

## CLH report

### Proposal for Harmonised Classification and Labelling

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

#### International Chemical Identification:

thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)

EC Number: 245-740-7

CAS Number: 23564-05-8

Index Number: 006-069-00-3

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Version number: 4

Date: 28 March 2018

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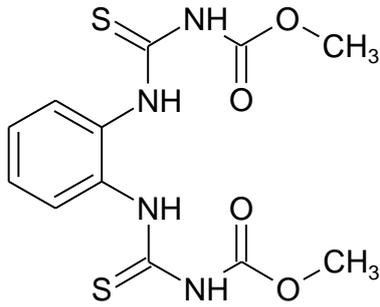
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## 1 IDENTITY OF THE SUBSTANCE

### 1.1 Name and other identifiers of the substance

**Table 1: Substance identity and information related to molecular and structural formula of the substance**

|  |   |
|--|---|
| <b>Name(s) in the IUPAC nomenclature or other international chemical name(s)</b>                             | IUPAC: dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate<br>CA: Carbamic acid, N,N'-[1,2-phenylenebis(iminocarbonothioyl)]bis-, C,C'-dimethyl ester |
| <b>Other names (usual name, trade name, abbreviation)</b>  | Topsin M  |
| <b>ISO common name (if available and appropriate)</b>  | Thiophanate-methyl  |
| <b>EC number (if available and appropriate)</b>  | 245-740-7   |
| <b>EC name (if available and appropriate)</b>  | Thiophanate-methyl  |
| <b>CAS number (if available)</b>   | 23564-05-8  |
| <b>Other identity code (if available)</b>  | 262 (CIPAC)   |
| <b>Molecular formula</b>   | C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> O <sub>4</sub> S <sub>2</sub>  |
| <b>Structural formula</b>  |   |
| <b>SMILES notation (if available)</b>  | <chem>S=C(Nc1ccccc1NC(=S)NC(=O)OC)NC(=O)OC</chem>   |
| <b>Molecular weight or molecular weight range</b>  | 342.40 g/mol  |
| <b>Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)</b> | Not applicable; thiophanate-methyl does not consist of stereoisomers  |
| <b>Description of the manufacturing process and identity of the source (for UVCB substances only)</b>        | Not relevant; thiophanate is not an UVCB substance  |
| <b>Degree of purity (%) (if relevant for the entry in Annex VI)</b>  | 95%   |

**1.2 Composition of the substance**

**Table 2: Constituents (non-confidential information)**

| Constituent<br>(Name and numerical<br>identifier) | Concentration range (%<br>w/w minimum and<br>maximum in multi-<br>constituent substances) | Current CLH in Annex VI<br>Table 3.1 (CLP)                                       | Current self- classification<br>and labelling (CLP)                             |
|---|---|--|---|
| Thiophanate-methyl                                | Min. 95%  | Acute Tox. 4*<br>Skin Sens. 1<br>Muta. 2<br>Aquatic Acute 1<br>Aquatic Chronic 1 | Acute Tox. 4<br>Skin Sens. 1<br>Muta. 2<br>Aquatic Acute 1<br>Aquatic Chronic 1 |

**Table 3: Impurities (non-confidential information) if relevant for the classification of the substance**

| Impurity<br>(Name and numerical identifier)            | Concentration range<br>(% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP)  | Current self-classification and labelling (CLP)   | The impurity contributes to the classification and labelling |
|--|--|--|---|--|
| 2,3-diaminophenazine (DAP)<br>CAS-No.: 655-86-7        | max. 0.5 ppm                                       | -  | 23 out of 30 notifiers has the following proposal:<br>Acute Tox. 4, H302<br>Skin Irrit. 2, H315<br>Eye Irrit. 2, H319<br>STOT SE 3., H335<br>(not provided)<br><br>GHS07<br>Wng<br><br>One further notifier proposes as above with the following addition:<br>Acute Tox. 4, H312<br>Acute Tox. 4, H332<br>STOT SE 3, H335<br>(Lungs)<br><br>One notifier has only<br>H302<br>GHS07<br>Wng | No   |
| 2-amino-3-hydroxyphenazine (HAP)<br>CAS-No.: 4569-77-1 | max. 0.5 ppm                                       | -  | -   | No   |
| Carbendazim<br>CAS-No.: 10605-21-7                     | max. 0.09%   | Muta. 1B, H340<br>Repr. 1B, H360FD<br>Aquatic Acute 1, H400<br>Aquatic Chronic 1, H410 | There are 25 aggregated notifications comprising approximately 500 notifiers. Most are proposal are identical to the current classifications. Some have not Aquatic Chronic 1, and one proposal has Carc. 2 (but not Muta. 1B and Repr. 1B) and STOT RE 2.  | No   |

*All other impurities are considered confidential (and are not relevant to the classification) and are only presented in the IUCLID-dossier section 1*

**Table 4: Additives (non-confidential information) if relevant for the classification of the substance**

| Additive (Name and numerical identifier) | Function | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) | The additive contributes to the classification and labelling |
|--|----------|---|---|---|--|
| Contains no additives                    |          |   |   |   |  |

**Table 5: Test substances (non-confidential information) (this table is optional)**

| Toxicology-studies |  |           |                 |         |
|--------------------|--|-----------|-----------------|---------|
| Study No.          | Study type   | Batch No. | Production date | Purity  |
| RD-9119            | A subchronic (3-month) oral toxicity study in the dog via capsule administration with Thiophanatemethyl  | TIF-1016  | 20.06.1989      | 96.55 % |
| RD-9207            | A chronic (1-year) oral toxicity study in the dog via capsule administration with Thiophanate-methyl   | TIF-1016  | 20.06.1989      | 96.55 % |
| RD-00828N          | Acute Neurotoxicity  | TIF-1016  | 20.06.1989      | 99.7 %  |
| RD-00634           | Subchronic Neurotoxicity   | TIF-1016  | 20.06.1989      | 99.7 %  |
| RD-73052           | Final report on the long-term oral toxicity studies of Thiophanate-methyl, Dimethyl 4,4'-o-phenylenebis (3-Thioallophanate) in beagle dogs for 24 months   | ni        | ni              | ni      |
| RD-73057           | Final report on the chronic oral toxicity studies of Thiophanate-methyl, Dimethyl 4,4'-o-phenylenebis (3-Thioallophanate) in rats of spraguedawley strain for 24 months  | ni        | ni              | ni      |
| RD-02805           | Phototoxicity  | TDE-021P  | 21.05.2004      | 98.52 % |
| RD-73062           | Mutagenic, cytogenetic and teratogenic studies on Dimethyl 4,4'OPhenylenebis (3-Thioallophanate), Thiophanate-methyl fungicide   | ni        | ni              | ni      |
| RD-9728            | Thiophanate-methyl technical: induction of micronuclei in cultured human peripheral blood lymphocytes  | TEE 2007  | 05.1995         | 98.23 % |
| RD-9729            | Thiophanate-methyl technical: study to determine the threshold of action for the induction of aneuploidy in cultured human peripheral blood lymphocytes  | TIF-1016  | 20.06.1989      | 96.39 % |
| RD-84109           | Gene mutation in chinese hamster V 79 cells with Thiophanate-methyl  | TM 767    | 26.02.1984      | 96.36%  |
| RD-9329            | Topsin-M - two generation oral (dietary administration) reproduction toxicity study in the rat (with one litter in the P and two litters in the F1 generation)   | TIF-01016 | ni              | 95.93 % |
| RD-9525            | Final addendum histopathology report and peer review pathology report to MRID 42899101. Topsin-M – two generation oral (dietary administration) reproduction toxicity study in the rat (with one litter in the | TIF-01016 | ni              | 95.93 % |

## CLH REPORT FOR THIOPHANATE-METHYL

| <b>Toxicology-studies</b> |  |                  |                          |               |
|---------------------------|--|------------------|--------------------------|---------------|
| <b>Study No.</b>          | <b>Study type</b>  | <b>Batch No.</b> | <b>Production date</b>   | <b>Purity</b> |
|                           | p and two litters in the F1 generation)  |                  |                          |               |
| RD-9120                   | Mutagenicity test on Topsin M technical in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells | 591              | ni                       | 95%           |
| RD-8195                   | Evaluation of pure Thiophanate-methyl in the primary rat hepatocyte unscheduled DNA synthesis assay  | CP 10B           | ni                       | ni            |
| RD-9160                   | 21-Day dermal study in rabbits with Thiophanate-methyl technical   | TIF-1016         | ni                       | ni            |
| RD-73058                  | The report on the carcinogenesis studies of Thiophanate-methyl, Dimethyl 4,4'-O-Phenylenebis (3-Thioallophanate) in mice of ICR-SLC strain for 24 months   | ni               | ni                       | ni            |
| RD-9083                   | Thiophanate-methyl - acute oral toxicity study in rats   | TIF-1016         | Ni Takaoka Plant         | 96.55%        |
| RD-9065                   | Thiophanate-methyl - reverse mutation study on bacteria  | TIF-1016         | Ni Takaoka Plant         | 96.55%        |
| RD-9084                   | Thiophanate-methyl - acute dermal toxicity study in rabbits  | TIF-1016         | Ni Takaoka Plant         | 96.55%        |
| RD-8711                   | Thiophanate-methyl - acute inhalation toxicity study in rats   | TP-544           | ni                       | 95.3%         |
| RD-8692                   | Thiophanate-methyl - primary dermal irritation study in rabbits  | TM-948           | ni                       | 96.2%         |
| RD-8691                   | Thiophanate-methyl - primary eye irritation study in rabbits   | TM-948           | ni                       | 96.2%         |
| RD-8924                   | Thiophanate-methyl - delayed contact hypersensitivity study in Guinea pigs   | TM-948           | ni                       | 96.2%         |
| RD-9059                   | Thiophanate-methyl - subchronic oral toxicity in rats  | TIF-1016         | Ni Takaoka Plant         | 96.55%        |
| RD-9347                   | Topsin M - skin sensitization study in Guinea-pigs   | TIF-1016         | Ni Takaoka Plant         | 96.55%        |
| RD-73055                  | Toxicological evaluation of Thiophanate-methyl (IV) - studies on the teratogenic effect of Thiophanatemethyl upon the fetus of ICR strain of mice          | ni               | ni                       | ni            |
| RD-73059                  | Toxicological evaluation of Thiophanate-methyl (V) – some pharmacological properties of a new fungicide, Thiophanate-methyl                                | ni               | ni                       | ni            |
| RD-73051                  | Toxicological evaluation of Thiophanate-methyl (I) - acute and subacute toxicity of Thiophanatemethyl  | ni               | ni                       | ni            |
| RD-73054                  | Toxicological evaluation of Thiophanate-methyl (III) - studies on the subchronic oral toxicity of Thiophanate-methyl in rats                               | ni               | ni                       | ni            |
| RD-73053                  | Toxicological evaluation of Thiophanate-methyl (II) - studies on the subchronic oral toxicity of Thiophanate-methyl in mice                                | ni               | ni                       | ni            |
| RD-73063                  | Effect of Thiophanate-methyl on reproductive function of multiple generations in the rat   | NF-44            | ni                       | ni            |
| RD-9957                   | Thiophanate-methyl – mouse micronucleus test   | TFB-2012         | 13.02.1996 Takaoka Plant | 97.28 %       |
| RD-8126                   | Teratology study of Thiophanatemethyl in rats  | TM-123           | ni                       | 97.2 %        |

