 15.5* | 15.2* | 14.6* | 14.9* |
| | W 52 | 15.9 | 16.4 | 16.2 | 16.5 | 16.1 | 16.2 | 14.9* | 15.3* |
| | W 77 | 15.9 | 16.6 | 15.4 | 16.4 | 16 | 16.3 | 14.4* | 15.6* |
| RBC ($10^6$/$cm^3$) | W 26 | 8.68 | 8.3 | 8.86 | 8.31 | 8.83 | 8.15 | 8.78 | 7.74* |
| | W 52 | 9.11 | 8.69 | 9.17 | 8.67 | 9.08 | 8.65 | 8.88 | 8.08* |
| | W 77 | 8.55 | 8.44 | 8.25 | 8.5 | 8.45 | 8.36 | 7.75* | 7.81* |
| | W 102 | 6.74 | 8.50 | 6.29 | 8.14 | 7.18 | 8.14 | 6.19 | 7.96* |
| Platelet count ($10^3$/$cm^3$) | W 26 | 392 | 381 | 403 | 399 | 387 | 408* | 480* | 476* |
| | W 52 | 529 | 464 | 462 | 448 | 520 | 479 | 622 | 530* |
| | W 77 | 432 | 376 | 485 | 394 | 462 | 405 | 618* | 470* |
| | W 102 | 456 | 366 | 463 | 402 | 563 | 413 | 640* | 464* |
| *: statistically significant (p<0,05) | | | | | | | | | |

Plasma urea concentrations (Table 52) were higher in rats treated with 9000 ppm (up to +96% in males on weeks 26, 52, 77 and 102; up to +62% in females on weeks 26 and 52) and in some rats treated with 1200 ppm (males on weeks 26 and 102 and females on weeks 52 and 77). Higher plasma phosphorus concentrations were observed in males given 9000 ppm (weeks 77 and 102: up to +31%) or 1200 ppm (week 102: +27%). Plasma butyrylCh-esterase and ACh-esterase activities were lower in females on all sampling occasions (up to -29% and -23% respectively), and in males (-62% and -52% respectively) on week 102, with a concentration of 9000 ppm each. Higher erythrocyte ACh-esterase activities were noted at the concentration of 9000 ppm on each sampling occasion in both sexes (males up to +39%, females up to +32%), and at 1200 ppm on weeks 26 (males) and 102 (females). Brain ACh-esterase activities were marginally higher in females given 9000 ppm (+8%).

Statistically significant changes were observed at urinalysis at some isolated sampling times but their amplitude were minimal, and/or they were only observed in one sex. They were therefore considered not to be biologically relevant.

Table 53: Blood chemistry findings in controls and carbetamide treated rats

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Sampling time** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Plasma urea | W 26 | 29 | 38 | 30 | 40 | 35 * | 40 | 36 * | 47* |
| | W 52 | 34 | 39 | 35 | 45 | 35 | 51* | 38* | 48* |
| | W 77 | 32 | 32 | 33 | 34 | 35 | 44* | 39* | 32 |
| | W 102 | 49 | 31 | 57 | 34* | 67* | 33 | 96* | 30 |
| Phosphorus | W 77 | 2.4 | 2.2 | 2.5 | 2.6* | 2.4 | 2.4 | 2.8* | 2.4 |
| | W 102 | 2.6 | 2.5 | 2.5 | 2.6 | 3.3* | 2.6 | 3.4* | 2.8 |
| Plasma butyrylCh-esterase | W 26 | 567 | 6787 | 637 | 6741 | 685 | 7006 | 547 | 4812* |
| | W 52 | 1523 | 7617 | 1535 | 7715 | 1646 | 7605 | 1368 | 5930* |
| | W 77 | 2226 | 7139 | 2133 | 7025 | 2525 | 6987 | 1761 | 6144* |
| | W 102 | 4552 | 7929 | 3415* | 7490 | 4206 | 7329 | 1723* | 6654* |
| Plasma ACh-esterase | W 26 | 773 | 3927 | 789 | 3896 | 827 | 4003 | 714 | 3019* |
| | W 52 | 1308 | 4828 | 1311 | 5064 | 1400 | 4602 | 1203 | 3708* |
| | W 77 | 1747 | 4896 | 1637 | 4650 | 1979 | 4666 | 1444 | 4107* |
| | W 102 | 3295 | 5418 | 2600 | 5094 | 3134 | 5205 | 1597* | 4616* |
| RBC ACh-esterase | W 26 | 2539 | 2323 | 2453 | 2338 | 2448 | 2491* | 2800* | 2560* |
| | W 52 | 2693 | 2265 | 2459* | 2215 | 2606 | 2400 | 3051* | 2872* |
| | W 77 | 3143 | 2946 | 3106 | 2973 | 3026 | 3056 | 3514* | 3454* |
| | W 102 | 1895 | 1984 | 2147 | 1897 | 2443* | 2006 | 2631* | 2624* |
| Brain ACh-esterase | Termination | 10220 | 10970 | 10310 | 10930 | 10530 | 11470 | 10610 | 11860* |

*: statistically significant ($p<0.05$)

Absolute and/or relative liver weights were compared to controls (Table 53). Relative liver weights were higher in the 9000 ppm group (+33% in males, +6% in females). Relative kidney weights were higher in males in the 9000 ppm group (+37%). Absolute and relative testes weights were considerably lower in rats given 9000 ppm (-50% and -36% respectively). Other changes were of limited amplitude, and not dose-related, and therefore considered as not treatment related.

Table 54: Mean absolute and relative organ weights in carbetamide treated rats for 2 years

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| **Sex** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Liver | | | | | | | | |
| Absolute weight (g) | 19.1 | 13.0 | 20.0 | 13.3 | 18.0 | 13.4 | 18.8 | 11.9* |
| Relative weights (%) | 4.49 | 4.11 | 4.87 | 4.22 | 4.45 | 4.39 | 5.95* | 4.36* |
| Kidneys | | | | | | | | |
| Absolute weight (g) | 4.13 | 2.82 | 4.29 | 2.85 | 4.1 | 2.93 | 4.23 | 2.54* |
| Relative weights (%) | 0.975 | 0.890 | 1.06 | 0.912 | 1.024 | 0.972 | 1.333* | 0.933 |
| Testes | | | | | | | | |
| Absolute weight (g) | 5.65 | - | 5.35 | - | 5.44 | - | 2.83* | - |
| Relative weights (%) | 1.327 | - | 1.322 | - | 1.330 | - | 0.884* | - |
| *: $p < 0.05$ | | | | | | | | |

The incidence of the following macroscopic lesions was significantly higher in rats given 9000 ppm compared to controls: granular kidneys, smaller and flaccid testes, smaller prostate and stomach wall lesions.

At microscopic examination, carbetamide given at the concentration of 9000 ppm was associated with liver lesions (mainly periacinar hepatocytic hypertrophy or fatty vacuolation) and thyroid follicular epithelial hypertrophy in both sexes, and with stomach wall lesions in males. Other thyroid follicule lesions and mesenteric lymph node lesions were observed at 9000 ppm and lower concentrations (Table 54).

Table 55: Group distribution of non-neoplastic findings at microscopic examination of rats treated for 2 years with carbetamide

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | | Males | Females | Males | Females | Males | Females | Males | Females |
| Liver | Periacinar hepatocytic hypertrophy | 2/39 | 1/50 | 4/32 | 0/45 | 3/36 | 3/45 | 35/40* | 42/46* |
| | Centriacinar hepatocytic hypertrophy | 2/39 | 0/50 | 0/32 | 3/45 | 0/36 | 6/45* | 1/40 | 1/46 |
| | Periacinar hepatocytic fatty vacuolation | 0/39 | 2/50 | 1/32 | 0/45 | 1/36 | 1/45 | 8 /40* | 23/46* |
| | Haemosiderin laden Kupffer cells | 0/39 | 0/50 | 1/32 | 0/45 | 2/36 | 0/45 | 7 /40* | 0/46 |
| Stomach | Ulceration | 1/39 | 1/50 | 4/32 | 1/45 | 4/36 | 3/45 | 10/40* | 0/46 |
| | Hyperkeratosis | 1/39 | 1/50 | 3/32 | 3/45 | 6/36 | 4/45 | 10/40* | 0/46 |
| | Acanthosis | 2/39 | 1/50 | 4/32 | 3/45 | 7/36 | 4/45 | 13 /40* | 0/46 |
| Mesenteric lymph nodes | Erythrophagocytosis in sinuses | 2/39 | 8/50 | 6/32 | 12/45 | 7/36 | 16/45* | 12/40* | 6/46 |
| | Histiocytosis | 0/39 | 7/50 | 4/32* | 6/45 | 5/36* | 6/45 | 7/40* | 3/46 |
| Thyroids | Intrafollicular cell debris | 26/39 | 4/50 | 21/32 | 13/45* | 25/36 | 10/45 | 38/40* | 36/46* |
| | Brown pigments in follicular cells | 0/39 | 1/50 | 7/32* | 11/45* | 23/36* | 21/45* | 29/40* | 44/46* |
| | Hypertrophy of follicular epithelium | 2/39 | 0/50 | 2/32 | 1/45 | 0/36 | 0 /45 | 12/40* | 6/46* |

*: $p < 0.05$

Senile nephropathy was more severe in males treated at 9000 ppm compared to controls. The opposite was observed for females (Table 55).

Table 56: Group distribution of severity of senile nephropathy in rats treated with carbetamide for 2 years

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | Females | Males | Females | Males | Females | Males | Females |
| Minimal | 0 | 13 | 0 | 8 | 0 | 7 | 0 | 32 |
| Slight | 8 | 19 | 2 | 21 | 5 | 20 | 1 | 9 |
| Moderate | 24 | 17 | 19 | 14 | 21 | 14 | 15 | 3 |
| Marked | 5 | 1 | 10 | 2 | 10 | 3 | 23 | 0 |
| Severe | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Total | 39 | 50 | 32 | 45 | 36 | 44 | 40 | 44 |

Various neoplastic lesions were observed, most of which were of the types and incidences commonly seen in F-344 rats of this age at the test laboratory (Table 57).

However, a concern has been expressed as to the malignant astrocytomas observed in the study: this relatively rare tumor at the two high-doses in females might be a substance-related effect. The incidence of the other observed tumors were not treatment-related (Table 57), be it whether among animals that died or were killed during the treatment period, or among animals that were killed at study termination.

The incidence of pre-neoplastic lesions was not increased in any organ.

Table 57: Group distribution of neoplastic findings at necropsy for rats given carbetamide for 2 years.

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | | M | F | M | F | M | F | M | F |
| Brain | M astrocytoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| | M oligodendroglioma | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | M ganglioneuroma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | M meningioma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pituitary | B adenoma | 14 | 29 | 15 | 23 | 12 | 31 | 7 | 21 |
| | M carcinoma | 2 | 1 | 1 | 4 | 0 | 1 | 2 | 1 |
| Salivary gland | M myoepithelial tumour | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Stomach | B squamous cell papilloma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Jejunum | M adenocarcinoma | 1 | 0 | 2 | 0 | 1 | 0 | 1 | 0 |
| Liver | B hepatocellular adenoma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M hepatocellular carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Pancreas | B exocrine cell adenoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B islet cell adenoma | 4 | 1 | 2 | 0 | 2 | 1 | 1 | 0 |
| | M islet cell carcinoma | 1 | 0 | 0 | 1 | 0 | 0 | 3 | 0 |
| Mesentery | B mesothelioma | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M mesothelial sarcoma | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| Caecum | B adenoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urinary bladder | B transitional cell papilloma | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Kidneys | B adenoma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | M sarcoma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M carcinoma | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 0 |
| Adrenals (cortex) | B adenoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Adrenals (medulla) | B phaeochromocytoma | 8 | 0 | 11 | 2 | 14 | 4 | 7 | 0 |
| | M phaeochromocytoma | 1 | 0 | 1 | 0 | 2 | 1 | 2 | 0 |
| | M ganglioneuroma | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lungs | M pulmonary carcinoma | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | M squamous cell carcinoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thyroids | B follicular cell adenoma | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 |
| | M follicular cell carcinoma | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 0 |
| | M parafollicular cell carcinoma | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 0 |
| Parathyroids | B adenoma | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| Skin | B fibroma | 10 | 0 | 6 | 2 | 8 | 0 | 3 | 0 |
| | B lipoma | 1 | 0 | 2 | 0 | 3 | 1 | 0 | 1 |
| | B basal cell tumour | 0 | 0 | 2 | 2 | 2 | 0 | 1 | 0 |
| | B sebaceous adenoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B squamous cell papilloma | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| | B keratoacanthoma | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | M squamous cell carcinoma | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | M Zymbal gland carcinoma | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | M sarcoma | 1 | 2 | 3 | 1 | 2 | 0 | 1 | 0 |
| | M haemangiosarcoma | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Eye | M sarcoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Bone | M osteosarcoma | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| Haemopoietic tissues | M monocytic leukaemia | 9 | 5 | 16 | 3 | 7 | 6 | 1 | 0 |
| | M histiocytic sarcoma | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Spleen | M haemangiosarcoma | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| Thymus | M fibrous histiocytoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Testes | B interstitial cell tumour | 50 | - | 56 | - | 54 | - | 46 | - |
| Preputial glands | B adenoma | 0 | - | 0 | - | 2 | - | 1 | - |
| Ovaries | B thecal cell tumour | - | 0 | - | 0 | - | 1 | - | 0 |

Table 57 (continued)

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | | M | F | M | F | M | F | M | F |
| Uterine cervix | M leiomyosarcoma | - | 0 | - | 0 | - | 1 | - | 0 |
| Uterus | M endometrial carcinoma | - | 1 | - | 1 | - | 0 | - | 2 |
| | M leiomyosarcoma | - | 0 | - | 1 | - | 1 | - | 0 |
| Vagina | M leiomyosarcoma | - | 0 | - | 1 | - | 0 | - | 0 |
| Clitoral gland | B adenoma | - | 1 | - | 0 | - | 2 | - | 0 |
| | M carcinoma | - | 0 | - | 0 | - | 0 | - | 1 |
| Mammary glands | B fibroadenoma | 4 | 16 | 4 | 11 | 1 | 12 | 1 | 5 |
| | M adenocarcinoma | 0 | 3 | 1 | 3 | 0 | 1 | 0 | 1 |
| Abdomen | M sarcoma | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Thorax | M mesothelial sarcoma | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| Miscellaneous | M carcinoma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M sarcoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

*: $p < 0.05$ ; B: benign ; M: malignant

***Malignant astrocytomas***

A concern has been expressed as to the malignant astrocytomas observed in the study: this relatively rare tumor was observed at the two high-doses in females (2/60). One of the two concerned animals died before the end of the study (week 103). The manufacturer submitted historical control data on the incidence of this tumor type from the Charles River laboratories and from the US National Toxicology Program (NTP) (Table 56). In addition, a second reference for historical control data from Charles River Laboratories (public and available online document) was added by the MSCA. The MSCA has also conducted an update of the available public NTP historical controls data submitted by the manufacturer.These data are provided in the dedicated IUCLID file.

In the historical control groups run in the Charles River laboratories and submitted by the manufacturer, the incidence of malignant astrocytomas ranged from 0/50 to 1/50 in F344 female rats. Only the historical range has been indicated by the manufacturer without detail on the number of studies examined, the overall incidence of the tumor or the in-life completion date of the studies. In another public document from Charles River laboratories which present the historical control data from dietary carcinogenicity studies run from 1980 to 1986, the overall incidence of astrocytoma in female F344 rats is 0.5% ranging from 0% to 2.9%

In the historical control data from dietary carcinogenicity studies run from 1998-2002 in the NTP database report, the overall incidence of astrocytoma in female F344 rats is 0.09% ranging from 0% to 2%.

Table 56: Occurrence of astrocytoma in female rats treated with carbetamide vs. control animals and historical background data from the Charles River laboratories and the US NTP.

| Historical control incidence | Controls | 9000 ppm |
|---|---|---|
| NTP database [3]<br>20 studies (1998-2002)<br>*Overall incidence*: 1/999 (**0.09%**)<br>*Historical range:* 0/50 to 1/50 (**0%-2%**) | | |
| Charles River laboratories database 1 [4]<br>*Historical range:* 0/50 to 1/50 (**0%-2%**) | Incidence: 0/60 (0%) | Incidence: 2/60 (3.3%) |
| Charles River laboratories database 2 [5]<br>13 studies (1980-1986) | | |

[3] http://ntp niehs nih.gov/ntp/research/database_searches/historical_controls/path/r_orlwr.txt (document available in the iuclid file)

[4] Charles River Laboratories (2007) Historical Histopathology Data – F344 Rats. Selected Neoplastic Brain Findings. 104-107 Week studies. Issued 5 November 2007.

[5] Charles River Laboratories (1990) Spontaneous Neoplastic Lesions in the CDF(F-344)/CrlBR Rat.( (document available in the iuclid file)

| | | |
|---|---|---|
| *Overall incidence*: 5/954 (0.5%)<br>*Historical range:* 0%-2,9% | | |

The manufacturer, who submitted a CLH report proposal to the French authorities in december 2012, suggested that these findings with incidences slightly higher than historical controls are of poor relevance for the human risk assessment. His argumentation is based on the fact that:

*" - There was no dose-effect relationship as this tumour type was not observed in any of the lower dose groups. Neither were benign astrocytomas - a necessary precursor to the malignant tumour - described in any of the rats investigated. If the astrocytoma were causally linked to carbetamide exposure, the benign precursors would be expected to also occur at increased frequencies.*

- *Malignant astrocytoma are a rare tumour type in the F344 rat. In historical control groups run in Charles River laboratories, the incidence of malignant astrocytomas ranged from 0/50 to 2/50 in males and from 0/50 to 1/50 in female rats. This incidence is comparable with historical control data for the Fischer 344 rat (US National Toxicology Program). The incidence observed in females treated with 9000 ppm Carbetamide (2/60) is therefore only slightly above the historical range for females and is most likely incidental.*
- *The fact that no astrocytomas were observed in male rats of any group is also indicative of an incidental rather than Carbetamide-induced tumour formation. Toxicokinetic studies with Carbetamide in the rat have not revealed a sex-specific metabolism that would explain a sex-specific pattern of carcinogenicity by Carbetamide.*
- *Furthermore, the Carbetamide dose of 9000 ppm was clearly in excess of the maximum tolerated dose (MTD). In the Carbetamide study in rats, 33% and 19% reductions in body weights compared to controls were observed in males and females, respectively. Under conditions of general toxicity, findings with incidences only marginally higher than reported for historical controls are, if truly treatment related, of little relevance for the assessment of risks for human health as the unphysiolgical conditions induced by sustained toxicity and secondary malnutrition can lead to diffuse effects on the body (including tumorigenicity) not specific to the test compound. In such cases, the hazard is not a property of the test compound and should not be a basis for classification."*

These comments were also submitted in the context of the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008 and are therefore presents in the final addendum to the DAR (vol. 3-B6.5.1, July 2010) .

Taking into account ECHA comments concerning the probable in depth analysis of the HCD required by RAC, MSCA has added the corresponding documents / publications when available in the iuclid file. In addition to that, the MSCA sought other references that are public for historical controls laboratory Charles River. The MSCA also conducted an update of the available public NTP historical controls data.

More precisely, concerning the historical control incidences for malignant astrocytomas (Table 56):

Since the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008, the online NTP historical control database was updated and amended. The reference and web link provided in the DAR is no longer available (http://ntp niehs.nih.gov/files/HistCont-2007-10-17-Rats_All_Routes.pdf, MSCA has decided to defer only the NTP HC data currently available online (footnote 8 , the corresponding document containing the full database is available in the iuclid file).

Overall, this does not change the interpretation, the incidence in the 9000ppm carbetamide female group is still higher than the historical control range obserbed in both NTP database references.

Charles River laboratories:

Because the historical control data from Charles River Laboratories submitted by the manufacturer (CR Laboratories 2007 footnote 6) were not detailed and the reference document is not available, MSCA has choosen to add a second reference for HCD from Charles River Laboratories which was public and available online (CR Laboratories 1990 footnote 7, referenced in chapter 7.2 and the corresponding document is available in the iuclid file).

Based on all these information, the MCSA does not support the argumentation provided above by the registrant. The incidence of malignant astrocytoma observed in female rats at the high dose is above the historical control range incidence of astrocytoma in female F344 rats from the Charles River laboratories and the NTP database reports (3.3% vs. 2% or 2.9%). Because this lesion is very rare and do not occur in control animals, MSCA is of opinion that a substance-related effect cannot be totally excluded.

Although toxicokinetic studies with carbetamide in the rat have not revealed a sex-specific metabolism, differences in effects were observed between males and females exposed to the 9000 ppm-carbetamide dose. These differences in effect concerns blood chemistry (lower PCV, Hb and RBC in female only) and AChE/BChE activities (increased brain AChE and plasma BChE activities in females only). In addition, male rats presented more severe nephropathies than female rats and stomach lesions were observed only in male rats.

