

201-15800B

**US EPA HPV Chemical Challenge Program**

**ROBUST SUMMARIES  
FOR  
CARBAMIC ACID, 1H-BENZIMIDAZOL-2-YL-, METHYL ESTER  
(CAS No. 10605-21-7)**

**Submitted By:**

Troy Corporation  
8 Vreeland Road  
PO Box 955  
Florham Park, NJ 07932-0955  
Phone: (201) 443-4200  
Fax: (201) 443-0257

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**Prepared By:**

RegNet Environmental Services  
1250 Connecticut Avenue, NW  
Suite 700  
Washington, DC 20036  
Phone: (202) 419-1500  
[www.regnet.com](http://www.regnet.com)

**January 11, 2005**

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**INTRODUCTION**

Troy Chemical Corporation (<http://www.troycorp.com/>) has prepared the following robust summaries for methyl 2-benzimidazole carbamate, otherwise known as 2-benzimidazolecarbamic acid, methyl ester (BCM) (CAS# 10605-21-7), as part of its contribution to the US Environmental Protection Agency's (EPA) High Production Volume (HPV) Challenge Program. The US HPV Challenge Program is directed at chemicals falling under the jurisdiction of the Toxic Substances Control Program and the initial list of HPV chemicals were identified from the 1990 TSCA Inventory Update. While BCM was on the HPV candidate list, Troy is currently not aware of any uses of this compound that is governed by TSCA. Troy's production of BCM is limited to pesticidal applications that are governed by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Nonetheless, as the goal of the HPV Challenge Program is to ensure that the American public has access to basic information about the chemicals manufactured in the US, Troy has provided these robust summaries.

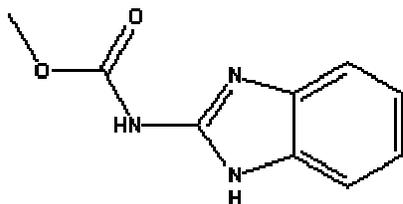
Background on BCM and FIFRA Registration

BCM is a free flowing, non-metallic, water-soluble powder preservative. It is intended for use in aqueous products up to 125°F (52° C) and a pH of 7 or greater.

Molecular Formula: C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>

Molecular Weight: 191.189

Chemical Structure:



The technical grade active ingredient is sold by Troy under the trade name Mergal BCM (FIFRA registration #365-81). Mergal BCM is a fungicide to be formulated into pesticides for the following uses: manufacture of paints, coatings, plasters, stuccos, sealants and fillers to inhibit or control the growth of fungi on paints, coatings, plasters, stuccos, sealants, fillers, architectural products, finishes and special purpose coatings. It and can be utilized to recover spoiled material.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

BCM, as the primary metabolite of thiophanate-methyl, was the subject of a review by EPA's Office of Pesticides as part of the FIFRA Reregistration Eligibility Decision (RED) program (Case 2680). As such, many of the studies contained in this robust summary submission were reviewed and discussed in the RED.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**1.0 MELTING POINT**

**Test Substance**

Identity: Carbendazim  
Technical Grade, Batch No. 010310, Active Ingredient Carbendazim, 99%

**Method**

Method/guideline followed: CCL SOP 10.20, Guideline 830.7200  
GLP: Yes  
Year: 2002

**Test Conditions**

Melting point was determined using a MelTemp II Capillary Melting Point Apparatus. Dried a portion of test substance in oven at 105°C for 2 hours. Charged a glass capillary tube with 3-4 mm of dried test substance, placed tube in apparatus receptacle and set power level to 4. Adjusted power level to 7 after 30 minutes.

**Results**

Melting point value in °C: Sample decomposed prior to melting (275°C)  
Decomposition: Yes, 75-314°C

At approximately 275°C the test substance turned brown and started to decompose, but showed no signs of melting. At 300°C the sample started to collapse and at 314° the sample became liquid.

**Conclusions**

Study author concludes sample decomposed prior to melting (275°C)

**Reliability**

Klimisch Code = 1, Reliable without restriction

Study certified as conducted in compliance Good Manufacturing Guidelines under 40 CFR 160 and according to standard methods under CCL SOP 10.20, Guideline 830.7200.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**References**

Case Consulting Laboratories, Inc., "Final Report - Physical and Chemical Characteristics of Carbendazim Technical: Color, Physical State, Odor, Stability, pH, UV/Visible Absorption, Melting Point, Bulk Density, Dissociation Constant, Octanol/Water Partition Coefficient, Solubility and Vapor Pressure," March 27, 2003, Case Study No. 650-48.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## **2.0 BOILING POINT**

### **Test Substance**

Identity: Carbendazim  
Technical Grade, HOE 17411

### **Method**

Method:	Guideline 63-6
GLP:	Not applicable
Year:	1992

### **Test Conditions**

### **Results**

Boiling point value in °C:	Not applicable
Remarks:	Not applicable

### **Conclusions**

Boiling point is only required if technical chemical is a liquid at room temperature (Pesticide Assessment Guidelines, Subdivision D, Page 64) BCM technical is not a liquid at room temperature. (Study author)

### **Reliability**

Boiling Point test was not applicable to this compound.

### **References**

Safepharm Laboratories Limited, BCM Technical Grade (HOE 17411)  
Determination of Melting Point/Melting Range, Page 36, Project ID 121/178(d),  
October 23, 1992.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

### **3.0 VAPOR PRESSURE**

#### **Test Substance**

Identity: Carbendazim  
Technical Grade, HOE 17411, Methyl Benzimidazole-2-carbamate

#### **Method**

Method: EEC Directive 67/548 Annex V A4 Vapour  
pressure Balance Method)  
GLP: Yes  
Year: 1993

#### **Test Conditions**

#### **Results**

Vapour Pressure value:  $3 \times 10^{-5}$  Pascals  
Temperature (°C): 25  
Decomposition: No

#### **Remarks**

The vapor pressure of sample Test material BCM Technical Grade (HOE 17411) was determined using the vapour pressure balance.

The solid sample was maintained below 194.5 °C during measurements and did not visibly appear to have changed at the temperature used. There was considerable (totaling around 50%) condensation onto the balance pan allowing correction of some data. The melting range is quoted as 307°C.

During pump down and in Run 1 there was some initial degassing of the sample. In Run 1 the mass differences rose from an initial ambient value of < 0.2ug at 25°C (equivalent to <  $1.6 \times 10^{-4}$  Pascal region) to 206 ug (equivalent to <  $2.7 \times 10^{-1}$  Pascals) at 192.75°C. Condensation was observed above 132°C but because of a considerable rise in mass difference when held at the highest temperature for some 5 minutes results were not corrected for this or indeed used to produce a room temperature vapour pressure by extrapolation. The considerable non-linearity of the vapour pressure relationship is evident on the plot presented.

In Run 2, earliest mass differences were lower by a factor around two than comparable data in Run 1 the lowest measurable uncorrected value - 0.1 ug at 92.8° C – yielding  $1.3 \times 10^{-4}$  Pascals. The whole data of Run 2 (corrected and

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

uncorrected mass differences and condensation data) again showed a kink and distinct steepening of the vapour pressure relationship towards higher temperatures in rather a similar manner to Run 1 making extrapolation in the case again unreliable.

In Run 3 the initial mass difference lay at 0.41 ug at 94.6°C i.e. larger than in the previous run but ultimately mass differences were rather smaller giving a flatter vapour pressure curve. The 6 corrected data of Run 3A although still kinked showed somewhat less erratic behavior than previous runs giving via extrapolation  $V_p(25^\circ\text{C}) = 4.734 \times 10^{-6}$  Pascals with slope around -3371K and calculated error range of about 50%.

The similarity of form of the results of all runs is noted as is the apparent absences of decomposition but in view of the large statistical error it is considered prudent to estimate a maximum vapour pressure via the imposition of the shallow slope of -2000K. This yields for the 94.6°C value of Run 3 to a whole number  $V_p(25^\circ\text{C}) = 3 \times 10^{-5}$  Pascals as a suggested maximum vapour pressure.

### **Conclusions**

Vapor Pressure (25°C) =  $3 \times 10^{-5}$  Pascals as the suggested maximum vapour pressure. (Study Author)

### **Reliability**

Klimisch Code = 1

### **Remarks**

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out as part of the UK GLP Compliance Programme. At the time of inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

### **References**

Safeparm Laboratories Limited, Derby, England, "BCM Technical, Product Chemistry, Determination of Vapour Pressure by Balance Method," Laboratory Project ID 121/177 December 1993

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

#### **4.0 PARTITION COEFFICIENT**

##### **Test Substance**

Identity: Carbendazim  
Technical Grade, HOE 17411

##### **Method**

Method: EPA Guideline 63-11 (Method Number 107,  
OECD Guideline, Section 1)  
GLP: Yes  
Year: 1992

##### **Remarks**

A preliminary test was performed to assess the approximate partition coefficient. A stock solution of BCM Technical Grade (HOE 17411) was prepared in water saturated n-octanol. Three tests were performed in duplicate. After separation, the concentration of test material in the aqueous phase was determined by HPLC.

##### **Test Conditions**

The peak heights of the pair of standards associated with a particular sample were corrected to nominal concentration and the mean value taken prior to the calculation of the sample concentration.

With the exception of the following deviation, the experimental procedure used complies with that specified in EPA Guideline 63-11 (Method Number 107, OECD Guideline, Section 1). This deviation has been considered not to have affected the integrity of the study.

##### **Results**

Log K<sub>ow</sub>:  $\text{Log}_{10} P_{ow} = 1.60.$   
Temperature ( $^{\circ}\text{C}$ ):  $23.0 \pm 0.5^{\circ}\text{C}$

The partition coefficient of BCM Technical Grade (HOE 17411) has been determined to be 40.0 at  $23.0 \pm 0.5^{\circ}\text{C}$ .  $\text{Log}_{10} P_{ow} = 1.60.$

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Sample No.	n-Octanol/water volume ratio	Partition coefficient	Log <sub>10</sub> P <sub>ow</sub>	Mean partition coefficient
1	1:2	41.0	1.61	41.3
2	1:2	41.6	1.62	41.3
3	1:1	39.9	1.60	39.8
4	1:1	39.8	1.60	39.8
5	2:1	40.0	1.60	38.8
6	2:1	37.7	1.58	38.8

Mean = 40.0      Log<sub>10</sub> P<sub>ow</sub> = 1.60      Std. Deviation = 1.35

### Conclusions

The partition coefficient of BCM Technical Grade (HOE 17411) has been determined to be 40.0 at 23.0 ± 0.5°C. Log<sub>10</sub> Pow = 1.60. (Study author)

### Reliability

K= 1, Reliable without restriction

Conducted according to EPA Guideline 63-11 (Method Number 107, OECD Guideline, Section 1) A general inspection for compliance with the Principles of Good laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Program. At the time of inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities. (Study Author)

### References

Safeparm Laboratories Limited, "BCM Technical Grade (HOE 17411) Determination of Partition Coefficient," Laboratory Project ID 121/178(i), TC0741, October 29, 1992.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**5.0 WATER SOLUBILITY**

- a. BCM Technical Grade (HOE 17411) Determination of Solubility

**Test Substance**

Identity: Carbendazim  
Technical Grade, HOE 17411, 2-(methoxycarbonylamino)-benzimidazole,  
Lot Number HOE 017411 of ZD 990010

**Method**

Method:	EPA Guideline 63-8 (based on OECD Method Number 105, Section 1)
GLP:	Yes
Year:	1992

**Test Conditions**

The determination was performed using the flask shaking method. A preliminary test was undertaken to determine the approximate water solubility. Based on this preliminary result, amounts of test material (such that approximately 60 times this saturation concentration would be present) were weighed into three separate flasks. After addition of glass double distilled water to the flasks, they were shaken at approximately 30°C and, after standing at 20°C for a period of not less than 24 hours, the contents of the flasks were centrifuged, filtered and the concentrations determined spectrophotometrically.

The sample solutions were diluted by a factor of 3.33 using 0.2% hydrochloric acid in acetonitrile. Standard solutions were prepared in 0.2% hydrochloric acid in acetonitrile at a nominal concentration of 5 mg/L. The absorbance of the standard and sample solutions were measured at 283 nm in cells of 10 mm path length using 0.2% hydrochloric acid in acetonitrile as the reference medium.

The absorbances of the pair of standards were corrected to nominal concentration and the mean value taken prior to the calculation of the sample concentration. With the exception of the following deviation, the experimental procedure complies with that specified in EPA Guideline 63-8 (Method number 105 OECD, Guideline Section 1):

Due to the low water solubility of BCM Technical Grade (HOE 17411) proved impractical to prepare samples at 5 times the saturation level. The test mixtures were prepared at approximately 60 times the saturation level. This deviation was not considered to have affected the integrity of the study. (Study author)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Results**

Value (mg/L) at temperature (°C):	1.29 x 10 <sup>-3</sup> g/100 ml (20.0°C) 12.9 mg/L (20.0°C)
pH value and concentration at temperature (°C):	Not determined
pKa value at 25 °C:	Not determined

The water solubility of BCM Technical Grade (HOE 17411) has been determined to be 1.29 x 10<sup>-3</sup> g/100 ml at a temperature of 20.0°C.

**Conclusions**

The water solubility of BCM Technical Grade (HOE174111) has been determined to be 1.29 x 10<sup>-3</sup> g/100 ml at a temperature of 20.0 ± 0.5 °C. (Study author)

**Reliability**

Klimisch Code = 1, Reliable without restriction

Conducted according to OECD Method Number 105. A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the laboratory as part of the UK GLP Compliance Programme. At the time of the inspection, no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities. (Study author)

**References**

Safeparm Laboratories Limited, Derby England, "BCM Technical Grade (HOE 17411) Determination of Solubility," Laboratory Project ID 121/178(f), November 17, 1992.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

b. Physical and Chemical Characteristics of Carbendazim Technical: Color, Physical State, Odor, Stability, pH, UV/Visible Absorption, Melting Point, Bulk Density, Dissociation Constant, Octanol/Water Partition Coefficient, Solubility and Vapor Pressure

**Test Substance**

Identity: Carbendazim  
Technical Grade, Batch 010310, Active ingredient Carbendazim, 99%

**Method:**

Method: pH determined by ASTM Method E-70-90

Dissociation constant spectral scans were collected at each pH change using the UV/Vis conditions listed for OPPTS Guideline 830.7050.

GLP: Yes  
Year: 2002

**Test Conditions**

pH: The pH of the test substance at 25° C was determined per CCL SOP 10.17. A 1% (w/v) mixture of the test substance in deionized water was used for this determination. This procedure is based on American Society for Testing and Materials (ASTM) Method No. E 70-90.

Dissociation Constant: The dissociation constant of the test substance was determined by the titration method. The pK is obtained from the titration curve. Dissolved a small amount of test substance in Buffer A, containing potassium dihydrogen phosphate, piperazine hexahydrate, chloroacetic acid, formic acid, ethylenediamine dihydrochloride, n-butylamine, hydrochloric acid and acetic acid. Adjusted the pH of the solution to 2.30 with 0.1 N HCL. The solution was contained in a beaker equipped with a magnetic stirring bar, pH electrode and plumbing to circulate the solution through a spectrophotometer cell. The pH of the solution was adjusted in increments of approximately 0.5 pH units with 50% NaOH until pH 12. Spectral scans were collected at each pH change using the UV/Visible conditions listed in OPPTS Guideline 830.7050.

The pKa was calculated for each pH using the following.

$$pK_a = pH + \log \left[ \frac{Abs_{ionized} - Abs_{actual}}{Abs_{actual} - Abs_{neutral}} \right]$$

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The dissociation constants are reported as the average pKa of the solutions bracketing the neutral solution.