## CLH REPORT FOR THIOPHANATE-METHYL

| <b>Toxicology-studies</b> |  |                  |                             |               |
|---------------------------|--|------------------|-----------------------------|---------------|
| <b>Study No.</b>          | <b>Study type</b>  | <b>Batch No.</b> | <b>Production date</b>      | <b>Purity</b> |
| RD-8125                   | Pilot teratology study of Thiophanatemethyl in rats  | TM-123           | ni                          | 97.2 %        |
| RD-9327                   | Thiophanate-methyl – combined chronic toxicity/ oncogenicity study in rats                                       | TIF-1016         | ni<br>Takaoka Plant         | 96.55 %       |
| RD-9247                   | Thiophanate-methyl - metabolism in rats. Supplemental report to Nisso EC-338 (MRID#42474802)                     | TIF-1016         | ni<br>Takaoka Plant         | 96.55 %       |
| RD-8642                   | Thiophanate-methyl - teratology study in the rabbit  | TIF-1016         | ni<br>Takaoka Plant         | 96.55 %       |
| RD-8642                   | Thiophanate-methyl - teratology study in the rabbit  | TM-948           | ni                          | 96.2 %        |
| RD-8641                   | Thiophanate-methyl - effects of oral administration upon pregnancy in the rabbit - 1. dosage range-finding study | TM-948           | ni                          | 96.2 %        |
| RD-9328                   | 18-month dietary oncogenicity study in mice with Topsin M  | TIF-1016         | ni<br>Takaoka Plant         | 96.55 %       |
| RD-9770                   | Oral (stomach tube) developmental toxicity study of Thiophanate-methyl in rabbits                                | TFB-2012         | 13.02.1996<br>Takaoka Plant | 97.28 %       |
| RD-9769                   | Oral (stomach tube and dietary) dosage-range developmental toxicity study of Thiophanate-methyl in rabbits       | TFB-2012         | 13.02.1996<br>Takaoka Plant | 97.28 %       |

| <b>Ecotoxicology studies</b> |  |                  |                        |               |
|------------------------------|--|------------------|------------------------|---------------|
| <b>Study No.</b>             | <b>Study type</b>  | <b>Batch No.</b> | <b>Production date</b> | <b>Purity</b> |
| RD-00041                     | Toxicity to arthropods   | ni               | ni                     | ni            |
| RD-II02074                   | The impact of Thiophanate-methyl on non-target flora and fauna   | ni               | ni                     | ni            |
| RD-00554                     | Addendum report: toxicity of Thiophanate-methyl to green algae: calculation of EbC50- and ErC50-values   | TM-502           | ni                     | 96.8%         |
| RD-73066                     | Acute oral LD50 - bobwhite quail - topsin M technical  | 77-126-3         | ni                     | 94 %          |
| RD-73067                     | Acute oral LD50 - mallard duck - topsin M technical  | 77-126-3         | ni                     | 94 %          |
| RD-9330                      | Thiophanate-methyl - acute toxicity to rainbow trout (oncorhynchus mykiss) under flow-through conditions | TIF-01016        | ni                     | 95.93 %       |
| RD-9651                      | Effect of Thiophanate-methyl on the mortality of the earthworm eisenia foetida                           | TEE 2007         | ni                     | 98.2 %        |
| RD-02475                     | Toxicity to activated sludge   | TEJ-096F         | 13.10.2005             | 98.08 %       |
| RD-73068                     | Eight-day dietary LC50 – bobwhite quail - technical topsin M   | 35-135-3         | ni                     | 94 %          |
| RD-73069                     | Eight-day dietary LC50 - mallard ducks - technical topsin M  | 35-135-3         | ni                     | 94 %          |
| RD-9009                      | The prolonged toxicity of Thiophanatemethyl to rainbow trout (Salmo gairdneri)                           | TP-569           | ni                     | 95.2%         |
| RD-9010                      | An assessment of the effects of Thiophanate-methyl on the reproduction of Daphnia magna                  | TP-569           | ni                     | 95.2%         |
| RD-08328                     | Acute fish toxicity  | TM-502           | ni                     | 96.8 %        |
| RD-8328                      | Acute toxicity study of Thiophanatemethyl in rainbow trout   | TM-502           | ni                     | 96.8 %        |
| RD-8693                      | Thiophanate-methyl: acute toxicity study in Daphnids   | TM-502           | ni                     | 96.8 %        |

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|             |  |           |                    |                   |
|-------------|--|-----------|--------------------|-------------------|
| RD-8304     | Toxicity study of Thiophanate-methyl in green algae  | TM-502    | ni                 | 96.8 %            |
| RD-00011    | Reproductive toxicity to birds   | TIF-01016 | ni                 | 95.93 %           |
| RD-00009    | Reproductive toxicity to birds   | TIF-01016 | ni                 | 95.93 %           |
| RD-99127    | Thiophanate-methyl - toxicity and reproduction study in bobwhite quail   | TIF-01016 | ni                 | 95.93 %           |
| RD-9229     | Thiophanate-methyl - acute toxicity to Daphnids ( <i>Daphnia magna</i> ) under flow-through conditions (FIFRA Guideline no.: 72-2) | TIF-01016 | ni                 | 95.93 %           |
| RD-84103    | The acute toxicity of Thiophanatemethyl to the earthworm <i>eisenia foetida</i>  | TM-803    | ni                 | 94.9 %            |
| RD-00627    | CM-0237 Acute Toxicity (LD50) to the Earthworm   | 31-02122  | ni                 | 98.6 %            |
| RD-II 02438 | Thiophanate-methyl: Inhibition of growth to the alga <i>Pseudokirchneriella subcapitata</i>  | TBC-035G  | 10.2000 and 3.2002 | 99.7 % and 98.2 % |
| RD-02368    | Toxicity to soil microflora  | TDE-021P  | 21.5.2004          | 98.52 %           |
| RD-99127    | Reproductive toxicity to birds   | TIF-01016 | ni                 | 95.93 %           |
| RD-03266    | Chronic toxicity to bees   | TBC-035G  | 2003.2002          | 97.9 %            |
| RD-02818    | Fish early stage toxicity  | TBC-035G  | 20.03.2002         | 97.9 %            |
| RD-8652     | Thiophanate-methyl - honey bee acute contact LD50  | TM-948    | ni                 | 96.2%             |
|             | Toxicity to arthropods   | ni        | ni                 | ni                |

## 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

### 2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

|   | Index No     | International Chemical Identification   | EC No     | CAS No     | Classification   |  | Labelling                                    |  |                                 | Specific Conc. Limits, M-factors | Notes |
|---|--------------|---|-----------|------------|--|--|--|--|---------------------------------|----------------------------------|-------|
|   |              |   |           |            | Hazard Class and Category Code(s)  | Hazard statement Code(s)                                       | Pictogram, Signal Word Code(s)               | Hazard statement Code(s)                               | Suppl. Hazard statement Code(s) |                                  |       |
| Current Annex VI entry                            | 006-069-00-3 | thiophanate-methyl (ISO)<br>1,2-di-(3-methoxycarbonyl-2-thioureido)benzene  | 245-740-7 | 23564-05-8 | Acute Tox. 4*<br>Skin Sens. 1<br>Muta. 2<br>Aquatic Acute 1<br>Aquatic Chronic 1                         | H332<br>H317<br>H341<br>H400<br>H410                           | GHS07<br>GHS08<br>GHS09<br>Wng               | H332<br>H317<br>H341<br>H410                           |                                 |                                  |       |
| Dossier submitters proposal                       | 006-069-00-3 | thiophanate-methyl (ISO); dimethyl(1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate) | 245-740-7 | 23564-05-8 | Acute Tox. 4<br>Skin Sens. 1<br>Modify Muta. 1B<br>Add STOT RE 2<br>Aquatic Acute 1<br>Aquatic Chronic 1 | Retain H332<br>H317<br>Modify H340<br>Add H373<br>H400<br>H410 | Retain GHS07<br>GHS08<br>GHS09<br>Modify Dgr | Retain H332<br>H317<br>Modify H340<br>Add H373<br>H410 | M factor 10<br>M factor 10      |                                  |       |
| Resulting Annex VI entry if agreed by RAC and COM | 006-069-00-3 |   |           |            |  |  |  |  |                                 |                                  |       |

**Table 7: Reason for not proposing harmonised classification and status under public consultation**

| <b>Hazard class</b>  | <b>Reason for no classification</b>  | <b>Within the scope of public consultation</b> |
|--|--|--|
| <b>Explosives</b>  | Data is conclusive but not sufficient for classification.                          | Yes  |
| <b>Flammable gases (including chemically unstable gases)</b>       | Hazard class not applicable  | No   |
| <b>Oxidising gases</b>   | Hazard class not applicable  | No   |
| <b>Gases under pressure</b>  | Hazard class not applicable  | No   |
| <b>Flammable liquids</b>   | Hazard class not applicable  | No   |
| <b>Flammable solids</b>  | Data is conclusive but not sufficient for classification.                          | Yes  |
| <b>Self-reactive substances</b>                                    | Data lacking   | No   |
| <b>Pyrophoric liquids</b>  | Hazard class not applicable  | No   |
| <b>Pyrophoric solids</b>   | Data (experience in handling) is conclusive but not sufficient for classification. | Yes  |
| <b>Self-heating substances</b>                                     | Data lacking   | No   |
| <b>Substances which in contact with water emit flammable gases</b> | Data (experience in handling) is conclusive but not sufficient for classification. | Yes  |
| <b>Oxidising liquids</b>   | Hazard class not applicable  | No   |
| <b>Oxidising solids</b>  | Data is conclusive but not sufficient for classification.                          | Yes  |
| <b>Organic peroxides</b>   | Hazard class not applicable  | No   |
| <b>Corrosive to metals</b>   | Data lacking   | No   |
| <b>Acute toxicity via oral route</b>                               | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Acute toxicity via dermal route</b>                             | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Acute toxicity via inhalation route</b>                         | Harmonised classification proposed   | Yes  |
| <b>Skin corrosion/irritation</b>                                   | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Serious eye damage/eye irritation</b>                           | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Respiratory sensitisation</b>                                   | Data lacking   | No   |
| <b>Skin sensitisation</b>  | Harmonised classification proposed   | Yes  |
| <b>Germ cell mutagenicity</b>                                      | Harmonised classification proposed   | Yes  |
| <b>Carcinogenicity</b>   | Harmonised classification proposed   | Yes  |
| <b>Reproductive toxicity</b>                                       | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Specific target organ toxicity-single exposure</b>              | Data conclusive but not sufficient for classification                              | Yes  |
| <b>Specific target organ toxicity-repeated exposure</b>            | Harmonised classification proposed   | Yes  |
| <b>Aspiration hazard</b>   | Data lacking   | No   |
| <b>Hazardous to the aquatic environment</b>                        | Harmonised classification proposed   | Yes  |
| <b>Hazardous to the ozone layer</b>                                | Hazard class not assessed in this dossier  | No   |

### 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Thiophanate-methyl has a harmonised classification in Acute Tox. 4\*, Skin sens. 1, Muta. 2, Aquatic Acute 1 and Aquatic Chronic 1. This has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC where it was classified as Xn; R20, R43, Muta. Cat 3; R68, R50-53 by the ECB as presented in Commission Directive 2001/60/EC.

The mutagenicity classification was discussed among the Specialised Experts in Arona on 1-2 September 1999. Thiophanate-methyl was considered together with the related compounds carbendazim and benomyl. Carbendazim and benomyl were classified in Cat 2 under DSD while for thiophanate-methyl *“the majority of the Specialised Experts stressed that the tabled information on toxicokinetic behaviour of thiophanate-methyl was supportive of a Category 3 classification. It was noted that the available documentation contained insufficient substantiation of the arguments in support of Category 2 raised by some Experts. In particular the rate of metabolism and excretion in the German monograph were not detailed enough to be taken into further consideration. ... On account of the suspicion that the available data set was incomplete, the majority of the Specialised Experts recommended a preliminary classification with Muta Cat 3; R40 based on the currently available evidence and to make an effort to obtain additional data related to toxicokinetic behaviour and mutagenicity. The substance could be revisited if these attempts were successful.”*

### 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Thiophanate-methyl is an active substance in the meaning of Regulation EC 1107/2009 and justification is not required (Article 36 CLP Regulation).