The maximum tolerable dose (MTD) is slightly exceeded at 9000 ppm carbetamide dose (equivalent to 400-500mg/kg day) in term of weight gain retardation (19% for the female instead of the generally acceptable 10%). In the other hand, a similar dose of 8000 ppm carbetamide (equivalent to 783-851 mg/kg/day) induced only a slight decrease in body weight gains (-7%) in female rats exposed orally during 28 days when compared to controls (28-d rat; West 1984). In addition, in chronic studies in rats with carbetamide, it has not been observed, at this dose, potential life-threatening lesions during microscopic examination of organs (e.g., necrosis). Thus, in the opinion of MSCA, there is no evidence of toxicity of a severity that would interfere with the interpretation of the study.

The increased incidence of this rare tumour (astrocytoma) in the females exposed to the high dose of carbetamide is slight but above the historical control range. For these reasons, a substance related effect cannot be excluded and a classification of carbetamide in category 2 (H351) is justified for the MSCA.In addition, this proposal is in agreement with the EFSA conclusion on Carbetamide which also specifically refers to an increased incidence of malignant astrocytoma in rats at the high dose as part of the basis for the proposal to classify for cancerogenicity.

- **Conclusion**

The long term toxic effects of carbetamide to rats for up to 104 weeks were evaluated in a chronic toxicity and carcinogenicity study by dietary administration at the concentrations of 0, 160, 1200 and 9000 ppm. The following effects were only observed at the highest concentration of 9000 ppm, unless marked "*" (effects also present at 1200 ppm):

- A transient staining of the coat, and a higher incidence of anterior subcapsular lenticular opacities at ophthalmoscopy,
- Lower body weight gain and food intake,
- Lower PCV, Hb and RBC (females only), and higher platelet counts compared to controls,
- Higher plasma urea*, plasma phosphorus* and RBC ACh-esterase activities*, and lower plasma butyrylCh- and ACh-esterase activities, higher brain Ach-esterase activities (female only).
- Higher relative liver and kidney (males only) weights, and lower absolute and relative testes weights,
- Granular kidneys, smaller and flaccid testes, smaller prostate and stomach wall lesions at macroscopic examination,
- Periacinar hepatocyte hypertrophy, fatty vacuolation and other liver lesions and thyroid follicular epithelial hypertrophy in both sexes, and stomach wall lesions and more severe nephropathies in males at microscopic examinations.
- Higher incidence of astrocytoma (female only). The increase incidence of this rare tumor is slight but above the historical control range. A substance related effect cannot be excluded.

There was no treatment-relation in other tumor incidence and nature, or in pre-neoplastic lesions frequency.

#### 4.10.1.1.2 Mice

- **Reference:** **Amyes SJ, Marris CJ, Brown PM, Virgo DM, Fowler JSL (1988): carbetamid**e: oncogenicity study in mice. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 87/RHA046/748 - Dates of work: 15/01/1985 to 01/02/1987.
- **Guidelines:** OECD N°453, 1981.
- **GLP standards:** Yes.
- **Deviations:** None.
- **Study acceptable:** Yes.
- **Test system:**

A total of 208 male and 208 female B6C3F1 mice (4-5 weeks old, bw ranging 16-23 g for males and 15-19 g for females) were randomly allocated to 3 groups of 52 males and 52 females given technical carbetamide (batches N°8404001, 8404002 and 85345, purity: 95.0 to 97.4%) in the diet at the concentrations of 160, 1200 or 9000 ppm for 2 years. Another group of 52 males and 52 females (with same characteristics) received the diet without carbetamide and served as a control group. The first treatment day was noted D0.

The homogeneity and stability of carbetamide in the diet were controlled. Achieved concentrations were controlled at regular intervals during the study.

The animals were observed at least once daily for clinical signs and mortality. Detailed observations and palpations were conducted weekly. Each animal was weighed on D0, at weekly intervals for the first 14 weeks and once every two weeks thereafter. Mean food consumption was calculated weekly, and achieved dosages were calculated at the same intervals as body weight for each group and sex. Water consumption was evaluated visually but not recorded.

Blood was sampled from 10 males and 10 females of each group on weeks 52, 78, 103 and 104 for haematological investigations (total and differential leucocyte counts).

All surviving mice were sacrificed after 104 weeks of treatment, weighed, and subjected to necropsy. The weights of designated organs (adrenals, brain, liver, kidneys, heart, lungs, testes and spleen, and abnormalities) were recorded, selected tissues were preserved and histological examinations were performed on specified tissue samples (adrenals, aorta, epididymides, marrow smear, blood smear, femoral bone with marrow and joint, harderian glands, tongue, heart, eyes with optic nerves, lungs, mammary glands caudal and cranial, trachea, gall bladder, oesophagus, thyroid, stomach, duodenum, thymus, jejunum, lymph node, ileum, caecum, spinal cord, skin, colon, salivary gland, liver, skeletal muscle, spleen, kidneys, seminal vesicles, urinary bladder, prostate, rectum, testes, uterus with cervix, ovary, pancreas, pituitary, sciatic nerve, brain, tumours and gross abnormalities) of all animals.

- **Results**

Homogeneity and stability of carbetamide in the diet under the use conditions were satisfying, as achieved dosages were within -10% and +10% of nominal values. The achieved test material concentrations (groups 2 to 4) were within -10% and +10% of the nominal values of 160, 1200 and 9000 ppm. Mean and extreme achieved dose-levels are summarized in Table 28.

Table 57: Mean and extreme values of carbetamide dose-levels

| **Concentration** | **160 ppm** | | **1200 ppm** | | **9000 ppm** | |
|---|---|---|---|---|---|---|
| **Sex** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Mean (mg/kg/day) | 20.1 | 22.7 | 150.3 | 172.1 | 1409 | 1578 |
| Minimum (mg/kg/day) | 14.9 | 14.3 | 103.5 | 102.3 | 1059 | 1077 |
| Maximum (mg/kg/day) | 34.5 | 43.0 | 257.5 | 323.1 | 2077 | 2520 |

Mortality was not treatment-related (Table 58). The trend was towards lower mortalities with increased concentrations.

Table 58: Summary of mortality in controls and carbetamide treated mice

| **Group** | **Not treated** | | **160 ppm** | | **1200 ppm** | | **9000 ppm** | |
|---|---|---|---|---|---|---|---|---|
| **Sex** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Total death/initial | 15/52 | 13/52 | 10/52 | 14/52 | 8/52 | 10/52 | 8/52 | 4/52 |
| Deaths weeks 1-54 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 2 |
| Deaths week 55-termination | 15 | 13 | 10 | 14 | 8 | 8 | 7 | 2 |

There was no treatment related clinical sign. On the contrary, clinical and ageing signs were less marked at the highest concentration, as was observed with mortality rates. Palpable swellings were mostly ventral and transient, and less frequent at the highest concentration.

Body weight gains were significantly lower in males and females given 9000 ppm, during all the study (up to -43% over all the study, see Table 59). From week 27 till study termination, food intake values were moderately lower (up to -7%, see Table 59) in males receiving carbetamide at all concentrations than in controls. There was no relation between treatment and food intake in females. Water intake was unaffected by treatment.

Table 59: Mean body weight gains in non-treated control and carbetamide treated mice

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| **Sex** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Weeks 0-13 (g) | 16.2 | 9.3 | 15.7 | 8.9 | 15.7 | 9.3 | 12.1 | 7.7 |
| % of controls | - | - | -3 | -4 | -3 | 0 | -25 | -17 |
| Weeks 0-52 (g) | 26.1 | 25.1 | 26.4 | 24.9 | 26.7 | 24.1 | 14.9 | 13.9 |
| % of controls | - | - | +1 | -1 | +2 | -4 | -43 | -45 |
| Weeks 0-104 | 24.0 | 31.8 | 22.9 | 31.4 | 24.3 | 32.7 | 13.6 | 18.6 |
| % of controls | - | - | -5 | -1 | +1 | +3 | -43 | -42 |

Examinations of blood smears did not reveal any treatment-related difference in total or differential leucocyte counts at any sampling time.

Relative liver weights were higher at 9000 ppm (higher absolute weight and +70% relative in males, +46% in females) compared to controls. Relative kidney weights were lower in males receiving 1200 or 9000 ppm (-6% and -13% respectively, absolute values lower too) but higher in females at the highest concentration (+21%) compared to controls (see Table 60). Other organ weight variations were noted but considered to be within the range of those observed in mice of this strain and age.

Table 60: Mean absolute and relative organ weights in controls and carbetamide treated mice

| Group | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|
| **Sex** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** |
| Liver | | | | | | | | |
| Absolute weight (g) | 2.8 | 2.1 | 2.9 | 2.0 | 2.6 | 2.2 | 3.6* | 2.2 |
| Relative weights (%) | 6.68 | 4.54 | 7.22 | 4.51 | 6.25 | 4.67 | 11.37* | 6.65* |
| Kidneys | | | | | | | | |
| Absolute weight (g) | 0.77 | 0.52 | 0.73* | 0.55 | 0.72* | 0.55 | 0.51* | 0.45* |
| Relative weights (%) | 1.815 | 1.113 | 1.753 | 1.237 | 1.709* | 1.155 | 1.582* | 1.343* |

*: $p < 0.05$

The only histologically correlated macroscopic findings (see Table 61) were liver masses in both sexes at 9000 ppm. Other treatment related findings at 9000 ppm were smaller seminal vesicles and some cases of ureteral distension or mass (non significant).

Table 61: Group distribution of macroscopic findings in mice treated with carbetamide for 2 years

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| **Sex** | | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| Liver | Mass | 25 | 9 | 23 | 12 | 27 | 16 | 43* | 18 |
| Seminal vesicles | Smaller size | 1 | - | 4 | - | 1 | - | 10* | - |
| Ureter | Distention | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Mass | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *: $p < 0.05$ | | | | | | | | | |

At microscopic examination (see Table 62), carbetamide given at the concentration of 9000 ppm was associated in both sexes with higher incidences of proliferative or hypertrophic lesions in the liver and the thyroids (these two types also being observed at 1200 ppm in males) and in the pituitary in females. carbetamide given at the concentration of 9000 ppm was also associated with neoplastic findings (see Table 63): higher incidences of hepatocytic tumours (in both sexes; present at 1200 ppm in females), cholangiocarcinoma (in males), thyroid adenomas and phaeochromocytomas (both in females).

Table 62: Group distribution of microscopic, non neoplastic findings in mice treated with carbetamide for 2 years

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | | M | F | M | F | M | F | M | F |
| Liver | Nodular hyperplasia | 9 | 3 | 9 | 4 | 18 | 8 | 22* | 6 |
| | Periacinar hepatocyte hypertrophy | 10 | 1 | 7 | 0 | 37* | 1 | 44* | 14* |
| | Hepatocytic erythrophagia | 1 | 6 | 1 | 4 | 21* | 0* | 24* | 5 |
| | Leucocytic foci | 17 | 19 | 23 | 17 | 21 | 12 | 33* | 23 |
| Thyroids | Follicular hyperplasia | 0 | 2 | 0 | 6 | 3 | 2 | 31* | 21* |
| | Coloidal cell debris | 3 | 4 | 7 | 6 | 10 | 8 | 44* | 40* |
| | Follicular cell hypertrophy | 0 | 5 | 0 | 2 | 7* | 8 | 38* | 40* |
| Pituitary | Focal hyperplasia | 0 | 4 | 0 | 3 | 0 | 7 | 0 | 13* |

*: $p < 0.05$

Table 63: Group distribution of neoplastic findings at necropsy in mice treated with carbetamide for 2 years

| Group | | Not treated | | 160 ppm | | 1200 ppm | | 9000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | | M | F | M | F | M | F | M | F |
| Pituitary | B adenoma | 0 | 4 | 0 | 6 | 0 | 6 | 0 | 9 |
| | M carcinoma | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | M pars nevrosa tumour | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Tongue | M basal cell carcinoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Liver | B hepatocellular adenoma | 12 | 1 | 7 | 4 | 12 | 7* | 27* | 22* |
| | B haemangioma | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M hepatocellular carcinoma | 16 | 3 | 10 | 4 | 9 | 2 | 22 | 6 |
| | M cholangiocellular carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | **3** | 0 |
| | M haemangiosarcoma | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 |
| Gall bladder | B adenoma | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pancreas | B islet cell adenoma | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mesentery | B haemangioma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Mesenteric lymph node | B haemangioma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Caecum | B adenoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Kidneys | B adenoma | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | M carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Adrenals (cortex) | B adenoma | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B spindle cell tumour | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adrenals (medulla) | B phaeochromocytoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | M phaeochromocytoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| Heart ventricle | M haemangiosarcoma | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lungs | B pulmonary adenoma | 5 | 0 | 2 | 0 | 3 | 1 | 1 | 0 |
| | M pulmonary carcinoma | 5 | 2 | 4 | 2 | 5 | 3 | 1 | 2 |
| Thyroids | B follicular adenoma | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| Skin | B squamous cell papilloma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | B keratoacanthoma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| | B haemangiopericytoma | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | B fibroma | 1 | 0 | 3 | 0 | 0 | 1 | 1 | 0 |
| | M sarcoma | 1 | 1 | 5 | 0 | 4 | 1 | 1 | 0 |
| | M haemangiosarcoma | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Harderian glands | B adenoma | 0 | 4 | 1 | 1 | 0 | 1 | 0 | 0 |
| | M carcinoma | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 |
| Bone | B fibroma | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | M osteosarcoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | M haemangiosarcoma | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haematopoietic tissues | M lymphocytic leukaemia | 1 | 0 | 2 | 4 | 0 | 1 | 0 | 0 |
| | M granulocytic leukaemia | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M lymphoma | 6 | 21 | 10 | 17 | 6 | 23 | 8 | 20 |
| | M histiocytic sarcoma | 1 | 2 | 1 | 2 | 1 | 5 | 1 | 0 |
| Spleen | B haemangioma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | M haemangiosarcoma | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Preputial glands | B keratoacanthoma | 0 | - | 1 | - | 0 | - | 0 | - |
| Ovaries | B granular cell tumour | - | 1 | - | 0 | - | 0 | - | 0 |
| | B adenoma | - | 1 | - | 0 | - | 1 | - | 0 |
| | B haemangioma | - | 1 | - | 0 | - | 0 | - | 0 |
| | B granulosa cell tumour | - | 1 | - | 0 | - | 0 | - | 0 |
| | M adenocarcinoma | - | 0 | - | 1 | - | 0 | - | 0 |
| Uterus | B haemangioma | - | 1 | - | 0 | - | 0 | - | 0 |
| | M endometrial sarcoma | - | 1 | - | 0 | - | 1 | - | 0 |
| | M schwannoma | - | 0 | - | 0 | - | 1 | - | 0 |
| Mammary glands | M adenocarcinoma | - | 1 | - | 0 | - | 1 | - | 0 |
| Abdomen | M granular cell tumour | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Miscellaneous | M adenocarcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | M osteosarcoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

*: $p < 0.05$

***Hepatocellular adenoma and carcinoma***

A statistically significant and dose dependant increase in hepatocellular neopslasm was observed in carbetamide-treated mice compared to controls.

The manufacturer, while submitting a CLH report proposal to the French authorities in december 2012, suggested that the liver carcinogenic effects in mice may be related to an induction of hepatic CYP450. According to this hypothesis, these effects would not be relevant to humans. In order to support this hypothesis, the manufacturer has submitted a mechanistic study, in which carbetamide was assayed for hepatic cytochrome P450 enzyme induction in the B6C3F1 mouse at the same dose levels as in the carcinogenicity study. The mechanistic study in which carbetamide was assayed for hepatic cytochrome P450 enzyme induction in mice was present in the final addendum to the DAR (vol. 3-B6.5.3, July 2010). The results showed that Carbetamide leads to sustained liver activation (see § 4.10.3.1), likely via interaction with the CAR or PXR-receptor. Key events and their human relevance are summarized as:

| | Experimentally demonstrated in mice | Human relevance | Remarks |
|---|---|---|---|
| Receptor activation (CAR, PXR) | Not investigated | Possible if occurring in mice | - |
| Induction of CYP2b forms of cytochrome P450 | Demonstrated | Possible if occurring in mice | CYP2B clearly the subfamily with strongest induction; early event (demonstrated at 2 weeks) |
| Liver weight increase | Demonstrated | Unlikely due to different sensitivity * | Demonstrated later than enzyme induction |
| Centrilobular hepatocellular hypertrophy | Demonstrated | Unlikely * | Demonstrated later than liver weight increase; in 28 d studies not observed, but in 90 d studies |
| Increased hepatocyte proliferation | Not investigated | Unlikely * | - |
| The appearance of adenomas and finally carcinomas | Demonstrated | Unlikely * | Not occurring in subchronic studies; only observed at end of lifespan |

*many examples of this mode of action have been investigated; conclusions were published (Holsapple et al., 2006; Deguchi et al., 2009; Cohen, 2010; Ross et al., 2010)

The MSCA considers that the proposed mode of action for the generation of livers tumors in mice is plausible considering the mechanistic data submitted. It is concluded that the hepatocellular adenoma and carcinoma observed in mice at the end of the natural lifespan after chronic liver activation are highly unlikely to be relevant for humans.

***Thyroid follicular adenoma***

Follicular adenomas of the thyroid gland were found in 5/52 female mice of the top dose group. The manufacturer submitted the historical control incidence of this tumor type in female B6C3F1 mice from dietary carcinogenicity studies compiled from the most recent studies (usually a 5-year period) through 1995 by the US National Toxicology Program. The incidence in the 9000ppm carbetamide female group is higher than the historical control range observed in the NTP database over 19 studies (Table 64).

Table 64: Occurrence of thyroid follicular adenomas in B3C3F1 female mice treated with carbetamide vs. control animals and historical background data from the US NTP.

| **Historical control incidence** | **Controls** | **9000 ppm** |
|---|---|---|
| NTP database* (1990-2000) [6]<br>19 studies<br>*Overall incidence*: 16/942 (**1.7%**)<br>*Historical range:* 0/48 to 3/51 (**0%-4%**) | Incidence: 0/52 (0%) | Incidence: 5/52 (**9.6%**) |

Since the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008, the online NTP historical control database was updated and amended. The reference and web link provided in the DAR is no longer available (http://ntp niehs.nih.gov/files/HistCont-2007-10-17-Rats_All_Routes.pdf (accessed 31 October, 2007), MSCA has decided to defer only the NTP HC data currently available online (footnote 8 and the corresponding document is available in the iuclid file).

[6] http://ntp niehs nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt (document available in the uiclid file)

Overall, this does not change the interpretation, the incidence in the 9000ppm carbetamide female group is still higher than the historical control range obserbed in both NTP database references

While submitting a CLH report proposal to the French authorities in december 2012, the manufacturer suggested that these findings are of poor relevance for the human risk assessment. His argumentation is presented below.

*« The causal connection between the induction of hepatic microsomal enzymes (as demonstrated for Carbetamide), and subsequent stimulation of the thyroid (with enhanced T4/T3 excretion) is well established in rodents. This has been summarised by, the IPCS framework of the WHO International programme on chemical safety (IPCS 2006) and it was concluded that the mode of action for phenobarbital (PB)-like P450 inducers was determined to be unlikely in humans after kinetic and dynamic factors were considered.*

*Carbetamide has been shown to possess an induction pattern similar to PB with CYP2A and CYP2B activities being most strongly induced (up to 10-fold) in female mice. It is thus likely that hyperplasia in both the thyroidal follicles and the pituitary are secondary to the induction of UGT activity in the liver.*

*This mechanism is not relevant for humans because of :*

- *The low inducibility of xenobiotic metabolism in human liver compared to rodents, especially at exposure levels encountered during the proposed use of Carbetamide-containing products.*
- *The much lower proportion of free T4/T3 in human serum that is a potential substrate for UGT and thereby a candidate for excess renal excretion.*

*Humans possess a high-affinity thyroxin-binding globulin (TBG) in plasma that carries >99% of the thyroid hormones in plasma. In adult mice, TBG expression is very low, and T4/T3 are mostly bound to low-affinity carriers like albumin and transthyretin. Thus, mice, like rats, are exquisitely sensitive to increased conjugation of thyroid hormones by chemically-induced phase-II metabolism.* »

The same comments has been also already submitted in the context of the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008.

All these comments/arguments are presents in the final addendum to the DAR (vol. 3-B6.5.2, July 2010) . The MCSA agrees with this argumentation and considers that thyroidal follicular adenomas as well as their likely non-neoplastic precursor should be disregarded for carcinogenicity classification.

***Cholangiocarcinoma***

Cholangiocarcinoma were observed in 3 male mice of the 9000 ppm dose group. The manufacturer submitted the historical control incidence of this tumour type in male B6C3F1 mice from dietary carcinogenicity studies mice from dietary carcinogenicity studies run from 1983-1986 at Charles River Laboratories and from the US National Toxicology Program (compilation studies from the most recent studies (usually a 5-year period) through 1995) while submitting a CLH report proposal to the French authorities in december 2012. Since the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008, the online NTP historical control database was updated and amended. The reference and web link provided in the DAR by the manufacturer is no longer available (http://ntp niehs.nih.gov/files/HistCont-2007-10-17-Rats_All_Routes.pdf (accessed 31 October, 2007)), MSCA has decided to defer only the NTP HC data currently available online (footnote 9 and the corresponding document is available in the iuclid file).