**Results**

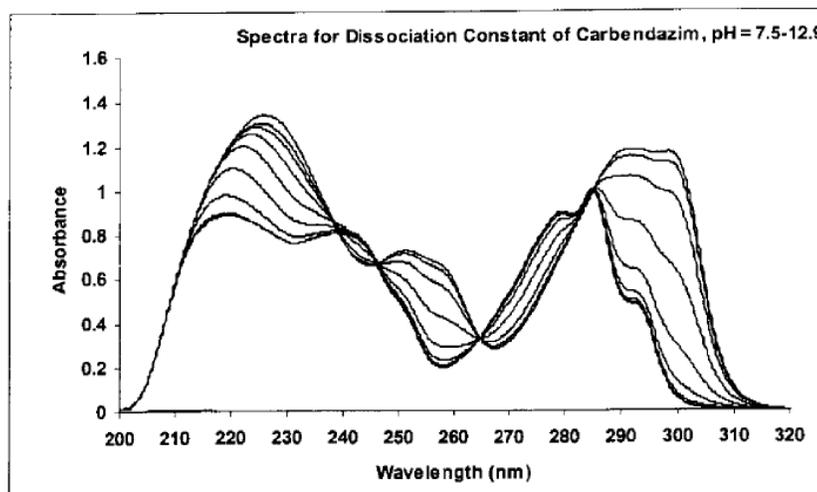
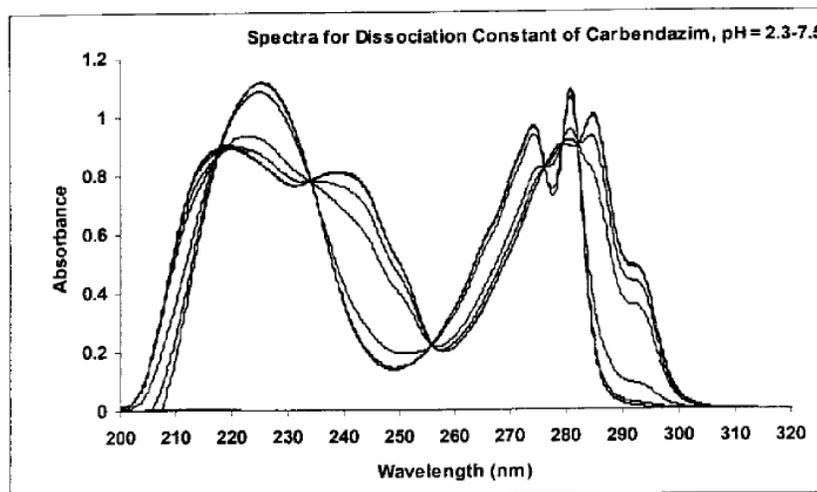
pH (25°C): 5.82  
 Dissociation Constant: See Figures 4 and 5 below.  
     pKa = 4.49  
     pKb = 10.62

**Figure 4. Dissociation Constant Data**

Absorbance at 290 nm					
Abs <sub>neutral</sub> =	0.022	Abs <sub>ionized</sub> =	0.5248		
Abs <sub>neutral</sub> =	0.5248	Abs <sub>ionized</sub> =	1.1736		
pH	Abs	dAbs/dpH	pKa	Average	
2.30	0.022				
2.89	0.0330	0.0186	4.54		
3.72	0.1021	0.0833	4.44		
4.84	0.3813	0.2493	4.44	4.49	
5.32	0.4659	0.1763	4.44		
6.36	0.5175	0.0496	4.53		
6.88	0.5225	0.0096	4.54		
7.49	0.5248	0.0038			
8.85	0.5353	0.0077	10.63		
9.51	0.5701	0.0527	10.63		
10.10	0.6684	0.1666	10.65	10.62	
10.70	0.8678	0.3323	10.65		
11.29	1.0590	0.3241	10.62		
11.89	1.1457	0.1445	10.54		
12.92	1.1736				

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Figure 5.** Dissociation Constant Data (Cont.)



**Conclusions**

pH(25°C) = 5.82. Dissociation Constants are pKa = 4.49, pKb = 10.62 (Study author)

**Reliability**

Klimisch Code = 1

The study was conducted in compliance with the Good Laboratory Practice Standards as set forth in Title 40 Part 160 of the Code of Federal Regulations of

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

the United States of America. Testing was conducted based on OECD Method Number 105 (Study Author)

**References**

Case Consulting Laboratories, Inc, Whippany, NJ, "Physical and Chemical Characteristics of Carbendazim Technical: Color, Physical State, Odor, Stability, pH, UV/Visible Absorption, Melting Point, Bulk Density, Dissociation Constant, Octanol/Water Partition Coefficient, Solubility and Vapor Pressure," Study No. 650-48, March 27, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## 6.0 PHOTODEGRADATION

### Test Substance

Identity: Carbendazim  
Technical Grade, HOE 17411, 2-(Methoxycarbonylamino)-benzimidazole,  
Lot Number HOE 017411 of ZD990010

### Method

Method/guideline followed:	EPA Guideline 161-2
Type:	Photostability
GLP:	Yes
Light source:	Simulated daylight
Light spectrum (nm):	See remarks under test conditions
Intensity of Sunlight:	See remarks under test conditions
Year:	1993

### Test Conditions

A 5 mg/L solution of BCM Technical Grade (HOE 17411) has been determined not to decompose after exposure to simulated daylight for 17 days at 25 °C ± 1°C.

Stock Solution: Stock Solutions of the test material were prepared at approximately 5 mg/L in distilled water, which contained a 0.5% v/v solvent of 0.2% hydrochloric acid in acetonitrile.

Test Solution: A portion of the stock solution was stored under simulated daylight at 25.0 ° ± 1°C for 17 days. The light source was a True-Life fluorescent lamp manufactured by Duro-lite Lamps Inc. USA.

Control Solution: A portion of stock solution was stored in the dark at 25.0 ± 1°C for 17 days. The concentration of the test material in each sample was determined by high performance liquid chromatography as follows:

Samples: The samples were diluted by a factor of 2 using 0.2% hydrochloric acid in acetonitrile.

Standards: Standard Solutions of Carbendazim (>99%) were prepared in 0.2% hydrochloric acid in acetonitrile: distilled water (1:1) at nominal concentrations of 2.5 mg/L. Conditions of analysis are listed below.

Analysis: The HPLC parameters were as follows:

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Column: Spherisorb ODS10 (250 mm x 4.6 mm i.d.)  
Flow rate: 1.5 ml/min.  
Mobile phase: water : acetonitrile : borate buffer\*\* (73:25:2)  
Injection volume: 25 µl.  
Wavelength: 254 nm  
\*\* Borate buffer: 200 ml of distilled water containing approximately 0.6 (pH 8.5) g of boric acid, 0.8 g of sodium chloride, 25 ml of sodium hydroxide (0.1 M).

The peak areas of the pair of standards associated with each sample were corrected to nominal concentration and the mean taken prior to the sample concentration being calculated. To minimize the possibility of microbial degradation of the test material, sterile distilled water was used and the glassware was autoclaved. The experimental procedure used complies with that specified in the EPA Guideline 161-2 (Study author.)

**Results**

Concentration of Substance: 5 mg/L  
Temperature (°C): 25°C ± 1°C  
Half-life t  $\frac{1}{2}$  (*preferred*): Not determined  
Degradation % after: 0% after 17 days  
Quantum yield: Not determined  
Indirect photolysis: Not determined  
Sensitiser (*type*): No  
Breakdown products: No

**Remarks**

Initial concentration expressed as % of control: 101  
Concentration after 8 days expressed as % of control: 99.9  
Concentration after 11 days expressed as % of control: 100  
Concentration after 17 days expressed as % of control: 101

The peak areas relating to the standard and sample solutions are detailed below:

<u>Solution</u>	<u>Peak area (units)</u>
Standard 2.61 mg/l	9.465 x 10 <sup>5</sup>
Standard 2.59 mg/l	9.244 x 10 <sup>5</sup>
Initial Sample A	9.824 x 10 <sup>5</sup>
Initial Sample B	9.741 x 10 <sup>5</sup>
Initial Control A	9.680 x 10 <sup>5</sup>
Initial Control B	9.633 x 10 <sup>5</sup>
Standard 2.57 mg/l	8.262 x 10 <sup>4</sup>

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Standard 2.29 mg/l	6.237 x 10 <sup>4</sup>
8 Day Sample A	7.650 x 10 <sup>4</sup>
8 Day Sample B	7.607 x 10 <sup>4</sup>
8 Day Control A	7.678 x 10 <sup>4</sup>
8 Day Control B	7.590 x 10 <sup>4</sup>
Standard 2.48 mg/l	7.325 x 10 <sup>4</sup>
Standard 2.46 mg/l	7.407 x 10 <sup>4</sup>
11 Day Sample A	7.888 x 10 <sup>4</sup>
11 Day Sample B	7.785 x 10 <sup>4</sup>
11 Day Control A	7.818 x 10 <sup>4</sup>
11 Day Control B	7.818 x 10 <sup>4</sup>
Standard 2.58 mg/l	6.700 x 10 <sup>4</sup>
Standard 2.54 mg/l	6.920 x 10 <sup>4</sup>
17 Day Sample A	7.687 x 10 <sup>4</sup>
17 Day Sample B	7.588 x 10 <sup>4</sup>
17 Day Control A	7.511 x 10 <sup>4</sup>
17 Day Control B	7.556 x 10 <sup>4</sup>

## Conclusions

A 5 mg/L solution of BCM Technical Grade (HOE 17411) has been determined not to decompose after exposure to simulated daylight for 17 days at 25.0 ± 1°C. (Study author)

## Reliability

Klimisch Code = 1

This study was conducted so as to conform with Good Laboratory Practices as published by the US EPA GLP standards as published in the Federal Register 40 CFR 160, Volume 54, No 158, August 17, 1989 with the following exception:

The GLP Compliance Statement signed by the Study Directors does not reference the 40CFR Part 160, but does reference Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989, and the Organization for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

## References

Safeparm Laboratories, Limited, Derby England, "Photostability Guideline No. 161-2, BCM Technical," Laboratory Project ID 121/182, February 8, 1993.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**7.0 STABILITY IN WATER**

**Test Substance**

Identity: Carbendazim  
Technical Grade, HOE 17411, 2-(Methoxycarbonylamino –benzimidazole,  
Lot number HOE 017411 of ZD990010.

**Method**

Method/guideline followed:	EPA Guideline 161-1
Type ( <i>test type</i> ):	Hydrolysis
GLP:	Yes
Year:	1993

**Test Conditions**

Duration:	24 days
Positive Controls:	No
Negative Controls:	No
Analytical procedures:	HPLC, See remarks

All buffer solutions and glassware were autoclaved to minimize the possibility of microbial degradation of the test material prior to the start of the test.

The composition of the buffer solutions is detailed below:

pH 5

Sodium acetate	13.7 g
Glacial acetic acid	6 ml
Distilled water	to 1 L

pH 7

Disodium hydrogen orthophosphate	0.04 M
Potassium dihydrogen orthophosphate	0.03 M

pH 9

Disodium tetraborate	0.05 M
Hydrochloric acid	0.02 M

Solutions of the test material were prepared at approximately 5 mg/l in the above buffer solutions which contained a 0.5% co-solvent of 0.2% hydrochloric acid in acetonitrile. These solutions were then maintained at  $25 \pm 1$  °C and the

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

concentration of the test material monitored over a suitable time period to establish the degree of hydrolysis.

Procedure: The concentration of the test material in each sample was determined by high performance liquid chromatography as follows:

Samples: The samples were diluted by a factor of 2 using 0.2% hydrochloric acid in acetonitrile.

Standards: Standard solutions of test material were prepared in 0.2% hydrochloric acid in acetonitrile:buffer solution\* (1:1) at nominal concentrations of 2.5 and 5 mg/l.

\*Specification detailed on page 9.

Analysis: The HPLC parameters were as follows:

Column	Spherisorb S10 ODS1 (250 mm x 4.6 mm i.d.)
Flow rate	1.5 ml/min
Mobile phase	water:acetonitrile:borate buffer** (73:25:2)
Injection volume	25 $\mu$ l
Wavelength	284 nm
** Borate buffer (pH 8.6)	200 ml of distilled water containing approximately 0.6 g of boric acid, 0.8 g of sodium chloride, 25 ml of sodium hydroxide (0.1 M).

A graph of the common logarithm of the concentration against time was plotted for pH 9 (see page 20) and an estimate of the rate constant and the half-life at the specified temperature obtained.

**Results**

Half-life ( $t(1/2)$ ): 1.56 x 10<sup>3</sup> hours at pH 9 (25 $\pm$  1 $^{\circ}$ C)  
Breakdown products: No

No hydrolysis was observed in pH 5 and 7 buffer solutions after 24 days at 25 $\pm$ 1 $^{\circ}$ C. The rate constant and half-life at 25 $^{\circ}$ C for pH 9 has been calculated from the graph of log<sub>10</sub> concentration (g/L) against time (hours).

Nominal Solution pH: 9  
Temperature: 25 $\pm$ 1 $^{\circ}$ C  
Estimated rate constant (hour<sup>-1</sup>): 4.44 x 10<sup>-4</sup>  
Estimated half-life (hours): 1.56 x 10<sup>3</sup>

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

It was established that the reactions at pH 9 are pseudo first order as the graph of  $\text{Log}_{10}$  concentration against time was a straight line.

The peak heights of the pair of standards associated with a particular sample were corrected to nominal concentration and the mean taken prior to the calculation of the sample concentration.

With the exception of the following deviation, the experimental procedure used complies with that specified in EPA Guideline 161-1:

No identification of the degradates in pH 9 buffer solution was made, although the anticipated products from the hydrolysis of carbendazim would be methanol and 2-(imino-oxy)-benzimidazole.