### 5 IDENTIFIED USES

Thiophanate-methyl is an active substance used in plant protection products. The main uses in EU Member States are intended for agriculture, horticulture and viticulture. The representative formulation Topsin M 500 SC is used as a fungicide on wine grapes, tomato, aubergine, leek, bean and wheat for the renewal of approval.

### 6 DATA SOURCES

Thiophanate-methyl was included in Annex I of Directive 91/414/EC by Commission Directive 2005/53/EC of 16 September 2005. Entry into Force of Annex I listing was 1st of March 2006. Annex I listing was extended to 31 October 2017 according to an amendment to Commission regulation No 540/2011 of 14 September 2012 (SANCO/10795/2013). Thiophanate-methyl is currently being evaluated under the following regulations for renewal of approval as an active substance in plant protection products:

- REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market
- COMMISSION IMPLEMENTING REGULATION (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market
- COMMISSION IMPLEMENTING REGULATION (EU) No 533/2013 of 10 June 2013 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of the active substances 1-methyl-cyclopropene, chlorothalonil, chlorotoluron, cypermethrin, daminozide, forchlorfenuron, indoxacarb, thiophanate-methyl and tribenuron-methyl

The data presented in this dossier has been submitted by the applicant as part of the renewal process. Some of the data was submitted and evaluated during the first approval while other data was submitted for the first time for the purpose of renewal of approval. All data is presented in the Renewal Assessment Report (RAR) prepared by Rapporteur Member State (RMS) Sweden which has been submitted to EFSA.

The summaries of toxicity data provided in this dossier have largely been taken from Volume 1 of the RAR. Annex I to this report consists of the more extensive study summaries presented in Volume 3 of the RAR.

## 7 PHYSICOCHEMICAL PROPERTIES

**Table 8: Summary of physicochemical properties**

| Property                                     | Value   | Reference   | Comment (e.g. measured or estimated)        |
|--|---|---|---|
| <b>Physical state at 20°C and 101,3 kPa</b>  | Solid (97%)   | Nakayama, K., (1990), Report No. RD-09024 (111-001)   |   |
| <b>Melting/freezing point</b>                | Decomposition before melting (165°C) (99.9%)  | Nakayama, K., (1992), Report No. RD-09216 (112-001)   |   |
| <b>Boiling point</b>                         | Not relevant (see melting point)  |   |   |
| <b>Relative density</b>                      | No data   |   |   |
| <b>Vapour pressure</b>                       | $> 9 \times 10^{-6}$ Pa at 20°C (99.9%)   | Higashida, S., Kobayashi, H. (1999), Report No. RD-IIM001 (115-005)   | Direct measurement at specified temperature |
| <b>Surface tension</b>                       | 72.2 mN/m at 20°C (86% of saturation concentration in water; 97.3%)   | Tanaka, T. (2001), Report No. RD-II01069 (116-001)  |   |
| <b>Water solubility</b>                      | 22.4 mg/L at 20°C (pH 4, phthalate buffer) (98.23%)<br>21.1 mg/L at 20°C (pH 5, phthalate buffer) (98.23%)<br>20.7 mg/L at 20°C (pH 6, phosphate buffer) (98.23%)<br>18.5 mg/L at 20°C (pH 7, phosphate buffer) (98.23%)<br>16.8 mg/L at 20°C (pH 7.5, phosphate buffer) (98.23%)<br>unstable at pH > 8 at 20°C (98.23%)<br>24.6 mg/L at 25°C (pH 6.3 distilled, pH 6.3 saturated) (>99%)<br>21.8 mg/L at 25°C (pH 5.1 distilled, pH 5.2 saturated) (99%) | Gomyo, T. (1996), Report No. RD-09629 (114-002)<br>Nakayama, K. (1992), Report No. RD-09219 (114-003)<br>Nomura, O., Nakashima, N. (1987), Report No. RD-08775 (114-004)<br>Soeda, Y., Shiotani, H. (1986), Report No. RD-08659 (114-007) |   |
| <b>Partition coefficient n-octanol/water</b> | log $P_{ow}$ = 1.40 at 25°C distilled water, pH was not reported) (99.88%)<br>log $P_{ow}$ = 1.53 (citrate-phosphate buffer, pH was not reported)<br>log $P_{ow}$ = 1.41 at pH 4 at 25°C (phthalate buffer) (98.23%)<br>log $P_{ow}$ = 1.45 at pH 5 (phthalate buffer) (98.23%)<br>log $P_{ow}$ = 1.47 at pH 6 (phthalate buffer) (98.23%)  | Shiotani, H. (1992), Report No. RD-09212 (114-005)<br>Soeda, Y., Shiotani, H. (1986), Report No. RD-08660 (114-006)<br>Gomyo, T. (1996), Report No. RD-09630 (114-008)  |   |

| Property   | Value   | Reference   | Comment (e.g. measured or estimated) |
|--|---|---|--------------------------------------|
| <b>Flash point</b>   | Not applicable. The active substance is a solid; its melting point is > 40 °C.  |   |                                      |
| <b>Flammability</b>  | Not highly flammable (98.2%)<br>In the preliminary test (EEC A.10) the flames extinguished immediately.                                       | Krips, H.J. (1996),<br>Report No. RD-09657 (142-001)  |                                      |
| <b>Explosive properties</b>  | Not explosive in the sense of the test method (EEC A.14). (97.9% technical)   | Pointer C. (2014),<br>Report No. RD-02837 (141-002)   |                                      |
| <b>Self-ignition temperature</b>   | No self-ignition (EEC A.16) of the test substance was observed (98.2%). The test substance had melted and changed into a black residue.       | Krips, H.J. (1996),<br>Report No. RD-09658 (142-002)  |                                      |
| <b>Oxidising properties</b>  | The technical material is not oxidizing in the sense of the test method (97.9%). No test substance/cellulose burned to completion (EEC A.17). | Pointer C. (2014),<br>Report No. RD-02837 (141-002)   |                                      |
| <b>Granulometry</b>  | No data   |   |                                      |
| <b>Stability in organic solvents and identity of relevant degradation products</b> | No data   |   |                                      |
| <b>Dissociation constant</b>   | pKa = 7.28 at 25°C (>99%)   | Ishihara, K. (1990),<br>Report No. RD-09016 (115-003) |                                      |
| <b>Viscosity</b>   | Not relevant since the substance is a solid with a melting point >> 40 °C.  |   |                                      |

## 8 EVALUATION OF PHYSICAL HAZARDS

### 8.1 Explosives

**Table 9: Summary table of studies on explosive properties**

| Method   | Results       | Remarks | Reference   |
|----------|---------------|---------|---|
| EEC A.14 | Not explosive |         | Pointer C. (2014),<br>Report No. RD-02837 (141-002) |

#### 8.1.1 Short summary and overall relevance of the information provided on explosive properties

*One negative study performed in accordance with EEC A.14 was provided. It could also be added that the substance does not contain any functional groups known to confer explosive properties.*

### 8.1.2 Comparison with the CLP criteria

*It is not evident from the CLP-guidance that a negative result from the EEC A.14 automatically means that it should not be classified as an explosive under CLP. Nevertheless, based on the structure, it seems that the waiving criteria for non-testing applies and a classification is thus not warranted.*

### 8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed. Data is conclusive but not sufficient for classification.

## 8.2 Flammable gases (including chemically unstable gases)

**Hazard class not applicable (thiophanate-methyl is not a gas)**

## 8.3 Oxidising gases

Hazard class not applicable (thiophanate-methyl is not a gas)

## 8.4 Gases under pressure

Hazard class not applicable (thiophanate-methyl is not a gas)

## 8.5 Flammable liquids

Hazard class not applicable (thiophanate-methyl is not a liquid)

## 8.6 Flammable solids

**Table 10: Summary table of studies on flammable solids**

| Method   | Results   | Remarks | Reference   |
|----------|---|---------|---|
| EEC A.10 | Not highly flammable. In the preliminary test the flames extinguished immediately |         | Krips, H.J. (1996), Report No. RD-09657 (142-001) |

### 8.6.1 Short summary and overall relevance of the provided information on flammable solids

One test performed in accordance with EEC A.10 was provided. The substance did not ignite in the preliminary screening test and is thus not regarded a highly flammable in the sense of the test method.

### 8.6.2 Comparison with the CLP criteria

The preliminary screening test in EEC A.10 and in CLP are identical. The substance should thus not be classified as a flammable substance under CLP.

### 8.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

## 8.7 Self-reactive substances

Data lacking

### 8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data has been provided addressing this property.

### 8.7.2 Comparison with the CLP criteria

No data has been provided that addresses this property. However, the structure of thiophanate-methyl does not contain any functional groups known to confer self-reactive properties (compared with Tables A6.1 and A6.2 in Appendix 6 to UN-MTC) . The waiving criteria in CLP therefore applies and no classification for self-reactive properties is warranted.

### 8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed due to lack of data.

## 8.8 Pyrophoric liquids

Hazard class not applicable (thiophanate-methyl is not a liquid).

## 8.9 Pyrophoric solids

Data lacking

### 8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No specific data derived in accordance with the recommended test method in CLP has been provided. However, thiophanate-methyl has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections)

### 8.9.2 Comparison with the CLP criteria

Based on experience in handling of thiophanate-methyl, it is not a pyrophoric solid (compare with example in CLP guidance section 2.10.7.2).

### 8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

## 8.10 Self-heating substances

**Table 11: Summary table of studies on self-heating substances**

| Method   | Results  | Remarks | Reference   |
|----------|--|---------|---|
| EEC A.16 | No self-ignition of the test substance was observed. The test substance had melted and changed into a black residue. |         | Krips, H.J. (1996), Report No. RD-09658 (142-002) |

### 8.10.1 Short summary and overall relevance of the provided information on self-heating substances

One negative study conducted in accordance with EEC A.16 was provided.

### 8.10.2 Comparison with the CLP criteria

Since no study has been provided in accordance with the recommended test method in CLP a full assessment cannot be made.

### 8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed due to lack of data.

## 8.11 Substances which in contact with water emit flammable gases

Data lacking

### 8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No specific data derived in accordance with the recommended test method in CLP has been provided. However, thiophanate-methyl has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

### 8.11.2 Comparison with the CLP criteria

Based on experience in handling of thiophanate-methyl, it is not a substance which in contact with water emit flammable gases (compare with CLP guidance section 2.12.3.2).

### 8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

## 8.12 Oxidising liquids

Hazard class not applicable (thiophanate-methyl is not a liquid).

## 8.13 Oxidising solids

**Table 12: Summary table of studies on oxidising solids**

| Method   | Results  | Remarks | Reference  |
|----------|--|---------|--|
| EEC A.17 | The technical material is not oxidizing in the sense of the test method.<br>No test substance/cellulose burned to completion |         | Pointer C. (2014), Report No. RD-02837 (141-002) |

### 8.13.1 Short summary and overall relevance of the provided information on oxidising solids

One test performed in accordance with EEC A.17 was provided. None of the test material:cellulose mixtures (2:1, 1:1, 1:2) burned to completion. The reference mixture (barium nitrate:cellulose, 3:2) burned vigorously to completion in 29 seconds.

### 8.13.2 Comparison with the CLP criteria

The test under EEC A.17 do not utilize the same reference standard as in the test recommended under CLP (potassium bromate) and the ratio of test substance:cellulose mixture to be tested according to the decision

logic in CLP is 1:1 and 4:1 whereas the available test only tested the 1:1 mixture (and 2:1, and 1:2). Nevertheless, since thiophanate-methyl only contains oxygen which is bonded to carbon or hydrogen the waiving criteria under CLP applies and the substance should not be classified for oxidising properties.

### 8.13.3 Conclusion on classification and labelling for oxidising solids

### 8.14 No classification is proposed. Data is conclusive but not sufficient for classification. Organic peroxides

Hazard class not applicable (thiophanate-methyl is not a organic peroxide).