Charles River lab. (1983-1986): The Charles River historical control data (footnote 10) have been submitted by the manufacturer for the resubmission of carbetamide (Regulation (EC) N°33/2008). However, only the reference has been provided by the manufacturer; no document is available.

The incidence in the 9000ppm carbetamide males is higher than the historical control range observed both in the Charles River Laboratories and the NTP database (table 65).

Table 65: Occurrence of liver cholangiocarcinoma in B3C3F1 male mice treated with carbetamide vs. control animals and historical background data from the Charles River laboratories and the US NTP.

| Historical control incidence | Controls | 9000 ppm |
|---|---|---|

| | | |
|---|---|---|
| NTP database (1990 - 2000)[7]<br>19 studies<br>*Overall incidence*: 1/946 (**0.1%**)<br>*Historical range:* 0/50 to 1/50 (**0%-2%)**<br><br>Charles river laboratories database (1983-1986) [8]<br>*Historical range:* 0/82 to 1/52 (**0%-1.9%**) | Incidence: 0/37(0%) | Incidence: 3/44 (**6.8%)** |

***Malignant phaeochromocytomas***

Malignant phaeochromocytomas were observed in 2/48 female mice of the 9000 ppm dose group. In the Charles River historical controls (dietary carcinogenicity studies run from 1979-1986), 2 malignant phaechromocytomas were observed in a total of 1255 female B6C3F1 mice examined, the incidence rate being 0.15%.In the NTP historical controls (studies compiled from the most recent studies (usually a 5-year period) through 1995), the incidence rate for malignant phaeochromocytomas was 0.2% (overall incidence) with a range from 0% to 2% (table 66). In the more contemporary historical control range submitted by the manufacturer (Eiben 2010) while submitting a CLH report proposal to the French authorities in december 2012., the incidence of 2/48 (4.2%) of malignant phaeochromocytomas incidence observed in the 9000 ppm dose group of female mice is on the upper limit of the historical range. Since the resubmission of carbetamide in the framework of Regulation (EC) N° 33/2008, the online NTP historical control database was updated and amended. The reference and web link provided in the DAR by the manufacturer is no longer available (http://ntp niehs.nih.gov/files/HistCont-2007-10-17-Rats_All_Routes.pdf (accessed 31 October, 2007)), MSCA has decided to defer only the NTP HC data currently available online (footnote 9 and the corresponding document is available in the iuclid file). An additional reference for HC data from Charles River Laboratories which was public and available online was added by MSCA in this CLH report (footnote 12, reference in chapter 7.2 and the corresponding document available in the iuclid file)

Table 66: Occurrence of malignant phaeochromcytomas in B6C3F1 female mice treated with carbetamide vs. control animals and historical background data

| **Historical control incidence** | **Controls** | **9000 ppm** |
|---|---|---|
| NTP database (1990-2000)[9]<br>19 studies<br>*Overall incidence*: 2/944 (**0.2%**)<br>*Historical range:* 0/50 to 1/50 (**0%-2%)**<br><br>Charles River laboratories database (1979-1986) [10]<br>*Overall incidence:* 2/1255 (**0.15%)**<br>*Historical range:* 1/26 to 1/69 (1.5-3.9%)<br><br>Eiben, 2010[11]<br>29 studies (1988-1998)<br>*Overall incidence:* 0.6%<br>*Historical range:* 0% - 4.2% | Incidence: 0/39 (0%) | Incidence: 2/48 **(4.2%)** |

It should be mentionned that cholangiocarcinoma and malignant phaechromocytoma were only observed at the top dose (9000 ppm) that led to a drastic (>40%) retardations in body weight development, indicating a clear exceedance of the maximum tolerable dose.

[7] http://ntp.niehs nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt (document available in the uiclid file)

[8] Charles River Laboratories (2007) Historical Histopatholy Data –B6C3F1 Mice-. Selected Liver abd adrenal findings. 91-106 Week studies. Issued 31 october 2007.

[9] http://ntp niehs nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt (document available in the uiclid file)

[10] Charles River Laboratories (1989) Spontaneous Neoplastic Lesions in the B6C3F1/ CrlBR mouse.( (document available in the iuclid file)

[11] Eiben R., 2011 : Frequency and time trends of spontaneous tumors in B6C3F1 mice oncogenicity studies over 10 years. Exp Toxic Pathol 2001; 53: 399-408

- **Conclusion**

The long term effects of Carbetamide to B6C3F1 mice for up to 104 weeks were evaluated in a carcinogenicity study by dietary administration to groups of 52 animals per sex at the concentrations of 0, 160, 1200 and 9000 ppm (Amyes *et al*., 1988b). Effects, when present, were observed at the two highest concentrations of 1200 and 9000 ppm and included:

- Lower body weight gain (males -43%, females -42%) and food intake at 9000 ppm
- Higher relative liver weights at 9000 ppm, and opposite trends relative changes in kidney weight between sexes (lower in males at 1200 and 9000 ppm, higher in females at 9000 ppm),
- Liver masses, smaller seminal vesicles, ureter distension or masses at 9000 ppm,
- Microscopic lesions at the concentration of 9000 ppm: proliferative or hypertrophic lesions in the liver and the thyroids (both also observed at 1200 ppm in males), and in the pituitary in females,
- Neoplastic findings at the concentration of 9000 ppm: hepatocytic tumours (in both sexes; present at 1200 ppm in females), cholangiocarcinoma (in males) and thyroid adenomas and phaeochromocytomas (both in females).

The increase incidence of hepatocellular carcinoma/adenoma and thyroid follicular adenoma in mice exposed to carbetamide can be explained by excessive enzyme induction. A Phenobarbital (PB) like MoA is postulated for carbetamide to explain liver and thyroid tumors observed in rodent. The mode of action for phenobarbital (PB)-like P450 inducers was determined to be unlikely in humans (Holsapple *et al.*, 2006; IPCS 2006).

In addition to the increased incidence of liver and thyroid tumors, which can be explained by excessive enzyme induction, an increased incidence of other tumor types were also observed in mice (liver cholangiocarcinoma and adrenal phaeochromocytoma). These tumor incidences are all above the available historical control ranges but were observed at doses clearly exceeding the MTD.

### 4.10.1.2 Carcinogenicity: inhalation

Carbetamide is not a volatile compound. The vapour pressure is $3 \times 10^{-7}$ Pa (20°C), and exposure via the inhalation route is low, a study is not required.

### 4.10.1.3 Carcinogenicity: dermal

Exposure to Carbetamide by the percutaneous route is not expected to be significant. Therefore, no such study has been conducted.

## 4.10.2 Human information

No data

## 4.10.3 Other relevant information

### 4.10.3.1 *Effect on liver cytochrome P450 enzyme activities*

Carbetamide was investigated for hepatic cytochrome P450 enzyme induction in the B6C3F1 mouse at the same dose levels as in the carcinogenicity study by Amyes et al. (1988b). Results of this study are shown below.

- **Reference:** **Bogaards J.J.**P., Grossouw D. (2006): Effect of carbetamide in the diet on cytochrome P450 enzyme activities in mouse liver. TNO Quality of life, Netherlands, Unpublished report No. 031.10506.
- **Test methods**: OECD of Good laboratory practice (as revised in 1997), Paris, ENV/MC/CHEM(98)17.
- **GLP standards**: Yes
- **Deviations**: None
- **Study acceptance**: Yes
- **Batch**: 020948
- **Purity**: 97.0%

- **Test system**

The aim of the study was to provide data on the possible induction of liver cytochrome P450 enzymes by carbetamide in mice. The animals were treated with Carbetamide incorporated in the diet at three dose levels: 160, 2000 and 9000 mg/kg diet.
The study comprised 4 groups of 10 female $B6C3F_1$ mice. The treatment period was 14 days. All animals were sacrificed after 2 weeks and their livers were collected weighted, immediately frozen with liquid nitrogen and stored at –18°C until the preparation of microsomes by ultracentrifugation. Enzyme activities were measured with cytochrome P450 substrates which are selective towards human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP 2D6, CYP2E1, CYP3A and CYP4A and compared with those of the untreated group.

- **Results**

No study- or test substance-related signs of toxicity or unusual behaviour were observed.

No differences were observed between the groups regarding body weights. Food consumption was significantly increased in the treated groups compared to the control group during the second week, probably due to contamination of the food container by excreta in one control animal cage and one low dose group cage.
The liver weights of the mid and high dose groups were significantly increased after the two-week dosing period.

A statistical analysis demonstrated significant differences between group means for all enzyme activities except for CYP3A. The results are showed in the table below.

| | Enzyme activities (group means ± SD) | | | |
|---|---|---|---|---|
| | Group A (control) | Group B (160 mg/kg diet) | Group C (2000 mg/kg diet) | Group D (9000 mg/kg diet) |
| CYP1A | 81.0 ± 8.1 | 100 ± 11.5* | 185 ± 20.2*** | 230 ± 22.8*** |
| CYP2A | 30.2 ± 3.4 | 33.6 ± 5.0 | 86.5 ± 22.5*** | 293 ± 31.6*** |
| CYP2B | 876 ± 101 | 1170 ± 241* | 2959 ± 325*** | 3825 ± 281*** |
| CYP2C | 191 ± 26.2 | 204 ± 14.3 | 223 ± 20.0*** | 251 ± 11.4*** |
| CYP2D | 725 ± 134 | 897 ± 86** | 1465 ± 148*** | 1636 ± 107*** |
| CYP2E | 2903 ± 295 | 2943 ± 274 | 3632 ± 273*** | 4545 ± 253*** |
| CYP3A | 1234 ± 173 | 1338 ± 113 | 1318 ± 112 | 1378 ± 101 |
| CYP4A | 194 ± 50.2 | 208 ± 59.1 | 263 ± 54.7** | 266 ± 30.3** |

Statistics: ANOVA + t-tests; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

- **Conclusion**

It can be concluded that carbetamide is an inducer of all measured CYP450 activities, with the exception of CYP3A. At the lowest concentration in the diet (160 mg/kg diet), a slight significant increase of enzyme activities was only found for CYP1A, CYP2B and CYP2D, whereas at the two highest concentrations (2000 and 9000 mg/kg diet), all the enzyme activities were increased, with the exception of CYP3A.

### 4.10.3.2 *Effect on cholinesterase activity*

In rats and dogs, investigations of plasma, erythrocyte and brain cholinesterases were confounding. Studies in dogs showed no decrease in cholinesterase activity, but overt signs of neurological toxicity were possibly indicative of carbamate toxicity. In rats, plasma cholinesterase and erythrocyte cholinesterase activity at high doses was generally decreased and increased, respectively. Brain chlolinesterase activity was not significantly affected or slightly increased by treatment at high dose levels. The relevance of such a variable effect is debatable.

### 4.10.4 Summary and discussion of carcinogenicity

The long term toxicity and carcinogenicity of carbetamide has been evaluated in rats and mice.

Carbetamide at 9000 ppm induced astrocytoma in female rats and was carcinogenic in mice at 1200 and 9000 ppm, with increased hepatocellular tumors (in both sexes; present at 1200 ppm in females), cholangiocarcinoma (in males), thyroid adenomas and phaeochromocytomas (both in females).

The increased liver weights in rats, the hyperplasia of thyroid epithelial cells in rats, and the carcinogenic effects in mice may be related to an induction of hepatic CYP450, and a secondary hyper-stimulation of the thyroid gland

correlated with an increased elimination of thyroid hormones by glucurono-conjugation in the liver. A mechanistic study supporting this hypothesis was submitted. In this study, carbetamide was assayed for hepatic cytochrome P450 enzyme induction in the B6C3F1 mouse at the same dose levels as in the carcinogenicity study. The results showed that carbetamide leads to an induction pattern similar to PB with CYP2A and CYP2B activities being most strongly induced. The mode of action for phenobarbital (PB)-like P450 inducers was determined to be unlikely in humans after kinetic and dynamic factors were considered (IPCS 2006 [12]).
The MSCA considers that the proposed mode of action for the generation of liver and thyroid tumors is plausible considering the mechanistic data submitted. Thus, hepatocellular carcinoma/adenoma as well as thyroidal follicular adenomas should be disregarded for carcinogenicity classification.

However, for the high dose of carbetamide (exceeding MTD) several rare tumors including carcinomas occurred in different tissues (brain astrocytoma, liver cholangiosarcoma and adrenal phaeochromocytoma) in mice and rats. The tumor incidences are all above the available historical control ranges.

Based on these data, the MSCA considers that a substance related effect cannot be excluded and a classification of carbetamide in category 2 (H351) is required.

### 4.10.5 Comparison with criteria

***Hepatocellular carcinoma/adenoma and thyroid follicular adenoma***

The incidence increase of hepatocellular carcinoma/adenoma and thyroid follicular adenoma in mice exposed to carbetamide can be explained by excessive enzyme induction. A Phenobarbital (PB) like MoA is postulated for carbetamide to explain liver and thyroid tumors observed in rodent. MSCA considers that thyroidal follicular adenomas as well as hepatocellular carcinoma/adenoma should be disregarded for carcinogenicity classification.

***Other tumors***

Several rare tumors including carcinomas were also observed in different tissues (brain astrocytoma, liver cholangiosarcoma and adrenal phaeochromocytoma) in mice and rats. The incidence of these tumors is all above the available historical control ranges but they were observed only for the high dose of carbetamide exceeding MTD. However, MSCA is of opinion that the possibility of a confounding effect is not justified. In the animals exposed to the high dose of carbetamide, no excessive toxicity (such as cell death –necrosis- with associated regenerative hyperplasia which can lead to tumor development) was observed in the organs in which tumors occurred.

Because these lesions are very rare and do not occur in control animals, MSCA is of opinion that a substance-related effect cannot be totally excluded. The Guidance on the application of Regulation (EC) N° 1272/2008, specify that *"If a test compound is only found to be carcinogenic at the highest dose(s) used in a lifetime bioassay, and the characteristics associated with doses exceeding the MTD (…), this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2 or no classification"*. MSCA supports a classification of carbetamide in Category 2.

In addition, Carbetamide was discussed in the PRAPeR 81 expert meeting and the EFSA conclusions were to consider carbetamide as a non-genotoxic carcinogen with the risk phrase R40 "Limited evidence of a carcinogenic effect" (Carc 2: H351)

The EFSA conclusion on carbetamide peer review (EFSA Journal 2010; 8(12):1913) was as following:
*"After long-term repeated exposure in rats and mice, carbetamide induced the same toxic effects as observed in the short-term studies. The relevant NOAEL for chronic toxicity was 6 mg/kg bw/day based on the 2 year rat study. Hepatocellular carcinoma and adenoma and thyroid follicular adenoma were seen in B6C3F1 mice at the high dose (exceeding MTD), however this mouse strain is known to be particularly sensitive to the induction of hepatocellular tumours. Proliferative or hypertrophic lesions in the liver, pituitary gland and the thyroid were seen at the two highest doses in mice. A mechanism study performed on B6C3F1 mice showed carbetamide to be an inducer of a variety of hepatic cytochrome P450 enzymes, which could support the proposed mechanism of hepatocarcinogenicity. In the high dose (exceeding MTD) several rare tumours including carcinomas occurred in different tissues (brain astrocytoma, liver cholangiocarcinoma and adrenal phaeochromocytoma) in mice and rats, the tumour incidences were all above the available historical control ranges. In addition the tumours were not sex-specific. Based on these data carbetamide is*

12 Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M (2006) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 2001 Oct;34(2):146-52.

*considered to be a non-genotoxic carcinogen and the risk phrase R40 "Limited evidence of a carcinogenic effect" is proposed.*

### 4.10.6 Conclusions on classification and labelling

Classification of carbetamide in category 2 (H351) is proposed based on several rare tumours occurring in different tissues (brain astrocytoma, liver cholangiocarcinoma and adrenal phaeochromocytoma) in mice and rats at the high dose (exceeding MTD.)

## 4.11 Toxicity for reproduction

Table 67: Summary table of relevant reproductive toxicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Rat multigeneration study, Dietary administration<br><br>CD rats (30/sex)<br><br>1000; 3000 & 7500 ppm | **Parental toxicity**<br>**NOAEL < 1000 ppm** (< 65 mg/kg),<br>**LOAEL=1000 ppm**<br>Higher liver weight in females but no liver histological lesions<br><br>**Reproductive effects**<br>**NOAEL = 3000 ppm** (208 mg/kg bw/day)<br>**LOAEL = 7500 ppm**<br>Decreased body weight gain of pups of all generations, and liver enlargement and centrolobular hepatocyte hypertrophy of F2B pups | **Guidelines:** not stated<br>**GLP:** Yes.<br>**Deviation**: not applicable<br>**Study acceptable:** Yes.<br>**Purity**: 95.1 –96.5% (batches 8404001 and 8404002) | Tesh et al., 1987 |
| Rat teratogenicity study, gavage<br><br>CD rats (20 females)<br><br>150; 450 & 1000 mg/kg bw/day | **Maternal toxicity**<br>**NOAEL = 450 mg/kg bw/day**.<br>**LOAEL = 1000 mg/kg bw/day**<br>Lower parental body weight gain at 1000 mg/kg bw/day<br><br>**Foetal toxicity and teratogenicity**<br>**NOAEL = 450 mg/kg bw/day**<br>**LOAEL = 1000 mg/kg bw/day**<br>Reduced foetal weight and foetal immaturity;<br>Complex foetal malformations at 1000 mg/kg bw/day.<br>Darkening of thyroid glands without effects on weight or maturity of the thyroid at 450 mg/kg bw/day | **Guidelines:** not stated<br>**GLP:** Yes.<br>**Deviation**: not applicable<br>**Study acceptable:** Yes.<br>**Purity**: 94.4% (batch 84 040 01) | Tesh et al., 1985 |
| Rabbit teratogenicity study, gavage<br><br>Rabbit New Zealand White<br><br>5, 40 and 320 mg/kg bw/day | **Maternal toxicity**<br>**NOAEL = 40 mg/kg bw/day**<br>**LOAEL = 320 mg/kg bw/day**<br>Transient lower maternal body weight gain<br><br>**Embryotoxicity and teratogenicity**<br>**NOAEL = 40 mg/kg bw/day,**<br>**LOAEL= 320 mg/kg bw/day**<br>Abortions and higher post-implantation losses | **Guidelines:** not stated<br>**GLP:** Yes.<br>**Deviation**: not applicable<br>**Study acceptable:** Yes.<br>**Purity**: 96.1% (batch 84 040 01) | Tesh et al., 1986 |

### 4.11.1 Effects on fertility

#### 4.11.1.1 Non-human information

- **Reference: Tesh JM, Wightman TJ, Fowler JSL (1987):** carbetamide: effects upon reproductive performance of rats treated continuously throughout two successive generations. Life Science Research, Ltd., Suffolk, UK - Unpublished report N° 8787/RHA047/469 - Dates of work: 05/11/1984 to 23/06/1986.
- **Guidelines:** Not stated.
- **GLP standards:** Yes.
- **Deviations:** Not applicable.
- **Study acceptable:** Yes.
- **Test system:**

A total of 120 male and 120 female CD rats (8 weeks old, bw ranging 184-219 g for males and 147-179 g for females) were randomly allocated to 3 groups of 30 males and 30 females given technical carbetamide (batches N° 8404001 and 8404002, respective purities: 95.1 (mean) and 96.5%) in the diet at the concentrations of 1000, 3000 or 7500 ppm throughout two successive generations. Another group of 30 males and 30 females (with same characteristics) received the diet without carbetamide and served as a control group. The first treatment day was noted D0. The homogeneity and

stability of carbetamide in the diet over the range of tested concentrations were validated in previous studies with concentrations of 160 up to 32000 ppm. Achieved concentrations were controlled at regular intervals up to study week 35.

- F0 generation animals were treated over a 13-week (92 days) pre-mating period, throughout a 3-week mating period, gestation and lactation of 2 litters (F1A and F1B). All females were allowed to deliver their litters and rear the pups for 25 days. F0 parents were killed and examined macroscopically after weaning of the F1B litters.
- At 4 days of age, some animals of the F1A litter were culled so as to reduce litter size to 4 animals per sex per litter. The remaining pups were reared until d-25 post partum and then sacrificed.
- At 4 days of age, some animals of the F1B litter were culled so as to reduce litter size to 4 animals per sex per litter. The remaining pups were reared and at d-25 post partum a second selection was performed: a total of 30 males and 30 females (when possible 1/sex/litter) were kept to form generation F1, whereas the remaining F1B pups were sacrificed.
- The same procedures and the same schedule as described for F0 generation were followed for F1 generation.