This deviation not considered to have affected the integrity of the study. (Study author)

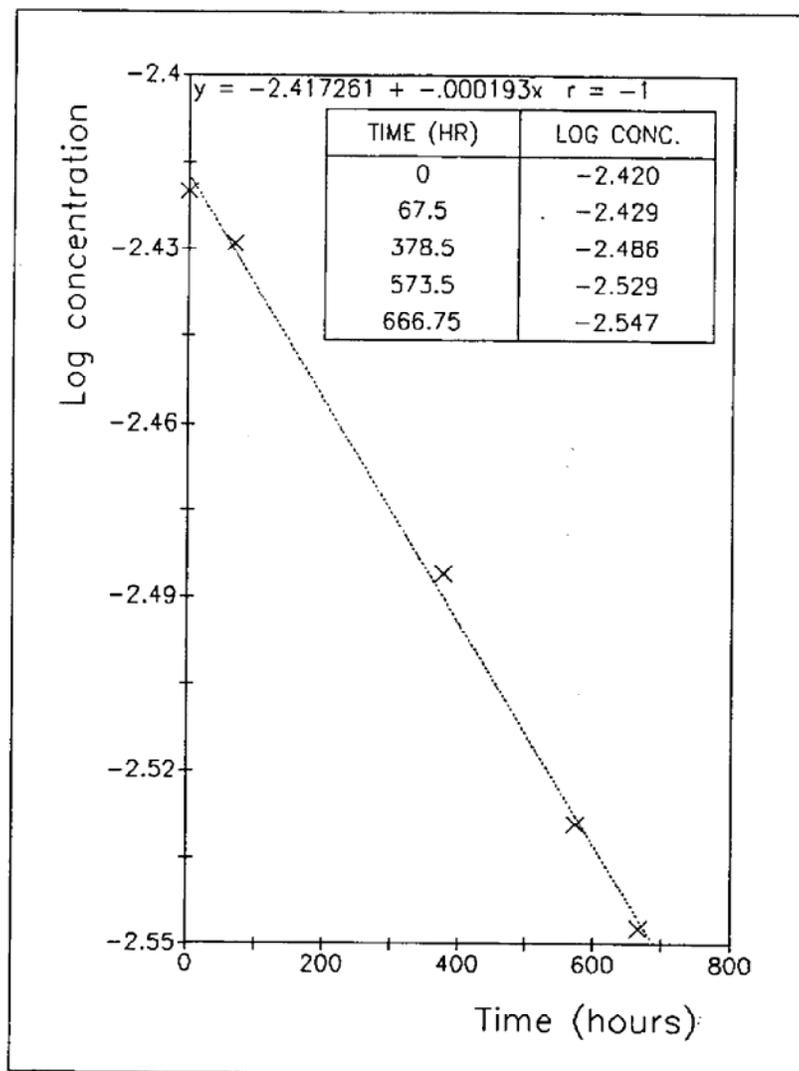
	pH 5	pH 7	pH 9
Concentration as weighed (g/l)	$5.04 \times 10^{-3}$	$5.04 \times 10^{-3}$	$4.03 \times 10^{-3}$
Concentration found initially (g/l)	$5.29 \times 10^{-3}$	$4.84 \times 10^{-3}$	$3.81 \times 10^{-3}$
Expressed as % of concentration as weighed	$105 \times 10^{-3}$	$96.1 \times 10^{-3}$	$94.4 \times 10^{-3}$
Concentration found at 67.5 hours (g/l)	$4.80 \times 10^{-3}$	$5.35 \times 10^{-3}$	$3.73 \times 10^{-3}$
Expressed as % of concentration as weighed	$95.3 \times 10^{-3}$	$106 \times 10^{-3}$	$92.4 \times 10^{-3}$
Concentration found at 379 hours (g/l)	$5.34 \times 10^{-3}$	$4.93 \times 10^{-3}$	$3.26 \times 10^{-3}$
Expressed as % of concentration as weighed	$106 \times 10^{-3}$	$97.8 \times 10^{-3}$	$80.9 \times 10^{-3}$
Concentration found at 574 hours (g/l)	$4.95 \times 10^{-3}$	$4.99 \times 10^{-3}$	$2.96 \times 10^{-3}$
Expressed as % of concentration as weighed	$98.1 \times 10^{-3}$	$98.9 \times 10^{-3}$	$73.3 \times 10^{-3}$
Concentration found at 667 hours (g/l)	---	---	$2.84 \times 10^{-3}$
Expressed as % of concentration as weighed	---	---	$70.4 \times 10^{-3}$

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**RESULTS (cont)**

pH 9 AT 25.0 ± 1.0°C

GRAPH OF LOG<sub>10</sub> CONCENTRATION (g/l) AGAINST TIME (HOURS)



**Conclusions**

No hydrolysis was observed in pH 5 and 7 buffer solutions after 24 days at 25± 1°C. The estimated half-life is 1.56 x10<sup>3</sup> hours at pH 9. (Study author)

**Reliability**

This study was conducted to conform with Good Laboratory Practices as published by the US EPA GLP Standards as published in the Federal Register, 40 CFR 160, Volume 54, No. 158, August 17, 1989 with the following exceptions:

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The GLP Compliance Statement signed by the Study Director does not reference the 40 CFR, Part 160, but does reference Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989, and the OECD ISBN 92-64-12367-9, Paris 1982. (Study Author)

**References**

Safeparm Laboratories Limited, Derby England, "BCM Technical Grade (HOE 17411) Hydrolysis," Laboratory Project ID 121/172, December 1, 1993.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## **8.0 TRANSPORTION BETWEEN ENVIRONMENTAL COMPARTMENTS**

### **Test Substance**

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, CAS 106-21-7

### **Method**

Test: Fugacity Level III

Fugacity Model, EPI Suite, Version 3.12.U.S. Environmental Protection Agency, 2004

Default values were assumed for environmental compartment descriptions, dimensions, and properties, advective and dispersive properties. Chemical-specific input parameters were: molecular weight (191.18 g/mol), vapor pressure (1.13E-6 mm Hg), log  $K_{ow}$  (0.40), melting point 302°C, aqueous solubility 8.0 mg/L, and a Henry's Law constant of  $3.5 \times 10^{-8}$  atm-m<sup>3</sup>/mol. Half-lives calculated by the model based on the properties of the test substance were: air 1.28 hr, water 900 hr, soil 1800 hr, and sediment 8100hr.

Emissions were assumed to be equally to air, water and soil.

### **Results**

Media:	air, water, soil, sediment
Estimated Distribution and Media Concentration (Level III):	Air = 0.09%
	Water = 51.4%
	Soil = 48.4%
	Sediment = 0.10%
Air:	half-life = 1.28 hr, emissions = 1000 kg/hr
Water:	half-life = 900 hr, emissions = 1000 kg/hr
Soil:	half-life = 1800 hr, emissions = 1000 kg/hr
Sediment:	half-life = 8100 hr, emissions = 0 kg/hr

### **Reliability**

Klimisch = 2, Reliable with restrictions  
Acceptable method. Some parameters calculated.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Reference**

Staples, Charles A., "Level Fugacity of Carbendazim Using EPI Suite, Version 3.12.U.S. Environmental Protection Agency , 2004," Unpublished Report for Troy Chemical, December 5, 2004.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## **9.0 BIODEGRADATION**

### **Test Substance**

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, > 98% pure

### **Method**

Method/guideline followed:	OECD 301D
Type ( <i>test type</i> ):	Aerobic
Year:	2002
Contact time:	28 days
Inoculum:	Fresh activated sludge

The biodegradability of carbendazim was determined as described under “Closed Bottle Test” in the OECD Guidelines 301D, for testing of chemicals: “Ready Biodegradability” using oxygen depletion as test criterion in a 28-day test. The method is in agreement with the EU Test Guideline C.4-E.

Two concentrations of the test substance, a control (blank medium), an inoculum activity control and a toxicity control were prepared with mineral medium, saturated with oxygen, placed in BOD bottles approximately 293 ml and incubated for 28 days in the dark at about 20°C, except for the inoculum activity and toxicity control which were incubated for 14 days. The oxygen concentration was determined at the beginning of the test and on days 7, 14, 21, and 28 of incubation.

The test substance was an off-white powder with a stated solubility in water of 8 mg/L at 24°C. The test substance was applied to an inert carrier (glass fibre filter) for introduction into the test system. A stock solution of carbendazim in methanol was prepared and an aliquot was placed on each filter. After evaporation of the methanol, the filters were placed in test bottles containing inoculated medium.

Two nominal concentrations of 0.75 and 1.87 mg/l were tested, corresponding to a Theoretical Oxygen Demand (ThOD) of 1.0 and 2.5 mg )<sub>2</sub>/ l, respectively. These concentrations were chosen because carbendazim (a fungicide) might be toxic to the inoculum. An inoculum was prepared from activated sludge taken from an oxidation ditch used to treat domestic sewage.

The inoculum activity appeared to be sufficient; the reference substance sodium acetate reached the 60% pass level of degradation within fourteen days. In a toxicity control with 1.87 mg/l of test substance and sodium acetate, no inhibition of the degradation of 4.04 mg/l sodium acetate was found.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

*Guideline*

The biodegradability test was conducted in accordance with the OECD Guideline 301D [1] and the EU Guideline C.4-E [2].

*Test substance concentrations*

The determination was performed with two concentrations of carbendazim: 0.75 and 1.87 mg.l<sup>-1</sup>, corresponding to a ThOD value of 1.0 and 2.5 mg O<sub>2</sub>.l<sup>-1</sup> respectively.

*Preparation of the biodegradation test bottles*

Due to the limited water solubility of the test substance, the test concentrations of carbendazim were prepared by adding a suitable amount of test substance stock solution in an organic solvent onto Whatman GF/C filters (Ø 47 mm), which were then inserted into the test bottles. For this purpose 0.0689 g of the test substance was dissolved in 250 ml methanol. Aliquots of 0.8 and 2 ml of this stock solution were applied to the filters, which after evaporation of the methanol, were placed in the test bottles. The total volume of each test bottle was 293 ml. The test concentrations obtained were 0.75 and 1.87 mg.l<sup>-1</sup> respectively.

In addition, an inoculum blank, containing a Whatman GF/C filter (Ø 47 mm) and inoculated mineral medium was prepared. For the activity control, an inoculum blank containing inoculated mineral medium only was used.

*Preparation of the activity control*

The final test concentration of sodium acetate was prepared by dissolving 0.4045 g of the reference substance in 100 ml of ultrapure water. From this stock solution a dilution was made in inoculated mineral medium to give a nominal test concentration of 4.04 mg.l<sup>-1</sup> sodium acetate.

*Preparation of the toxicity control*

A dilution of the reference substance stock solution (described above) was prepared in inoculated mineral medium and placed in the test bottles. A filter with the test substance was placed in each test bottle. The toxicity control contained 1.87 mg.l<sup>-1</sup> carbendazim and 4.04 mg.l<sup>-1</sup> sodium acetate.

**Test Conditions**

Inoculum (concentration and source): Fresh activated sludge

Characterization of bacterial inoculum: A sample of activated sludge was taken from an oxidation ditch situation in the municipality of Hazerwoude, the

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Netherlands. The oxidation ditch is used to treat domestic wastewater. The activated sludge was transported in a plastic bottle and aerated until use.

Concentration of test chemical:	0.75 and 1.87 mg/l
Vehicle used:	Methanol
Temperature of incubation (°C):	20°C
Dosing procedure:	Inert carrier gas
Sampling frequency:	Days 0, 7, 14, 21 and 28
Appropriate controls and blank system used:	Yes
Analytical method used to measure biodegradation:	Oxygen concentration measured with Oxygen probe
Method of calculating measured concentrations:	Arithmetic mean

The pH of the medium remained fairly constant during the test (7.0 – 7.3). The temperature measured in one of the bottles of each treatment varies between 19.0 and 20.6°C during the test.

Deviations from the protocol: The test substance concentrations of respectively 0.75 and 1.87 mg/L are corresponding with a Theoretical Oxygen Demand of respectively 1.0 and 2.5 mg/L. However they deviate from the test substance concentrations that are mentioned in the protocol. The concentrations were chosen because carbendazim (a fungicide) might be toxic to the inoculum.

The amount of NH<sub>4</sub>Cl in Nutrient stock solution A2 was 1.5 g instead of 0.5 g.

These deviations are assumed not to have influenced the results of the test. (Study author)

*Test series*

The following test series were prepared:

Inoculum blank

- Concentration : 0 mg.l<sup>-1</sup>
- Number of replicate bottles per sample point : 4
- Oxygen concentration determined after : 0, 7, 14, 21, 28 days.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Inoculum blank with filter

- Concentration : 0 mg.l<sup>-1</sup>
- Number of replicate bottles per sample point : 4
- Oxygen concentration determined after : 0, 7, 14, 21, 28 days.

Test substance

- Concentrations : 0, 0.75 and 1.87 mg.l<sup>-1</sup>
- Number of replicate bottles per sample point : 4
- Oxygen concentration determined after : 0, 7, 14, 21, 28 days.

Inoculum activity control

- Concentration of the reference substance : 4.04 mg.l<sup>-1</sup>
- Number of replicate bottles per sample point : 4
- Oxygen concentration determined after : 0, 7 and 14 days.

Toxicity control

- Concentration of reference substance : 4.04 mg.l<sup>-1</sup>
- Concentration of test substance : 1.87 mg.l<sup>-1</sup>
- Number of replicate bottles per sample point : 4
- Oxygen concentration determined after : 0, 7 and 14 days.

*Incubation and measurements*

The filled BOD bottles were closed and incubated at about 20°C in the dark after measurement of the oxygen concentration in one bottle of each series with an oxygen electrode. Thereafter a separate set of four bottles was sacrificed for oxygen measurements at each time-point.

The temperature in one bottle of each treatment was measured at each time-point indicated above.

The pH in one bottle of each treatment was measured at the start of the test and after 28 days of incubation, except for the control bottles with sodium acetate and the toxicity controls with sodium acetate and test substance that were measured after 14 days of incubation.

# Robust Summaries, CAS No. 10605-21-7

## Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester

Calculations:

Calculations were performed as given in the test Guidelines [1] and [2]. The oxygen depletion (in  $\text{mg O}_2\text{.l}^{-1}$ ) in each test bottle after 7, 14, 21 and 28 days was calculated by subtracting each measured oxygen concentration from the mean oxygen concentration measured at the start of the test. The oxygen depletion due to the test or control substance at each time was calculated by subtracting the mean oxygen consumption in the blanks from that in the bottle under consideration. These crude values were then converted to values per mg substance (BOD). The percentage biodegradation of the test substance was calculated as  $\text{BOD/ThOD} \times 100$ .

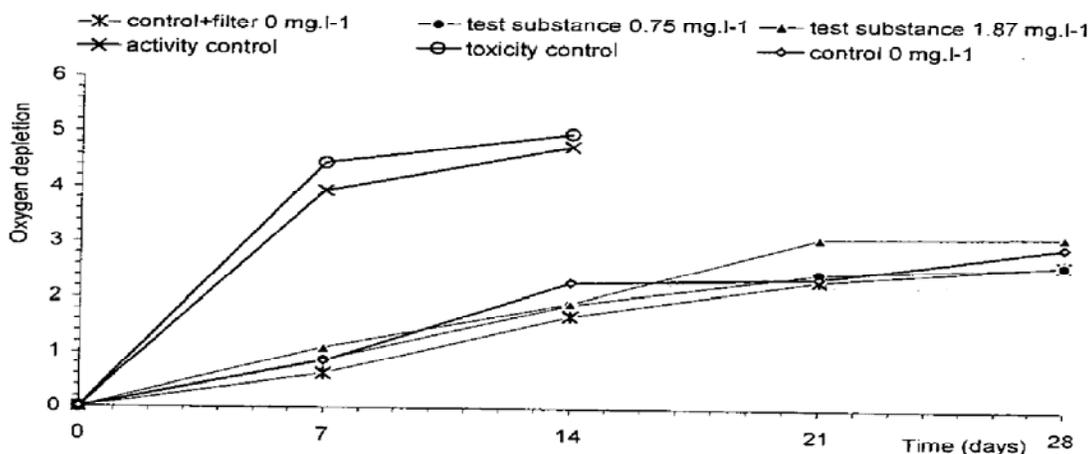
### Results

Degradation % after time: 22-42% at 21 days

After 21 days of incubation, the Biochemical Oxygen Demand (BOD) of the test substance at a concentration of 1.87 mg/l varied between 0.29 and 0.55  $\text{mg O}_2$  /mg. Based on the ThOD value, this corresponds to a maximum biodegradability (corrected for the inoculum control with filter) varying between 22 and 41%.

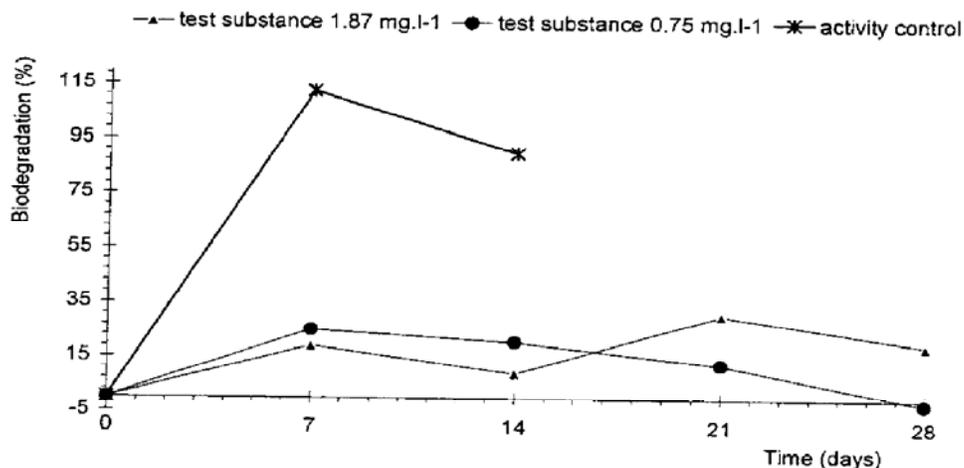
The measured data resulting from the determination of the oxygen concentrations in the various bottles of the inoculum activity and toxicity control bottles, as well as the biodegradation test are given in Annex B. The calculated results are given in Annex C.