### 8.15 Corrosive to metals

No data has been provided addressing this property.

#### 8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data has been provided addressing this property. Thiophanate-methyl does contain acidic or basic functional groups (pka=7.3) and should thus be considered for classification in this class according to the CLP-guidance. However, the substance is a high melting substance which also means that it is currently difficult to test with the available test method.

#### 8.15.2 Comparison with the CLP criteria

No data has been provided and a thorough assessment can thus not be made.

#### 8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to lack of data.

## 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

| Test substance<br>Test type<br>Method<br>Guideline                                      | Route of exposure | Species<br>Strain<br>Sex<br>No./group<br>Vehicle  | Exposure Period  | Doses   | Main findings   | Reference              |
|---|-------------------|---|--|---|---|------------------------|
| <sup>14</sup> C-thiophanate-methyl<br><br>Thiophanate-methyl<br><br>EPA 85-1<br><br>GLP | Oral (gavage)     | Rat<br><br>Fisher 344<br><br>M, F<br><br>Group B, C, D, D2:<br>5/sex/group<br><br>Group B2: 3 | Group B, B2 <sup>1</sup> , D and D2 <sup>2</sup> :<br>one single dose<br><sup>14</sup> C-thiophanate-methyl.<br><br>Group C: one single dose<br>thiophanate- | Low dose:<br>Group B: 13 mg/kg bw.<br>Group B2: 13.3 mg/kg bw.<br>Group C: 14 mg/kg bw (thiophanate-methyl), 10 mg/kg bw ( <sup>14</sup> C-thiophanate- | Low dose (groups B, B2):<br>C <sub>max</sub> : 1.7-4.2 µg eq/g<br>T <sub>max</sub> : 1-3 hours post dosing.<br>T <sub>1/2</sub> (initial): 1.6-2.8 h<br>Excretion: ~99.5% within 96 hours.<br>Radioactivity mainly excreted with urine (70%) and faeces (30%)<br>Main residues: 5-OH-carbendazim-S (urine), 4-OH- | RAR Vol 3<br>B.6.1.1.1 |

<sup>1</sup> Group B2 was established since the metabolic pattern of group B slightly differed from that of group C.

<sup>2</sup> Group D2 was established since Group D due to technical difficulties was given only 140 mg/kg bw instead of the intended 170 mg/kg bw.

CLH REPORT FOR THIOPHANATE-METHYL

|   |               |                                  |   |   |  |                        |
|---|---------------|----------------------------------|---|---|--|------------------------|
| Acceptable  |               | M.                               | methyl repeated for 14 days, then one single dose <sup>14</sup> C-thiophanate-methyl. | methyl)<br><u>High dose:</u><br>Group D: 140 mg/kg bw.<br>Group D2: 171 mg/kg bw. | thiophanate-methyl (feces).<br>Distribution: Highest residue levels in liver, thyroid and kidney.<br><br><u>Pre-administration low dose (group C):</u><br>C <sub>max</sub> : 1.7-4.2 µg eq/g<br>T <sub>max</sub> : 1-3 hours post dosing.<br>T <sub>1/2(initial)</sub> : 1.6-2.8 h<br>Excretion: ~99.5% within 96 hours.<br>Radioactivity mainly excreted with urine (70%) and faeces (30%)<br>Main residues: 5-OH-carbendazim-S (urine), thiophanate-methyl (faeces).<br>Distribution: Highest residue levels in liver, thyroid and kidney.<br><br><u>High dose (groups D, D2):</u><br>C <sub>max</sub> : 14-27 µg eq/g<br>T <sub>max</sub> : 2-7 hours post dosing<br>T <sub>1/2(initial)</sub> : 2.4-7.8 h<br>Excretion: ~99.5% within 96 hours.<br>Radioactivity mainly excreted with faeces (70%) and urine (30%).<br>Main residues: 5-OH-carbendazim-S (urine), thiophanate-methyl (faeces).<br>Distribution (96 h post dosing): Highest residue levels in liver, thyroid and kidney.<br>No sex differences. |                        |
| <sup>14</sup> C-thiophanate-methyl<br>OECD 417<br>GLP<br>Acceptable | Oral (gavage) | Rat Fisher 344/NCrHsd M, F 9/sex | One single dose   | 14 mg/kg bw   | Blood, males(females):<br>C <sub>max</sub> : 4.7(4.8) µg eq/g<br>T <sub>max</sub> : 2 h<br>T <sub>1/2(initial)</sub> : 3.4(4.6) h<br>T <sub>1/2(terminal)</sub> : 12.4(10.5) h<br>AUC <sub>inf</sub> : 43(47) µg h/g<br><br>Plasma:<br>C <sub>max</sub> : 5.9(5.4) µg eq/g<br>T <sub>max</sub> : 2 h<br>T <sub>1/2(initial)</sub> : 3.5(5.1) h<br>T <sub>1/2(terminal)</sub> : 8.7(8.9) h<br>AUC <sub>inf</sub> : 52(56) µg h/g<br><br>Blood/plasma ratio: ~0.7 up to 24 h, thereafter ~1 at 48 h<br><br>No sex differences.   | RAR Vol 3<br>B.6.1.1.1 |
| <sup>14</sup> C-thiophanate-methyl<br>OECD 417<br>GLP<br>Acceptable | Oral (gavage) | Rat Fisher 344/NCrHsd M, F 6/sex | One single dose   | 14 mg/kg bw   | Oral bioavailability: 89%<br>Excretion: 99.6% complete after 48 h. Excretion was driven by urine (47%) and bile (40%). 7% was recovered from faeces.<br>No sex differences.  | RAR Vol 3<br>B.6.1.1.1 |
| <sup>14</sup> C-thiophanate-  | Oral          | Rat Wistar                       | One single dose   | 24.2 mg/rat (~65 mg/kg bw)  | 60% of the administered radioactivity was excreted via   | RAR Vol 3<br>B.6.1.1.1 |

## CLH REPORT FOR THIOPHANATE-METHYL

|  |                   |                          |   |   |  |                        |
|--|-------------------|--------------------------|---|---|--|------------------------|
| methyl<br>Supportive   |                   | M                        |   |   | faeces. 30% was found in urine.  |                        |
| <sup>14</sup> C-thiophanate-methyl<br>Supportive   | Oral (via diet)   | Rat<br>Wistar<br>F<br>15 | Repeated dose<br>20 consecutive<br>days | 42 ppm (~2.25<br>mg/kg bw)  | Excretion: Each day ~90% of<br>the ingested radioactivity was<br>excreted in urine (54%) and<br>faeces (35%).<br>Distribution: Highest residue<br>levels in GI-tract, thyroid,<br>adrenals and liver.  | RAR Vol 3<br>B.6.1.2.1 |
| <sup>14</sup> C-thiophanate-methyl<br>Thiophanate-methyl<br>EPA 85-1<br>GLP<br>Acceptable  | Oral<br>(gavage)  | Mice<br>CD 1<br>M<br>5   | One single<br>high dose                 | 171 mg/kg bw  | Excretion: 99.9 within 48<br>hours.<br><br>Radioactivity was excreted<br>mainly with faeces (73%) and<br>urine (26%).<br><br>Main residues: 5-OH-<br>carbendazim-S (urine),<br>thiophanate-methyl (faeces).<br><br>No significant differences in the<br>relative excretion to faeces and<br>urine when compared to rats.                   | RAR Vol 3<br>B.6.1.1.2 |
| I: [ <sup>14</sup> C-thio-<br>ureido]-<br>thiophanate-<br>methyl<br><br>II: [ <sup>35</sup> S-thio-<br>ureido]-<br>thiophanate-<br>methyl<br><br>III: [ <sup>14</sup> C-<br>methyl]-<br>thiophanate-<br>methyl<br><br>IV: [ <sup>14</sup> C-<br>phenyl]-<br>thiophanate-<br>methyl<br><br>Supportive | Oral<br>(gavage)  | Mice<br>dd-Y<br>M        | One single<br>dose                      | I: 2 mg/mouse<br>(~100 mg/kg<br>bw)<br>II: 1 mg/mouse<br>(~50 mg/kg bw)<br>III: 3 mg/mouse<br>(~150 mg/kg<br>bw)<br>IV: 1.1<br>mg/mouse (~55<br>mg/kg bw) | T <sub>max</sub> : 2-3 h<br><br>Excretion: Majority of<br>radioactivity within 24 hours.<br><br>Radioactivity was excreted<br>mainly with urine (70-89%,<br>depending on labelling) and<br>faeces (18-29%, depending on<br>labelling).<br><br>Distribution (3 h post dosing):<br>Highest residue levels in GI-<br>tract, liver and kidney. | RAR Vol 3<br>B.6.1.1.2 |
| <sup>14</sup> C-thiophanate-methyl<br>Supportive   | Oral<br>(capsule) | Dog<br>Beagle            | One single<br>dose                      | 9.85 mg/dog<br>(~1 mg/kg bw)  | Radioactivity was excreted<br>mainly with urine (74%) and<br>faeces (14%).   | RAR Vol 3<br>B.6.1.1.3 |

### 9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The toxicokinetics of thiophanate-methyl was studied following oral exposure via gavage or feed in rats, mice and dogs following low and/or high dose administration. The data from the acute- and repeated dose toxicity studies indicated that toxicity following exposure to thiophanate-methyl via the dermal route was not of concern compared to oral administration, hence studies on absorption, distribution, metabolism and excretion following exposure via the skin is not required.

### Time-concentration profile

The time-concentration profiles for thiophanate-methyl was investigated following oral administration of single low and high doses in male and female rats. Peak concentrations in blood were reached after 1-4 hours after administration. The majority (>99.5%) of the thiophanate-methyl related radioactivity was eliminated from blood within 48 (low dose) or 96 hours (high dose). The time-concentration profile of thiophanate-methyl related radioactivity in blood and plasma after low dose administration is depicted in Figure 9.1.-1. Similar parameters were obtained in both male and female rats indicating that there are no sex differences in the toxicokinetics of thiophanate-methyl.

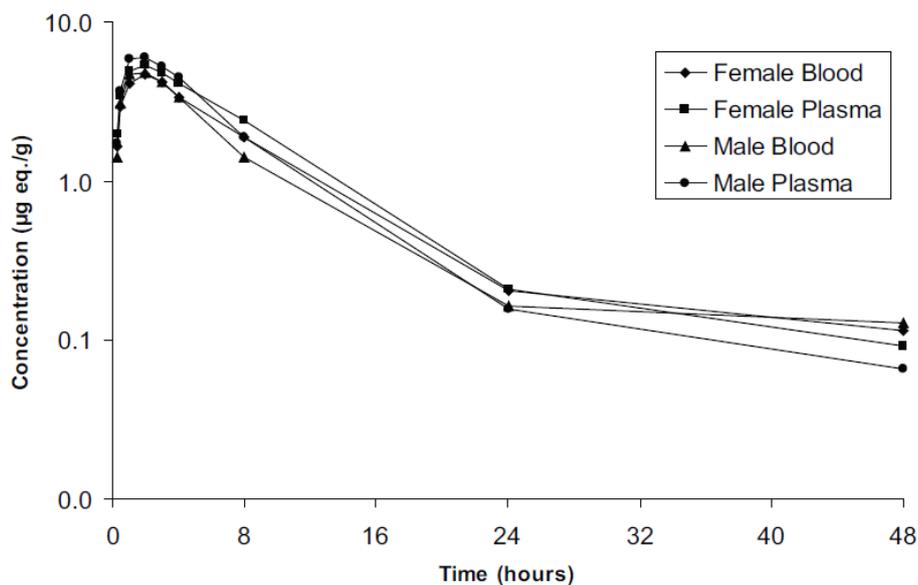


Figure 9.1.-1 Concentration-time profiles in blood and plasma after single oral low dose administration of  $^{14}\text{C}$ -thiophanate-methyl.