In parents F0 and F1, all animals were observed daily for mortality and clinical signs, body weights were recorded weekly (and more frequently during gestation and lactation in females), food intake was recorded weekly until the first pairing (pairing A), and water intake was assessed visually, vaginal smears were taken from 10 days before pairing A till detection of a vaginal plug, pre-coital intervals were recorded (each female having at most 21 days to achieve mating), durations of gestations were recorded, parturition was followed up until completion, gross necropsy was conducted on decedents and all remaining animals after weaning of litter B, followed by organ weighing (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thyroids, uterus and vagina) and histological examinations (adrenals, all tumours, brain, marrow smear, caecum, duodenum, epididymides, eye with optic nerve, heart, ileum, kidneys, liver, lungs, cervical and mesenteric lymph nodes, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, seminal vesicles, spleen, stomach, testes, thymus when present, thyroids, urinary bladder, uterus and vagina) of 10 animals/sex (only in groups 1 and 4 except for livers).

In offspring F1A, F1B, F2A and F2B, at birth, each litter was evaluated for alive and dead pups, decedents were examined for macroscopic abnormalities, pups were examined on day 1 post-partum, pups were weighted and sexed on days 1, 4, 7, 14, 21 and 25 post-partum, pups of litters F1A and F2A were subjected to gross necropsy and organ weighing, pups of litters F1B and F2B were evaluated for physical development (individual dates of onset of pinna unfolding, hair growth, tooth eruption and eye opening), among F2B pups, 10 animals/sex were subjected to necropsy after weaning, followed by organ weighing and histological examinations (only in groups 1 and 4 except for livers).

In pups culled for litter reduction at day 4 post-partum, F1A pups were not examined, F1B, F2A and F2B pups were examined for macroscopic abnormalities, and F1B and F2B pups were also fixed and examined after skeletal coloration.

- **Results**

The diet's stability was satisfying, as the achieved test material concentrations (groups 2 to 4) were within -10% and +10% of the nominal values of 1000, 3000 and 7500 ppm (except on 2 sampling times for the 1000 ppm) over 14 sampling occasions.

Parents F0 and F1:

Mean and extreme achieved dose-levels for F0 and F1 parents are summarized in Table 68.

Table 68: Mean and extreme values of carbetamide dose-levels (mg/kg/day) in F0 and F1 parents before pairing

| **Sex** | **Male** | | | **Female** | | |
|---|---|---|---|---|---|---|
| **Concentration** | **1000** | **3000** | **7500** | **1000** | **3000** | **7500** |
| **F0 parents** | | | | | | |
| Mean | 65 | 194 | 506 | 74 | 221 | 570 |
| Minimum | 48 | 142 | 385 | 58 | 171 | 480 |
| Maximum | 103 | 310 | 784 | 100 | 295 | 711 |
| **F1 parents** | | | | | | |
| Mean | 82 | **253** | 676 | 91 | 284 | 749 |
| Minimum | 50 | 157 | 423 | 62 | 199 | 530 |
| Maximum | 164 | 524 | 1393 | 162 | 497 | 1338 |

Mortality was not treatment-related. There were three deaths: one F0-group 2 male was humanely killed, one F0-group 2 female was found dead and one F1-group 1 animal was killed because of incorrect sexing (Table 69).

Table 69: Mortality in all groups of F0 and F1 parent rats treated with carbetamide

| **Sex** | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration** | **0** | **1000** | **3000** | **7500** | **0** | **1000** | **3000** | **7500** |
| **F0 parents** | | | | | | | | |
| Number of deaths | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Week of death | | 17 of treatment | | | | 3 post-coitum | | |
| **F1 parents** | | | | | | | | |
| Number of deaths | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Week of death | | | | | 8 after selection | | | |

There were no treatment-related clinical signs.

Body weights (and often bw gains) were lower in both sexes given 7500 ppm (F0-group 4: -20 to -23% for the first 13 weeks) than in controls (Table 70). This was correlated with lower food intake in group 4 (F0: -6% of controls, overall). Water intake was reduced in F0-group 4 males (-11% of controls, overall).

Table 70: Body weight gains in all groups of F0 and F1 parent rats before pairing, and of females during each gestation and lactation

| **Sex** | | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | | **0** | **1000** | **3000** | **7500** | **0** | **1000** | **3000** | **7500** |
| **F0 parents** | | | | | | | | | |
| Mean body weight gain | Weeks 0-13 | 368 | 354 | 353 | 296* | 145 | 145 | 139 | 111* |
| | Gestation A | - | - | - | - | 119 | 124 | 120 | 108* |
| | Lactation A | - | - | - | - | -1 | 21* | 20* | 25* |
| | Gestation B | - | - | - | - | 123 | 133 | 119 | 114* |
| | Lactation B | - | - | - | - | -12 | 1 | 13* | 14* |
| **F1 parents** | | | | | | | | | |
| Mean body weight gain | Weeks 0-13 | 442 | 428 | 444 | 390* | 213 | 212 | 203 | 189* |
| | Gestation A | - | - | - | - | 123 | 133 | 124 | 104* |
| | Lactation A | - | - | - | - | 0 | 4 | 14* | 21* |
| | Gestation B | - | - | - | - | 121 | 126 | 118 | 99* |
| | Lactation B | - | - | - | - | 5 | 4 | 11* | 19* |

*: $p<0.05$

Oestrous cycles, pre-coital intervals, mating, conception rate, fertility index and gestation index were not affected by treatment and there were no cases of dystocia. Gestation length was normal in all F0 groups, but minimally longer in F1-groups 3 and 4 for the first pregnancy (A), and F1-group 3 for the second (B), compared to controls (71 and Table 72).

Table 71: Data on reproduction in all groups of F0 and F1 parents

| **Sex** | | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | | **0** | **1000** | **3000** | **7500** | **0** | **1000** | **3000** | **7500** |
| **F0 parents** | | | | | | | | | |
| Regular oestrous cycles | | - | - | - | - | 29/30 | 26/30 | 27/30 | 25/30 |
| Mating A | Precoital interval within 4 d | - | - | - | - | 27/30 | 26/30 | 28/30 | 27/29 |
| | Conception rate (%) | 93 | 86 | 75 | 100 | 93 | 87 | 77 | 100 |
| | Fertility index (%) | 87 | 83 | 70 | 97 | 93 | 87 | 77 | 97 |
| | Gestation length within 23 d | - | - | - | - | 26/28 | 25/26 | 23/23 | 25/29 |
| | Gestation index (%) | - | - | - | - | 100 | 100 | 100 | 100 |
| Mating B | Precoital interval within 4 d | - | - | - | - | 28/30 | 25/29 | 27/29 | 28/29 |
| | Conception rate (%) | 93 | 89 | 83 | 87 | 90 | 90 | 83 | 87 |
| | Fertility index (%) | 90 | 86 | 80 | 87 | 90 | 87 | 80 | 87 |
| | Gestation length within 23 d | - | - | - | - | 23/27 | 24/26 | 17/24 | 18/26 |
| | Gestation index (%) | - | - | - | - | 100 | 96 | 96 | 100 |
| **F1 parents** | | | | | | | | | |
| Regular oestrous cycles | | - | - | - | - | 27/28 | 29/30 | 24/30 | 26/30 |
| Mating A | Precoital interval within 4 d | - | - | - | - | 25/29 | 29/30 | 23/29 | 29/30 |
| | Conception rate (%) | 85 | 87 | 64 | 100 | 86 | 87 | 66 | 100 |
| | Fertility index (%) | 79 | 87 | 60 | 100 | 86 | 87 | 63 | 100 |
| | Gestation length within 23 d | - | - | - | - | 22/25 | 24/26 | 15/19 | 24/30 |
| | Gestation index (%) | - | - | - | - | 100 | 100 | 95 | 100 |
| Mating B | Precoital interval within 4 d | - | - | - | - | 28/29 | 29/29 | 25/30 | 29/29 |
| | Conception rate (%) | 93 | 90 | 81 | 97 | 93 | 90 | 80 | 97 |
| | Fertility index (%) | 90 | 90 | 73 | 97 | 93 | 90 | 80 | 97 |
| | Gestation length within 23 d | - | - | - | - | 24/27 | 25/27 | 20/24 | 27/29 |
| | Gestation index (%) | - | - | - | - | 96 | 100 | 100 | 100 |

Table 72: Duration of gestation of female rats treated with carbetamide

| Group | Number of pregnant animals | Gestation length (days) | | | | Number of live litters |
|---|---|---|---|---|---|---|
| | | 22.5 | 23 | 23.5 | 24 | |
| F0-F1A | | | | | | |
| 1 | 28 | 0 | 13 | 13 | 2 | 28 |
| 2 | 26 | 1 | 17 | 7 | 1 | 26 |
| 3 | 23 | 0 | 7 | 16 | 0 | 23 |
| 4* | 29 | 0 | 6 | 19 | 4 | 29 |
| F0-F1B | | | | | | |
| 1 | 27 | 11 | 12 | 3 | 1 | 27 |
| 2 | 25 | 11 | 13 | 1 | 0 | 25 |
| 3 | 23 | 3 | 14 | 6 | 0 | 23 |
| 4 | 26 | 5 | 13 | 8 | 0 | 26 |
| F1-F2A | | | | | | |
| 1 | 25 | 6 | 12 | 3 | 0 | 25 |
| 2 | 26 | 0 | 13 | 1 | 1 | 26 |
| 3** | 19 | 0 | 3 | 4 | 0 | 18 |
| 4** | 30 | 0 | 10 | 5 | 1 | 30 |
| F1-F2B | | | | | | |
| 1 | 27 | 0 | 16 | 8 | 2 | 0 |
| 2 | 27 | 0 | 13 | 12 | 0 | 1 |
| 3* | 24 | 0 | 7 | 13 | 4 | 0 |
| 4 | 29 | 2 | 12 | 13 | 2 | 0 |

*: significantly different from controls, p<0.05 Mann-Withney
**: significantly different from controls, p<0.01 Mann-Withney

At necropsy, there was no treatment-related and histologically confirmed macroscopic abnormality.

Absolute and/or relative liver weights were higher in both sexes compared to controls at various dose-levels (relative values in group 4 males: F0+17%, F1+22%; in females of groups 2 to 4: up to +21% for both generations) (Table 44).

Bone marrow smears showed no treatment-related change. Histopathology showed higher incidences of periacinar (centrolobular) hepatocytic hypertrophy in both sexes in groups 3 and 4 (Table 73).

Table 73: Relative liver weights and incidence of hepatocytic hypertrophy in all groups of F0 and F1 parents

| **Sex** | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration** | **0** | **1000** | **3000** | **7500** | **0** | **1000** | **3000** | **7500** |
| **F0 parents** | | | | | | | | |
| Relative liver weights (%) | 3.32 | 3.39 | 3.45 | 3.87* | 3.82 | 4.15* | 4.37* | 4.61* |
| Incidence of periacinar hepatocytic hypertrophy | 0/10 | 0/10 | 4/10* | 4/10* | 0/10 | 0/10 | 9/10* | 10/10* |
| **F1 parents** | | | | | | | | |
| Relative liver weights (%) | 3.31 | 3.47 | 3.49 | 4.03* | 3.91 | 4.18* | 4.41* | 4.74* |
| Incidence of periacinar hepatocytic hypertrophy | 0/10 | 1/10 | 6/10* | 9/10* | 0/10 | 0/10 | 5/10* | 10/10* |

*: p<0.05

Pups F1A, F1B, F2A and F2B:

For all four litters, offspring condition and appearance, litter size, sex ratio and live birth index, viability at day 4 (culling) and 25 (weaning) were not affected by treatment (Table 74). Offspring body weight gains till weaning were lower in group 4 of all litters compared to controls (up to -16%).

Table 74: Data on litters F1A, F1B, F2A and F2B

| Time point | At birth (d-1) | | | | Before litter reduction (d-4) | | At weaning (d-25) |
|---|---|---|---|---|---|---|---|
| **Mean values of parameter:** | **Sex ratio M:F** | **Litter size** | **Live birth index (%)** | **Pup weight (g)** | **Litter size** | **d-4/birth viability index (%)** | **Pup weight (g)** |
| **F1A pups** | | | | | | | |
| 0 ppm | 1:1.04 | 12.2 | 95 | 6.4 | 11.7 | 93 | 74.9 |
| 1,000 ppm | 1:1.25 | 13.4 | 99 | 6.4 | 12.3 | 89 | 73.5 |
| 3,000 ppm | 1:0.94 | 12.0 | 100 | 6.5 | 11.8 | 99 | 75.0 |
| 7,500 ppm | 1:1.01 | 11.8 | 99 | 6.4 | 11.4 | 98 | 65.1* |
| **F1B pups** | | | | | | | |
| 0 ppm | 1:0.99 | 12.8 | 98 | 6.5 | 12.2 | 93 | 78.5 |
| 1,000 ppm | 1:0.93 | 14.7 | 99 | 6.4 | 13.6 | 93 | 76.4 |
| 3,000 ppm | 1:1.00 | 12.4 | 100 | 6.7 | 11.1 | 89 | 75.7 |
| 7,500 ppm | 1:0.99 | 12.0 | 99 | 6.6 | 11.1 | 94 | 66.1* |
| **F2A pups** | | | | | | | |
| 0 ppm | 1:1.14 | 11.7 | 99 | 6.4 | 10.8 | 93 | 69.1 |
| 1,000 ppm | 1:1.07 | 13.1 | 99 | 6.4 | 11.7 | 83 | 71.8 |
| 3,000 ppm | 1:0.99 | 11.8 | 100 | 6.5 | 10.8 | 86 | 67.9 |
| 7,500 ppm | 1:1.01 | 11.3 | 100 | 6.3 | 10.9 | 91 | 59.3* |
| **F2B pups** | | | | | | | |
| 0 ppm | 1:0.98 | 12.5 | 99 | 6.5 | 12.1 | 98 | 69.9 |
| 1,000 ppm | 1:1.21 | 12.9 | 9 | 6.5 | 11.9 | 91 | 70.6 |
| 3,000 ppm | 1:0.83 | 11.3 | 96 | 6.6 | 10.4 | 88 | 67.9 |
| 7,500 ppm | 1:0.95 | 10.7 | 100 | 6.6 | 10.5 | 95 | 61.7* |

*: $p<0.05$

Physical development (F1B and F2B only) was not affected by treatment.

At necropsy of all litters, and at skeletal examination of F1B and F2B pups, there were no treatment-related abnormalities.

F2 pups: bone marrow smears showed no treatment-related change.

F2B pups: lower absolute and/or relative spleen weights in both sexes in group 4 males (-22%), but this was considered to be due to lower body weights; higher absolute and/or relative liver weights in both sexes compared to controls in groups 3 and 4 (up to +23% and +18%, in males and females respectively). Histopathology showed higher incidences of periacinar hepatocytic hypertrophy in both sexes in group 4 (Table 75).

Table 75: Relative spleen and liver weights and incidence of hepatocytic hypertrophy in all groups of F2B pups

| **Sex** | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration** | **0** | **1000** | **3000** | **7500** | **0** | **1000** | **3000** | **7500** |
| **F2B pups** | | | | | | | | |
| Relative spleen weights (%) | 0.537 | 0.498 | 0.503 | 0.418* | 0.503 | 0.486 | 0.470 | 0.452 |
| Relative liver weights (%) | 5.36 | 5.46 | 5.99* | 6.61* | 5.25 | 5.71 | 6.08* | 6.17* |
| Incidence of periacinar hepatocytic hypertrophy | 0/10 | 0/10 | 0/10 | 6/10* | 0/10 | 0/10 | 0/10 | 6/10* |

*: $p<0.05$

- **Conclusion**

Rats were treated for 2 generations with carbetamide in the diet at the concentrations of 0, 1000, 3000 and 7500 ppm.

In parents, body weights of the F0 generation were lower in both sexes at the highest dose level (max -20% when compared to controls for the first 13 weeks) than in controls, correlated with a minimally lower (-6%) food intake. Gestation length was minimally longer in F1-groups 3 and 4 for the first pregnancy (A), and F1-group 3 for the second (B), compared to controls, but this was not associated with changes in litter size, foetal viability or birth weight. Relative liver weights were higher than controls in males of the F0 and F1 generations treated at the highest dose-level and in females treated at all dose-levels. These changes in organ weights were associated with centrilobular hepatocyte hypertrophy in males and females treated at 3000 and 7500 ppm.

Offspring body weight gains till weanling were lower in all litters of the highest dose-level group (up to -16%) when compared to controls, and higher absolute and/or relative liver weights were observed in male and female pups of the F2B generation treated at the highest dose-level, in association with increased incidence of centrilobular hepatocyte hypertrophy in males and females treated at 3000 and 7500 ppm.

Higher mean liver weight was observed in all adult female groups of F0 and F1 generations, whatever the dose-level, and therefore no NOAEL could be determined for parental toxicity. However, the effects observed at the lowest dose-level were minimal and not associated with lesions at histopathological examination of the liver.

Reproductive parameters were not affected by the treatment, except lower body weight gain up to weanling in all litters treated at the highest dose-level, and liver enlargement associated with centrilobular hepatocyte hypertrophy in F2B pups treated at the highest dose-level. Therefore, the LOAEL for parental toxicity was 1000 ppm, and the NOAEL for toxicity to reproduction was 3,000 ppm (approximately 208 mg/kg/day for combined sexes).

#### 4.11.1.2 Human information

No data

### 4.11.2 Developmental toxicity

#### 4.11.2.1 Non-human information

#### 4.11.2.1.1 Rat

| | |
|---|---|
| **Reference:** | **Tesh JM, Deans CF, Rigg J, Tesh SA, Wilby OK (1985): carbetami**de - teratology study in the rat. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 85/RH0029/136 - Dates of work: 25/07/1984 to 11/01/1985. |
| **Guidelines:** | Not stated. |
| **GLP standards:** | Yes. |
| **Deviations:** | Not applicable. Blood sampled animals were examined at the same time point as other females and included in all examinations. |
| **Study acceptable:** | Yes. |
| **Test system:** | |

A total of 66 pregnant adult female CD rats (age not mentionned, bw ranging 202-236 g) were allocated to 3 groups given technical carbetamide (batch 84 040 01, purity: 94.4%) by gavage at the dose-levels of 150, 450 or 1000 mg/kg/day (20 females/groups and 6 blood sampled satellite females in lowest dose group). Another group of 20 females (with same characteristics) received the vehicle alone: 10 mL/kg of a 1% (w/v) aqueous methylcellulose mucilage. Four of them were blood sampled. Blood sampled animals were examined at the same time and using the same methods as other females. The treatment period was from day 6 to 15 of gestation.

The homogeneity and stability of carbetamide in the dosing mixtures were controlled. Achieved plasmatic concentrations of carbetamide and his metabolite (29835 RP) were evaluated in this study for the dose-level of 150 mg/kg/day on day 15 of gestation.

The animals were observed daily for clinical signs and mortality. Each animal was weighed on days 1, 3, 6 to 15, 18 and 21 of gestation. Individual food and water intake were recorded at 2 to 4 day intervals throughout gestation. All animals were sacrificed on day 21 by carbon dioxide asphyxiation and subjected to necropsy. Macroscopic examination

including detailed reproductive tract recordings (corpora lutea, implantation and resorption sites, live and dead foetus were counted, and foetus distribution in horns was recorded) was performed on all adults. Each foetus and placenta was weighed and examined for macroscopic abnormalities. Foetuses were also sexed, examined by free-hand sectioning and stained for skeletal abnormalities.

- **Results**

Achieved carbetamide concentrations in dosage forms were satisfying (within ±10% of intended concentrations, none detected in controls).

Plasmatic concentration measures in treated females suggested that plasmatic Tmax for the test material was around or inferior to, ½ hour. Plasmatic carbetamide levels, and to a lesser extent those of 29835 RP, in females treated at 150 mg/kg/day (Table 76) showed important inter-individual variations (up to 15-fold) ½ hour and 1 hour post-treatment. As metabolite maximal concentrations were 5 times that of test material, this demonstrates that carbetamide was rapidly absorbed and metabolized.

Plasmatic concentrations of carbetamide in controls were at most <0.5 µg/mL. The results of the preliminary study were not given so plasmatic concentrations in the two other groups could not be assessed.

Table 76: Test material dose-levels (mg/kg/day), and test material and metabolite 29835 RP plasmatic concentrations on day 15 of gestation (µg/mL) for controls and the low dose group

| Group | | 1 | | | 2 | | |
|---|---|---|---|---|---|---|---|
| Target dose-level (mg/kg/day) | | 0 | | | 150 | | |
| Value | | Min. | Mean | Max. | Min. | Mean | Max. |
| carbetamide | Cmax (µg/ml) | ND | ND | ND | 1.4 | 9.8 | 20.8 |
| Metabolite* | Cmax (µg/ml) | ND | <0.5 | <0.5 | 32.1 | 52.3 | 64.6 |

ND: none detected; * carbetamide metabolite: 29835 RP

No deaths occurred during the study. The only observable clinical sign was occasionally increased salivation in females treated at 1000 mg/kg/day.

Body weight gain was lower in females treated at 1000 mg/kg/day. Respective values during 21 days of gestation for females treated at 0, 150, 450 and 1000 mg/kg/day were +152, +153, +148 and +141 g.

There were no significant differences in food or water intake, apart from a transiently higher water intake during the first 6 days of treatment in females given 1000 mg/kg/day.