The results of the test are illustrated in Figure 1 (i.e. by the oxygen depletion in the control and test bottles over time) and Figure 2 (i.e. by the % biodegradability based on the ThOD).



**Figure 1** The mean oxygen depletion ( $\text{mg O}_2\text{.l}^{-1}$ ) in the inoculated mineral medium biodegradation test with two concentrations of carbendazim.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**



**Figure 2** The biodegradation (% of ThOD) in the inoculated mineral medium biodegradation test with two concentrations of carbendazim.

Results for each time period %:

Tables 2 and 3 show the relevant mean Biochemical Oxygen Demand (BOD) values and the percentage biodegradation of the carbendazim concentrations of 0.75 and 1.87 mg.l<sup>-1</sup>, calculated from the ThOD<sub>NH3</sub> (1.34 mg O<sub>2</sub>.mg<sup>-1</sup>) of the test substance.

**Table 2** Biodegradation of carbendazim (0.75 mg.l<sup>-1</sup>) expressed as the BOD (mg O<sub>2</sub>.mg<sup>-1</sup>) and as percentage of its ThOD.

Time Days	BOD mg O <sub>2</sub> .mg <sup>-1</sup>	% Biodegradation
7	0.34 (0.30-0.37)	25 (22-27)
14	0.28 (0.07-0.62)	21 (5-46)
21	0.17 (0.12-0.19)	13 (9-14)
28	-0.02 (-0.09-0.10)	-2 (-7-7)

**Table 3** Biodegradation of carbendazim (1.87 mg.l<sup>-1</sup>) expressed as the BOD (mg O<sub>2</sub>.mg<sup>-1</sup>) and as percentage of its ThOD.

Time (days)	BOD mg O <sub>2</sub> .mg <sup>-1</sup>	% Biodegradation
7	0.26 (0.23-0.28)	19 (17-21)
14	0.12 (-0.12 - 0.34)	9 (-9-26)
21	0.41 (0.29-0.55)	31 (22-41)
28	0.27 (0.18-0.35)	20 (14-26)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Breakdown products: No

**Conclusions**

Carbendazim was considered not to have exceeded the pass level of 60% ThOD removal within 28 days and a 14 day window, classifying it as not readily biodegradable. Carbendazim may, on the basis of this result, be considered inherently biodegradable under the appropriate test conditions. (Study author)

**Reliability**

Klimisch Code = 1

The study was conducted according to OECD 301D.  
The study was carried out in accordance with OECD Principles of Good Laboratory Practice. (Study author)

**References**

TNO, The Netherlands, "Determination of the ready biodegradability of carbendazim in a Closed Bottle Test (OECD Guidelines No. 301D, EU C.4-E)," TNO Study number 01-4003/04, September 9, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## 10.0 ACUTE TOXICITY TO FISH

### Test Substance

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, CAS Reg. Number 10605-21-7, Batch No. 010310

### Method

Method/guideline followed:	OECD Guideline no. 203 and EU Guideline No. C.1.
Test type:	Semi-static
GLP:	Yes
Year:	2002
Species/Strain/Supplier:	<i>Brachydanio rerio</i> from a commercial hatchery Atlanta, Hellevoetsluis, the Netherlands
Analytical Monitoring:	The concentration of carbendazim in test medium was determined using HPLC with UV detection. Quantitation of carbendazim was obtained by comparing the peak areas in the chromatograms of the study samples with those in the chromatograms of calibration solutions.
Exposure period:	96 hours
Statistical methods:	NA

The test was conducted in accordance with the OECD Guideline 203 and the EU Guideline No. C.1. The duration of the test was 96 hours. The test was carried out under a 16 hour light/8 hour dark regime with transition periods of ca. 30 minutes in a temperature controlled room. The temperature in the control medium was measured at the beginning of the test, at each replacement time in the newly prepared control vessel and the corresponding “spent” control vessel and at the end of the test was controlled to be  $24 \pm 1^\circ\text{C}$ .

As a preliminary range finding test indicated that no adverse effects were to be expected at 10 mg/l (this concentration is above the water solubility of the substance), only one concentration of carbendazim was tested in a limit test. DSWL-E was used as a control medium, triethylene glycol (TEG) was used as a carrier to dose the test substance and therefore a solution containing 100 ug/l TEG was used as a solvent control. New concentrated solutions were prepared daily throughout the test period.

The test was performed in 2 liter all glass beakers, each containing 1.5 liter of exposure medium; the beakers were covered with a watch glass and coded with

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

the study number and test substance concentration. One beaker containing 10 fish was used for each exposure medium. The fish were not fed. The exposure media were slightly aerated and replaced daily. The fish were added randomly, using a random table.

The pH and oxygen concentration of the exposure media were measured at the beginning and at the end of the test as well as at each replacement time (in the spent media just before replacement and in the newly prepared media just after dosing.)

The dead and living animals were counted (and, if applicable, the dead ones removed) after 3, 24, 48, and 96 hours. At the same time the conditions (swimming behavior, color and any other visually observable morphological or behavioral criterion) of the survivors was compared with those of the control animals.

**Test Conditions**

Test fish: *Brachydanio rerio*.

The average total length and weight ( $\pm$  standard deviation) of 10 fish of the batch used (randomly taken at the start of the test) were  $2.2 \pm 0.22$  cm and  $0.098 \pm 0.030$  g respectively. The fish was cultured under the circumstances of the test.

Dilution water source: The dilution water was DSWL\_E, a synthetic medium prepared from ground water, which has proven to be suitable for the culture of *Brachydanio rerio*. (See Table in Test Condition Remarks for composition of DSWL\_E.)

Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity):

Hardness (As  $\text{CaCO}_3$ ): 219 mg/l

Total Organic Carbon: 2.97 mg/l

As a preliminary range-finding test indicated that no adverse effects were to be expected at  $10 \text{ mg.l}^{-1}$  (this concentration is above the water solubility of the substance), only one concentration of carbendazim was tested in a limit test. DSWL-E was used as a control medium; triethylene glycol (= TEG) was used as a carrier to dose the test substance and therefore a solution containing  $100 \text{ }\mu\text{g.l}^{-1}$  TEG was used as a solvent control. New concentrated solutions were prepared daily throughout the test period.

Concentrations dosing rate, flow-through rate, in what medium:

Vehicle/solvent and concentrations: Triethylene glycol (TEG) 100 ug/l.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Stability of the test chemical solutions: Not reported  
 Exposure vessel type: 2 liter all glass beakers, each containing 1.5 liter of exposure medium; the beakers were covered with a watch glass.  
 Number of replicates, fish per replicate: 1 replicate, 10 fish per replicate.

Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed:

pH value: 7.9 – 8.2  
 Lowest measured oxygen concentration: 8.1 mg O<sub>2</sub> /l

Test temperature range: 24.5 – 25.4° C  
 Method of calculating mean measured concentrations: Arithmetic mean

Single samples were taken from only the concentration tested, and not duplicate samples. Throughout the test, particles floating on the surface were observed at a dosed amount of 10 mg/l.

Measured vs. nominal concentrations of test substance:

**Table 1** *Chemical analysis of carbendazim in the exposure media during the acute toxicity test with the zebrafish Brachydanio rerio.*

Nominal concentration in mg.l <sup>-1</sup>	Measured concentrations in mg.l <sup>-1</sup>				Average %
	t = 0h		t = 24h		
	mg.l <sup>-1</sup>	%	mg.l <sup>-1</sup>	%	%
0	< 0.05	-	< 0.05	-	
10	5.6	56	4.3	43	50

**Results**

Nominal concentrations (as mg/L): 1,10  
 Measured concentrations (as mg/L): <0.05, 4.3-5.6  
 Unit (results expressed in what unit): mg/L  
 Element value:  
 Statistical results (as appropriate): NA  
 Biological observations: No effects  
 Table showing cumulative mortality: No mortality  
 Lowest test substance concentration causing 100% mortality: No mortality, > 10 mg/l (nominal), > 5mg/l (measured)  
 Mortality of controls: 0  
 Abnormal responses: 0

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Reference substances (if used) – results: NA

Any observations that might cause a difference between measured and nominal values: Throughout the test undissolved material was present on the surface of the Carbendazim exposure medium; the controls were clear.

With respect to the survival and the condition of the test animals there were no adverse effects observed throughout the test. It is therefore concluded that carbendazim is not acutely toxic to *Brachydanio rerio* within its water solubility. The summarized results of the ecotoxicity test are:

Parameter (h = hours)	Effect	Nominal concentration (mg.l <sup>-1</sup> )	Measured concentration (mg.l <sup>-1</sup> )
3h LC50	mortality	> 10	> 5.0
24h LC50	mortality	> 10	> 5.0
48h LC50	mortality	> 10	> 5.0
72h LC50	mortality	> 10	> 5.0
96h LC50	mortality	> 10	> 5.0
96h LC100	mortality	> 10	> 5.0
96h NOEC	mortality	≥ 10	≥ 5.0
96h NOEC	condition	≥ 10	≥ 5.0

As required by the Dutch act on animal experimentation, the design of the study protocol was reviewed and agreed to by the TNO animal ethics committee.

**Table B1** Number of fish and their condition in the exposure media of carbendazim during the test<sup>1)</sup>.

Time (h)	Nominal concentration of test substance (mg.l <sup>-1</sup> )		
	0	0 <sup>2)</sup>	10
0	10a	10a	10a
3	10a	10b	10b
24	10a	10b	10b
48	10a	10b	10b
72	10a	10b	10b
96	10a	10b	10b

1) The following codes are used to denote condition:

- a) Condition of all fish, visually assessed, normal (= good).
- b) Condition of all fish, visually assessed, equal to that of the control fish.

2) Solvent control = 0.1 ml TEG per litre of dilution water

## Conclusions

With respect to the survival and to the condition of the test animals, there were no adverse effects observed throughout the test. It is therefore concluded that carbendazim is not acutely toxic to *Brachydanio rerio* within its water solubility. (Study author)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Reliability**

Klimisch Code = 1

The study was conducted according to OECD Guideline no. 203 and was carried in accordance with the OECD Principles of Good Laboratory Practice. (Study author)

**References**

TNO, The Netherlands, "Semi-static acute toxicity test with carbendazim and the zebra fish *Brachydanio rerio* (Guidelines: OECD no. 203 and EU no. C.1)," TNO Study Number 01-4003/01, May 6, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## 11.0 TOXICITY TO AQUATIC PLANTS

### Test Substance

Identity: Carbendazim, 2-(methoxycarbonylamino)-benzimidazole  
CAS 10605-21-7, Batch 010310, >98%

### Method

Method/guideline followed: OECD 201  
GLP: Yes  
Year: 2002

#### Species/strain # and source:

The fresh-water green alga *Selenastrum capricornutum* (CCAP 278/4)<sup>J1</sup>, which belongs to the order of *Chlorococcales* (class *Chlorophyceae*), was used as the test organism.

The culture was supplied by the CCAP, The Freshwater Biological Association, the Ferry House, Far Sawrey, Ambleside, Cumbria LA22 OLP, England.

A preculture of algae in the exponential growth phase was prepared as detailed in OECD Guideline no. 201 [1], using the medium described in 2.4.

This organism is the preferred species for regulatory testing.

Element basis: growth rate, biomass (area under growth curve),  
growth yield  
Exposure period: 72 hours  
Analytical monitoring: Yes

The toxicity of Carbendazim to the fresh water alga *Selenastrum capricornutum* was determined in a 72 hour growth inhibition test according to the Guidelines OECD No. 201 and EU C.3, and in compliance with the OECD Principles of GLP.

A range-finding test was conducted as a simplified version of the OECD Guidelines No. 201 prior to the actual test.

Carbendazim is an off white powder with a stated solubility of 8 mg/l in water. The concentrations of carbendazim tested were 0, 0.33, 1.0, 3.3 10 and 33 mg/l and were added to the algal medium with the solvent carrier triethylene glycol (TEG).

The test was carried out in triplicate with six controls (three containing algae only and three containing algae and TEG) and a single background concentration series containing test substances without algae.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The algal growth was determined by electron particle counting after 22.5, 47.5 and 71.5 hours incubation.

The EC values with respect to the growth yield and logistic growth ( $E_yC$  values) were calculated by means of a parametric model developed by Kooijman *et al.* assuming an error proportional to the cell density; a summary of the method is given in Annex B. This calculation method is based on the assumptions given in the OECD Guidelines and has been validated by international ring tests.

EC values with respect to the area under the growth curve ( $E_bC$  values) were calculated by the method given in the OECD guideline. The values were calculated by linear interpolation of a plot of the percentage reduction in growth ( $I_A$ ) against the log concentration of the test substance.

**NOEC**

The NOEC (no-observed-effect concentration) was determined as the highest concentration at which no (statistically) significant inhibition was observed:

- An effect was considered significant, when the average inhibition of the growth rate was higher than 5 %.
- Statistical significance was determined with a one tailed t-test ( $\alpha=5\%$ ).

**NEC**

In addition model calculations were carried out using the DEBtox software package according to the Dynamic Energy Budgets Theory developed by Kooijman and Bedaux [9]. Model parameters for population growth and their asymptotic standard deviation and correlation coefficients were estimated. The NEC (no-effect concentration [8,9]), was calculated from the Profile Ln Likelihood function.

Deviations from the protocol:

- The initial algal cell concentration was lowered to  $0.3 \times 10^4$  cells/ml in order to prevent an exceeding of pH increase.
- The amount of organic carrier used was 200 ul/l (not 100 ul/l) because it was no possible to suspend the highest dose of test substance in that volume of triethylene glycol.
- These deviations are assumed not to have affected the results of the study. (Study author)

The test included analytical determination of a low, middle and high test substance concentration at the state and end of the test. Samples were taken from nominal

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

test concentrations of 0, 0.33, 3.3 and 33 mg/l. The concentration of carbendazim in test medium was determined using High Performance Liquid Chromatography (HPLC) with UV detection. Quantitation of carbendazim was obtained by comparing the peak areas in the chromatograms of the study samples with those in the chromatograms of calibration solutions.

The pH was measured at the start (medium without alga) and after 71.5 hours in all cultures. The morphology of the algae was examined visually with the aid of a microscope at the start and end of the test. The light intensity at two different culture positions was measured at the start of the test.

**Test Conditions**

Test Organism:

**The test organism will be the fresh water green alga *Selenastrum capricornutum* (strain No. CCAP 278/4). The culture was supplied by the CCAP, The Freshwater Biological Association, the Ferry House, Far Sawrey, Ambleside, Cumbria LA22 0LP, England. It belongs to the class of *Chlorophyceae* and will be cultured according to the Guideline [1].**

Test temperature range: The temperature was measured to be 23.6 – 24.6°C (mean 23.9°C)

Growth/test medium chemistry (Hardness):

The medium was prepared from concentrated stock solutions in ultra pure water (Annex A). It was sterilized by micropore filtration and contained 150 mg.l<sup>-1</sup> NaHCO<sub>3</sub> (not 50 mg.l<sup>-1</sup> as specified in the OECD Guideline [1], this in order to improve the buffer capacity of the medium). Furthermore, the medium contained Fe-citrate, because the growth of the algae would be erratic in the absence of complexed iron.

Hardness, mg equivalent CaCO<sub>3</sub>.l<sup>-1</sup> :

$$=2.497 [\text{Ca, mg.l}^{-1}] + 4.118[\text{Mg, mg.l}^{-1}] = 24.2$$

Water chemistry in test (pH) in at least one replicate of each concentration (at start and end of the test): At the start of the test the pH of the algal medium containing different test substance concentrations was found to be

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

At the end of the test, with and without algal cells, the pH was found to have remained stable during the test (pH 8.1 – 8.3).