### Absorption

In rats, the absorption from the gastro-intestinal tract of low doses of thiophanate-methyl was rapid and represented approximately 89% of the administered dose, based on excretion in urine, bile and faeces. A clear shift from mainly urinary excretion at low doses to predominantly faecal excretion following high dose administration of thiophanate-methyl was detected, suggesting a decrease in oral absorption at high doses. The results from measurements of radioactivity in blood in the same studies provide some support to the statement - there seems to be a less than proportional increase of the AUC in relation to the increase in dose following high dose administration of thiophanate-methyl - but the level of detail in the studies was too low for any conclusion to be drawn. Another explanation to the observation may be that excretion of thiophanate-methyl via bile increases at high doses compared to low doses, meaning that the fraction thiophanate-methyl absorbed by the oral route would be independent of dose. Unfortunately, there are no studies available on excretion via bile following high dose administration of thiophanate-methyl to help elucidate this matter.

### Distribution

Thiophanate-methyl is widely distributed in the body. In rats, at 96 hours post dosing (single low-, repeated low-, and single high dose administration) thiophanate-methyl related radioactivity was mainly found in thyroid, liver, and kidneys. Thiophanate-methyl was also present in testes and ovaries at levels above or similar to the ones in blood. In male mice, after administration of a single low dose, the highest radioactivity was found in liver, followed by kidney and blood. Low levels of radioactivity was also found in thyroid and testis.

### **Metabolism**

The results of the metabolism studies in rat showed that neither the dose level nor the duration of exposure resulted in a major change in the metabolites, identified in urine. The most important urinary metabolite at both low and high doses was 5-OH-carbendazim-S which accounted for up to 42% of the total radioactivity following single low dose administration. The formation of 5-OH-carbendazim-S is proposed to occur via two competing pathways; one with carbendazim (MBC in table 9.1-2) as an intermediate. The proposed metabolic pathway of thiophanate-methyl in rats is depicted in Figure 9.1-2. The biotransformation routes in mice were found to be similar. In rats, there were an indication of possible gut microfloral enzyme induction, and/or possible biotransformation enzyme induction following repeated exposure, as indicated by the lower residue levels in excreta following repeated dosing relative to single low dose administration.

### **Excretion**

Excretion of thiophanate-methyl in rats, mice and dogs occurred rapidly via urine and faeces. The excretion was almost complete within 24 to 96 hours depending on dose level and species, and the excretion patterns were comparable between the three species. Excretion via expired CO<sub>2</sub> was negligible in rats. At low doses, most thiophanate-methyl was excreted with urine (70% and 74 % in rats and dogs, respectively), and to a lesser extent via faeces (30% and 14% in rats and dogs, respectively). After high dose administration the relationship was reversed and most thiophanate-methyl was recovered from faeces (70% and 73% in rats and mice, respectively), and to a lesser extent via urine (30% and 26% in rats and mice, respectively). During repeated low dosing of rats, every day during the treatment about 90% of the administered radioactivity was excreted via urine (54%) and faeces (35%). Hence, there is no indication that thiophanate-methyl accumulates in the body. Excretion via bile was investigated in one study in rats following single low dose administration. Excretion was found to be driven by both urine (47%) and bile (40%). Only 7% of the administered radioactivity was recovered from faeces.



MBC = carbendazim

Figure 9.1.-2. Proposed metabolic pathway of thiophanate-methyl in rats.

## 10 EVALUATION OF HEALTH HAZARDS

### Acute toxicity

#### 10.1 Acute toxicity - oral route

Table 14: Summary table of animal studies on acute oral toxicity

| Test substance/<br>Route                           | Vehicle            | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose<br>levels   | LD <sub>50</sub> /LC <sub>50</sub> | Reference            |
|--|--------------------|--|--------------------------------------|------------------|------------------------------------|----------------------|
| Thiophanate-methyl<br><br>Oral<br><br>EPA OPP 81-2 | Distilled<br>water | EPA OPP 81-1<br>(similar to<br>OECD 401) | Rat<br>Crj:CD(SD)<br>M, F<br>5/sex   | 5000<br>mg/kg bw | >5000 mg/kg bw                     | RAR Vol 3<br>B.6.2.1 |

M: male; F: female

**Table 15: Summary table of human data on acute oral toxicity**

No data.

**Table 16: Summary table of other studies relevant for acute oral toxicity**

No data.

**10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity**

An acute oral toxicity study was conducted according to GLP and EPA OPP 81-1. Five Crj:CD(SD) rats/sex received a single oral gavage treatment with thiophanate methyl in distilled water at 5000 mg/kg bw (10 mL/kg bw) and were then observed for 14 days. No mortalities or treatment-related changes were noted during the study period.

**10.1.2 Comparison with the CLP criteria**

According to Regulation (EC) No 1272/2008 (CLP), classification in Acute Tox. 4 (the lowest classification) is required for substances with oral LD<sub>50</sub> of 300-2000 mg/kg bw. The LD<sub>50</sub> for oral toxicity was above 2000 mg/kg bw and thiophanate-methyl thus does not fulfil the classification criteria for acute oral toxicity.

**10.1.3 Conclusion on classification and labelling for acute oral toxicity**

No classification is proposed for thiophanate-methyl.

**10.2 Acute toxicity - dermal route**

**Table 17: Summary table of animal studies on acute dermal toxicity**

| Test substance/<br>Route                             | Vehicle            | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose<br>levels   | LD <sub>50</sub> /LC <sub>50</sub> | Reference            |
|--|--------------------|--|--------------------------------------|------------------|------------------------------------|----------------------|
| Thiophanate-methyl<br><br>Dermal<br><br>EPA OPP 81-2 | Distilled<br>water | EPA OPP 81-2<br>(similar to<br>OECD 402) | Rabbit<br>Kbs:JW<br>5/sex            | 2000<br>mg/kg bw | >2000 mg/kg bw                     | RAR Vol 3<br>B.6.2.2 |

M: male; F: female

**Table 18: Summary table of human data on acute dermal toxicity**

No data.

**Table 19: Summary table of other studies relevant for acute dermal toxicity**

No data.

**10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity**

An acute dermal toxicity study was conducted according to GLP and EPA OPP 81-2. Five 5 Kbs:JW rabbits/sex were treated topically with thiophanate methyl in distilled water at 2000 mg/kg bw, or with water alone (control group), for 24 hours under occlusive conditions (5 mL/kg bw), and were then observed for 14 days. No mortalities was noted during the study period. There was erythema on the treatment area for two days in seven rabbits. No other effects were noted.

### 10.2.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), classification in Acute Tox. 4 (the lowest classification) is required for substances with a dermal LD<sub>50</sub> of 1000-2000 mg/kg bw. The LD<sub>50</sub> for dermal toxicity was above 2000 mg/kg bw and thiophanate-methyl thus does not fulfil the classification criteria for acute dermal toxicity.

### 10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed for thiophanate-methyl.

## 10.3 Acute toxicity - inhalation route

**Table 20: Summary table of animal studies on acute inhalation toxicity**

| Test substance/<br>Route   | Vehicle | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose levels   | LD <sub>50</sub> /LC <sub>50</sub>                                     | Reference            |
|--|---------|--|--------------------------------------|---|--|----------------------|
| Thiophanate-methyl<br><br>Inhalation<br>(4 hr, whole body,<br>aerosol)<br><br>EPA OPP 81-3 | -       | EPA OPP 81-3<br>(similar to<br>OECD 403) | Rat<br>Crj:CD(SD)<br>M, F<br>5/sex   | 0.5 mg/L<br>(females<br>only), 1.0,<br>1.5, 1.6 and<br>1.9 mg/L | LC <sub>50</sub> M: 1.7<br>mg/L<br><br>LC <sub>50</sub> F: 1.9<br>mg/L | RAR Vol 3<br>B.6.2.3 |

M: male; F: female

**Table 21: Summary table of human data on acute inhalation toxicity**

No data.

**Table 22: Summary table of other studies relevant for acute inhalation toxicity**

No data.

### 10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

An acute inhalation toxicity study was conducted according to GLP and EPA OPP 81-3. Five Crj:CD(SD) rats/sex/group were exposed to a dust aerosol (MMAD: 3.7-4.5 µm) of thiophanate-methyl at concentrations of 0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L, or to clean air (control group), for 4 hours, and then observed for 14 days. Exposure was via whole body inhalation. During the study period, 5/5 males and 3/5 females died at 1.9 mg/L, and 1/5 females died at 1.0 mg/L. The calculated 4-hour LC<sub>50</sub> values were 1.7 mg/L for males and 1.9 mg/L for females.

### 10.3.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP), classification in Acute Tox. 4 (the lowest classification) is required for substances with an inhalation LC<sub>50</sub> of 1.0-5.0 mg/L. The LC<sub>50</sub> for inhalation toxicity was 1.7-1.9 mg/L and thiophanate-methyl thereby fulfils the criteria for classification with Acute Tox 4; H332.

### 10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification in **Acute Tox 4; H332** is proposed for thiophanate-methyl.

**10.4 Skin corrosion/irritation****Table 23: Summary table of animal studies on skin corrosion/irritation**

| Test substance/<br>Route       | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose level  | Remarks           | Reference            |
|--------------------------------|--|--------------------------------------|---|-------------------|----------------------|
| Thiophanate-methyl<br><br>Skin | EPA OPP 81-4<br>(similar to<br>OECD 404) | Rabbit<br>New Zealand<br>M<br>6      | 500 mg thiophanate-<br>methyl moistened with<br>water | No skin reactions | RAR Vol 3<br>B.6.2.4 |

**Table 24: Summary table of human data on skin corrosion/irritation**

No data.

**Table 25: Summary table of other studies relevant for skin corrosion/irritation**

No data.

**10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation**

In a study performed in accordance with GLP and EPA OPP 81-4 (similar to OECD 404), 6 male New Zealand White rabbits each received dermal treatments with 0.5 g of thiophanate-methyl moistened with water for 4 hours under occlusive conditions, and skin reactions were then scored for erythema and oedema formation according to the Draize method at 0, 0.5, 1, 24, 48 and 72 hours. No skin reactions were observed.

**10.4.2 Comparison with the CLP criteria**

Not relevant as no effects were observed.

**10.4.3 Conclusion on classification and labelling for skin corrosion/irritation**

No classification is proposed for thiophanate-methyl.

**10.5 Serious eye damage/eye irritation****Table 26: Summary table of animal studies on serious eye damage/eye irritation**

| Test substance/<br>Route      | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose level | Remarks   | Reference            |
|-------------------------------|--|--------------------------------------|------------|---|----------------------|
| Thiophanate-methyl<br><br>Eye | EPA OPP 81-5<br>(similar to<br>OECD 405) | Rabbit<br>New Zealand<br>M<br>9      | 100 mg     | 1 h: Conjunctival<br>redness in 7/9 rabbits<br>(grade 1-2)<br>24 h: Conjunctival<br>redness in 1/9 rabbits<br>(grade 1) | RAR Vol 3<br>B.6.2.5 |

**Table 27: Summary table of human data on serious eye damage/eye irritation**

No data.

**Table 28: Summary table of other studies relevant for serious eye damage/eye irritation**

No data.

### **10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation**

In a study performed in accordance with GLP and EPA OPP 81-4 (similar to OECD 405), 0.1 g of thiophanate-methyl was placed in the conjunctival sac of the left eye of 9 male New Zealand White rabbits. The treated eyes of 3 rabbits were washed 2 minutes after the treatment for 30 seconds, and then irritation was scored at 1, 24, 48 and 72 hours after treatment. Conjunctival redness (grade 1-2) was seen in 7/9 rabbits (grade 1-2) at the 1-hour reading and in 1/9 rabbits at the 24-hour reading (in the eye irritation study).