Necropsy of adults showed no treatment-related finding.

Litters presented no treatment-related finding in terms of number of pregnancy rate (100% of all females), implantations, live and dead foetuses, pre- and post-implantation losses, and mean placental weight. Mean foetal bodyweight was markedly lower when females were treated at 1000 mg/kg/day (-18% compared to controls) (Table 77).

Foetuses from the 1000 mg/kg/day group showed higher incidences of signs of immaturity (dilatation of brain ventricles, space between body wall and organs, subcutaneous haemorrhagies and reduced ossification of cranial bones, sternebrae, vertebrae, metacarpals/metatarsals and pubic bones), which can be interpreted as a sign of toxicity. They also presented higher incidences of darkened thyroid glands, several arterial or cardiac defects (no historical control data), elongated genital tubercle, imperforate anus and vestigial/absent tail. Their skeletal lesions also included fused sternebrae, higher number of ribs and presacral vertebrae and asymmetric pelvis (Table 48).

At the intermediate dosage some observations were considered to be indicative of a marginal adverse fetal response to Carbetamide. One foetus of 88 foetuses (1.11 %) of the mid dose group showed darkening of thyroid glands. In the offspring of high dose dams, 15 of 86 foetuses showed this effect (17.4%). The manufacturer argues that: "*This effect can be regarded as an indication of minimal treatment-related effect in the mid dose. Offspring of the mid dose, however, developed normally and weight and maturity were unaffected, which is an indication for normal function of the thyroids. In addition, results from the multi-generation study conducted in the same laboratory do not support the*

*findings in the thyroids of the developmental toxicity study. Therefore, it is concluded that the mid dose level is the NOAEL for the developmental toxicity of Carbetamide in the rat."*

The other abnormalities observed in foetuses from the 450 mg/kg/day treated females were of the types and frequencies usually recorded in foetuses of this strain of rats, and were not considered by the RMS to be treatment related.

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

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

- **Conclusion**

Carbetamide, when given orally to pregnant rats from days 6 to 15 of gestation, was associated with maternal embryotoxic and teratogenic adverse effects at the highest dose-level of 1000 mg/kg/day:

- Lower body weight gain in adults,
- Reduced foetal weight (-18% when compared to controls),

- Generalized foetal immaturity, characterized by soft tissue variations and ossification retardation,
- Several foetal abnormalities including complex malformations associating elongated genital tubercle, imperforate anus and vestigial/absent tail, and cardiovascular malformations.

At the dose-level of 450 mg/kg/day, no adverse effects were observed for dams, and the abnormalities observed in foetuses were of the types and frequencies usually recorded in foetuses of this strain of rats. Only one foetus showed darkening of thyroid glands without effects on weight or maturity of the thyroid.

Carbetamide was therefore clearly teratogenic in rats at the materno-toxic dose level of 1000 mg/kg/day. The no-effect level for this study was 150 mg/kg/day. The no adverse effect level for this study was 450 mg/kg/day for maternal, foetal toxicity and teratogenicity.

### 4.11.2.1.2 Rabbit

- **Reference:** **Tesh JM, Ross FW, Wightman TJ, Wilby OK (1986): carbetami**de - teratology study in the rabbit. Life Science Research, Ltd., Suffolk, UK - Unpublished report No. 85/RH0030/733 - Dates of work: 01/04/1985 to 13/07/1985.
- **Guidelines:** Not stated.
- **GLP standards:** Yes.
- **Deviations:** Not applicable.
- **Study acceptable:** Yes.
- **Test system:**

A total of 49 pregnant adult female New Zealand White rabbits (21-27 weeks old, bw ranging 3.80-5.43 kg) were allocated to 3 groups given technical carbetamide (batch 84 040 01, purity: 96.1%) by gavage at the dose-levels of 5, 40 or 320 mg/kg/day (15 females/group and 4 blood sampled satellite females in lowest dose group). Another group of 15 females (with same characteristics) received the vehicle alone (10 mL/kg of a 1% (w/v) aqueous methylcellulose solution). The treatment period was from days 6 to 19 of gestation.

The homogeneity and stability of carbetamide in the dosing mixtures were controlled. Achieved plasmatic concentrations of carbetamide and his metabolite (29835 RP) were evaluated in satellite females (5 mg/kg/day) on day 19 of gestation, after which the latter were killed and subjected to limited gross necropsy.

Each day all females were observed for clinical signs/mortality and weighed. Individual food and water intake were recorded over 5 consecutive periods over the whole gestation (defined by days 5/6, 12/13, 19/20 and 23/24). All animals were sacrificed on day 29 (or immediately after detection of an abortion) and subjected to necropsy. Macroscopic examination including detailed reproductive tract recordings (uterus were weighted; corpora lutea, implantation and resorption sites, live and dead foetuses were counted, and foetus distribution in horns was recorded) was performed on all females. Each foetus and placenta was weighed and examined for macroscopic abnormalities. Foetuses were also sexed, examined by necropsy and stained for skeletal abnormalities.

- **Results**

Achieved carbetamide bolus concentrations were satisfying (within $\pm$10% of intended concentrations, none detected in controls).

Plasma concentrations measured in females treated at 5 mg/kg/day showed that plasmatic Tmax was ½ hour for the carbetamide and metabolite 29835 RP (Table 78). Plasmatic carbetamide levels were up to 6 times lower than that of his metabolite, demonstrating that absorption and/or metabolization were rapid.

Table 78: carbetamide and metabolite 29835 RP plasmatic concentrations on day 19 of gestation (µg/mL) for the low dose group adult females (5 mg/kg/day)

| Group | | 2 | | |
|---|---|---|---|---|
| Target dose-level (mg/kg/day) | | 5 | | |
| Value | | Min. | Mean | Max. |
| carbetamide | Cmax (µg/mL) | ND | 0.16 | 0.47 |
| | Tmax (hour) | - | 0.5 | 0.5 |
| Metabolite* | Cmax (µg/mL) | 1.40 | 2.15 | 2.98 |
| | Tmax (hour) | 0.5 | 0.5 | 0.5 |

ND: none detected (<0.1 µg/mL); * carbetamide metabolite: 29835 RP

There were no treatment-related deaths. Clinical signs were only observed in the highest dose group (320 mg/kg/day): unsteadiness, inactivity, increased respiratory rate.

Body weight loss was observed during the first 4 days of treatment at 320 mg/kg/day (Table 52). Yet it was recovered so body weight gain over the whole gestation was not influenced by the treatment. Food and water intake showed the same variations.

Necropsy of adults showed no treatment-related finding apart from accentuated lobular pattern of the liver.

Two high dose group females (320 mg/kg/day) aborted, without any specific finding at necropsy. The same group showed a higher incidence of post-implantation loss (almost 4-fold compared to control values (Table 81). The other litter parameters were unaffected by treatment, except for a lower litter size in the highest dose-level group (-26% when compared to controls), but within historical control values.

There was an increased incidence of skeletal abnormalities in foetuses from 320 mg/kg/day treated females (Table 81): signs of immaturity (incomplete ossification of vertebrae and long bones), higher number of ribs and presacral vertebrae.

The manufacturer submitted the historical control occurrence of a 13$^{th}$ pair of ribs and incomplete ossification of vertebrae in rabbit offspring from 86 studies with 8655 foetuses from the testing laboratory (Table 79 and 80). However it is not known if the HCD are contemporary to the Tesh *et al.* study as no information was given regarding the time by which the studies were performed.

Table 79: Occurrence of a 13$^{th}$ pair of ribs in offspring treated with carbetamide vs. control animals and historical backgroung data of the laboratory (%)

| Controls | 5 mg/kg | 40 mg/kg | 320 mg/kg | Historical mean (8655 foetuses) | Historical range (86 studies) |
|---|---|---|---|---|---|
| 27.9 | 27.7 | 40.9 | 86.3 | 35.91 | 11.9-61.0 |

Table 80: Occurrence of incomplete ossification of vertebrae in offspring treated with carbetamide vs. control animals and historical backgroung data of the laboratory (%)

| | Controls | 5 mg/kg | 40 mg/kg | 320 mg/kg | Historical mean (8655 foetuses) | Historical range (86 studies) |
|---|---|---|---|---|---|---|
| Incomplete ossification of vertebrae | | | | | | |
| Cervical | 3.9 | 1.0 | 7.1 | 23.2 | 2.11 | 0.0-9.9 |
| Thoracic | 0.8 | 0 | 1.6 | 0 | 0.75 | 0.0-11.5 |
| Caudal | 0.8 | 0 | 0 | 0 | 0.07 | 0.0-1.3 |
| Pooled data | 5.5 | 1.0 | 8.7 | 23.2 | 2.93 | 0.0-22.7 |

The occurrence of a 13$^{th}$ pair of ribs in the offspring of dams treated at 40 mg/kg was 40.9% compared to 27.9% in control animals and 27.7% at 5 mg/kg (but still in the range of HCD). Thus, the incidence of 13$^{th}$ pair of ribs at 40 mg/kg is slightly above the mean value for the spontaneous occurrence (35.9%). Furthermore there is a clear dose response relationship.

The incidence of incomplete ossification of cervical vertebrae in the high dose group is above the historical range, however this dose level causes maternal toxicity.

Nine foetus of 127 foetuses (7.1 %) of the 40 mg/kg bw group showed an incomplete ossification of cervical vertebrae. Twenty two of 95 foetuses (23.2%) in the offspring of high dose dams and 5 of 129 foetuses (3.9%) in controls showed this effect. Thus, a dose response relationship for this effect has been observed and at the intermediate 40mg/kg bw dosage these observations could be considered indicative of a adverse fetal response to Carbetamide. Moreover, although the incidence of incomplete ossification of cervical vertebrae observed at 40 mg/kg bw is comprised in the historical range (0 to 9.9%), it is above the mean value for the spontaneous occurrence (7.1% vs. 2.11%).

Other abnormalities were of the types and frequencies usually recorded in foetuses of this strain of rabbits.

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

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

- **Conclusion**

When given by oral route to pregnant rabbits during the organogenesis period, carbetamide induced at the highest dose-level of 320 mg/kg/day:

- Transiently lower body weight gain in adults,
- Abortions (2/15 females) and higher incidence of post-implantation loss,
- Some signs of foetal immaturity, including delayed ossification of vertebrae and long bones,
- Benign foetal abnormalities: supernumerary 13$^{th}$ and 14$^{th}$ ribs and presacral vertebrae.

No maternal toxicity was observed at lower doses, but incomplete ossification of vertebrae and surnumerary ribs were observed in foetus of dams treated at 40 mg/kg/day. Although these increases are in the range of the HCD submitted by the manufacturer, a clear dose response relationship was observed from the 40 mg/kg dose. Moreover, information is lacking regarding the time range of the studies.

The NOAEL for maternal toxicity was 40 mg/kg/day, and embryotoxic effects were observed at 40 and 320 mg/kg/day, resulting in a NOAEL for foetal toxicity of 5 mg/kg/day.

### 4.11.2.2 Human information

No data

### 4.11.3 Other relevant information

No data

### 4.11.4 Summary and discussion of reproductive toxicity

Carbetamide was well tolerated when given in the diet to rats for 2 generations at the concentrations of 0, 1000, 3000 and 7500 ppm:

- In parents, body weights of the F0 generation were lower in both sexes at the highest dose level (max -20% when compared to controls for the first 13 weeks) than in controls, correlated with a minimally lower (-6%) food intake. Gestation length was minimally longer in F1-groups 3 and 4 for the first pregnancy (A), and F1-group 3 for the second (B), compared to controls, but this was not associated with changes in litter size, foetal viability or birth weight. Relative liver weights were higher than controls in males of the F0 and F1 generations treated at the highest dose-level and in females treated at all dose-levels. These changes in organ weights were associated with centrilobular hepatocyte hypertrophy in males and females treated at 3000 and 7500 ppm.
- Offspring body weight gains till weaning were lower in all litters of the highest dose-level group (up to -16%) when compared to controls, and higher absolute and/or relative liver weights were observed in male and female pups of the F2B generation treated at the highest dose-level, in association with increased incidence of centrilobular hepatocyte hypertrophy in males and females treated at 3000 and 7500 ppm.

Higher mean liver weight was observed in all adult female groups of F0 and F1 generations, whatever the dose-level, and therefore no NOAEL could be determined for parental toxicity. However, the effects observed at the lowest dose-level were minimal and not associated with lesions at histopathological examination of the liver.

Reproductive parameters were not affected by the treatment, except lower body weight gain up to weanling in all litters treated at the highest dose-level, and liver enlargement associated with centrilobular hepatocyte hypertrophy in F2B pups treated at the highest dose-level. Therefore, the LOAEL for parental toxicity was 1000 ppm, and the NOAEL for toxicity to reproduction was 3,000 ppm (approximately 208 mg/kg/day for combined sexes).

Carbetamide, when given orally to pregnant rats from days 6 to 15 of gestation, was associated with maternal embryotoxic and teratogenic adverse effects at the highest dose-level of 1000 mg/kg/day:

- Lower body weight gain in adults,
- Reduced foetal weight (-18% when compared to controls),
- Generalized foetal immaturity, characterized by soft tissue variations and ossification retardation,
- Several foetal abnormalities including complex malformations associating elongated genital tubercle, imperforate anus and vestigial/absent tail, and cardiovascular malformations.

At the dose-level of 450 mg/kg/day, no adverse effects were observed for dams, and the abnormalities observed in foetuses were of the types and frequencies usually recorded in foetuses of this strain of rats, and not considered by the MCSA to be treatment related. However, one foetus at the dose-level of 450 mg/kg/day showed darkening of thyroid glands without effects on weight or maturity of the thyroid.

Carbetamide was therefore clearly teratogenic in rats at the materno-toxic dose level of 1000 mg/kg/day. The NOAEL of carbetamide was 450 mg/kg/day for maternal, foetal toxicity and teratogenicity.

When given by oral route to pregnant rabbits during the organogenesis period, carbetamide induced at the highest dose-level of 320 mg/kg/day:

- Transiently lower body weight gain in adults,
- Abortions (2/15 females) and higher incidence of post-implantation loss,
- Some signs of foetal immaturity, including delayed ossification of vertebrae and long bones,
- Benign foetal abnormalities: supernumerary $13^{th}$ and $14^{th}$ ribs and presacral vertebrae.

No maternal toxicity was observed at lower doses, but incomplete ossification of vertebrae and surnumerary ribs were observed in foetus of dams treated at 40 mg/kg/day. Although these increases are in the range of the HCD submitted by the manufacturer, a clear dose response relationship was observed from the 40 mg/kg dose. Moreover, information is lacking regarding the time range of the studies.

The NOAEL for maternal toxicity was 40 mg/kg/day, and embryotoxic effects were observed at 40 and 320 mg/kg/day, resulting in a NOAEL for foetal toxicity of 5 mg/kg/day.

The overall NOAEL for reproductive effects of carbetamide is 5 mg/kg/day, based on the teratogenicity study in rabbits.

### 4.11.5 Comparison with criteria

***Category 1 Known or presumed human reproductive toxicants***

> Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Application of the classification criteria of Annex I to Regulation (EC) 1272/2008 to the available body of reproductive toxicity data for Carbetamide indicate that a classification into category 1A can be ruled out because the substance is not known to cause reproductive toxicity in humans. Furthermore, there is no a evidence that Carbetamide adversely affects sexual function and fertility or development in the absence of other toxic effects.

***Category 2 Suspected human reproductive toxicants***

> Substances are classified in Category 1 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.
> Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

At the dose level of 1000 mg/kg/day, carbetamide induced severe abnormalities in foetuses (generalised fetal immaturity and high incidence of abnormalities including complex malformations associating elongated genital tubercle, imperforate anus and vestigial/absent tail, and cardiovascular malformations). At this dose level, only a slight reduction in maternal weight gain during late gestation was observed; therefore Carbetamide can be considered as a teratogen agent in rats.

Moreover, several findings observed both in rats (dark thyroid) and rabbits (dose related skeletal abnormalities: incomplete ossification and supernumerary ribs) may suggest a slight effect of carbetamide on the development at dose levels without maternal toxicity. According to classification criteria of Annex I to Regulation (EC) 1272/2008, MCSA proposed that carbetamide should be classified as a category 2, H361d: Suspected of damaging the unborn child.

### 4.11.6 Conclusions on classification and labelling

Classification reproductive toxicity cat. 2 (H361d) is proposed based on the developmental effects observed in rats at dose levels where maternal toxicity is not strong enough to explain the findings. In addition, several findings observed both in rats (dark thyroid) and rabbits (dose related skeletal abnormalities: incomplete ossification and supernumerary ribs) could suggest a slight effect of carbetamide on the development at dose levels without maternal toxicity.

## 4.12 Other effects

### 4.12.1 Non-human information

#### 4.12.1.1 Neurotoxicity

No acute, subchronic or post-natal neurotoxicity study was performed. Neuromuscular signs including hind limb unsteadiness, drowsiness and tremors were observed together with signs of neurovegetative dysfunctions during repeated dose toxicity studies in dogs. These signs were transient and did not progress, but rather attenuated when the study progressed. These studies failed to reveal any effect of carbetamide on the nervous system.

As far as delayed neurotoxicity following acute exposure is concerned, one study was performed in hens. In order to administer high doses of carbetamide, scavengers were administered at the same time against the effects related to its acute inhibition of Acetyl-Choline esterase.

- **Reference:** **Ross DB, Roberts NL, Philipps CNK, Majeed SK, Prentice DE (1978):** The acute oral toxicity (LD50) and assessment of neurotoxic effects of carbetamide on the domestic hen. Rhône-Poulenc Agro, Centre de Recherche, Lyon, France - Unpublished report No. RNP 94/78991 - Dates of work: 00/05/1978 to 27/11/1978.
- **Guidelines:** US-EPA Draft Guideline "Acute delayed neurotoxicity" (1977). OECD guideline 418 is based on the procedure applied in this study.
- **GLP standards:** No.
- **Deviations:** Not applicable.
- **Study acceptable:** Yes.
- **Test system:**

Technical carbetamide (batch MAG 73, purity: 95.8%) was given orally to adult hens (12-14 months old, bw ranging 1.80-2.72 kg) in corn oil (40% w/v).

A preliminary dose range finding study tested dose-levels of 500, 1000, 1500, 2000 and 5000 mg/kg/day (which was the maximal practicable single oral dose). No adverse effects were observed on clinical signs,mortality or body weights. 5 birds were given the vehicle only, and 10 were given carbetamide at 5000 mg/kg in order to confirm the absence of toxicity.

In the neurotoxicity test, 5 groups of 10 birds were given single oral doses of respectively the vehicle only, positive control Tri-ortho-cresyl-phosphate (TOCP, 20% in vehicle, 500 mg/kg), and 500, 1250 or 5000 mg/kg of carbetamide. Carbetamide treated hens were protected by co-administration of pyridine-2-aldoxime methane sulphonate (50mg/kg) and atropine (10 mg/kg) to compensate for potential acute effects of the cholinesterase inhibition induced by the test article. The study lasted 21 days.

Each day hens were observed for mortality, clinical signs, and ataxia was assessed using a scale ranging from 0 (absence) to 8 (unable to stand). At study termination all birds were sacrificed and samples of spinal cord and peripheral nerve were taken for histological examination.

- **Results**

There were no treatment-related deaths either in the preliminary tests or in the neurotoxicity test. The acute oral LD50 of carbetamide is over the maximal practicable dose-level of 5000 mg/kg.

General clinical signs, neurotoxic signs and bodyweights were not treatment-related. There were no positive ataxia score on any occasion at any carbetamide dose-level. TOCP caused marked body weight loss and ataxia from day 13 (Table 82).

Table 82: Body weight changes and ataxia scores in controls and carbetamide treated groups

| **Group** | | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|---|
| **Treatment** | | **Vehicle** | **TOCP** | **carbetamide*** | | |
| **Dose-level (mg/kg)** | | **-** | **500** | **500** | **1250** | **5000** |
| Mean body weight change (g) days 0-21 | | -61 | -429 | +84 | +73 | +71 |
| Mean Ataxia score | before day 13 | 0 | 0 | 0 | 0 | 0 |
| | days 13-15 | 0 | 3.4 | 0 | 0 | 0 |
| | days 16-18 | 0 | 4.4 | 0 | 0 | 0 |
| | days 19-21 | 0 | 4.8 | 0 | 0 | 0 |

* including protection with pyridine-2-aldoxime methane sulphonate (50 mg/kg) and atropine (10 mg/kg)

At necropsy, no treatment-related changes were detected in hens treated with carbetamide.

- **Conclusion**

carbetamide given as a single oral dose to hens, simultaneously with atropine and pyridine-2-aldoxime methane sulphonate caused no mortality, clinical sign, body weight loss or neurotoxic effect at any dose-level, up to the maximal achievable dose-level of 5000 mg/kg. The NOAEL of carbetamide is 5000 mg/kg in this study.

#### 4.12.1.2 Immunotoxicity

No data

#### 4.12.1.3 Specific investigations: other studies

No data

#### 4.12.1.4 Human information

A medical surveillance is available from a Carbetamide production site in France (Decalf, 2000).