Stock solutions preparation (vehicle, solvent, concentrations): Stock solutions were prepared in the carrier solvent Triethylene glycol.

Light levels and quality during exposure: The light intensity was measured to be 84-105  $\mu\text{mol s}^{-1} \cdot \text{m}^{-2}$  and was within the required limits.

Test design (number of replicates, concentrations): 3 replicates of test substance at 0.33, 3.3 and 33 mg/l; 3 replicates of control with algae only; 3 replicates of control with algae and carrier (TEG).

**Results**

Element value:  $E_rC_{50}$ ,  $E_yC_{10}$ ,  $E_yC_{50}$ ,  $E_yC_{90}$ ,  $E_bC_{10}$ ,  $E_bC_{50}$ ,  $E_bC_{90}$ , NOEC, NEC (mg/l)

Based on the nominal and measured concentrations of the test substance carbendazim the following results were obtained:

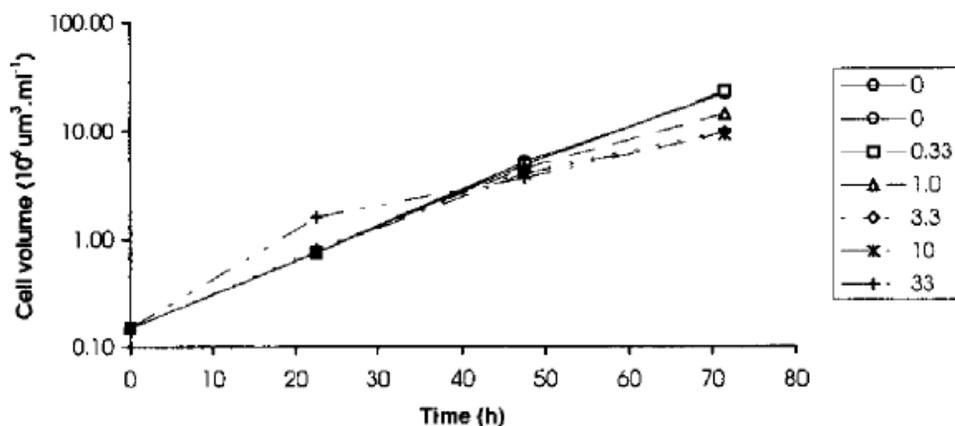
Parameter	Dimension	Value (95% confidence limit)
NOEC	$\text{mg.l}^{-1}$	1.0
NEC	$\text{mg.l}^{-1}$	0.33 (0.09 – 0.47)
$E_rC_{50}$	$\text{mg.l}^{-1}$	23 (4.4 – 120)
$E_yC_{10}$	$\text{mg.l}^{-1}$	0.06
$E_yC_{50}$	$\text{mg.l}^{-1}$	0.68 (0.31 – 1.5)
$E_yC_{90}$	$\text{mg.l}^{-1}$	>3.3 (extrapolated: 7.2)
$E_bC_{10}$	$\text{mg.l}^{-1}$	0.5 (0.33 – 1.0) <sup>a)</sup>
$E_bC_{50}$	$\text{mg.l}^{-1}$	>3.3 (extrapolated: 4.5 )
$E_bC_{90}$	$\text{mg.l}^{-1}$	>3.3

<sup>a)</sup> range between tested concentrations

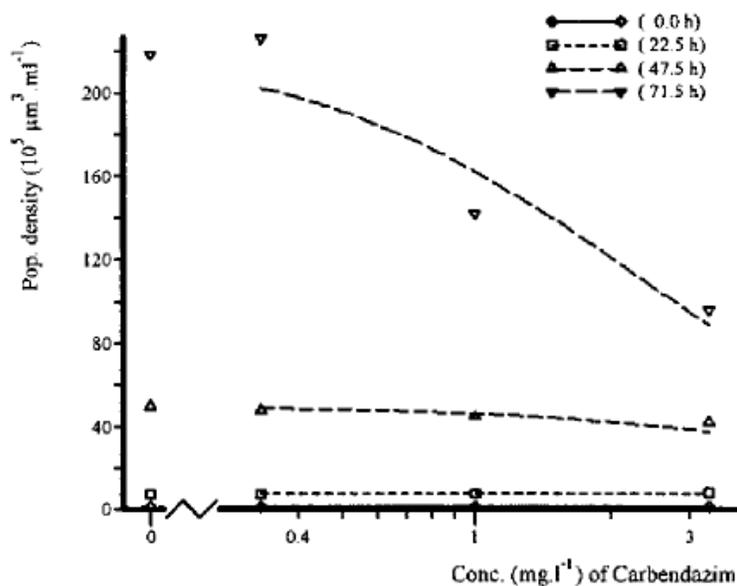
Was control response satisfactory: Yes

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Biological observations:



**Figure 1** Growth curves of *Selenastrum capricornutum* at different concentrations of carbendazim



**Figure 2** Carbendazim concentration-effect curves for *Selenastrum capricornutum* after different exposure periods. The lines are calculated with a model assuming an effect on the growth yield.

The test fulfilled the validity criteria of sufficient growth and a minimum increase of test medium pH.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The EC<sub>50</sub> with respect to the growth yield (E<sub>y</sub>C<sub>50</sub>) was found to be 0.68 mg/l with a 95% confidence interval of 0.31 – 1.5 mg/l.

Model calculations with respect to effect on the growth rate revealed an extrapolated E<sub>r</sub>C<sub>50</sub> value of 23 mg.l<sup>-1</sup>, with a 95% confidence interval of 4.4 - 120 mg.l<sup>-1</sup>. This result demonstrated that an effect on the growth rate could not be defined very well. As expected the E<sub>b</sub>C<sub>50</sub> value was lower than the E<sub>r</sub>C<sub>50</sub> value.

The effect of carbendazim on the algal growth manifested itself mainly at the end of the test and the effect did not increase at concentrations above the solubility in water. Therefore the E<sub>y</sub>C-values were calculated from the data of the concentrations tested lower than the solubility in water (i.e. < 10 mg.l<sup>-1</sup>). These calculations confirmed a significant effect on the biomass rather on the growth rate in this test. However, the E<sub>y</sub>C-values are outside the scope of the applied guidelines. The E<sub>b</sub>C values are, however, defined by the applied guidelines as the biomass (algal growth) related endpoints. Therefore the E<sub>b</sub>C<sub>50</sub> value of 4.5 mg.l<sup>-1</sup> is considered to represent the best approximation of the toxicity of carbendazim to algae.

With regard to the analysis of the test substance concentration, the following results were obtained:

- The measured concentrations at the highest tested nominal concentration (33 mg/l) were in the range 9.42 - 12.2 mg/l at the start and the end of the test respectively. These values are at or above the stated solubility of carbendazim in water (8 mg/l at 24°C). The results obtained could be best explained by the solubility of carbendazim in water.
- The measured concentrations of the other samples were found to be 71-97% of the nominal concentrations at the start of the test and 100-118% of the nominal concentrations at the end of the test.
- The average measured concentration of carbendazim during the test with exclusion of the nominal concentration higher than the solubility in water was 96.5% and the substance remained stable during the test. Therefore nominal concentrations were used to report the test results as defined by the EU C.3 Guideline.
- The apparent increase in the measured test substance concentrations during the test may be connected with the temperature dependent solubility of carbendazim in water.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

*Table E2 Concentration of carbendazim in the study samples*

Sample codes <sup>1)</sup>	Dose level (mg/l)	Sampling time (hours)	Concentration measured (mg/l)
01-4003-03/1	0	0	< 0.05
01-4003-03/2	0.33	0	0.32
01-4003-03/3	3.3	0	2.34
01-4003-03/4	33	0	9.42
01-4003-03/5	0	approx. 72	<0.05
01-4003-03/6	0.33	approx. 72	0.39
01-4003-03/7	3.3	approx. 72	3.30
01-4003-03/8	33	approx. 72	12.2

1) Samples were received on 16 November 2001 and analyzed on 19 November 2001. Until analysis the samples were stored at 2 - 10°C.

## Conclusions

The inhibiting effect of carbendazim on the growth of the algal cultures mainly manifests itself in a reduced growth towards the end of the test. The explanation of this effect is probably a relative slow transfer of test substance from the aqueous phase into the algal cells. Furthermore the analytical results indicate that there may be a slow dissolution of carbendazim in the test medium and a slow transfer of carbendazim from the solid to the aqueous phase may contribute to this effect. In the models developed by Kooijman et al. [4], an effect on the growth yield (thus on the growth or biomass at the end of the test) is expressed by the  $E_yC$ -values. However, the  $E_yC$ -values also represent an extrapolation of the present data to a calculated yield beyond the exposure period of 72 h. This calculation therefore confirms that there is a significant effect by carbendazim on the biomass in this test, but the exact values are outside the scope of the applied test guidelines. The  $E_yC50$  value is comparable to the NOEC/NEC values. The relative high  $E_rC50/E_bC50$  ratio of > 7.3 in this test compared with an average value of 1.8 in international ring-tests [6,7] is also an indication for a biomass related effect of carbendazim.

The growth (or biomass) related endpoint defined by the applied guidelines is the  $E_bC50$  value. The  $E_bC50$  value of 4.5 mg.l<sup>-1</sup> is therefore considered to represent the best approximation of the toxic effect of carbendazim on algae. The  $E_rC50$  value is higher than the solubility of carbendazim in water.

Reliability: K=1

The study was carried out in accordance with the OECD Principles of Good Laboratory Practice. Characterization and verification of the test substance

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

identity and properties are, however, the responsibility of the sponsor. (Study author) Sponsor provided certification regarding the identity, batch number and purity. Test substance was >98% pure.

**References**

TNO, “The Netherlands, Determination of the effect of carbendazim on the growth of the fresh water green alga *Selenastrum capricornutum* (OECD Guidelines No. 201 and EUC.3),” Unpublished study for Troy Corporation, Study No. 01-4003/03, May 16, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

## 12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

### Test Substance

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, CAS RN 10605-21-7, Purity >98%,  
Batch number 010310

### Method

Method/guideline followed:	Experimental, OECD Guideline no. 202 and EU Guidelines no. C.2
Test type:	Static
GLP:	Yes
Year:	2002
Analytical Monitoring:	HPLC/UV
Species/Strain:	<i>Daphnia magna</i>
Exposure period:	48 hours
Statistical methods:	EC50 values and their confidence interval were calculated by means of a parametric model developed by Kooijman, S.A.L.M. (1996). For these calculations the combined mobility data per test concentration were used.

The test was carried out as a static test with 4x5 Daphnias (fourfold exposure media) for the control medium and each concentration and the exposure duration was 48 hours. The test substance was dosed from concentrated solutions in triethyleneglycol (TEG). The nominal concentrations tested for carbendazim were 0.056, 0.1, 0.18, 0.32, 0.56 and 1.0 mg /l, together with a control and a TEG control.

DSWL-E was used as a control medium and triethyleneglycol (TEG) as a carrier to dose the test substance and therefore a solution of 0.10 ml TEG per liter of dilution water was used as a solvent control.

The mobile and if applicable the immobile animals were counted after 24 hours and at the end of the test according to the definition given in OECD Guideline no. 202 (animals not able to swim within 15 seconds after gentle agitation of the test containers are considered to be immobile). At the same time the condition (swimming behavior, color, or any other visually observable morphological or behavioral criterion) of the mobile animals was compared with that of the control animals. The condition of the Daphnia from the exposure media of 0.18 mg/l and higher and from 0.10 mg/l and higher were also checked under a microscope at t=24h and at the end of the test.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Test Conditions**

Test organisms:

Source, supplier, any pretreatment, breeding method:

The test organism used was the fresh-water crustacean *Daphnia magna*, cultured in the laboratory under standard conditions. *Daphnia magna* has been cultured in the dilution water, used for the test (see Annex A).

Every week a number of cultures is started with ca. 150 Daphnias of the same age (about one day) in ca. 4 litres of dilution water. The cultures are fed daily with ca.  $4 \times 10^9$  algal cells (*Chlorella*) and ca. 0.13 grams of yeast per 4 litre. The medium is completely replaced at least once a week; at the same time all young born are removed. The cultures are kept at  $20 \pm 1^\circ\text{C}$  under a 16h light and 8h dark regime with transition periods of ca. 30 minutes. After 4 weeks the cultures are discarded.

Age at study initiation: 1 day  
Control group: same

Stock solutions preparation and stability: A solution of 0.10 ml TEG per liter of dilution water was used as a solvent control. At end of the test and the test concentration appeared to be between 87% and 110% of the nominal concentrations (average 98%).

Test temperature range:  $20 \pm 1^\circ\text{C}$

Exposure vessel type (*e.g., size, headspace, sealed, aeration, number per treatment*): The test was performed in 150 ml all-glass beakers, each containing 100 ml of exposure medium. Four beakers containing 5 Daphnia each were used for each exposure medium. The exposure media were not aerated or replaced.

Dilution water source: The dilution water was DSWL-E, a synthetic medium prepared from ground water. Its composition is provided below. DSWL-E has proven to be suitable for the culture of *Daphnia magna*.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Dilution water chemistry:

**Annex A      Composition of the synthetic medium (DSWL-E)**  
**used in the test**

The nominal composition is as described below:

Na <sup>+</sup>	1.19	mmol.l <sup>-1</sup>
K <sup>+</sup>	0.20	mmol.l <sup>-1</sup>
Ca <sub>2</sub> <sup>2+</sup>	1.36	mmol.l <sup>-1</sup>
Mg <sub>2</sub> <sup>2+</sup>	0.73	mmol.l <sup>-1</sup>
Cl <sup>-</sup>	2.72	mmol.l <sup>-1</sup>
SO <sub>4</sub> <sup>2-</sup>	0.73	mmol.l <sup>-1</sup>
HCO <sub>3</sub> <sup>-</sup>	1.39	mmol.l <sup>-1</sup>

This medium is prepared by the addition of several salts to ground water from a locality near Linschoten (the Netherlands). The ground water contains several other trace elements (<< 1 mg.l<sup>-1</sup>). Media prepared from it have proved to be suitable for growing several species of water organisms. The equilibrium pH of the medium, after aeration, should be 8.3-8.5, but is usually slightly less, i.e. 8.0-8.2. The hardness, expressed as CaCO<sub>3</sub>, is about 210 mg.l<sup>-1</sup>.

The medium is prepared in large amounts (10,000 l) and the following components of the batch used for this test were checked by chemical analysis and found to be:

Na <sup>+</sup>	1.37	mmol.l <sup>-1</sup>
K <sup>+</sup>	0.19	mmol.l <sup>-1</sup>
Ca <sup>2+</sup>	1.38	mmol.l <sup>-1</sup>
Mg <sup>2+</sup>	0.81	mmol.l <sup>-1</sup>
Cl <sup>-</sup>	2.66	mmol.l <sup>-1</sup>
SO <sub>4</sub> <sup>2-</sup>	0.64	mmol.l <sup>-1</sup>

Some traces of minerals were added (as salts) to the batch used together with some EDTA, resulting in the following final element and EDTA concentrations.

B	46.25	μmol.l <sup>-1</sup>
Mn	1.82	μmol.l <sup>-1</sup>
Li	7.22	μmol.l <sup>-1</sup>
Rb	0.59	μmol.l <sup>-1</sup>
Sr	0.57	μmol.l <sup>-1</sup>
Br	0.16	μmol.l <sup>-1</sup>
Mo	0.26	μmol.l <sup>-1</sup>
Cu	0.10	μmol.l <sup>-1</sup>
Zn	0.10	μmol.l <sup>-1</sup>
Co	0.04	μmol.l <sup>-1</sup>
I	0.02	μmol.l <sup>-1</sup>
Se	0.01	μmol.l <sup>-1</sup>
V	0.05	μmol.l <sup>-1</sup>
Fe	0.36	μmol.l <sup>-1</sup>
Na <sub>2</sub> EDTA	0.67	μmol.l <sup>-1</sup>

The hardness, expressed as CaCO<sub>3</sub>, was 219 mg.l<sup>-1</sup>.  
The total organic carbon content was 2.97 mg.l<sup>-1</sup>.