### **10.5.2 Comparison with the CLP criteria**

According to Regulation (EC) No 1272/2008 (CLP)table 3.3.2, a substance should be classified in Category 2 (irritating to eyes) if it produces “*at least in 2/3 animals, a positive response of:*

*-corneal opacity  $\geq 1$  and/or*

*-iritis  $\geq 1$  and/or and/or*

*-conjunctival redness  $\geq 2$  and/or*

*-conjunctival oedema (chemosis)  $\geq 2$*

*calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.”*

These criteria were not fulfilled in the study performed with thiophanate-methyl.

### **10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation**

No classification is proposed for thiophanate-methyl.

## **10.6 Respiratory sensitisation**

No data.

## 10.7 Skin sensitisation

**Table 29: Summary table of animal studies on skin sensitisation**

| Test substance<br>Test type<br>Method<br>Guideline | Route of exposure      | Species<br>Strain<br>Sex<br>No./group<br>Vehicle   | Exposure Period  | Doses   | Main findings  | Reference            |
|--|------------------------|--|--|---|--|----------------------|
| Thiophanate-methyl<br><br>GPMT<br><br>Acceptable   | Intradermal<br>/Dermal | Guinea pig<br><br>Hartley<br><br>F<br><br>10/group<br><br>Freund's complete adjuvant/White vaseline. | The duration of dermal induction was 48 hours, carried out 7 days after intradermal induction.<br><br>The duration of challenge was 24 hours, carried out another 14 days later.<br><br>The experiment was repeated under the same conditions. | Intradermal induction: 3.5% (mildly irritant).<br><br>Dermal induction: 42% (non-irritant).<br><br>Challenge: 42% (non-irritant). | Challenge: 10/10 animals with positive skin reactions.<br><br>Repeated study:<br>Challenge: 10/10 animals with positive skin reactions.<br><br>Sensitiser. | RAR Vol 3<br>B.6.2.6 |
| Thiophanate-methyl<br><br>Supportive               | Intradermal            | Guinea pig<br><br>Hartley<br><br>14<br><br>Aqueous Arabic gum suspension.                            | Intradermal: 10 applications (3 per week).<br>Challenge: 14 days after induction in a treated and non-treated area.  | Intradermal induction: 1%<br><br>Intradermal challenge: 1%  | Diameter of erythema caused by sensitisation was slightly bigger than erythema caused by primary irritation.<br><br>Weak sensitiser.                       | RAR Vol 3<br>B.6.2.6 |

**Table 30: Summary table of human data on skin sensitisation**

No data.

**Table 31: Summary table of other studies relevant for skin sensitisation**

No data.

### 10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The skin sensitisation potential of thiophanate-methyl was assessed in two studies in guinea pig. Both studies showed that thiophanate-methyl have skin sensitising properties. In the guinea pig maximization test (GPMT), considered acceptable, 100% of the animals showed positive skin reactions following intradermal induction to thiophanate-methyl. When the study was repeated, the response rate was identical.

The second test was considered supportive due to limitations in scope and reporting. The study was not performed in accordance with GLP nor with any specific guideline. It measured the diameter of erythema caused by sensitisation compared to erythema caused by primary irritation. The response rate was not reported. The diameter of erythema caused by thiophanate-methyl was slightly bigger than that of the erythema caused by primary skin irritation. A big difference (up to 4-fold) was noted for the positive control. Thiophanate-methyl showed weak sensitising properties under the conditions of the study. The weaker reactions in the second test may be due to the use of lower induction and challenge doses and/or the use of a

different vehicle (Aqueous Arabic gum suspension compared to FCA). Thiophanate-methyl has currently a harmonised classification as a skin sensitiser (Skin Sens. 1, H317).

### 10.7.2 Comparison with the CLP criteria

The Regulation (EC) No 1272/2008 (CLP) allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. For Category 1, when an adjuvant Guinea pig test method is used, a response in at least 30% of the animals is considered positive. This criteria is fulfilled by thiophanate-methyl which had a response rate of 100% following the use of a 3.5% intradermal induction dose. Classification into sub-categories is only allowed if data are sufficient (CLP Annex I 3.4.2.2.1.1). The criteria for sub-categorisation based on results from Guinea pig maximisation tests are given below.

**Table 32: Criteria for sub-category classification of skin sensitisers.**

| Sub-category | Assay                        | Response   |
|--------------|------------------------------|--|
| 1A           | Guinea Pig Maximisation Test | ≥ 30 % responding at ≤ 0,1 % intradermal induction dose or<br>≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose         |
| 1B           | Guinea Pig Maximisation Test | ≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or<br>≥ 30 % responding at > 1 % intradermal induction dose |

According to Table 43, thiophanate fulfils the criteria for subcategorisation in category 1B (≥ 30 % responding at > 1 % intradermal induction dose). However, the Guidance on the application of the CLP criteria, section 3.4.2.2.2, states that subcategorisation in 1B is only allowed if category 1A can be excluded. For thiophanate-methyl, there is a very high response rate following the use of a high intradermal induction dose. It is therefore possible that the use of a lower dose would result in a response rate which fulfills the criteria for category 1A. Subcategorisation of thiophanate-methyl is therefore not proposed.

### 10.7.3 Conclusion on classification and labelling for skin sensitisation

Thiophanate-methyl has a harmonised classification as a skin sensitiser (**Skin Sens. 1, H317**). No change is proposed.

## 10.8 Germ cell mutagenicity

**Table 33: Summary table of mutagenicity/genotoxicity tests in vitro**

| Test substance<br>Test system<br>Method  | Organism/Strain(s)   | Concentrations tested (range) | Result   |          | Remark | Reference                                     |
|--|--|-------------------------------|----------|----------|--------|---|
|  |  |                               | +S9      | -S9      |        |   |
| Thiophanate-methyl<br><br>Bacterial Reverse Mutation Test (Ames test)<br>OECD TG 471 | <i>Salmonella typhimurium</i><br>TA100<br>TA1535<br>TA1537<br>TA98<br><i>Escherichia coli</i><br>WP2uvrA | 39.1 - 5000 µg/plate          | Negative | Negative |        | RAR Vol 3<br>B.6.4.1.1<br>Doc. No.<br>557-006 |
| Thiophanate-methyl<br><br>Mammalian  | HPRT locus V79<br>Chinese hamster cells  | 6.25 - 100 µg/ml              | Negative | Negative |        | RAR Vol 3<br>B.6.4.1.2<br>Doc. No.<br>557-003 |

## CLH REPORT FOR THIOPHANATE-METHYL

|  |                              |   |                  |          |   |  |
|--|------------------------------|---|------------------|----------|---|--|
| Cell Gene Mutation Test  |                              |   |                  |          |   |  |
| OECD TG 476  |                              |   |                  |          |   |  |
| Thiophanate-methyl (Topisn M*)<br><br>Mammalian Chromosome Aberration Test                         | Chinese hamster ovary cells  | 100 - 400 µg/ml (without S9)<br>250 – 1000 µg/ml (with S9-mix)                        | Negative         | Negative |   | RAR Vol 3<br>B.6.4.1.3<br>Doc. No. 557-004 |
| OECD TG 473  |                              |   |                  |          |   |  |
| Thiophanate-methyl<br><br>Unscheduled DNA Repair Synthesis (UDS test)                              | Rat primary hepatocytes      | 5 – 1000 µg/ml  | Negative         | Negative |   | RAR Vol 3<br>B.6.4.1.4<br>Doc. No. 557-005 |
| OECD TG 482  |                              |   |                  |          |   |  |
| Thiophanate-methyl<br><br>Micronucleus test<br><br>Similar to OECD 487                             | Human peripheral lymphocytes | Up to 47.5 µg/mL (-S9 mix)<br>Up to 550 µg/mL (+S9 mix)                               | Negative         | Positive | Evidence for aneuploidy (-S9 mix)   | RAR Vol 3<br>B.6.4.1.5<br>Doc. No. 557-001 |
| Thiophanate-methyl<br><br>Micronucleus test<br>Examination of four chromosomes<br><br>No guideline | Human peripheral lymphocytes | Range-finding study:<br>8.5 - 200 µg/mL<br>Main study:<br>0.05 - 39.0 µg/mL (-S9 mix) | Not investigated | Positive | The purpose of the study was to establish a threshold for aneugenicity.<br><br>Result: the threshold was found to be 6 µg/mL. | RAR Vol 3<br>B.6.4.1.6<br>Doc. No. 557-002 |

\* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95%.

**Table 34: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo**

| Test substance<br>Test system<br>Method  | Species<br>Strain<br>Sex<br>No./group | Frequency of application                           | Dose levels              | Results  | Remark  | Reference  |
|--|---------------------------------------|--|--------------------------|----------|---|--|
| Thiophanate-methyl<br>Chromosomal aberration (micronucleus test)<br><br>OECD 474<br><br>Acceptable | B6D2F1 mice M, F<br>5/group           | Single dose  | 500, 1000, 2000 mg/kg bw | Positive | The incidence in micronuclei was 4-fold higher than in the concurrent negative control group, but 6-fold lower than in the positive control group exposed to carbendazim. | RAR Vol 3<br>B.6.4.2.1<br>Doc. No. 557-007<br><br>Study evaluated in DAR |
| Thiophanate-methyl<br><br>Chromosomal aberration   | CrI:CD-1 (ICR) mice M<br>5/group      | Administered twice at an interval of approximately | 500, 1000, 2000 mg/kg bw | Negative | Data confirming bone marrow exposure not presented.   | RAR Vol 3<br>B.6.4.2.2<br>Doc. No. 557-016                               |

CLH REPORT FOR THIOPHANATE-METHYL

|   |  |   |   |  |  |  |
|---|--|---|---|--|--|--|
| (micronucleus test)<br>OECD 474<br>Low reliability  |  | 24 hours                                  |   |  | There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.  | Study submitted for the purpose of renewal   |
| Thiophanate-methyl<br><i>In vivo</i> genotoxicity studies: micronucleus test, chromosome aberration analysis, comet assay<br>Supportive | Lizards ( <i>Podarcis sicula</i> ) captured in the wild<br>Control group 9 M & 9 F<br>Exposed groups 10 M & 10 F                                 | Administered by spraying twice weekly     | 100 ml of 1.5 % thiophanate-methyl solution used for spraying twice weekly during 15, 30 or 40 days.        | Positive for all genotoxic effects tested                      | Information on the purity of the test substance is not available. The results may be relevant as supportive information for the assessment of the genotoxic potential of thiophanate-methyl.                         | RAR Vol 3 B.6.4.2.3<br>Doc. No. (892-017)<br><br>Study submitted for the purpose of renewal          |
| Thiophanate-methyl<br><i>In vivo</i> cytogenetic assay (i.p)<br>Dominant lethal assay (i.p)<br>Supportive                               | Wistar rats ICR mice<br>Cytogenicity assay:30m/dose<br><br>DL: 10m/dose group/week mated with 3 females. Repeated 8 weeks (240 females in total) | Cytogenicity assay: 5 d<br>DL:single dose | Cytogenicity assay: 0, 62.5, 125, 250, 500 and 1000 mg/kg bw<br><br>DL: 0, 8, 40, 200, 400 and 500 mg/kg bw | Cytogenicity assay:<br>Inconclusive<br><br>DL:<br>Inconclusive | Deficiencies with respect to reporting (lack of individual data) and study design (lack of positive controls)  | RAR Vol 3 B.6.4.3.1<br>Doc. No. RD-73062 (551-006)<br><br>Study evaluated in DAR                     |
| Thiophanate-methyl<br>Spermatogonial chromosomal abberation test<br>Low reliability   | CrI:CD-1 (ICR) mice M<br>6/group (5 analysed for chromosomal aberrations)  | Single dose                               | 500, 1000, 2000 mg/kg bw  | Negative   | Data confirming target cell exposure not presented.<br><br>There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.                               | RAR Vol 3 B.6.4.3.2<br>Doc. No. RD-03956 (557-012)<br><br>Study submitted for the purpose of renewal |
| Thiophanate-methyl<br>Spermatogonial chromosomal abberation test (Pretest)<br>Low reliability   | CrI:CD-1 (ICR) mice M<br>10/group for negative control and test substance, 4/group for positive control/group                                    | Single dose                               | 2000 mg/kg bw   | Negative   | Data confirming target cell exposure not presented.<br><br>There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.<br><br>Non-GLP compliant test | RAR Vol 3 B.6.4.3.2<br>RD-03888 (557-018)<br><br>Study submitted for the purpose of renewal          |