There were no clinical abnormalities observed, which were attributable to Carbetamide production.

### 4.12.2 Summary and discussion

No further effects noted.

### 4.12.3 Comparison with criteria

Not applicable

### 4.12.4 Conclusions on classification and labelling

Not applicable

# 5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of Carbetamide were assessed in the Draft Assessment Report and additional report prepared in the context of the possible inclusion of Carbetamide in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005, Addendum 1, September 2007, and Additional report, July 2010, RMS France) as well as the conclusion regarding the peer review of the pesticide risk assessment of the active substance Carbetamide (EFSA Journal 2010;8(12):1913).

## 5.1 Degradation

Table 83: Summary of relevant information on degradation

| Method | Result | Remarks | Reference |
|---|---|---|---|
| Hydrolysis study | pH 3 and 6: stable (≤ 10 % degraded after 1 month at 25°C)<br>pH 9, 25°C: $DT_{50}$= 21 days (1st order)<br>pH 9, 35°C: $DT_{50}$ = 7 days (1st order) | $^{14}$C-Carbetamide, purity 98.5% | Buys, M., Chabassol, Y., Laurent, M. (1979) |
| Molar absorption coefficient,<br>OECD 101 | No light absorption at λ > 290 nm.<br>Photolysis not expected, no study available, not required. | UV visible spectrum | Jendrzejczak NH, Turier GP, and Adrian PP. (1992) |
| Ready biodegradability<br>OECD 301 D | Not readily biodegradable | test units maintained at 20°C for 28 days | Voigt, Günther. (1989) |
| | | | Dengler, D. (2009) |
| Water/ sediment study | DissT50/DissT90 water = 8/94.5 and 12.8/499 days (DFOP kinetics)<br>DissT50 sediment = 150 and 424 days (SFO kinetics)<br>DT50/DT90 system = 33.6/1017 days and 81/3650 days (FOMC kinetics) | Test performed with Sandy clay loam and loam type sediment<br>Dark aerobic conditions, 20°C | Ayliffe, J.M., Hardy, I.A.J. (1996) |
| | | | O'Brien (2006) |
| Photochemical transformation in air | DT50air = 2.151 hours | Atkinson method | Tiemann J (2003) |
| Volatilization from soil surface<br>BBA - Part IV, 6-1 (July 1990) | No considerable volatilisation amounts of Carbetamide from soil or plant surfaces (maximum 9% disappearance within 24 hours) | Product Pradone Kombi (containing 50% Carbetamide and 25%Dimefuron) | Offizorz, P., Knoch, E. (1992) |
| Adsorption/Desorption of carbetamide | mean adsorption constant (1/n) = 0.93<br>mean Koc = 88.6 mL/g | Test performed on four soils | Reeves G.L., Savage E.A. (1985) |

**Hydrolysis (Buys, M., Chabassol, Y., Laurent, M. (1979))**

No guidelines were mentioned in the study and the study was performed before OECD 111 was published. However, the study is considered well performed and is acceptable.

The stability of Carbetamide in water depends on the temperature and pH of solution. In the hydrolysis study at pH 9, Carbetamide is degraded fairly quickly ($DT_{50}$ of 21 days at 25°C and 6 days at 35°C). Aniline and 10 810 RP are the main degradation products reaching respectively 16% and 40% of the applied radioactivity at 25°C. At pH 6 and pH 3, Carbetamide is hydrolysed slowly (less than c.a. 10% being degraded after 1 month at 25°C, $DT_{50}$ not determined) and the main degradation products are respectively 10 810 RP (max <5% at pH 6) and 12 913 RP (max <10% at pH 3) at 25°C.

**Photolysis in water**
The molar absorption coefficient of Carbetamide at $\lambda > 295$ nm was found to be less than 10 $mol^{-1}$ $cm^{-1}$ (Jendrzejczak NH, Turier GP, and Adrian PP. (1992)). Photodegradation in water is therefore not expected to be a significant process.

## 5.1.1 Biodegradation

### 5.1.1.1 Biodegradation estimation

Not relevant as screening and simulation tests are available (see below).

### 5.1.1.2 Screening tests

The biodegradability of Carbetamide was investigated in two ready biodegradability studies according to OECD guideline 301 D (closed bottle test, Voigt, G (1989) and Dengler, D. (2009)).

- **Voigt, G (1989)**

**Methods**
The degradation of carbetamide was investigated in activated sludge originating from a municipal water treatment plant. The oxygen demand of carbetamide treated inocula were determined and compared to blank (without any substance) and reference item inocula. The oxygen demand of the test medium without sludge was also tested. The concentration of carbetamide was 20 mg/L, the reference item sodium acetate was tested at 10 mg/L.
The test units were maintained at 20°C for 28 days.
The oxygen demand in each treatment group was determined for up to 28 days in duplicate on each sampling occasion.

**Results**
The theoretical oxygen demand of the carbetamide test units was 35.2 mg $O_2$/L. After an incubation period of 28 days, the actual mean oxygen demand for the carbetamide inocula was 2.5 mg $O_2$/L, which is equal to 7.1% of the theoretical demand. The oxygen consumption of the untreated inoculum in test medium was 1 mg $O_2$/l. No oxygen consumption was found in the blank test medium.

**Conclusion**
Carbetamide was not readily biodegradable under test conditions, since only 7.1 % of the TOD was consumed after 28 days.

- **Dengler, D. (2009)**

**Guidelines**: OCDE Guideline for testing of chemicals N° 301 D: Ready biodegradability
EC method C.4-E (92/69/EEC), biological degradability, determination of the ready degradability, part VI, closed bottle test

**Materials and methods**
The ready biodegradability of the carbetamide tech. (batch No. 20080741) was estimated in a mineral test medium at a concentration of 2 mg/L which was inoculated with micro-organisms from a municipal wastewater treatment plant (Pforzheim, Germany).

The test solutions were kept in glass bottles closed and filled with aerated test medium. The closed bottles are maintained at 20 ± 2°C and in the dark. Degradation was determined by measuring dissolved oxygen over a 28-d period. The amount of oxygen taken up by the microbial population during biodegradation of the test item, corrected for uptake by the blank inoculum run in parallel, was expressed as a percentage of the ThOD (theoretical oxygen demand).

A control with inoculum, but without test item was run in parallel for the determination of oxygen blank. Additionally, in order to check the procedure, Na-Benzoate was used as a degradable reference item at a concentration of 2 mg/L, along with a toxicity control with Carbetamide tech. and 2 mg/L Na-Benzoate.

One vessel from each treatment group was analysed immediately after application. Subsequently, one bottle (of each treatment) was removed and analysed for O2 after 4 days, two bottles after 11 days and three bottles (from each treatment) after 7, 14, 21, and 28 days. Measurement of O2 consumption was done with a WTW Microprocessor Oximeter OXI 340 and a calibrated electrode. Each test assay was measured twice.

This corrected depletion was divided by the concentration (mg/L) of the test item, to obtain the specific BOD as mg oxygen per mg test item. The percentage biodegradation was calculated by dividing the specific BOD by the theoretical specific oxygen demand (ThOD), calculated from the molecular formula in accordance with the OECD guideline.

**Findings**
The initial bacterial cell numbers of the inoculum were determined to be 1.16 x 105 cells in each test vessel.

The theoretical oxygen demands (ThOD) for the test item, for Na-benzoate and for the toxicity control were 2.30 mg O2/mg test item, 1.67 mg O2/ mg reference item and 1.99 mg O2/mg substance mixture, respectively. On average, the initial oxygen contents of the aerated test media were 8.86 mg/L for the inoculum blank, 8.96 mg/L for the reference item, 8.91 mg/L for the test item and 9.03 mg/L for the toxicity control.

A maximum degradation of 3.3% was determined for the carbetamide tech. after an incubation period of 11 days. The degradation of the toxicity control was > 25% after 14 days (calculated for the mixture “Na-benzoate, 2 mg/L + carbetamide tech., 2 mg/L”). Toxic effects of carbetamide tech. can be excluded, since the degradation of the toxicity control was > 25% after 14 days (calculated for the mixture "Na-benzoate, 2 mg/L + Carbetamide tech., 2 mg/L"). The degradation of the reference item sodium benzoate reached 65.3% within the first 14 days. The residual O2 content did not fall below 0.5 mg/L at any time. The average O2 consumption in the inoculum blank after 28 days was < 1.5 mg/L O2. Therefore, the test can be considered as valid.

**Conclusion:**
The criterion for ready biodegradation of > 60% removal of the ThOD (theoretical oxygen demand) within 28-days period was not reached. Therefore, carbetamide tech. cannot be considered as readily biodegradable under the conditions of this closed bottle test.

### 5.1.1.3 Simulation tests

Degradation in water/sediment
The route and rate degradation of $^{14}$C-Carbetamide were examined in two aquatic systems (River and Stream) under aerobic conditions over a 100-day period in the **Ayliffe J.M. and Hardy I.A.J. study (1996)**.

- **Ayliffe J.M. and Hardy I.A.J. (1996)**.

**Materials and methods:**
The route and rate degradation of $^{14}$C-Carbetamide were examined in two aquatic systems (River and Stream) under aerobic conditions over a 100-day period in the study of Ayliffe and Hardy, 1996). The route of degradation of this study was presented in the monography of December 2005 about Carbetamide.

Two water-sediment systems were established containing natural sediment and water source. The physical and chemical characteristics of the aquatic systems are given below.

Table 84: Physical and chemical characteristics of the aquatic systems (Ayliffe and Hardy, 1996)

| Characteristics | System | |
|---|---|---|
| | River Roding | Manningtree Stream |
| Sediment characteristics | | |
| Particle size distribution (%)<br>Sand<br>Silt<br>Clay | <br>47.69<br>25.77<br>26.54 | <br>40.14<br>43.53<br>16.31 |
| Soil texture (USDA Classification) | Sandy clay loam | Loam |
| pH (water) | 7.7 | 6.2 |
| Organic carbon | 2.8 | 6.5 |
| Cation exchange capacity (CEC) (mEq/100g) | 75 | 17.3 |
| Microbial biomass at start (µg C/g) | 294 | 263 |
| Microbial biomass at end (µg C/g) | 70 | 199 |
| Water characteristics | | |
| Water hardness (mg/L as $CaCO_3$) | 349 | 327 |

| Characteristics | System | |
|---|---|---|
| | River Roding | Manningtree Stream |
| Total organic carbon (ppm) | 64.04 | 32.73 |
| pH (at time of application) | 7.90 | 6.19 |
| Temperature (°C) | 13.3 | 9.5 |
| Redox potential (mV) | 170 | 179 |
| Oxygen content just below surface (%) | 93 | 45 |
| Oxygen content sediment level (%) | 85 | 13 |

The sediments were sieved through a 2 mm. Sediments and water phase were stored separately at 4°C in the dark until use. Seven weeks prior to application of the test substance, the test units were filled with sediment and water resulting in layers of 2.5 and 6.0 cm, respectively. A continuous stream of $CO_2$-free air was passed trough the water and the test units were maintained at 20 ± 2°C in the dark for acclimatisation until test start.

At test start, 0.180 mg of $^{14}$C-Carbetamide (batch, specific activity 1.0 GBq/mmol; radiochemical purity: 99.0%) solved in methanol (220µL) was added to surface water of each test units, corresponding to a treatment rate of 2.0 kg a.s./ha.

**Results**
The maximum concentration of Carbetamide in the sediment (38% of applied radioactivity) was observed at 14 days.
With HPLC[13] measurements, Carbetamide-COOH was detected at a maximum of 6.55% of applied radioactivity in the whole system of Manningtree system.
With TLC[14] measurements, Carbetamide-COOH was detected at a maximum of 5.1% of applied radioactivity in the whole system of River Roding at 2 days and 7 days.

After 14 days, 31.7-33.2% of active radioactivity of carbetamide was found in sediment.

**Mineralization and non extractable residues**

Table 85: Mineralisation and extractable residues (Ayliffe and Hardy, 1996)

| Water / sediment system | pH water phase | pH sed | Mineralization max % at the end of the study | | Non-extractable residues in sed. max % at the end of the study | |
|---|---|---|---|---|---|---|
| River Roding | 7.90 | 7.7 | 29.18 | 100 | 30.01 | 100 |
| Manningtree | 6.19 | 6.2 | 19.87 | 100 | 25.65 | 100 |

As mentioned above, the degradation rates calculated in this study are not presented here since updated kinetic fitting were performed in O'Brien 2006 (see below).

- **O'Brien, 2006**

**Methods**
The kinetic evaluation of O'Brien, 2006 can be regarded as the key study for the degradation of carbetamide in water/sediment system and hence for classification and labelling. Degradation rates were derived for water-sediment data by analysis of level-I kinetic modelling of data from River Roding and Manningtree Stream water-sediment systems according to FOCUS Kinetics guidance document[15].

**Results**
Concerning the water column of River Roding and of Manningtree Stream, the best fit was obtained using DFOP[16] kinetics. Less than 10% of initial concentration was reached within the experimental period and the FOMC[17] curve

[13] HPLC: High-performance liquid chromatography
[14] TLC; Thin layer chromatography
[15] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp
[16] DFOP : Double First-Order in Parallel
[17] FOMC : First Order Multi-Compartment

trends above the final data point values while both DFOP and HS[18] curves match the data well up to and through this 10% amount. For the sediments of River Roding and Manningtree Stream, the best fit was obtained using SFO[19] kinetics. Concerning the whole system of River Roding and of Manningtree Stream, the best fit was obtained using FOMC kinetics.

Table 86: Evaluation of level-I kinetic modelling data for River Roding and Manningtree Stream (O'Biren, 2006)

| Parameters | River Roding | | | Manningtree Stream | | |
|---|---|---|---|---|---|---|
| | Water (DFOP) | Sediment (SFO) | Whole system (FOMC) | Water (DFOP) | Sediment (SFO) | Whole system (FOMC) |
| DissT50 [d] | 8.0 | 150 | 33.6 | 12.8 | 424 | 81.0 |
| DissT90 [d] | 94.5 | 499 | 1017 | >100 | 1409 | 3650 |

### 5.1.2 Summary and discussion of degradation

Carbetamide is stable to hydrolysis at pH 3 and pH 6, but degrades quickly at pH 9 (DT50 = 21 days at pH 9, 25°C). Based on the molar absorption coefficient of Carbetamide, photolysis in water is not expected to be a significant process of dissipation of Carbetamide in aquatic systems.

The rate and route of degradation of carbetamide has been investigated in 2 water-sediment systems under dark aerobic conditions. In both systems, Carbetamide exhibited moderate to medium persistence (DT50 whole system 34 to 81 days, DT90 > 1000 days, FOMC kinetics), forming no major metabolites.

## 5.2 Environmental distribution

### 5.2.1 Adsorption/Desorption

- **Reeves G.L., Savage E.A. (1985)**

**Methods**
The adsorption/desorption of [$^{14}$C]-carbetamide (Batch 0883, specific activity 27 mCi/mmol; radiochemical purity 94%) was investigated in loamy sand, loam, sandy loam and clay loam soils (pH 6.8 – 7.8, OC content 0.99% - 3.06%) in a batch adsorption study conducted according to OECD 106,

To determine the rate of adsorption, [$^{14}$C]-carbetamide was dissolved in 0.01M calcium chloride aqueous solution at concentrations of 0.01, 0.1, 1.0 and 10 µg a.s./mL. Ten mL of these solutions were added to 2.5 g dry soil each in duplicate. The test tubes were sealed and maintained agitated for 24 h at 20 °C in the dark. The tubes were then centrifuged at 800 x g for 10 minutes and radio-analysis of each supernatant was performed by LSC. Desorption of carbetamide was investigated by re-suspending the soil pellets in 10 mL aqueous $CaCl_2$–solution for 20 minutes. The tubes were then centrifuged and the supernatant analysed as described above. This procedure was repeated four times.
After the last desorption cycle, the soil pellets were air dried for 24 hours and soil residues were investigated in triplicate by combustion and radio-analysis.
TLC analyses were carried out on selected adsorbates and desorbates, and on methanol extracts of the air dried loamy-sand soil to control the stability of carbetamide during the test. TheFreundlich adsorption parameters and the $K_{OC}$ values were calculated.

**Results**
The average overall recovery of radioactivity was 96.3 - 99.4%. All samples were acceptable, except one clay loam sample, which was contaminated, giving falsely high recovery, and was eliminated from analysis.

The TLC demonstrated that carbetamide represented c.a. 80 to 95% of the total radioactivity, with two exceptions (79% and 64%). In most cases, no other peak was however identified on the TLC, demonstrating the lack of significant degradation of carbetamide.

[18] HS : Hockey Stick
[19] SFO: Single First Order

Carbetamide adsorption is positively correlated with the soil silt content. This adsorption however, is readily reversible, as shown by the values of the second and third desorption cycles, where stronger adsorption by soil containing a high percentage of sand becomes apparent. The organic carbon content of the soil appears to have little or no influence on the adsorption of carbetamide.

For carbetamide, the Freundlich adsorption constant Kf was determined to be 0.67 - 1.86 mL/g. The adsorption coefficient (1/n) was found between 0.93 – 1 and corresponding $K_{OC}$ was 59.5 - 118.2 mL/g. These results indicate a weak adsorption to soil particles, whatever the soil type with mean adsorption constant (1/n) of 0.93, and mean Koc of 88.6 mL/g.
Desorption K values were considerably higher than those for adsorption, and increased with each desorption cycle.

### 5.2.2 Volatilisation

Carbetamide has low vapour pressure ($3.10^{-7}$ Pa at 20° C) and relatively low Henry law constant (1.93 .10-8 at 20°C). It is therefore not expected to volatise from plants and soil surfaces in significant amounts. In laboratory experiments, volatilisation represented 9% and 3% AR from plant and soil surface within 24 hours, respectively (Offizorz P., Knoch E. (1992)). Oxidative photodegradation in air is very rapid ($DT_{50}$ Atkinson 2.151 hours, calculations based on structural data using version 1.9 of AOP Win, Tiemann J (2003)). Therefore long range transport is not expected.

### 5.2.3 Distribution modelling

Please refer to previous chapters.

## 5.3 Aquatic Bioaccumulation

### 5.3.1 Aquatic bioaccumulation

#### 5.3.1.1 Bioaccumulation estimation

There is no indication for a bioaccumulation potential of Carbetamide (log POW of 1.78 at pH = 7).

#### 5.3.1.2 Measured bioaccumulation data

There is no indication for a bioaccumulation potential of Carbetamide (log Pow of 1.78 at pH = 7). No BCF study was conducted, since it is required if log POW >3.

### 5.3.2 Summary and discussion of aquatic bioaccumulation

Carbetamide is not considered to have potential for bioaccumulation (log POW = 1.78).

Carbetamide has a log POW of 1.78 (pH = 7) and hence it is not considered to have potential for bioaccumulation. No BCF study was conducted, since it is required if log POW >3.

## 5.4 Aquatic toxicity

Table 87: Summary of relevant information on aquatic toxicity

| Method* | Species | Results | Remarks | Reference |
|---|---|---|---|---|
| OECD 203 | *Oncorhynchus mykiss* | LC50 (96 h) > 100 mg/L<br>NOEC (96 h) = 50 mg/L<br>(nominal) | Purity: 96.5%- | Memmert, U. &<br>Knoch, E. (1994a), Document |

| | | | | |
|---|---|---|---|---|
| | | | | No: RCC452901 |
| OECD 203 | *Cyprinus carpio* | LC50 (96 h) > 100 mg/L<br>NOEC (96 h) = 46 mg/L<br>(nominal) | Purity: 96.5%- | Memmert, U. &<br>Knoch, E. (1994b), Document<br>No: RCC452903 |
| OECD 203 | *Oncorhynchus mykiss* | LC50 (96 h) > 100 mg/L<br>NOEC (96 h) = 100 mg/L<br>(nominal) | Study conducted with Carbetamide-COOH<br>Purity: 99.0% | Gonsior, G. (2008), Document<br>No: S08-01260 |
| OECD 204 | *Oncorhynchus mykiss (*formerly *Salmo gairdneri)* | NOEC (21 d) = 32 mg/L<br>(nominal) | Purity: 95.0% | Douglas, M. T. et al. (1989), Document<br>No: RNP 300/89578 |
| OECD 202 | *Daphnia magna* | EC50 (48 h) = 81 mg/L<br>NOEC (48 h) = 13.3 mg/L<br>(nominal) | Purity: 96.8% | Knacker, Th. &<br>Hilt, J. (1989), Document<br>No: BE-ET-01-89-02-DAK-1 |
| OECD 202 | *Daphnia magna* | EC50 (48 h) > 100 mg/L<br>NOEC (48 h) = 10 mg/L<br>(nominal) | Study conducted with Carbetamide-COOH<br>Purity: 99.0% | Bormann, K. (2008), Document<br>No: S08-00873 |
| OECD 202 | *Daphnia magna* | NOEC (21 d) = 1.0 mg/L<br>(nominal) | Purity: 96.5% | Memmert, U. &<br>Knoch, E. (1994c), Document<br>No: RCC 452905 |
| EEC 92/69/EEC C.3 | *Scenedesmus subspicatus* | EbC50 (72 h) = 158 mg/L<br>ErC50 (72 h) = 305 mg/L<br>NOECr (72 h) = 125 mg/L<br>(nominal) | Purity: 98.8% | Scheerbaum, D. (2003), Document<br>No: SSO88411 |
| EEC 92/69/EEC C.3 | *Navicula pelliculosa* | EbC50 (72 h) = 128 mg/L<br>ErC50 (72 h) = 212 mg/L<br>NOECr (72 h) = 50 | Purity: 98.8% | Scheerbaum, D. (2003), Document<br>No: SVO88411 |

| | | | | |
|---|---|---|---|---|
| | | mg/L<br>(nominal) | | |
| OECD 201 | *Desmodesmus subspicatus* | EC50 (72 h) > 100 mg/L<br>NOECr (72 h) = 100 mg/L<br>(nominal) | Study conducted with Carbetamide-COOH<br>Purity: 99.0% | Dengler, D. (2008), Document<br>No: S08-00886 |
| OECD 221 | *Lemna minor* | EbC50 (7 d) = 629 mg/L<br>ErC50 (7 d) = 301 mg/L<br>NOECr (7 d) = 100 mg/L<br>(nominal) | Purity: 98.8% | Scheerbaum, D. (2003), Document<br>No: TLA88411 |
| OECD 221 | *Lemna gibba* | Fronds,<br>EbC50 (7 d) = 110.8 mg/L<br>ErC50 (7 d) > 200 mg/L<br>EyC50 (7 d) = 102.7 mg/L<br>NOECr (7 d) = 25 mg/L<br>(nominal) | Study conducted with Carbetamide-COOH<br>Purity: 99.0% | Dengler, D. (2008), Document<br>No: S08-00896 |
| OECD 219 | *Chironomus riparius* | NOEC (28 d) = 640 mg/L<br>(nominal) | Study conducted with Carbetamide-COOH<br>Purity: 99.0% | Gonsior, G. (2008), Document<br>No: S0801671 |

* during all tests, the temperature and pH parameters were within the acceptable range of the respective guideline.