Lighting (quality, intensity and periodicity): 16 h light and 8 h dark with transition period of ca. 30 minutes.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Water chemistry:

The summarized results of the measurements in the exposure media were:

Variation of pH value : 7.9 – 8.0  
 Lowest measured oxygen concentration : 9.2 mg O<sub>2</sub>.l<sup>-1</sup>  
 Variation of temperature measured in one of the control vessels : 19.3 – 19.6°C

It is not likely that any of these parameters affected the reliability of the test.

Element (unit) basis: Immobilization, mortality  
 Test design: 4 replicates, 5 Daphnia per replicate  
 Method of calculating mean measured concentration: Arithmetic mean  
 Exposure period: 48 hours

Analytical monitoring: The actual concentration of carbendazim in the exposure media was determined by HPLC with UV detection. Therefore samples of the exposure media of 0.10, 0.32 and 1.0 mg/l and the solvent (TEG) control were taken at the start of the test just after dosing and the concentration appeared to be between 96% and 110% of the nominal concentration (average 102%). In order to test the stability, the same media and the control were sampled again at the end of the test and the concentration appeared to be between 87% and 110% of the nominal concentrations (average 98%). The average percentage present during the test was 100%. Therefore it is allowed for this study to express the test substance concentrations as nominal values.

**Table 1** Chemical analysis of carbendazim in the exposure media during the acute toxicity test with *Daphnia magna*.

Nominal concentration mg.l <sup>-1</sup>	Measured concentrations				Average %
	t= 0h		t= 48h		
	mg.l <sup>-1</sup>	%	mg.l <sup>-1</sup>	%	
0 <sup>1)</sup>	< 0.05	-	< 0.05	-	-
0.10	0.11	110	0.11	110	110
0.32	0.32	100	0.32	97	98
1.0	0.96	96	0.87	87	92
Average % ± St. dev.		102 ± 7.2		98 ± 12	100 ± 9.3

1) solvent control (= TEG)

All exposure media were completely clear (visually assessed) throughout the test. The *Daphnia* were not fed during the test.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Results**

Nominal concentrations (mg/L): 0, 0.056, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l

Measured concentrations (mg/L): 0, 0.056, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l

The average percentage present during the test was 100%. Therefore it is allowed for this study to express the test substance concentrations as nominal values. (Study author)

EC50, EL50, LC0, LL0, at 24, 48 hours: Results expressed in mg/l

The results of the test were in mg.l<sup>-1</sup> (95 confidence interval in brackets):

<b>Parameter (h = hours)</b>	<b>Effect</b>	<b>Concentration</b>
24h EC50	mobility	0.92 (0.70 – 1.2)
48h EC50	mobility	0.16 (0.12 – 0.21)
48h EC100	mobility	0.56
48h NOEC	mobility	0.10
48h NOEC	condition	0.056

There was no immobility in the DSWL-E control and at 0.056 and 0.10 mg/l; from 0.18 mg/l immobility and mortality were observed. In the solvent control one daphnia died, however, this is allowed according to the guideline. From 0.10 mg/l effects with respect to the condition of the test animals, such as slow swimming were observed. At 0.056 mg/l one slow swimming animal was present; this is considered to be an accidental case, and is not taken into account for the estimation of the NOEC for condition. The NOEC values for immobility and condition are therefore respectively 0.10 and 0.056 mg/l respectively. The slope of the concentration –effect curve (inverse of parameter B of the model) was 2.9 (2.1-4.6.) the results of the test, expressed as EC50, EC100 and NOEC values are summarized under “Results” above.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Biological observations:

**Table B1** Number of mobile animals in the control and exposure media of carbendazim<sup>1)</sup>

Time (h)	Concentration of test substance (mg.l <sup>-1</sup> )							
	0	0 <sup>2)</sup>	0.056	0.10	0.18	0.32	0.56	1.0
0	20	20	20	20	20	20	20	20
24	20	20	20	20	18	13	15	12
48	20	19	20	20	3	2	0	0

1) Data on the condition of the test animals in each test vessel used are given in Table B2.

2) Solvent control (= TEG)

Was control response satisfactory?: Yes

### Conclusions

24 hour EC50 (mobility): 0.92 (0.70 – 1.22)  
48 hour EC50 (mobility): 0.16 (0.12- 0.21)  
48 hours EC50 (mobility): 0.56  
48 hours NOEC (mobility): 0.10  
48 hour NOEC (condition): 0.056 (Study Author)

### Reliability

Klimisch = 1

Reliable without restriction. OECD Guideline no. 202 and EU Guidelines no. C.2  
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice. (Study author)

### References

TNO, The Netherlands, “Static acute toxicity test with carbendazim and the crustacean species *Daphnia magna* (Guidelines OECD no. 202 and EU no. C.2),” TNO Study number 01-4003/02, May 6, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**13.1 ACUTE TOXICITY - ORAL**

**Test Substance**

Identity (purity): Carbendazim  
1320103200L, Referenced as test material LX1132-01 in the study

**Method**

Method/guideline followed:	EPA Guideline 81-1 (OPP)
Type (test type):	Acute oral toxicity
GLP:	Yes
Year (study performed):	1989
Species/Strain:	Rat/Harlan Sprague Dawley
Sex:	Male and female
No. of animals per sex per dose:	5
Vehicle:	2% w/v aqueous carboxymethyl cellulose (CMC)
Route of administration:	Oral intubation (gavage)

**Test Conditions:**

Age:	Young adult
Doses:	5050 mg/kg
Doses per time period:	1 initial dose
Volume administered or concentration:	Administered as a 25% w/v concentration in 2.0% w/v CMC.
Post dose observation period:	14 days

Healthy albino rats were released from quarantine and five males and five females were selected for testing. The animals were fasted for at least 16 hours prior to treatment. Food was again made available *ad libitum* immediately after treatment for the duration of the study.

All animals were treated with a 5050 mg/kg (20.2 ml/kg) of a 25.0% w/v concentration of the test material in 2.0% w/v CMC. The dose was administered by oral intubation using an appropriately sized syringe and stainless steel ball-tip intubation needle. The animals were returned to their cages immediately after treatment.

Observations for mortality and signs of pharmacologic and/or toxicologic effects were made at least three times on the day of treatment and at least once daily thereafter for 14 days (day of treatment considered day 0). Individual body

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

weights were recorded just prior to treatment and on days 7 and 14. A gross necropsy examination was conducted on each animal at termination of the study.

**Results**

Value:	> 5050 mg/kg
Number of deaths at each dose level:	No mortality in the study
Time of death:	NA

Description, severity, time of onset and duration of clinical signs at each dose level: The only prominent in-life observation was piloerection, which occurred in all of the five male rats at hour six after the administration of the dose; the effect was not seen after one day or for the remainder of the study. All females appeared normal for the duration of the study.

Necropsy findings, included doses affected, severity and number of animals affected: Unremarkable

Potential target organs (if identified in the report):

If both sexes tested, results should be compared: Transient piloerection in all five male rats observed only at hour 6. No effects were seen in females.

Observations for pharmacologic and/or toxicologic signs were made frequently throughout the study. The only prominent in-life observation was piloerection, which occurred in all of the five male rats at hour six after the administration of the dose; the effect was not seen after one day or for the remainder of the study. All females appeared normal for the duration of the study. The gross necropsy examination conducted on all animals at termination of the study revealed no observable abnormalities in any of the animals. No animals died during the study. The acute oral LD50 for the test compound is greater than 5050 mg/kg (20.2 ml/kg) when administered as a 25.0% w/v concentration in 2.0% w/v CMC to albino rats.

**Conclusions**

The acute oral LD50 for actual LX1132-01 (Carbendazim), as indicated by the data, is greater than 5050 mg/kg (20.2 ml/kg) when administered as a 25.0% w/v concentration in 2.0% w/v CMC to albino rats. (Study Author)

**Reliability**

Klimisch Code: 1. The study is reliable without restriction.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The study was conducted according to EPA Guideline 81-1 (OPP). This study was conducted in accordance with Good Laboratory Practices as published by the US EPA Good Laboratory Practice Standards as published in the Federal Register 40 CFR 160, Volume 54 No. 158. (Study Author)

**References**

Still Meadow Inc., Sugarland Texas, “Acute Oral Toxicity Study in Rats Guideline No. 81-1 – BCM Technical,” Laboratory Project ID 6274-89, August 8, 1989.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**14.0 GENETIC TOXICITY IN VIVO**

**Test Substance**

Identity: Carbendazim  
>98%, 2-(methoxycarbonylamino)-benzimidazole, CAS RN 10605-21-7, Batch number 010310

**Method**

Method/guideline followed:	OECD 474
Type:	Bone marrow micronucleus test
GLP:	Yes
Year (study performed):	2002
Species:	Mice
Strain:	Charles River, CD-1
Sex:	27 Male; 27 Female
Route of administration:	Oral
Doses/concentration levels:	2000 mg/kg-bw
Exposure period:	1 gavage dose
Statistical methods:	Pooled error variance t-tests; Separate variance t-test

**Test Conditions:**

Age at study initiation:	Not reported
No. of animals per dose:	10 Males, 10 Females
Vehicle:	Corn oil
Duration of test:	48 hours
Frequency of treatment:	One gavage dose
Sampling times and number of samples:	24 hours (5 per sex) 48 hours (5 per sex)
Control groups and treatment:	
Negative control:	Corn Oil
Positive control:	Mytomycin C, 0.75 mg/kg-bw
Organs examined at necropsy:	Bone marrow
Criteria for evaluating results:	See remarks (test condition)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The limit dose level of 2000 mg/kg-bw was based on the results of a dose-range finding acute toxicity test with Carbendazim, performed prior to the start of the main micronucleus test.

The route of administration, by gavage, was selected because it is a common dosing route for a micronucleus test.

After a fasting period of 2 hours, 10 male mice and 10 female mice were treated once by gavage with Carbendazim (2000 mg/kg-bw). The negative control group, consisting of 10 males and 10 females, were treated in a similar way with corn-oil (the vehicle). A positive control group, consisting of 5 males and 5 females, was given a single intraperitoneal injection with the mutagen mitomycin C (0.75 mg/kg-bw). At 24 hours after treatment, 10 animals of the negative control group (5/sex), 10 animals of the test group (5/sex) and 10 animals (5/sex) of the positive control group were sacrificed. At 48 hours after treatment, 10 animals of the negative control group (5/sex) and 10 animals of the test group (5/sex) were sacrificed.

Signs of reactions to treatment were recorded twice during the first 4 hours after treatment, and once daily thereafter. At the designated time point after dosing, all animals were killed by cervical dislocation. Bone marrow was immediately collected into foetal calf serum and processed into glassdrawn smears according to the method described by Schmid (1976). Two bone marrow smears per animal were prepared, air-dried, fixed in methanol. One smear was stained with a solution of May-Grünwald and Giemsa. The unstained smear was stored in the archives of the TNO Nutrition and Food Research.

The slides were randomly coded by a person not involved in the scoring of slides. Slides (one slide per animal) were read by moving from the beginning of the smear (label end) to the leading edge in horizontal lines taking care that areas selected for evaluation were evenly distributed over the whole smear.

The numbers of polychromatic and normochromatic erythrocytes (PE and NE, respectively) were recorded in a total of 1000 erythrocytes (E) per animal; if micronuclei were observed, these were recorded as micronucleated polychromatic erythrocytes (MPE) or micronucleated normochromatic erythrocytes (MNE).

Once a total number of 1000 E (PE + NE) had been scored, an additional number of PE was scored for the presence of micronuclei, if possible, until a total number of 2000 PE had been scored. If possible, the incidence of MPE was recorded in a total of 2000 PE per animal and the number of MNE was recorded in the number of NE.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Summary of experimental design: Micronucleus Test with Carbendazim</b>					
test substance: Carbendazim			vehicle: corn-oil		
route: by gavage			dosing volume: 20 ml/kg-bw		
treatment	Dose level (mg/kg-bw)	group code/ colour code	scheduled killing following treatment (h)	number and sex of animals	coding of animals
neg. control (corn-oil)	-	A/white	24	5 males	2, 4, 6, 8, 10
			24	5 females	1, 3, 5, 7, 9
			48	5 males	12, 14, 16, 18, 20
			48	5 females	11, 13, 15, 17, 19
test substance: Carbendazim	2000	B/blue	24	5 males	22, 24, 26, 28, 30
			24	5 females	21, 23, 25, 27, 29
			48	5 males	32, 34, 36, 38, 40
			48	5 females	31, 33, 35, 37, 39
positive control (Mitomycin C <sup>#</sup> )	0.75	C/green	24	5 males	42, 44, 46, 48, 50
			24	5 females	41, 43, 45, 47, 49

# Derived from Sigma Co. (St Louis, USA); single intraperitoneal injection (10 ml/kg-bw)

Deviations from the protocol:

- The animal arrival dates of the dose-range finding acute toxicity test and main micronucleus test were 21 November 2001 and 5 December 2001, respectively, instead of 9 January 2002 and 30 January 2002, respectively.
- The experimental start dates of the dose-range finding acute toxicity test and main micronucleus test were 27 November 2001 and 11 December 2001, respectively, instead of 15 January 2002 and 5 February 2002, respectively.

The deviations from the protocol did not adversely affect the integrity or validity of the study. (Study author)

**Results**

Genotoxic effects: Positive  
 Remarks: No clinical abnormalities were observed at the dose tested (2000 mg/kg-bw.)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

At 24 hours after treatment, the incidences of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE) in male and female mice, treated with the test substance Carbendazim, were clearly statistically significantly higher than those found in the negative controls.

At 48 hours after treatment, the incidences of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE) in male and female mice, treated with the test substance Carbendazim, could not be analysed because an insufficient number of PE was available in the bone marrow. Therefore, the study clearly indicates that treatment with the test substance Carbendazim resulted in cytotoxicity to bone marrow cells. The positive control group differed significantly from the negative control; this demonstrates the sensitivity of the test system.

At both sacrifice times of 24 hours and 48 hours after treatment, for both the males and females of group B, treated with the test substance Carbendazim, a clearly significant difference in the number of PE was observed, when compared with the negative control group A. This indicates that Carbendazim reached the target cells of the bone marrow and that treatment with Carbendazim resulted in cytotoxicity to bone marrow cells of both male and female mice.

<b>body weights of the animals prior to treatment</b>				
treatment group	Dose level (mg/kg-bw)	sex m/f	n	body weight (g) mean ± SEM
				day 0
A= negative control (corn-oil)	-	m	10	31.7 ± 0.4
		f	10	25.6 ± 0.5
B=Test substance: Carbendazim	2000	m	10	32.5 ± 0.5
		f	10	26.2 ± 0.2
C=positive control (mitomycin C*)	0.75	m	5	32.0 ± 0.8
		f	5	25.8 ± 0.4

# Sigma; single intraperitoneal injection (10 ml/kg-bw)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The results of the statistical analysis are summarized in Table 1 and 2 and were as follows:

**For males:**

At time point 24h, group B differed significantly from the negative control group A for MPE ( $p < 0.05$ ) and for PE ( $p < 0.001$ ). The positive control group C differed significantly from the negative control group A for MPE ( $p < 0.01$ ). At time point 48h, group B differed significantly from the negative control group A for PE ( $p < 0.01$ ).