|  |                                       |             |                          |          |   |  |
|--|---------------------------------------|-------------|--------------------------|----------|---|--|
|  |                                       |             |                          |          | Guideline: not applicable   |  |
| Thiophanate-methyl<br>Micronucleus test in germ cells<br>No guideline<br>Low reliability                     | CrI:CD-1 (ICR) mice M<br>5/group      | Single dose | 500, 1000, 2000 mg/kg bw | Negative | Data confirming target cell exposure not presented.<br><br>There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.<br><br>In addition, micronuclei in erythrocytes were scored according to OECD 474; no increase in micronucleated polychromatic erythrocytes was observed | RAR Vol 3 B.6.4.3.3 No. RD-10093 (557-017)<br><br>Study submitted for the purpose of renewal |
| Thiophanate-methyl<br>Micronucleus test in germ cells (Pretest)<br>No GLP<br>No guideline<br>Low reliability | CrI:CD-1 (ICR) mice M<br>4 or 5/group | Single dose | 2000 mg/kg bw            | Negative | Data confirming target cell exposure not presented.<br><br>There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.<br><br>Two of the four positive control substances did not induce an increase in the frequency of micronucleated spermatids.                             | RAR Vol 3 B.6.4.3.3 No. RD-03931 (557-011)<br><br>Study submitted for the purpose of renewal |

**Table 35: Summary table of human data relevant for germ cell mutagenicity**

No data.

**10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity**

The genotoxicity studies performed with thiophanate-methyl are thoroughly described in Annex I (Volume 3 to the RAR).

Thiophanate-methyl has been investigated for genotoxicity *in vitro* and *in vivo*.

Thiophanate-methyl tested negative in the bacterial reverse mutation test (Ame’s test), mammalian cell gene mutation test, chromosome aberration test and unscheduled DNA synthethis test, however the *in vitro* micronucleus test was positive. A follow-up study was made to establish a threshold effect for the aneugenic effect noted in the micronucleus test.

The *in vivo* studies also indicate an aneugenic potential, as concluded at the first approval and which is in accordance with the current classification in Muta. 2 for aneugenicity in somatic cells. However, the DS is of the opinion that there is also evidence of clastogenicity. Information with some relevance for genotoxicity was found in the literature search performed by the applicant (see Vol 3, B.6.10). Data consists of a non-GLP study including a combined comet assay, micronucleus test and chromosome aberration test performed with thiophanate-methyl in lizards (*Podarcis sicula*). In the study lizards captured in the wild were sprayed twice weekly with a 1.5 % concentration of thiophanate-methyl (in water) for 15, 30 or 40 days and the results obtained indicated a potential for clastogenicity, since DNA damage and chromosome aberrations due to single or double strand breaks were increased compared to controls and correlated with exposure time. Due to the lack of a specification or information on the purity of the test substance it is not possible to exclude that effects could be due to any impurities present in the solution. Therefore, this study alone is considered to be of limited value for the hazard assessment.

However, stronger evidence for a clastogenic effect of thiophanate-methyl is available in the first mouse micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). The results of the study are presented in the study summary in Annex I and are summarized below:

**Table 36: Incidence of micronucleated cells per 2000 immature erythrocytes examined**

| Sampling time | Treatment (compound) | Dose (mg/kg bw) | Incidence |
|---------------|----------------------|-----------------|-----------|
| 24 hours      | Vehicle control      | 0               | 1.3       |
|               | Thiophanate-methyl   | 500             | 4.2**     |
|               | Thiophanate-methyl   | 1000            | 3.8**     |
|               | Thiophanate-methyl   | 2000            | 6.3**     |
|               | Carbendazim          | 1000            | 24.5**    |
| 48 hours      | Vehicle control      | 0               | 1.2       |
|               | Thiophanate-methyl   | 500             | 3.1**     |
|               | Thiophanate-methyl   | 1000            | 5.0**     |
|               | Thiophanate-methyl   | 2000            | 5.6**     |

\*\* Statistically significant (nonparametric method of analysis, based on permutation with one-sided probabilities); p < 0.001

**Table 37: Summary of centromeric staining results**

| Treatment (compound)                          | Dose (mg/kg bw) | Centromere-positive micronuclei (indicative of aneugenicity) | Centromere-negative micronuclei (indicative of clastogenicity) |
|---|-----------------|--|--|
| Thiophanate-methyl                            | 2000            | 34 %   | 66 %   |
| Carbendazim (positive control aneugenicity)   | 1000            | 68 %   | 32 %   |
| Mitomycin C (positive control clastogenicity) | 12              | 24 %   | 76 %   |

This was concluded by the DS after re-evaluation of the results, as presented below. Not presented in the previous version of this document, nor in the DAR, this study included centromeric staining of micronuclei, enabling a distinction between a clastogenic effect (centromere-negative micronuclei) and an aneugenic effect (centromere-positive micronuclei). The proportion of centromere-negative micronuclei observed following exposure to carbendazim, thiophanate-methyl and mitomycin C, respectively, was 32%, 66% and 76%, respectively. The result demonstrates that the proportion of centromere-negative micronuclei observed after thiophanate-methyl exposure was very close to the proportion observed after exposure to the known clastogen mitomycin C and distinctly different from the proportion observed after exposure to the known aneugen carbendazim. The result is a strong piece of evidence that thiophanate-methyl has clastogenic properties.

A new micronucleus study of thiophanate-methyl in the bone marrow of mice (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016) was submitted by the applicant as additional data during the Pesticide Peer Review (Evaluation

table, data requirement 2.2). Since no statistically significant increase or dose-dependent relationship were observed in micronucleated polychromatic erythrocytes, the study author concluded that the result of the study was negative. However, no data demonstrating bone-marrow exposure were presented and, therefore, the negative result of the study is considered to be of low reliability. The reliability can be further questioned on account of the 4-fold increase in the incidence of micronucleated polychromatic erythrocytes observed in the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). This study used the mouse strain B6D2F1 and, in addition to the increase in micronuclei, substantial amounts of thiophanate-methyl and carbendazim were established in blood plasma following administration of thiophanate-methyl. In the new study a different mouse strain was used (CrI:CD-1 (ICR)), and the negative result obtained gives reason to be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl.

In connection with this analysis it is relevant to mention that the applicant is of the opinion that the result of the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007) should not be concluded to be positive, since the increase in micronuclei might be within the range of historical negative control values. The view of the RMS is that the concurrent negative control group has the strongest weight in comparisons with results in exposed groups. In studies where a small increase was observed in one exposed group only, i.e. the result was not reproducible, it would be relevant to compare the result with historical negative control values to conclude if the observed increase should be considered to have occurred by chance only. However, in the first study (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016), a 4-fold increase in micronuclei was observed both in the experiment using 24 h sampling time and the experiment using 48 h sampling time. Moreover, in both experiments the increases in micronuclei were dose related. This clearly demonstrates that the observed effect occurred as a result of exposure to thiophanate-methyl and was not a chance finding. Accordingly, the new study (RAR Vol 3, B.6.4.2.2 Doc No. 557-016) should be ascribed low weight in the evaluation of the genotoxicity of thiophanate-methyl.

As thiophanate-methyl has been demonstrated to be mutagenic in the bone marrow of mice, with evidence for both aneugenic and clastogenic effects, this effect can be suspected to occur also in germ cells. Therefore, an assessment of the potential of thiophanate-methyl to induce mutations in germ cells is considered necessary and is made below.

A dominant lethal test performed as part of a mutagenic, cytogenetic and teratogenic study is available. In the cytogenicity part of the study, metaphase chromosomes from the bone marrow as well as from spermatogonial cells were investigated, with no indication of an increase in chromosome aberrations. Neither was there an indication of treatment-related dominant lethal mutations in male mice. However, the study suffered from many deficiencies including the lack of individual data and a positive control to verify appropriate functioning of the test. Thus, based on this result, it is difficult to exclude that the aneugenic effects of thiophanate-methyl observed in somatic cells would not occur in germ cells. Overall, the result is considered inconclusive.

Although the dominant lethal assay is recommended as a method to follow up positive results observed in *in vivo* genotoxicity tests in somatic cells, the test is generally regarded to have low sensitivity and this is even recognised in the OECD guideline 478. Furthermore, it is noted that carbendazim, which is concluded to be a germ-cell mutagen (currently classified Muta. 1B, see section on classification) was also negative in dominant lethal tests (DRAR on carbendazim, 2009).

Additional data was requested by EFSA during the Peer review (Evaluation table, data requirement 2.2) and consisted of four new studies testing mutagenic effects of thiophanate-methyl in germ cells of mice, one spermatogonial chromosome aberration study with a preceding pretest and a micronucleus study in germ cells with a preceding pretest. All four studies were concluded to be negative by the study author, since no statistically significant increase or dose-dependent relationship were observed in chromosome aberrations or micronuclei. However, no data demonstrating target-cell exposure were presented and, therefore, the negative results of the studies have low reliability. The reliability can be further questioned by the arguments presented above for the new bone marrow micronucleus test by (RAR Vol 3, B.6.4.2.2 Doc. No. 557-016), i.e. that the strain CrI:CD-1 (ICR) was used and that there is reason to be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl. Accordingly, all four studies should be ascribed low weight in the evaluation of the genotoxicity of thiophanate-methyl in germ cells.

The applicant also performed a specific literature search only focused at aneugenicity (see RAR Vol 3, B.6.10 in Annex I). No information was found.

### 10.8.2 Comparison with the CLP criteria

At present, thiophanate-methyl is classified as Muta. 2; H341 based on a translation of the classification Muta. Cat. 3 established under Council Directive 67/548/EEC, now replaced by Regulation (EC) No 1272/2008. Carbendazim is classified as Muta. 1B; H340.

Criteria for Classification in category 1A can only be fulfilled if results are based on:

*- positive evidence from human epidemiological studies.*

This type of data is not available for thiophanate-methyl thus **classification in category 1A is not supported.**

Classification in **category 1B** is based on:

*-positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or*

*-positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or*

*-positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.*

Classification in **category 2** is based on:

*-Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*

*-Somatic cell mutagenicity tests in vivo, in mammals; or*

*-Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.*

Considering that there is no robust data on germ cell genotoxicity available for thiophanate-methyl, it may be argued that the existing data do not fulfil the criteria for classification in category 1B. However, in the Guidance on the Application of the CLP Criteria Version 4.1 – June 2015 (and in the current draft of this guidance document in the ongoing updating process) the following is stated: “*It could be argued that in a case where in vivo mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, then that substance should also be considered as a Category 1B mutagen. Germ cell mutagens as the spermatogonia are generally not protected from substance exposure by the blood-testes barrier formed by the Sertoli cells. In such circumstances the relevant criteria are as follows:*

*-positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, **or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells**” (text highlighted in bold by the RMS is of particular relevance for the classification of thiophanate-methyl, as discussed below).*

With respect to the above it is of importance to note that, according to data in the section on toxicokinetics (Vol 3, section B.6.1.1.1), low levels of labelled “parent equivalents” (i.e. below 0.5 ppm but in a range comparable to residue levels in the blood) are found in testis and ovaries of rats administered a single dose thiophanate-methyl. In rats administered repeated doses of the substance the levels in ovaries were initially

higher (at 3 hours) than the levels in blood. Exposure of testis was also demonstrated in mice receiving a single dose of thiophanate-methyl. Overall, this indicates that gonads are exposed to the substance or its metabolites but no accumulation seems to occur. Moreover, according to data in the section on toxicokinetics, urinary metabolites carbendazim and 5-OH-carbendazim-S are formed to an extent of  $\leq 1\%$  and 42% of the total administered radioactivity respectively. It is reasonable to conclude that thiophanate-methyl, which is systemically available, detected in the gonads of rats and mice, and mutagenic (clastogenic and aneugenic) in the bone marrow of mice after oral administration has the ability to interact with the genetic material of germ cells.