### 5.4.1 Fish

#### 5.4.1.1 Short-term toxicity to fish

The acute toxicity of Carbetamide and Carbetamide-COOH to fish is summarised in Table 88.

Table 88: Acute toxicity of Carbetamide and Carbetamide-COOH to fish

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration (h), tested substance | Endpoint | Value (mg/L) | |
| OECD 203 | *Oncorhynchus mykiss* | static | 96, Carbetamide | $LC_{50}$<br>NOEC | > 100<br>50 | Memmert, U. & Knoch, E. (1994a), Document No: RCC452901 |
| OECD 203 | *Cyprinus carpio* | static | 96, Carbetamide | $LC_{50}$<br>NOEC | > 100<br>46 | Memmert, U. & Knoch, E. (1994b), Document No: RCC452903 |
| OECD 203 | *Oncorhynchus mykiss* | semi-static | 96, Carbetamide-COOH | $LC_{50}$<br>NOEC | > 100<br>100 | Gonsior, G. (2008), Document No: S08-01260 |

#### 5.4.1.2 Long-term toxicity to fish

The chronic toxicity of Carbetamide to fish is summarised in Table 89.

Table 89: Long-term toxicity of Carbetamide to fish

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration (d) | Endpoint | Value (mg/L) | |
| OECD 204 | *Salmo gairdneri* | static | 21 | NOEC | 32 | Douglas, M. T. et al. (1989), Document No: RNP 300/89578 |

### 5.4.2 Aquatic invertebrates

#### 5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of Carbetamide and Carbetamide-COOH to invertebrates is summarised in Table 90.

Table 90: Acute toxicity of Carbetamide and Carbetamide-COOH to invertebrates

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration (h), tested substance | Endpoint | Value (mg/L) | |
| OECD 202 | *Daphnia magna* | static | 48, Carbetamide | $EC_{50}$<br>NOEC | 81<br>13.3 | Knacker, Th. and Hilt, J. (1989), Document No: BE-ET-01-89-02-DAK-1 |
| OECD 202 | *Daphnia magna* | static | 48, Carbetamide -COOH | $EC_{50}$<br>NOEC | > 100<br>10 | Bormann, K. (2008), Document No: S08-00873 |

The acute study with invertebrates *Daphnia magna* can be regarded as the key study for the aquatic toxicity of Carbetamide and hence for classification and labelling. Therefore the study is presented in more detail below:

**Acute toxicity of Carbetamide to *Daphnia magna***

**Author:** Knacker Th., Hilt J. (1989)
**Report:** A study of the acute immobilisation to Daphnia of Carbetamide. Rhone-Poulenc Agro.
**Report No.:** BE-ET-01-89-02-DAK-1; unpublished
**Guidelines:** OECD 202
**Deviations:** Separate analytical controls: no verification of test substance concentration during the in vivo study.
**GLP/GEP:** Yes
**Validity:** Acceptable
**Material and methods:**
Test substance: Carbetamide, batch OP 3820, purity: 96.8%

The acute toxicity of Carbetamide in *Daphnia magna* was investigated under static conditions for 48 hours. Daphnids 5 to 18 hours of age were taken from the laboratories stock culture. The same climatic conditions were maintained during the test period.

Test solutions were prepared with nominal concentrations of Carbetamide of 2.2, 5.4, 13.3, 32.7, 80 and 100 mg/L using reconstituted water (pH 7.6, hardness: 179 mg/L as $CaCO_3$, 96 % $O_2$-saturation). An untreated control was tested in parallel. At test start the daphnids were placed into the prepared test media. Two test units per concentration level were used containing ten animals each. The test units were maintained at 21 ± 1 °C in the dark.

Test media were analysed for Carbetamide. Daphnids were observed for immobilisation 24 and 48 hours after introduction into the test media.

**Findings:**

Measured concentrations of Carbetamide were 4.48, 10.6, 23.8, 72.8 and 94.7 mg a.s./L. The test media were oxygen saturated (95 to 99 %) and the pH was ranging from 7.7 to 8.0.

The immobilization of daphnids after 24 and 48 hours are shown in the following table:

Table 91: Cumulative acute immobilisation* (%) observed in *Daphnia magna* exposed to Carbetamide

| **Concentration (mg/L)** | | **24 h** | **48 h** |
|---|---|---|---|
| nominal | actual | | |
| Control | 0 | 0 | 0 |
| 2.2 | n.d. | 0 | 0 |
| 5.4 | 4.48 | 0 | 0 |
| 13.3 | 10.6 | 0 | 0 |
| 32.7 | 23.8 | 0 | 5 |
| 80.0 | 72.8 | 0 | 40 |
| 100 . | 94.7 | 100 | 100 |

* mean of two replicates
n.d. not determined

**Conclusion:**

The NOEC and LOEC (48 h) were 13.3 and 32.7 mg/L, respectively. The 48 h $EC_{50}$ value was recalculated at 81 mg/L (nominal concentration).

The concentration is not measured with nominal concentrations of Carbetamide of 2.2 mg/L. However, there is no impact on the validity of the test and on the results because the endpoints concentrations (EC50, LOEC and NOEC) are above the checked concentrations (≥ 5.4 mg/L).

### 5.4.2.2 Long-term toxicity to aquatic invertebrates

The long-term toxicity of Carbetamide to invertebrates is summarised in Table 92.

Table 92: Long-term toxicity of Carbetamide to invertebrates

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration (d) | Endpoint | Value (mg/L) | |
| OECD 202 | *Daphnia magna* | semistatic | 21 | NOEC | 1.0 | Memmert, U. & Knoch, E. (1994), Document No: RCC 452905 |

The chronic study with invertebrates *Daphnia magna* can be regarded as the key study for the chronic aquatic toxicity of Carbetamide and hence for classification and labelling. Therefore the study is presented in more detail below:

**Chronic toxicity of Carbetamide to *Daphnia magna***

**Author:** Memmert U., Knoch E. (1994)
**Report:** Influence of carbetamide on survival and reproduction of *Daphnia magna* in a semistatic test (21 days). RCC Umweltchemie GmbH, Rossdorf, Germany.
**Report No.:** RCC 452905; unpublished
**Guidelines:** OECD 202, part II (1991)
**Deviations:** None.
**GLP/GEP:** Yes
**Validity:** Acceptable
**Material and methods:**
In the first study (RCC 452905), groups of 10 daphnids (first instars of Daphnia magna, ≤ 24h old) were treated with technical carbetamide (batch 9336001, purity: 96.5%) under semi-static conditions, at nominal concentrations of 0 (controls), 0.32, 1.0, 3.2, 10.0 and 32.0 mg/L. Mortality and health of adult daphnids and number of young were recorded three times per week, just before each treatment renewal, until study termination on day 21.

Water characteristics (pH, oxygen) were recorded at least three times per week. The concentrations of the test material were measured on treatment renewal on days 0, 9 and 19 and inside treatment periods on days 12 and 21. They are reported in study RCC 452906.

## Findings:

Water characteristics were regular and within standard ranges.

Achieved test material concentrations were validated by the results of concurrent study RCC 452906 (see Table 93): they were within + 20% of all nominal a.i. concentrations.

Table 93: Concentrations of carbetamide along the study in exposure solutions

| **Nominal concentration (mg/L)** | | **Measured a i. concentration: % of nominal a i. concentration** | | | | | |
|---|---|---|---|---|---|---|---|
| **Test material** | **Active ingredient** | **Day 0[a]** | **Day 9[a]** | **Day 12[b]** | **Day 19[a]** | **Day 21[b]** | **Mean + SD** |
| 1.0 | 0.97 | 77.8 | 80.9 | 80.2 | 79.9 | 85.1 | 81.3 + 3.2 |
| 10.0 | 9.65 | 101.7 | 104.1 | 103.1 | 107.7 | 109.6 | 105.5 + 3.3 |
| 32.0 | 30.88 | 104.0 | 95.6 | 99.1 | 103.4 | 105.1 | 101.6 + 3.8 |

a: data at treatment introduction or renewal, determined on one sample/day
b: data at the end of treatment periods, mean calculated on two samples/day

Survival rates at the end of the 21-day period were reduced at the three highest concentrations (no statistical test available) of 3.2 to 32.0 mg/L (see Table 31). Global health was not affected by the treatment at any concentration.

Released offspring were first observed on day 9, except for the highest concentration at which they were first observed on day 12. Aborted eggs were observed occasionally at 10 mg/L, and frequently at 32 mg/L (see Table 31). Total alive offspring production at the end of the study, and mean alive offspring per surviving adult were both significantly ($p<0.05$) lower in the group treated at the highest concentration of 32 mg/L.

Table 94: Chronic toxicity of carbetamide to *Daphnia magna* – survival rate of adults and cumulated alive offspring production on day 21

| Test material nominal concentration (mg/L) | Survival rate of adults (%) | Total alive offspring | Mean alive offspring per surviving adult |
|---|---|---|---|
| 0 (control) | 90 | 1250 | 128 |
| 0.32 | 90 | 1184 | 125 |
| 1.0 | 100 | 1219 | 122 |
| 3.2 | 70 | 1061 | 116 |
| 10.0 | 70 | 997 | 121 |
| 32.0 | 50 | 467* | 53* |

*: statistical differences ($p<0.05$)

**Conclusion:**

Taking into account the 23% increase in mortality at 3.2 mg/L compared to controls, it is proposed a parental NOEC of 1.0 mg/L. For reproduction, the NOEC was 10.0 mg/L.

The concentration is not measured with nominal concentrations of Carbetamide of 0.32 mg/L. However, there is no impact on the validity of the test and on the results because the endpoints concentrations (LOEC and NOEC) are above the checked concentrations (≥ 1 mg/L).

The measured concentrations for the test material at the nominal concentration of 1 mg/L are slightly below 80 % of the nominal concentration the Days 0 and 19 but are above 80 % of the nominal concentration the Days 9, 12 and 21. Moreover, mean measured concentration is above 80 % of the nominal concentration. Nominal concentration can therefore be considered for endpoints.

### 5.4.3 Algae and aquatic plants

The toxicity of Carbetamide and Carbetamide-COOH to algae and aquatic plants is summarised in Table 95.

Table 95: Long-term toxicity of Carbetamide and Carbetamide-COOH to algae and aquatic plants

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration, tested substance | Endpoint | Value (mg/L) | |
| EEC 92/69/EEC C.3 | *Scenedesmus subspicatus* | static | 72 h, Carbetamide | $E_bC_{50}$<br>$E_rC_{50}$<br>NOECr | 158<br>305<br>125 | Scheerbaum, D. (2003), Document No: SSO88411 |
| EEC 92/69/EEC C.3 | *Navicula pelliculosa* | static | 72 h, Carbetamide | $E_bC_{50}$<br>$E_rC_{50}$<br>NOECr | 128<br>212<br>50 | Scheerbaum, D. (2003), Document No: SVO88411 |
| OECD 201 | *Desmodesmus subspicatus* | static | 72 h, Carbetamide -COOH | $EC_{50}$<br>NOECr | > 100<br>100 | Dengler, D. (2008), Document No: S08-00886 |
| OECD 221 | *Lemna minor* | static | 7 d, Carbetamide | $E_bC_{50}$<br>$E_rC_{50}$<br>NOECr | 629<br>301<br>100 | Scheerbaum, D. (2003), Document No: TLA88411 |
| OECD 221 | *Lemna gibba* | static | 7 d, Carbetamide -COOH | Fronds,<br>$E_bC_{50}$<br>$E_rC_{50}$<br>$E_yC_{50}$<br>NOECr | <br>110.8<br>460.5<br>102.7<br>25 | Dengler, D. (2008), Document No: S08-00896 |

### 5.4.4 Other aquatic organisms (including sediment)

The toxicity of Carbetamide-COOH to sediment dwelling organism is summarised in Table 96.

Table 96: Long-term toxicity of Carbetamide-COOH to sediment dwelling organism

| Guideline / Test method | Species | Exposure | | Results | | Reference |
|---|---|---|---|---|---|---|
| | | Design | Duration (d) | Endpoint | Value (mg/L) | |
| OECD 219 | *Chironomus riparius* | static | 28 | NOEC | 640 | Gonsior, G. (2008), Document No: S08-01671 |

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

Conclusion of environmental classification according to Regulation (EC) No 1272/2008

In aquatic toxicity studies, the lowest $EC_{50}$ value was obtained for invertebrates at Carbetamide concentration of 81 mg/L. Therefore carbetamide does not require an acute classification ($EC_{50}$ > 1 mg/L).

In aquatic toxicity studies, the lowest NOEC value was obtained for invertebrates at Carbetamide concentration of 1 mg/L (based on parental mortality). The parental mortality is considered to be relevant for the population as the mortality of adults could influence the reproduction rate of the population.

Carbetamide is not considered to have a potential for bioaccumulation (log $K_{OW}$ of 1.78 at pH = 7).

Carbetamide cannot be considered as rapidely degradable.

Therefore carbetamide should be considered as chronic category 2 (NOEC = 1 mg/L for this not rapidely deagradable substance).

Classification according to Regulation (EC) No 1272/2008 is required.

Chronic Category 2

GHS Pictogram

Signal Word

-

Hazard Statement

H411: Toxic to aquatic life with long lasting effects.

Precautionary Statement Prevention

P273: Avoid release to the environment

Precautionary Statement Response

P391: Collect spillage.

Precautionary Statement Disposal

P501: Dispose of container in accordance with current local/regional regulation.

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

Carbetamide does fulfil the criteria for classification.

Classification: Chronic Category 2, according to Regulation (EC) No 1272/2008 is required.

## 6 OTHER INFORMATION

No other information.

## 7 REFERENCES

### 7.1 References Identity

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Anonymous | 2000a | Explosivity of Carbetamide technical<br>EBRC Consulting GmbH, Hannover, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. not stated<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Anonymous | 2000b | Oxidizing properties of Carbetamide technical<br>EBRC Consulting GmbH, Hannover, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. not stated<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Bär, C. | 2008a | Water Solubility of Carbetamide-COOH<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-02435-L1_PCSB, S08-02435<br>GLP: yes<br>Published: no | yes | FSG |
| Bär, C. | 2008b | Dissociation Constant of Carbetamide-COOH in Water<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-02434-L1_PCDC, S08-02434<br>GLP: yes<br>Published: no | yes | FSG |
| Chabassol, Y., Giraud, J.P. | 1989 | Carbetamide-Vapor Pressure Curve<br>Rhône-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. AG/CRLD/AN/8916243<br>GLP: yes<br>Published: no | yes | FSG |
| Courtier, M., Caude, M.C. | 1984 | 11 561 R.P. : Carbetamide<br>Rhône-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. 322001<br>GLP/GEP: no<br>Published: no | yes | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Cousin, J. | 1993 | Determination of the dissociation constant of Carbetamide by potentiometric titration<br>Rhône-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. R&D/CRLD/AN9315482<br>GLP: yes<br>Published: no | yes | FSG |
| Cousin, J., Valcarce, M.H. | 1993 | Carbetamide Active Ingredient - Physical and Chemical Characteristics - dissociation constant<br>Rhône-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. 93-07, R&D/CRLD/AN 9315725<br>GLP: yes<br>Published: no | yes | FSG |
| Messerschmidt, S. | 2006 | Solubility of Carbetamide in two Organic Solvents<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20061264/01-PSBO<br>GLP: yes<br>Published: no | yes | FSG |
| Schneider, V. | 2002 | Determination of the Water Solubility of Carbetamide<br>UCL Umwelt Control Labor, Köln, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. PR02/006<br>GLP: yes<br>Published: no | yes | FSG |
| Schnell, R. | 2005a | Carbetamide purified<br>Feinchemie Schwebda GmbH, Eschwege, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. not applicable<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Schnell, R. | 2005b | Carbetamide technical<br>Feinchemie Schwebda GmbH, Eschwege, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. not applicable<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Smeykal, H. | 2001 | Carbetamide techn., 20010412 - Auto-Flammability (solids-determination of relative self-ignition temperature)<br>Sicherheitstechnik Siemens Axiva GmbH & Co. KG, Frankfurt<br>Feinchemie Schwebda GmbH<br>Report-no. 20010934.01<br>GLP: yes<br>Published: no | yes | FSG |
| Smeykal, H. | 2005 | Carbetamide 030749 - Boiling point A.2. (OECD 103)<br>Siemens AG, Prozess-Sicherheit, Frankfurt am Main, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20050353.01<br>GLP: yes<br>Published: no | yes | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Smeykal, H. | 2008 | Carbetamide-COOH Batch No.: RP 10810 - Vapour pressure A.4. (OECD 104)<br>Siemens AG, Prozess-Sicherheit, Frankfurt am Main, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20080222.01<br>GLP: yes<br>Published: no | yes | FSG |
| Tiemann, J. | 2005a | Carbetamide - Point 2.13 Explosive properties of the active substance<br>GAB Consulting GmbH, Lamstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 111118-A2-0213-01<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Tiemann, J. | 2005b | Carbetamide - Point 2.15 Oxidizing properties of the active substance<br>GAB Consulting GmbH, Lamstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 111118-A2-0215-01<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Walter, D. | 2001a | Solubility of Carbetamide technical in Organic Solvents<br>ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20011240/01-PBSO<br>GLP: yes<br>Published: no | yes | FSG |
| Walter, D. | 2001b | Flammability (solids) of Carbetamide technical<br>ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20011240/01-PCFS<br>GLP: yes<br>Published: no | yes | FSG |
| Walter, D. | 2001c | Surface Tension of Carbetamide technical<br>ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20011240/01-PCST<br>GLP: yes<br>Published: no | yes | FSG |
| Walter, D. | 2002a | Melting Temperature of Carbetamide<br>ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20021074/01-PCMP<br>GLP: yes<br>Published: no | yes | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Walter, D. | 2002b | Relative Density of Carbetamide technical<br>ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 2001124/01-PCRD<br>GLP/GEP: no<br>Published: no | yes | FSG |
| Walter, D. | 2005a | Relative density of Carbetamide<br>GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn<br>Feinchemie Schwebda GmbH<br>Report-no. 20051156/01-PCRD<br>GLP: yes<br>Published: no | yes | FSG |
| Walter, D. | 2005b | Surface tension of Carbetamide<br>GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn<br>Feinchemie Schwebda GmbH<br>Report-no. 20051156/01-PCST<br>GLP: yes<br>Published: no | yes | FSG |
| Wilfinger, W. | 2005c | Partition Coefficent of Carbetamide<br>GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn<br>Feinchemie Schwebda GmbH<br>Report-no. 20051156/01-PCPC<br>GLP: yes<br>Published: no | yes | FSG |