**For females:**

At time point 24h, group B differed significantly from the negative control group A for MPE ( $p < 0.001$ ) and for PE ( $p < 0.001$ ). The positive control group C differed significantly from the negative control group A for MPE ( $p < 0.01$ ). At time point 48h, group B differed significantly from the negative control group A for PE ( $p < 0.01$ ).

**Table 1**

<b>Group mean <math>\pm</math> S.D. numbers of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE)</b>					
treatment group	Dose level (mg/kg-bw)	sex/time point (h)	n	MNE/NE	MPE/2000 PE
A=negative control (corn-oil)	-	males / 24	5	0.2 $\pm$ 0.4	2.2 $\pm$ 0.8
		males / 48	5	0.8 $\pm$ 0.8	2.0 $\pm$ 0.7
		females / 24	5	0.4 $\pm$ 0.9	2.0 $\pm$ 0.7
		females / 48	5	1.0 $\pm$ 0.7	2.4 $\pm$ 1.3
B=Test substance: Carbendazim	2000	males / 24	5	2.0 $\pm$ 0.7	93.6 $\pm$ 50.3*
		males / 48	5	5.6 $\pm$ 3.2	not analysable
		females / 24	5	1.8 $\pm$ 1.1	81.0 $\pm$ 24.1***
		females / 48	5	7.2 $\pm$ 4.5	not analysable
C=positive control (Mitomycin C)	0.75	males / 24	5	0.8 $\pm$ 0.4	57.4 $\pm$ 16.8**
		females / 24	5	0.6 $\pm$ 0.5	42.2 $\pm$ 12.2**

Means and standard deviations:

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Table 2**

<b>Group mean <math>\pm</math> S.D. numbers of polychromatic erythrocytes (PE) per 1000 erythrocytes (E)</b>				
<b>treatment group</b>	<b>Dose level (mg/kg-bw)</b>	<b>sex/time point (h)</b>	<b>n</b>	<b>PE / 1000 E</b>
<b>A=negative control (corn-oil)</b>	-	males / 24	5	435.6 $\pm$ 79.8
		males / 48	5	508.8 $\pm$ 66.5
		females / 24	5	433.8 $\pm$ 79.9
		females / 48	5	427.0 $\pm$ 62.2
<b>B=Test substance: Carbendazim</b>	2000	males / 24	5	224.8 $\pm$ 100.4**
		males / 48	5	28.4 $\pm$ 12.1***
		females / 24	5	154.8 $\pm$ 27.5***
		females / 48	5	29.2 $\pm$ 10.4***
<b>C=positive control (Mitomycin C)</b>	0.75	males / 24	5	410.8 $\pm$ 22.8
		females / 24	5	407.6 $\pm$ 31.3

Means and standard deviations:  
 \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

**Conclusions**

From the results obtained in this micronucleus test with the test substance Carbendazim, it is concluded that Carbendazim was genotoxic and cytotoxic for the bone marrow cells of both male and female mice, under the conditions of this study. It is further concluded that the test substance Carbendazim has the potential to induce damage to the mitotic spindle apparatus of the bone marrow cells under the conditions of this study. (Study Author)

**Reliability**

Klimisch Code 1

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

This study was conducted in compliance with:

- the EEC protocol B.12 (Mutagenicity: Micronucleus Test) of council Directive 67/548/EEC, adopted December 29, 1992,
- the OECD guideline 474, Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test, adopted 21 July 1997

The study was conducted according to the OECD Principles of Good Laboratory Practice (as revised in 1997), Organisation for Economic Co-operation and Development (OECD), Paris; ENV/MC/CHEM (98) 17.

**References**

TNO, The Netherlands, “Micronucleus test with Carbendazim in mice,” TNO Study No. 3404/10, March 12, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**15.0 GENETIC TOXICITY IN VITRO**

(A) Chromosome Abbreviation

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, MERGAL BCM, CAS RN 10605-21-7, Batch no. 010310, Purity > 98%

**Method**

Method/guideline followed: OECD 473  
Type: Mammalian cell gene mutation assay  
System of testing: Non bacterial  
GLP: Yes  
Year (study performed): 2002  
Species/Strain or cell type: Chinese Hamster Ovary Cells  
Metabolic activation: With and without S9  
Species and cell type: Chinese Hamster ovary cells

Concentrations tested:

Test I: 0, 0.05, 0.1, 0.2, 0.4, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 80, 100, 200 ug/ml

Test II: 0, 1, 2.5, 5, 10, 30, 50, 75, 100, 150, 200 ug/ml

Statistical Methods: Fisher's exact probability test (two-sided)

**Test Conditions**

Frequency of Dosing:

Test 1: 1/8 h

Test 2: 18/18 h, 32/32h (without S9-mix)  
4/18 h, 4/32 h (with S9-mix)

Positive and negative control groups: Yes

Negative Control (vehicle): DMSO

Positive Control: Mitomycin C (in the absence of the S9-mix);  
Cyclophosphamide (in the presence of the S9-mix)

Number of metaphases analyzed: 200 per concentration

Solvent: DMSO

Precipitation concentration if applicable: 100 ug/ml in DMSO

Criteria for evaluating results:

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

The test substance Carbendazim was examined for its potential to induce structural chromosomal aberrations in Chinese hamster ovary cells, in both the absence and presence of a metabolic activation system (S9-mix).

Two independent chromosomal aberration tests were conducted. In the first test, in both the presence and absence of S9-mix, the treatment/harvesting times were 1/18 h (pulse treatment). In the second test, in the absence of S-9-mix, the treatment/harvesting times were 18/18 h (continuous treatment and 32/32 h (continuous treatment). In the second test, in the presence of S9-mix, the treatment/harvesting times were 4/18h (pulse treatment) and 4/32 h (pulse treatment).

The highest concentration tested was based on toxicity of the test substance to the cells.

In both chromosomal aberration tests, the negative (vehicle) controls were within the historical range and the positive control substances mitomycin C (in the absence of the S9-mix) and cyclophosphamide (in the presence of the S9-mix) and cyclophosphamide (in the presence of the S9-mix) induced the expected increases in the incidence of structural chromosomal aberrations. This demonstrates the validity and sensitivity of the test system.

Deviations from the protocol:

- During the analysis of the mitotic index of the second test, many polyploid cells were observed in the cultures treated with the test substance Carbendazim. The number of polyploid cells per 100 metaphases per culture and the percentage of polyploid cells per 200 metaphases are reported in the tables of this report.
- At the later sampling time of 32 hours, in both the absence and presence of S9-mix, at least three concentrations of the test substance together with the negative control were analysed for the induction of chromosomal aberrations, instead of one concentration.

## **Results**

In the first chromosomal aberration test, Carbendazim did not induce a statistically significant increase in the number of cells with chromosomal aberrations at any of the dose levels analyzed when compared with the negative control values.

In the second (independent) chromosomal aberration test, in the absence of S9-mix (in both continuous treatment groups of 18/18 hours and 32/32 hours) and in the presence of S9-mix (in the pulse treatment group of 4/18 hours), Carbendazim induced a statistically significant increase in the number of cells with chromosomal aberrations over the concurrent control frequencies.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

In the second (independent) chromosomal aberration test, clear evidence of polyploidy (abnormal number of chromosomes) was demonstrated at all the time-points and concentrations analyzed. Although the purpose of the chromosomal aberration test is to detect structural chromosomal aberrations, the number of polyploidy cells per concentration were counted and the results were included in the tables of this report.

<b>Table 1: Chromosomal aberration test with Carbendazim in the presence of S9-mix: mitotic index (Test 1)</b>					
treatment time:			4 h		
harvesting time:			18 h		
treatment	dose (µg/ml)	number of cells scored	mitotic index		
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring
negative control (DMSO)	0	1000	15.4	100	+
		1000	14.1		+
test substance	200	1000	12.3	78	+
		1000	10.7		+
	100	1000	8.5	58	+
		1000	8.6		+
	50	1000	11.0	79	+
		1000	12.3		+
	25	1000	9.8	68	-
		1000	10.4		-
cyclophosphamide	3	1000	7.4	48	+
		1000	6.8		+

+ selected cultures  
 - not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Table 2: Chromosomal aberration test with Carbendazim in the absence of S9-mix: mitotic index (Test 1)</b>					
treatment time:			4 h		
harvesting time:			18 h		
treatment	dose (µg/ml)	number of cells scored	mitotic index		
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring
negative control (DMSO)	0	1000	10.3	100	+
		1000	9.5		+
test substance	100	1000	9.5	98	+
		1000	9.9		+
	50	1000	3.8	37	+
		1000	3.6		+
	25	1000	11.1	119	+
		1000	12.5		+
	12.5	1000	12.5	128	-
		1000	12.9		-
	6.25	1000	13.5	133	-
		1000	12.9		-
mitomycin C	0.1	1000	8.4	90	+
		1000	9.4		+

+ selected cultures  
 - not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Table 3: Chromosomal aberration test with Carbendazim in the absence of S9-mix: mitotic index (Test 2)</b>					
treatment time:			18 h		
harvesting time:			18 h		
treatment	dose (µg/ml)	number of cells scored	mitotic index		
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring
negative control (DMSO)	0	1000	7.5	100	+
		1000	8.0		+
test substance	200	1000	9.0	117	+
		1000	9.2		+
	100	1000	9.1	114	+
		1000	8.6		+
	50	1000	8.1	103	+
		1000	7.9		+
	30	1000	9.5	121	-
		1000	9.3		-
	10	1000	8.8	112	-
		1000	8.5		-
	5	1000	7.4	98	-
		1000	7.8		-
	2.5	1000	5.8	79	+
		1000	6.4		+
mitomycin C	0.05	1000	4.9	63	+
		1000	4.8		+

+ selected cultures  
 - not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Table 4: Chromosomal aberration test with Carbendazim in the absence of S9-mix: mitotic index (Test 2)</b>					
treatment time:			32 h		
harvesting time:			32 h		
treatment	dose (µg/ml)	number of cells scored	mitotic index		
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring
negative control (DMSO)	0	1000	9.3	100	+
		1000	9.1		+
test substance	100	1000	10.2	108	+
		1000	9.7		+
	75	1000	7.3	79	-
		1000	7.3		-
	50	1000	10.3	109	-
		1000	9.8		-
	30	1000	9.2	102	-
		1000	9.5		-
	10	1000	7.7	84	-
		1000	7.8		-
	5	1000	7.6	85	-
		1000	8.0		-
	2.5	1000	6.6	72	+
		1000	6.7		+
	1	1000	8.7	95	+
		1000	8.8		+

+ selected cultures  
 - not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Table 5: Chromosomal aberration test with Carbendazim in the presence of S9-mix: mitotic index (Test 2)</b>						
treatment time:			4 h			
harvesting time:			18 h			
treatment	dose (µg/ml)	number of cells scored	mitotic index			
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring	
negative control (DMSO)	0	1000	17.4	100	+	
		1000	17.7		+	
test substance	200	1000	17.3	98	+	
		1000	17.2		+	
	150	1000	14.4	83	+	
		1000	14.6		+	
	100	1000	17.8	100	+	
		1000	17.3		+	
	50	1000	12.9	76	-	
		1000	13.8		-	
	30	1000	13.9	79	-	
		1000	13.9		-	
	10	1000	15.0	84	-	
		1000	14.6		-	
	cyclophosphamide	3	1000	4.4	25	+
			1000	4.2		+

+ selected cultures

- not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

<b>Table 6: Chromosomal aberration test with Carbendazim in the presence of S9-mix: mitotic index (Test 2)</b>					
treatment time:			4 h		
harvesting time:			32 h		
treatment	dose (µg/ml)	number of cells scored	mitotic index		
			percentage of cells in mitosis	percentage of treated versus negative control value	selection for chromosomal aberration scoring
negative control (DMSO)	0	1000	9.8	100	+
		1000	10.0		+
test substance	200	1000	12.7	129	+
		1000	12.8		+
	150	1000	8.6	86	-
		1000	8.5		-
	100	1000	8.2	81	+
		1000	7.8		+
	50	1000	10.6	106	+
		1000	10.3		+
	30	1000	11.8	119	-
		1000	11.7		-
	10	1000	9.2	92	-
		1000	9.0		-

+ selected cultures  
 - not selected cultures

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Conclusions**

It is concluded that Carbendazim is a suspect clastogenic compound and has the potential to inhibit mitotic processes and to induce numerical chromosomal aberrations, under the conditions in this study. (Study author)

**Reliability**

Klimisch Code 1

The protocol had been drafted in accordance with:

- the OECD guideline 473, Genetic Toxicology: *In Vitro* Mammalian Chromosome Aberration Test, adopted 21 July 1997,
- the EEC protocol B.10 (Mutagenicity: *in vitro* Mammalian Cytogenetic Test) of Council Directive 67/548/EEC, adopted December 29, 1992.

The study was conducted according to the OECD Principles of Good Laboratory Practice (as revised in 1997), Organisation for Economic Co-operation and Development (OECD), Paris; ENV/MC/CHEM(98)17.

**References**

TNO, The Netherlands, "Chromosomal Aberration Test with Carbendazim in Cultured Chinese Hamster Ovary cells," TNP Study number 3402/13, February 15, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

(B) Bacterial reverse mutation assay

**Test Substance**

Identity: Carbendazim  
2-(methoxycarbonylamino)-benzimidazole, CAS Reg. Number  
10605-21-7, Batch #010310, Purity >98%

**Method**

Method/guideline followed:	OECD Guideline 471, EEC protocol of Council Directive 2000/32/EG, B.13/14(19 May 2000)
Type:	Bacterial reverse mutation assay
System of testing:	Bacterial
GLP:	Yes
Year (study performed):	2002
Species/Strain or cell type and or cell line (bacterial or non-bacterial):	<i>Salmonella typhimurium</i> , Strains TA 1535, TA 1537, TA 98, TA100 <i>Escherichia coli</i> Strain WP2 <i>uvrA</i>
Metabolic activation:	Yes. S9 metabolic activation was used in this study.
Statistical Methods:	Arithmetic mean and standard deviation.

**Test Conditions**

Number of replicates:	3 replicates per concentration
Solvent:	DMSO
Negative control:	Solvent, DMSO
Positive Controls:	

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

In all assays the reference mutagens used as positive controls and their dose levels were:

positive control mutagens		
strain	in the absence of the S9-mix	in the presence of the S9-mix
TA 1535	sodium azide: 1.0 µg/plate	2-aminoanthracene: 2.0 µg/plate
TA 1537	9-aminoacridine: 80 µg/plate	benzo(a)pyrene: 4.0 µg/plate
TA 98	2-nitrofluorene: 2.0 µg/plate	2-aminoanthracene: 2.0 µg/plate
TA 100	sodium azide: 1.0 µg/plate	2-aminoanthracene: 2.0 µg/plate
WP2 <i>uvrA</i>	N-ethyl-N-nitrosourea: 100 µg/plate	2-aminoanthracene: 80 µg/plate

The test substance Carbendazim was examined for mutagenic activity in the bacterial reverse mutation test using four histidine-requiring *Salmonella typhimurium* strains and one tryptophan-requiring *Escherichia coli* strain, and a liver fraction of Arcolor 1254-induced rats for metabolic activation (S9-mix).

Two assays were performed with all stains in both the absences and the presence of S9-mix with five different concentrations of the test substance, ranging from 62-5000 ug/plate in the first assay and from 313 to 5000 ug/plate in the second assay. The test substance was suspended in DMSO. The test substance precipitated into the agar at the doses starting at 556 ug/plate. At the highest dose a slight precipitate was observed on the agar plates. Negative controls (solvent) and positive controls were run simultaneously with the test substance.

On the day of use, aliquots of S9 were thawed and mixed with a NADPH generating system. The final concentrations of the various ingredients in the S9-mix were: MgCl<sub>2</sub> 8 mM; KCl 33 mM; G-6-P 5 mM; NADP 4 mM; sodium phosphate 100 mM (pH 7.4), 46 mM NaCl, and S9 10 %. The S9-mix was kept on ice until use.

## Results

Cytotoxic concentration: No cytotoxicity observed

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Genotoxic effect: Positive in *Salmonella typhimurium* strains TA 1537 and TA98 with metabolic activation at the highest concentration (5000 ug/plate).

The test substance Carbendazim was not toxic to any strain, as was evidenced by the absences of a decrease in the mean number of revertant colonies.

In the first assay Carbendazim caused a two-fold or greater increase in the mean number of revertants in two *Salmonella typhimurium* strains in the presence of the S9-mix at the highest dose. Carbendazim caused a 1.8 and 1.98-fold increase respectively, in the mean number of revertants compared to the background spontaneous reversion rate observed with the negative control. Also, tentative dose-effect relationships are observed. Therefore, Carbendazim was considered to be mutagenic under the conditions employed in this study. In all other strains Carbendazim did not cause an increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative control.

The positive controls gave the expected increase in the number of his\* and trp\* revertants in both the absence and the presence of the S9-mix.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Table 1: Bacterial reverse mutation test with Carbendazim (first assay).

Dose ug/pl		TA 1535		TA 1537		TA 98		TA 100		E-COLI	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0		20	19	9	11	38	29	131	117	20	23
		18	15	13	12	30	33	153	124	27	27
		20	12	9	12	31	40	131	121	33	27
	Mean	19	15	10	12	33	34	138	121	27	26
	Sd	1	4	2	1	4	6	13	4	7	2
62		22	12	11	8	24	31	131	103	25	31
		27	19	9	14	30	42	107	102	28	26
		22	19	18	18	24	39	134	120	28	24
	Mean	24	17	13	13	26	37	124	108	27	27
	Sd	3	4	5	5	3	6	15	10	2	4
185		27	24	19	13	23	31	115	122	19	25
		24	18	14	13	17	28	123	102	31	28
		16	11	7	9	20	29	123	101	25	22
	Mean	22	18	13	12	20	29	120	108	25	25
	Sd	6	7	6	2	3	2	5	12	6	3
556		14	18	7	9	26	40	139	144	23	20
		23	16	11	17	25	45	134	106	27	24
		26	15	9	17	22	40	105	129	29	26
	Mean	21	16	9	14	24	42	126	126	26	23
	Sd	6	2	2	5	2	3	18	19	3	3
1667		23	18	8	17	26	45	123	106	25	18
		17	26	12	15	41	55	97	86	25	35
		17	17	16	27	29	42	117	126	25	27
	Mean	19	20	12	20	32	47	112	106	25	27
	Sd	3	5	4	6	8	7	14	20	0	9
5000		27	23	23	28	24	94	153	174	33	29
		22	21	15	19	33	110	132	153	21	36
		28	12	11	25	36	80	136	156	27	38
	Mean	26	19	16	24	31	95	140	161	27	34
	Sd	3	6	6	5	6	15	11	11	6	5
Pos.C.		407	466	773	231	1300	1496	602	1643	201	564
		416	416	998	166	1350	1370	506	1751	224	575
		391	495	1157	200	1294	1227	715	1616	251	500
	Mean	405	459	976	199	1315	1364	608	1670	225	546
	Sd	13	40	193	33	31	135	105	71	25	41

Mean : Average number of revertants per plate

Sd : Standard deviation

S9 : Liver homogenate from rats treated with aroclor

Pos.C. : Positive control; for actual concentrations of reference mutagens see text

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Table 2: Bacterial reverse mutation test with Carbendazim (second assay).

Dose ug/pl		TA 1535		TA 1537		TA 98		TA 100		E-COLI	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0		20	17	18	24	32	51	123	117	29	33
		18	19	14	15	35	44	132	131	27	31
		22	17	14	11	38	45	142	143	22	31
	Mean	20	18	15	17	35	47	132	130	26	32
	Sd	2	1	2	7	3	4	10	13	4	1
313		26	15	13	25	20	60	155	134	38	25
		15	11	12	16	23	41	152	148	26	25
		17	9	18	20	20	29	128	122	22	33
	Mean	19	12	14	20	21	43	145	135	29	28
	Sd	6	3	3	5	2	16	15	13	8	5
625		24	15	16	21	26	38	152	143	28	18
		18	18	11	26	36	53	120	144	23	31
		18	7	15	29	27	34	154	143	31	41
	Mean	20	13	14	25	30	42	142	143	27	30
	Sd	3	6	3	4	6	10	19	1	4	12
1250		25	17	12	28	23	40	124	144	36	35
		27	12	7	18	29	57	128	120	20	30
		28	9	7	13	29	48	107	120	36	38
	Mean	27	13	9	20	27	48	120	128	31	34
	Sd	2	4	3	8	3	9	11	14	9	4
2500		23	20	14	19	15	64	144	134	34	30
		22	15	7	18	23	47	146	154	41	37
		17	15	12	20	31	61	156	149	42	37
	Mean	21	17	11	19	23	57	149	146	39	35
	Sd	3	3	4	1	8	9	6	10	4	4
5000		28	18	16	30	23	100	141	140	35	37
		25	23	11	29	33	83	106	138	35	35
		22	15	17	33	32	97	148	154	29	30
	Mean	25	19	15	31	29	93	132	144	33	34
	Sd	3	4	3	2	6	9	23	9	3	4
Pos.C.		485	396	765	313	1125	806	523	1197	190	840
		475	460	832	333	1269	889	539	1332	218	936
		504	423	875	336	1251	1097	529	1346	214	972
	Mean	488	426	824	327	1215	931	530	1292	207	916
	Sd	15	32	55	13	78	150	8	82	15	68

Mean : Average number of revertants per plate

Sd : Standard deviation

S9 : Liver homogenate from rats treated with aroclor

Pos.C. : Positive control; for actual concentrations of reference mutagens see text

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Conclusions**

It is concluded that the results obtained with the test substance Carbendazim in *Salmonella typhimurium* strains TA 1535, TA 1537, TA98 and TA100 and in the *Escherichia coli* strain WP2 *uvrA*, in both the absence and the presence of the S9-mix, indicate that Carbendazim was mutagenic under the conditions employed in this study. (Study author)

**Reliability**

Klimisch Code 1 (Reviewer)

GLP, OECD Guideline 471, EEC protocol of Council Directive 2000/32/EG, B.13/14(19 May 2000)

The study was carried out in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), Paris, ENV/MC/CHEM (98)17. (Study Author)

**References**

TNO, The Netherlands, "Bacterial reverse mutation test with Carbendazim," TNO Study No. 3405/18, February 28, 2002.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**16.0 REPEATED DOSE TOXICITY**

**Test Substance**

Identity (purity): Carbendazim, (99% pure)

**Method:**

Test type:	Combined chronic toxicity and carcinogenicity study
GLP:	Not known, assume yes
Year ( <i>study performed</i> ):	1976
Species:	Rat
Strain:	Wistar
Route of administration:	Oral, feed
Duration of test:	2 year
Doses/concentration levels:	0, 150, 300, 2000 mg/kg diet. 2000 mg/kg diet concentration was increased to 5000 mg/kg after one week, then to 10,000 mg/kg after two weeks for the remainder of the study
Sex:	60 Male/ 60 female per group
Exposure period:	2 years
Frequency of treatment:	Daily
Control group and treatment:	20 Male/ 20 Female
Post exposure observation period:	Daily observation

**Test Conditions**

In a 2-year rat study, groups of Wistar rats (60 males and 60 females/group) were administered carbendazim (99% pure) in the diet at levels of 0, 150, 300, and 2000 mg/kg diet for two years. The dose of 2000 mg/kg was increased to 5000 mg/kg after one week and then to 10,000 mg/kg after two weeks for the remainder of the study. Animals were examined daily for clinical signs of toxicity. Body weight and food consumption were measured regularly throughout the study. Hematological (peripheral blood), blood chemistry (orbital sinus) and urinalysis evaluations were conducted periodically during the study. All animals were subjected to complete gross necropsy and selected organs were weighed. Tissues were examined microscopically in 20 males and 20 female rats in the control and high dose groups. All tumors and gross abnormalities were also examined histologically.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

Test Subjects:

Age at study initiation: Not reported  
No. of animals per sex per dose: 60 Males/ 60 females

Study Design:

Clinical observations performed:

Body weight, food consumption (regularly)  
Hematological (peripheral blood, cold chemistry (orbital sinus),  
urinalysis (periodically)  
Gross necropsy: (termination)  
Organ weight and examination macroscopic and microscopic  
(termination)

**Results**

NOAEL (NOEL): Not reported  
LOAEL (LOEL): Not reported  
Statistical results (as appropriate): Not reported

There were no differences between test groups and control animals concerning clinical signs of toxicity or food consumption. Body weights were significantly reduced in low dose males from week 88 to term and in high-dose females from week 12 to term. Urinalyses were comparable among all groups. Of the hematological parameters examined, hemoglobin was depressed in high-dose females at weeks 26, 52, and 103 and hematocrit was depressed in high dose females at week 103.

There were no compound related effects in males. Serum glutamic oxaloacetic transaminase (SGOT) activity was decreased in high dose males at term, but not in females. High dose females had increased serum glutamic pyruvic transaminase (SGPT) activity and decreased total serum protein at study termination. There were no compound-related effects on organ weights except for increased relative liver weights in high dose females. There were also no compound related effects on mortality, (50% at week 76 in control males and at week 92 in treated males). There was 50% mortality in control and low dose females at week 88 and in mid and high dose females at 92-96 weeks. Survival at termination of the study was similar in all groups. There were no histological differences between control and treated groups except for an increased incidence of diffuse proliferation of parafollicular cells of the thyroid in the high dose females. (WHO reviewer of Til et al, 1976a)

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Reliability**

Klimisch Code = 2. The study is reliable with restrictions.

Unpublished study by Til et al, 1976(a) not available for review. However, the World Health Organization summarized the study in its Environmental Health Criteria Document for Carbendazim (1993) without noting concern. (Reviewer Comment)

**References**

WHO "Environmental Health Criteria 149 Carbendazim" 1993, Summary of unpublished study by Til H.P., Koellen C. and van der Heijden C.A.(1976a), "Combined chronic toxicity and carcinogenicity study with carbendazim in rats." The Hague, Central Institute for Nutrition and Food Research (TNO) (prepared for BASF AG, Ludwigshafen and Hoechst AG, Frankfurt).

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**17.0 REPRODUCTIVE TOXICITY**

**Test Substance**

Identity (purity): Carbendazim

**Method**

Type:	Three Generation Reproduction Study
GLP:	Not known, assume Yes
Year ( <i>study performed</i> ):	1972
Species:	Rat
Strain:	Chr-CD
Route of administration:	Oral, diet
Doses/concentration levels:	0, 100, 500, 5,000 and 10,000 mg/kg (diet)
Sex:	3 male/ 16 female per group Except high dose 3 male/20 female
Control group and treatment:	3 male/ 16 female
Frequency of treatment:	Daily (dietary exposure)
Duration of test:	3 generation
Statistical methods:	Not reported

**Test Conditions**

Groups of Chr-CD rats (3 male and 16 female rats per group, except that the high dose group contained 20 females) were fed carbendazim in the diet at dose levels of 0, 100, 500, 5,000 and 10,000 mg/kg and subjected to a standard two litter per generation, three-generation reproduction study. The parental animals were fed the experimental diet at 21 days of age and mated to produce the F1 litter at 100 days of age. The number of matings, pregnancies and number of young in each litter at birth was recorded. The litters were culled to 10 pups/litter on day 4. The number of the live pups was recorded on days 4, 12 and 21 as was pup weight at weaning. The parents were mated again to produce the F1B litters. The F1B litters were maintained on the respective diets for 110 days and then mated to produce the F2A and F2B litters. The F3A and F3B litters were produced similarly. Gross and histopathological examinations of selected tissues and organs were performed on two males and two females in each of five F3B litters from the control, 5,000 and 10,000 mg/kg groups. Reproduction indices, including mating, fecundity, fertility, gestation, viability and lactation, were calculated and compared with control values.

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**Conclusions**

Carbendazim was without effects on fertility, gestation, viability and lactation. However, the average litter weights at weaning were reduced in all generations fed 5,000 and 10,000 mg/kg. Histopathological examination of F3B weanlings did not reveal any effects that were considered compound related. (Study author)

**Reliability**

Klimisch: Code =2. The study is reliable with restriction.

This is an unpublished study by Sherman (1972), not available for review. However, the World Health Organization summarized the study in its Environmental Health Criteria Document for Carbendazim (1993) without noting concern. (Reviewer Comment)

**References**

WHO "Environmental Health Criteria 149 Carbendazim," 1993 Summary of unpublished study by Sherman H. (1972) "Long Term feeding studies in rats and dogs with 2-benzimidazole-carbamic acid, methyl ester(INE-965) (50% and 70% MBC wettable powder formulations) Parts I and II." Newark, Delaware, EI Dupont de Nemour and Co., Inc. Haskell Laboratory (Unpublished report No. HLR 195-72).

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

**18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY**

**Test Substance**

Identity (purity): Carbendazim

**Method**

Method/guideline followed:	Not reported
GLP:	Not reported
Year ( <i>study performed</i> ):	1987
Species:	Rats
Strain:	CrI: CDBR
Route of administration:	Oral, gavage
Doses/concentration levels:	0, 5, 10,20 and 90 mg/kg per day
Sex:	Female (maternal dosing)
Exposure period:	Days 7 – 16 of gestation
Frequency of treatment:	Once per day
Control group of treatment:	Yes
Duration of test:	22 days
Statistical methods:	Not reported

**Test Conditions**

Carbendazim, in a 0.5% aqueous suspension of methyl cellulose was administered by gavage to CrI:CDBR rats (25/dose group) on days 7-16 of gestation at daily doses of 0,5,10, 20 or 90 mg/kg per day.

**Results**

NOAEL (NOEL) maternal toxicity:	20 mg/day
NOAEL (NOEL) developmental toxicity:	10 mg/day
Actual dose received by dose level by sex:	Female (0, 5, 10, 20, 90 mg/kg)
Statistical results, as appropriate:	Not reported

Maternal toxicity was seen only at the highest dose in the form of depressed weight gain during the dosing periods and prior to sacrifice on day 22. Mean values for liver weight and liver-to-body weight ratio were increased. Decreased pregnancy rate was observed at the highest dose. An increase in the incidence of early resorptions per dam, decreased litter size and the total resorption of three litters occurred at the highest doses, only the reduction in females per litter being significant. Significant reductions in mean fetal weight were observed at both 20 and 90 mg/kg per day. A significant increase in the incidence of fetal malformations was also seen at 90 mg/kg per day. The malformations consisted

**Robust Summaries, CAS No. 10605-21-7**  
**Carbamic acid, 1H-benzimidazol-2-yl-, methyl ester**

primarily of hydrocephaly, microphthalmia, anophthalmia, malformed scapulae and axial skeletal malformations (vertebral, rib and sternebral fusions, exencephaly, hemivertebrae and rib hyperplasia).

**Conclusion**

The no-observed-effects levels (NOEL) for the dam and fetus were 20 and 10 mg/per day respectively. (Study Author)

**Reliability**

Klimisch Code=2, Reliable with Restriction

This is an unpublished study by Alverez (1987) not available for review. However, the World Health Organization summarized the study in its Environmental Health Criteria Document for Carbendazim (1993) without noting concern. (Reviewer Comment)

**References**

WHO "Environmental Health Criteria 149 Carbendazim," 1993 Summary of unpublished study by Alverez L.(1987) "Teratogenicity study of INE 965 (carbendazim) in rats." Newark, Delaware, E.I. Du Pont de Nemours and Co., Inc. Haskell Laboratory (Unpublished report No. HLR 281-87).