## 7.2 References Human Health Hazard

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Ashkenazi A, Ramachandran J, Capon DJ. | 1989 | Nature. 1989 Jul 13;340(6229):146-50.<br>Acetylcholine analogue stimulates DNA synthesis in brain-derived cells via specific muscarinic receptor subtypes.<br>GLP: no<br>Published: yes | | |
| Amyes, S.J., Marris, C.J., Brown, P.M., Lee, P., Virgo, D.M., Fowler, J.S.L. | 1988 | Carbetamide - Combined Oncogenicity and Toxicity Study in Rats<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 87/RHA058/749<br>GLP: yes<br>Published: no | yes | FSG |
| Amyes, S.J., Marris, C.J., Brown, P.M., Lee, P., Virgo, D.M., Fowler, J.S.L. | 1988 | Carbetamide - Combined Oncogenicity and Toxicity Study in Rats<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 87/RHA058/749<br>GLP: yes<br>Published: no | yes | FSG |
| Amyes, S.J., Marris, C.J., Brown, P.M., Virgo, D.M., Fowler, J.S.L. | | Carbetamide - Oncogenicity Study in Mice<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 87/RHA046/748<br>GLP: yes<br>Published: no | yes | FSG |
| August, M. | 2006a | In vitro Assessment of the Clastogenic Activity of Carbetamide in Cultured Human Peripheral Lymphocytes<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20079/06<br>GLP: yes<br>Published: no | yes | FSG |
| August, M. | 2006b | Mutagenicity Study of Carbetamide in the Mouse Lymphoma Forward Mutation Assay - in vitro -<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20080/06<br>GLP: yes<br>Published: no | yes | FSG |
| August, M. | 2006c | Micronucleus test of Carbetamide in bone marrow cells of the NMRI mouse by oral administration<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20081/1/06<br>GLP: yes<br>Published: no | yes | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| August, M. | 2006d | Micronucleus test of Carbetamide in bone marrow cells of the NMRI mouse by oral administration - pharmacokinetic evaluation -<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 20081/2/06<br>GLP: yes<br>Published: no | yes | FSG |
| Benazet, Cartier | 1977 | Carbetamide (11561 R.P.) - Etude de l´Activeté Mutagène Vis-à-Vis de Salmonella Typhymurium<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. RP/RD/CNG N° 19231<br>GLP/GEP: no<br>Published: no | no | FSG |
| Bogaards, J.J.P., Grossouw, D. | 2006 | Effect of carbetamide in the diet on cytochrome P450 enzyme activities in mouse liver<br>TNO Chemistry, Zeist, The Netherlands<br>Feinchemie Schwebda GmbH<br>Report-no. 6950, V6950<br>GLP: yes<br>Published: no | yes | FSG |
| Buys, Depaire, Laurent | 1972 | Metabolism of Carbetamide (11561 R.P.) in Rat<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. RP-D.S. An. Nord N° 2012<br>GLP/GEP: no<br>Published: no | no | FSG |
| Buys, M., Fournel, Heusse, Laurent, M. | 1974 | Metabolism Study of 14C - Ring-Labelled Carbetamide in Rat<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. S.U.C.R.P.-D.S.Ph/D.S.An. Nord<br>GLP/GEP: no<br>Published: no | no | FSG |
| Cifone, M.A. | 1984 | Evaluation of Carbetamide Technique in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay<br>Litton Bionetics, Inc., Kensington, Maryland, USA<br>Feinchemie Schwebda GmbH<br>Report-no. 20991<br>GLP: yes<br>Published: no | no | FSG |
| Charles River Laboratories | 2007 | Historical Histopatholy Data –B6C3F1 Mice-. Selected Liver abd adrenal findings. 91-106 Week studies. Issued 31 october 2007<br>GLP: not applicable<br>Published: no | no | - |
| Charles River Laboratories | 1989 | Spontaneous Neoplastic Lesions in the B6C3F1/ CrlBR mouse.<br>GLP: not applicable<br>Published: yes<br>(document available in the iuclid file) | no | - |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Charles River Laboratories | 2007 | Charles River Laboratories (2007) Historical Histopathology Data – F344 Rats. Selected Neoplastic Brain Findings. 104-107 Week studies. Issued 5 November 2007.<br>GLP: not applicable<br>Published: no | no | - |
| Charles River Laboratories | 1990 | Spontaneous Neoplastic Lesions in the CDF(F-344)/CrlBR Rat.<br>GLP: not applicable<br>Published: yes<br>(document available in the iuclid file) | no | - |
| Cordier, A., Bonneau, D. | 1984 | Carbetamide (11561 R.P.) in Vitro Mutagenic Activity on Five Ames´Tester Strains of Salmonella Typhimurium Using the Mammalian-Microsomes / Plate Incorporation Assay<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. ST/CRV/Tox. N°22048-E<br>GLP/GEP: no<br>Published: no | no | FSG |
| Cordier, A., Fournier, E. | 1984 | Carbetamide (11561 R.P.) - Micronucleus Test in Mice by the Oral Route<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. ST/CRV/Tox. N°22145<br>GLP: yes<br>Published: no | no | FSG |
| Cummins, H.A. | 1988a | Carbetamide: Acute Oral Toxicity in the Rat<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 88/0559<br>GLP: yes<br>Published: no | no | FSG |
| Cummins, H.A. | 1988b | Carbetamide: Acute Percutaneous Toxicity Study in the Rat<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 88/0560<br>GLP: yes<br>Published: no | no | FSG |
| Cummins, H.A., Gardner, J.R. | 1984 | Carbetamide: Acute Oral Toxicity in the Mouse<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 84/RH0039/462<br>GLP: yes<br>Published: no | no | FSG |
| Cummins, H.A., Gardner, J.R. | 1985 | Carbetamide: Delayed Contact Hypersensitivity Study in Guinea-Pigs<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 85/RHA059/319<br>GLP: yes<br>Published: no | no | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Danks, A. | 1985a | Carbetamide - 4-week Preliminary Toxicity Study in Oral Administration to Beagle Dogs<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 84/RH0035/670<br>GLP: yes<br>Published: no | no | FSG |
| Danks, A. | 1985b | Carbetamide - 13-week Toxicity Study in Oral Administration to Beagle Dogs<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 85/RHA048/425<br>GLP: yes<br>Published: no | no | FSG |
| Danks, A. | 1987 | Carbetamide: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 Weeks<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 86/RHA062/486<br>GLP: yes<br>Published: no | no | FSG |
| Eiben R., | 2011 | Frequency and time trends of spontaneous tumors in B6C3F1 mice oncogenicity studies over 10 years. Exp Toxic Pathol 2001; 53: 399-408<br>GLP: not applicable<br>Published: yes<br>(document available in the iuclid file) | no | - |
| European Food Safety Authority (EFSA) | 2010 | Conclusion on the peer review of the pesticide risk assessment of the active substance carbetamide<br>European Food Safety Authority (EFSA), Parma, Italy<br>EFSA Journal 2010;8(12):1913<br>GLP: not applicable<br>Published: yes<br>http://www.efsa.europa.eu/fr/efsajournal/doc/1913.pdf<br>(document available in the iuclid file) | no | - |
| Flügge, C. | 2008 | UNSCHEDULED DNA SYNTHESIS (UDS) TEST OF CARBETAMIDE BY ORAL ADMINISTRATION TO CD® RATS<br>- in vivo / in vitro study<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 22839<br>GLP: yes<br>Published: no | yes | FSG |
| Guizzetti M, Costa P, Peters J, Costa LG. | | **Acetylcholine** as a mitogen: muscarinic receptor-mediated proliferation of rat astrocytes and human **astrocytoma** cells.<br>Eur J Pharmacol. 1996 Feb 22;297(3):265-73.<br>GLP: no<br>Published: yes | no | |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Haferkorn, J. | 2008 | EXAMINATION OF CARBETAMIDE IN THE SKIN SENSITISATION TEST IN GUINEA PIGS ACCORDING TO MAGNUSSON AND KLIGMAN (MAXIMISATION TEST)<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 22838<br>GLP: yes<br>Published: no | yes | FSG |
| Hoorn, A.J.W. | 1985 | Mutagenicity Evaluation of Carbetamide Technique in the Mouse Lymphoma Forward Mutation Assay<br>Litton Bionetics, Inc., Kensington, Maryland, USA<br>Feinchemie Schwebda GmbH<br>Report-no. E-9265 / E-9361<br>GLP: yes<br>Published: no | no | FSG |
| Ivett, J. | 1984a | Mutagenicity Evaluation of Carbetamide Technical ref. 04040.02 in an In Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells<br>Litton Bionetics, Inc., Kensington, Maryland, USA<br>Feinchemie Schwebda GmbH<br>Report-no. 20990<br>GLP: yes<br>Published: no | no | FSG |
| Ivett, J. | 1984b | Mutagenicity Evaluation of Carbetamide Technical ref. 04040.02 in an In Vitro Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells<br>Litton Bionetics, Inc., Kensington, Maryland, USA<br>Feinchemie Schwebda GmbH<br>Report-no. 20990 E-9265<br>GLP: yes<br>Published: no | no | FSG |
| Laurent, M., Buys, M. | 1973 | Metabolism of Carbetamide in Rat<br>Rhône-Poulenc, Vitry-sur-Seine, France<br>Feinchemie Schwebda GmbH<br>Report-no. S.U.C.R.P.-D.S. An Nord N°2272<br>GLP/GEP: no<br>Published: no | no | FSG |
| Leuschner, J. | 2002 | 14-Day Kinetics and Metabolism Study of 14C-Labelled Carbetamide after Repeated Oral Administration to Sprague-Dawley Rats<br>LPT Lab. of Pharm. and Tox. GmbH & Co. KG, Hamburg, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 14139/01<br>GLP: yes<br>Published: no | yes | FSG |
| Lewis, R.J. Sr | 2004 | *Sax's Dangerous Properties of Industrial Materials. 11th Edition.* (ed) Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 3013] **PEER REVIEWED**<br>GLP: not applicable<br>Published: yes | no | - |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| National Toxicological Program (NTP) | 1999 | *Historical control tumor incidence (B3C3F1 mice)*<br>http://ntp niehs nih.gov/ntp/research/database_searches/historical_controls/path/m_orlfd.txt<br>GLP: not applicable<br>Published: yes<br>(document available in the uiclid file) | no | - |
| National Toxicological Program (NTP) | 1999 | *Historical control tumor incidence (F344 rats)*<br>http://ntp niehs nih.gov/ntp/research/database_searches/historical_controls/path/r_orlfd.txt<br>GLP: not applicable<br>Published: yes<br>(document available in the uiclid file) | no | - |
| Ross, D.B., Roberts, N.L., Philipps, C.N.K., Majeed, S.K., Prentice, D.E. | 1978 | The Acute Oral Toxicity (LD50) and Assessment of Neurotoxic Effects of Carbetamide in the Domestic Hen<br>Rhône-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. RNP 94/78991<br>GLP/GEP: no<br>Published: no | no | FSG |
| Spielmann, H & Gerbracht, | 2001 | The use of dogs as second species in regulatory testing of pesticides. Part II: Subacute, subchronic and chronic studies in the dog Arch Toxicol.;75, 1-21.<br>GLP: not applicable<br>Published: yes | no | - |
| Shane Cox Gad | 2009 | *Drug Safety Evaluation, Second Edition,*<br>Appendix E: Common Vehicles for Nonclinical Evaluation of Therapeutic Agents<br>Ed. John Woley & Sons, Inc.<br>GLP: not applicable<br>Published: yes | no | - |
| Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M | 2006 | IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 2001 Oct;34(2):146-52.<br><br>GLP: not applicable<br>Published: yes | no | - |
| Smith, K.D., Cummins, H.A. | 1988a | Carbetamide: Acute Dermal Irritation/Corrosion Test in the Rabbit<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. LSR Report 88/0561<br>GLP: yes<br>Published: no | no | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Smith, K.D., Cummins, H.A. | 1988b | Carbetamide: Acute Eye Irritation/Corrosion Test in the Rabbit<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 88/0562<br>GLP: yes<br>Published: no | no | FSG |
| Tesh, J.M., Deans, C.F., Rigg, J., Tesh, S.A., Wilby, O.K. | 1985 | Carbetamide - Teratology Study in the Rat<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 85/RH0029/139<br>GLP: yes<br>Published: no | no | FSG |
| Tesh, J.M., Ross, F.W., Wightman, T.J., Wilby, O.K. | 1986 | Carbetamide - Teratology Study in the Rabbit<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 85/RH0030/733<br>GLP: yes<br>Published: no | yes | FSG |
| Tesh, J.M., Wightman, Fowler, J.S.L. | 1987 | Carbetamide - Effects Upon Reproductive Performance of Rats Treated Continuously Throughout Two Successive Generations<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 87/RHA047/469<br>GLP: yes<br>Published: no | no | FSG |
| West, H.A. | 1984 | Carbetamide: Preliminary Toxicity Study in Dietary Administration to CD Rats<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 84/RH0034/395<br>GLP: yes<br>Published: no | no | FSG |
| West, H.A. | 1985 | Carbetamide -13-week Toxicity Study in Dietary Administration to CD Rats<br>Life Science Research, Ltd., Suffolk, UK<br>Feinchemie Schwebda GmbH<br>Report-no. 85/RH0031/139<br>GLP: yes<br>Published: no | no | FSG |

## 7.3 References Environmental Hazard

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Ayliffe, J.M., Hardy, I.A.J. | 1996 | Carbetamide: Degradation and Retention in Water/Sediment Systems<br>Rhône-Poulenc Agro, Centre de Recherche, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. 201059<br>GLP: yes<br>published: no | yes | FSG |
| Buys, M., Chabassol, Y., Laurent, M. | 1979 | Carbetamide-Study of Hydrolysis<br>Rhone-Poulenc Agro, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. RP/RD/CNG-An No. 3614-E<br>GLP: no<br>published: no | no | FSG |
| Dengler, D. | 2009 | Assessment of the Ready Biodegradability of Carbetamide tech. with the Closed Bottle Test<br>Eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S09-00243<br>GLP: yes<br>Published: no | yes | FSG |
| Jendrzejczak NH, Turier GP, and Adrian PP. | 1992 | carbetamide-UV-Visible Spectrum<br>Rhône-Poulenc Agro, Centre de Recherche, Lyon, France<br>Feinchemie Schwebda GmbH<br>Report-no. AG/CRLD/AN/9116899<br>GLP: yes<br>published: no | no | FSG |
| O'Brien | 2006 | Calculation of environmental fate endpoints in surface water and sediment or Carbetamide according to recommendations of the work group on degradation kinetics of FOCUS<br>GAB Consulting GmbH<br>Feinchemie Schwebda Gmbh, Report No. : 111118-A3-090203-01<br>GLP, unpublished | no | FSG |
| Offizorz, P., Knoch, E. | 1992 | Investigation of the Evaporation of Pradone Kombi From Soil and Dwarf Runner Bean<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC Project 281406<br>GLP: yes<br>published: no | no | FSG |

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Reeves G.L., Savage E.A. | 1985 | Carbetamide-14C: Adsorption/Desorption on Four Soils<br>May and Baker Ltd, Essex, UK<br>Feinchemie Schwebda GmbH<br>Report-no. AG. Tech. 947<br>GLP: no<br>published: no | no | FSG |
| Tiemann J. | 2003 | carbetamide: Estimation of the Photochemical Oxidative Degradation<br>GAB Consulting GmbH, Lamstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 111118-A2-0210-01<br>GLP: no<br>Published: no | Yes | FSG |
| Voigt, G. | 1989 | Prüfung auf Biologische Abbaubarkeit n. OECD 301 D Prüfsubstanz: Carbetamid<br>Ökolimna - Ges. für Ökol. & Gew. GmbH, Burgwedel, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. 29<br>GLP: no<br>published: no | no | FSG |

## 7.4 Ecotoxicology

| Author(s) | Year | Title<br>Testing Facility<br>Owner / Source (where different from owner)<br>Report No<br>GLP or GEP status (where relevant)<br>Published or not | Data protec-tion claimed<br><br>yes/no | Owner |
|---|---|---|---|---|
| Borrmann, K. | 2008 | Assessment of toxic effects of Carbetamide-COOH on Daphnia magna using the 48 h acute immobilisation test<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-00873-L1_AADm, S08-00873<br>GLP: yes<br>Published: no | yes | FSG |
| Dengler, D. | 2008a | Testing of toxic effects of Carbetamide-COOH to the single cell green alga Desmodesmus subspicatus<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-00886-L1_AADs, S08-00886<br>GLP: yes<br>Published: no | yes | FSG |
| Dengler, D. | 2008b | Assessment of toxic effects of Carbetamide-COOH on the duckweed Lemna gibba in a semi static test<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-00896-L1_AALg, S08-00896<br>GLP: yes<br>Published: no | yes | FSG |

| **Author(s)** | **Year** | **Title**<br>**Testing Facility**<br>**Owner / Source (where different from owner)**<br>**Report No**<br>**GLP or GEP status (where relevant)**<br>**Published or not** | **Data protec-tion claimed**<br><br>**yes/no** | **Owner** |
|---|---|---|---|---|
| Douglas, M.T., Sewell, I.G., Macdonald, I.A. | 1989 | The Prolonged Toxicity of Carbetamide to Rainbow Trout (Salmo gairdneri)<br>Huntingdon Life Sciences Ltd., Huntingdon, UK<br>Feinchemie Schwebda GmbH<br>Report-no. RNP 300/89578<br>GLP: yes<br>Published: no | no | FSG |
| Gonsior, G. | 2008a | Acute toxicity testing of Carbetamide-COOH in rainbow trout (Oncorhynchus mykiss) (Teleostei, Salmonidae)<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-01260-L1_AAOm, S08-01260<br>GLP: yes<br>Published: no | yes | FSG |
| Gonsior, G. | 2008b | Assessment of side effects of Carbetamide-COOH on the larvae of the midge, Chironomus riparius with the laboratory test method<br>eurofins-GAB GmbH, Niefern-Öschelbronn, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. S08-01671-L1_ASCr, S08-01671<br>GLP: yes<br>Published: no | yes | FSG |
| Knacker, Th., Hilt, J. | 1989 | A Study of the Acute Immobilisation to Daphnia of Carbetamide<br>Battelle Europe, Frankfurt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. BE-ET-01-89-02-DAK-1<br>GLP: yes<br>Published: no | no | FSG |
| Knoch, E. | 1994a | Determination of the Concentration of Carbetamide in Test Water<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452902<br>GLP: yes<br>Published: no | yes | FSG |
| Knoch, E. | 1994b | Determination of the Concentration of Carbetamide in Test Water<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452904<br>GLP: yes<br>Published: no | yes | FSG |
| Knoch, E. | 1994c | Determination of the Concentration of Carbetamide in Test water<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452906<br>GLP: yes<br>Published: no | yes | FSG |
| Memmert, U., Knoch, E. | 1994a | Acute Toxicity of Carbetamide to rainbow trout (Oncorhychus mykiss) in a static test (96 hours)<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452901<br>GLP: yes<br>Published: no | yes | FSG |

| **Author(s)** | **Year** | **Title**<br>**Testing Facility**<br>**Owner / Source (where different from owner)**<br>**Report No**<br>**GLP or GEP status (where relevant)**<br>**Published or not** | **Data protec-tion claimed**<br><br>**yes/no** | **Owner** |
|---|---|---|---|---|
| Memmert, U., Knoch, E. | 1994b | Acute Toxicity of Carbetamide to Common Carp (Cyprinus Carpio) in a Static Test (96 Hours)<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452903<br>GLP: yes<br>Published: no | yes | FSG |
| Memmert, U., Knoch, E. | 1994c | Influence of Carbetamide on Survival and Reproduction of Daphnia Magna in a Semistatic Test (21 days)<br>RCC Umweltchemie GmbH, Roßdorf, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. RCC 452905<br>GLP: yes<br>Published: no | yes | FSG |
| Scheerbaum, D. | 2003a | Carbetamide Tech. Alga, Growth Inhibition Test with Desmodesmus subspicatus, 72 h (formerly Scenedesmus subspicatus)<br>Dr. U. NOACK Lab. für angew. Biologie, Sarstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. SSO88411<br>GLP: yes<br>Published: no | yes | FSG |
| Scheerbaum, D. | 2003b | Carbetamide Tech. Alga, Growth Inhibition Test with Navicula pelliculosa, 72 h<br>Dr. U. NOACK Lab. für angew. Biologie, Sarstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. SVO88411<br>GLP: yes<br>Published: no | yes | FSG |
| Scheerbaum, D. | 2003c | Carbetamide Tech. Aquatic Plant Toxicity Test, Lemna minor, Static<br>Dr. U. NOACK Lab. für angew. Biologie, Sarstedt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. TLA88411<br>GLP: yes<br>Published: no | yes | FSG |
| US EPA | 1998 | **ASSESSMENT OF THYROID FOLLICULAR CELL TUMORS**<br>EPA/630/R-97/002<br>GLP : not applicable<br>Published : yes | no | - |
| Zietz, E. | 1991 | A Study of the Acute Toxicity of Carbetamide to Daphina According to OECD Guideline No. 202, Part I - Supporting Analyses of Concentrations in Test Water Samples<br>Battelle Europe, Frankfurt, Germany<br>Feinchemie Schwebda GmbH<br>Report-no. BE-ET-01-89-02-DAK-01<br>GLP: yes<br>Published: no | no | FSG |