### **10.8.3 Conclusion on classification and labelling for germ cell mutagenicity**

Thiophanate-methyl is currently classified Muta. 2; H341 based on a translation from the classification established under the Dangerous Substances Directive. As the substance is mutagenic (clastogenic and aneugenic) *in vivo* in somatic cells (bone marrow), systemically available and detected in gonads of rats and mice, the data is considered to indicate “the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells” and classification of thiophanate-methyl in **Muta. 1B – H340** is therefore proposed.

10.9 Carcinogenicity

Table 38: Summary table of animal studies on carcinogenicity

| Test substance<br>Route<br>Duration of study                                     | Species<br>Strain<br>Sex<br>No./group<br>Vehicle | Dose levels  | Main findings   | Reference            |
|--|--|--|---|----------------------|
| Thiophanate-methyl<br>Oral<br>(dietary)<br>24 months<br>Acceptable               | Rat<br>Fischer-344<br>60/sex/dose                | 0, 75, 200, 1200 and 6000 ppm (equivalent to 0, 3.3, 8.8, 54.4 and 280.6 mg/kg bw/day for males and 0, 3.8, 10.2, 63.5 and 334.7 mg/kg bw/day for females.)          | <u>1200 ppm:</u><br>↓ Bw (9-16%)<br>↑ Thyroid weights (24% and 27% in males and females, respectively)<br>↑ Liver weight (25% and 24% in males and females, respectively)<br>↑ kidney weight (20% and 8% in males and females, respectively)<br><br>- Morphological and functional changes in the thyroid, kidneys and hepatocellular hypertrophy and occurrence of lipofuscin (indicating a liver cell damage, accelerated nephropathy and lipidosis of the adrenal cortex.<br>↑ Follicular cell hyperplasia and hypertrophy (M: 38%, p<0.01, F: 47%, p<0.001)<br>↑ Follicular cell adenomas (M: 7%, not statistically significant, M: 20%, p<0.05)<br>-Haematological changes (↓ red blood cell count, ↓ MCV, ↓ MCH, ↓ MCHC)<br>- Effects on clinical chemistry<br><br><u>More pronounced effects at 6000 ppm:</u><br>↑ Mortality rate 6000 ppm (53/55 males died, cause of death was mainly accidents, nephropathy, thyroid tumours and leukaemia)<br>↑ Follicular cell hyperplasia and hypertrophy (M: 97%, F: 98%, p<0.001)<br>↑ Follicular cell adenomas (20% in males, p<0.01, 3% in females)<br>- ↑ Follicular cell adenocarcinomas (M: 5%, not statistically significant. F: 0%) | RAR Vol 3<br>B.6.5.1 |
| Thiophanate-methyl<br>Oral<br>(dietary)<br>24 months<br>Supportive               | Rat<br>Sprague-Dawley<br>35/sex/dose             | 0, 10, 40, 160 and 640 ppm (equivalent to 0, 0.5, 2, 7 and 30 mg/kg bw/day for males and 0, 0.5, 2, 8 and 34 mg/kg bw/day for females.)                              | <u>640 ppm:</u><br>↓ Bw gain<br><br>- histopathological changes in the testes (reduction in spermatogenesis) and the thyroid (follicular epithelium hypertrophy, decrease of colloidal substance)   | RAR Vol 3<br>B.6.5.2 |
| Thiophanate-methyl<br>(Topsin M*)<br>Oral<br>(dietary)<br>18 month<br>Acceptable | Mouse<br>Crl:CD-1<br>(ICR)BR<br>60/sex/dose      | 0, 150, 640, 3000, and 7000 ppm (equivalent to 0, 23.7, 98.6, 467.6 and 1078.8 mg/kg bw/day for males and 0, 28.7, 123.3, 557.9 and 1329.4 mg/kg bw/day for females) | <u>3000 ppm (m) 640 (f):</u><br>↓ Bw (11-17%)<br>↑ Liver weight (24-82%)<br>↑ Thyroid weight (110%)<br>↑ Heart weight (f: 23-39%)<br><br>↑ Hepatocellular centrilobular hypertrophy ↑ Hepatocellular adenomas (M: 50% of terminal necropsies (p<0.05) at 3000 ppm; 69% of terminal necropsies (p<0.05) at 7000 ppm. F: 24% of terminal necropsies (p<0.05) at 3000 ppm; 59% of terminal necropsies (p<0.05) at 7000 ppm)  | RAR Vol 3<br>B.6.5.3 |

|   |  |   |  |                      |
|---|--|---|--|----------------------|
| Thiophanate-methyl<br>Oral<br>(dietary)<br>24 month<br>Supportive | Mouse<br>ICR-SLC<br>50/sex/dose                              | 0, 10, 40, 160, and<br>640 ppm (equivalent<br>to 0, 1.2, 4.4, 20 and<br>82 mg/kg bw/day for<br>males and 0, 1.3, 5.0,<br>19 and 82 mg/kg<br>bw/day for females) | <u>640 ppm:</u><br>↓ Bw gain (10%)<br><br>No increase in the incidence of neoplasia at<br>any dose level.  | RAR Vol 3<br>B.6.5.4 |
| Thiophanate-methyl<br>Oral<br>(dietary)<br>24 month<br>Supportive | Beagle dogs<br>5/sex/dose,<br>4/sex/dose at<br>highest dose. | to 0, 2, 19, 50 and<br>250 mg/kg bw/day<br>in capsules  | <u>250 mg/kg bw/day:</u><br>Slight retardation of growth in both sexes. A<br>moderate hypertrophy of the thyroid was noted<br>in males and females at 50 and 250 mg/kg<br>bw/d based on organ weight and<br>histopathological examination. | RAR Vol 3<br>B.6.5.5 |

\* The test substance was referred to as "Topsin M". According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 96.6 and 95.9 % in the two batches used in the study.

**Table 39: Summary table of human data on carcinogenicity**

No data.

**Table 40: Summary table of other studies relevant for carcinogenicity**

No data.

**10.9.1 Short summary and overall relevance of the provided information on carcinogenicity**

The data available to assess this endpoint include five long-term studies. These are thoroughly described in Annex I (Volume 3 to the RAR).

Two of the studies are considered acceptable for classification purposes whereas the remaining three are regarded as supportive information only. All studies were thoroughly assessed in the first DAR of 1997. For this assessment, the studies considered acceptable are presented in detail whereas the studies considered supportive are only briefly described. In rats, an increased incidence of thyroid follicular cell hypertrophy and hyperplasia as well as an increased incidence of thyroid follicular cell adenomas were noted.

**Table 41: Thyroid changes**

|  | Incidence of thyroid follicular cell (FC) changes (%) |               |                |                 |                 |
|--|---|---------------|----------------|-----------------|-----------------|
| <b>Males</b>                           | <b>0 ppm</b>  | <b>75ppm</b>  | <b>200 ppm</b> | <b>1200 ppm</b> | <b>6000 ppm</b> |
| Diffuse FC hyperplasia/<br>hypertrophy | 0/60 (0)  | 0/58 (0)      | 0/60 (0)       | 23/60 (38)**    | 58/60 (97)***   |
| Focal FC hyperplasia                   | 3/60 (5)  | 2/58(3)       | 2/60 (3)       | 3/60 (5)        | 15/60 (25)**    |
| FC adenoma                             | 1/60 (2)  | 0/58 (0)      | 0/60 (0)       | 4/60 (7)        | 12/60 (20)**    |
| FC adenocarcinoma                      | 0/60 (0)  | 0/60 (0)      | 0/60 (0)       | 0/60 (0)        | 3/60 (5)        |
| <b>Females</b>                         | <b>0 ppm</b>  | <b>75 ppm</b> | <b>200 ppm</b> | <b>1200 ppm</b> | <b>6000 ppm</b> |
| Diffuse FC hyperplasia/<br>hypertrophy | 1/60 (2)  | 1/59 (2)      | 0/60 (0)       | 28/60 (47)***   | 59/60 (98)***   |
| Focal FC hyperplasia                   | 0/60 (0)  | 1/59 (2)      | 0/60 (0)       | 4/60 (7)        | 8/60 (13)*      |
| FC adenoma                             | 0/60 (0)  | 0/59 (0)      | 0/60 (0)       | 1/60 (2)        | 2/60 (3)        |
| FC adenocarcinoma                      | 0/60 (0)  | 0/59 (0)      | 0/60 (0)       | 0/60 (0)        | 0/60 (0)        |

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Mortality was high in the high dose-males as 53/55 of them died on study. 8 of these were killed in extremis at weeks 11 and 12 as they showed a fracture of the nasal bone and subsequent dyspnoea (rhinorrhagia) which was attributed to the feeding stations (not test substance related). The main causes of death noted in males of the same dose group were nephropathy (22 rats), thyroid follicular cell tumours (10 rats) and leukaemia (6 rats). The severe nephropathy was associated with hyperplasia of the parathyroid,

demineralization of the bone and metastatic calcification in various organs. It should be noted that leukaemia is common in the F344 rat. At this dose level, the MTD was exceeded.

The adenomas were considered related to treatment and assumed to result from effects on thyroid hormone production or release, a conclusion based on the results from a mechanistic study including the following experiments:

- Measurement of T3, T4 and TSH, drug intake, liver and thyroid weights and total cholesterol in serum. Measurements of enzyme induction: cytochrome P450, cytochrome b5, NADPH-cytochrome c reductase, UDP-glucuronosyltransferase) and microsomal protein (rat)
- Additional administration of thiophanate-methyl with daily T4 supplementation, measurements of body weight, thyroid and liver weights and total cholesterol levels (rat)
- Peroxidase activity measurements (pig) using PTU as a reference substance
- Proliferation of liver cells (mice and rats) using PB as a reference substance

As the increase of thyroid follicular cell adenomas was only statistically significant in high dose males and the dose seems to have been above the maximum tolerated dose, the tumours are not considered to demonstrate a carcinogenic potential of thiophanate-methyl.

There were no thyroid tumours noted in mice but a statistically significant increase of hepatocellular adenomas was observed in animals males and females administered dietary concentrations of >3000 ppm thiophanate-methyl. In addition, one hepatocellular carcinoma was observed in the second lowest dose and in the top dose male group. In the high dose group, there was a concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis which is not considered to be test-substance related and the MTD is therefore not considered to have been reached in this study.

The previous evaluation considered this effect the tumours to result from the induction of the cytochrome P450 drug metabolising system, an effect known to occur in mice following exposure to xenobiotics. This conclusion, which was shared by the study author, was based on the results from the mechanistic study showing an increased liver weight and increased proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. However, for this review, this data is not considered to demonstrate that the hepatocellular adenomas observed arise due to a phenobarbital-like mode of action lacking human relevance (see section 10.12).

**Table 42: Incidence of mouse hepatocellular adenomas.**

| Dose level (ppm)       | Incidence of hepatocellular adenomas (%) |             |                       |                |                 |
|------------------------|--|-------------|-----------------------|----------------|-----------------|
|                        | 0  | 150         | 640                   | 3000           | 7000            |
| <b>Males</b>           |  |             |                       |                |                 |
| Unscheduled necropsies | 0/10 (0)                                 | 0/11 (0)    | 0/14 (0)              | 2/16 (12.5)    | 6/24 (25)       |
| Terminal necropsies    | 4/40 (10)                                | 8/39 (20.5) | 7/36 (19.4)           | 17/34 (50) **  | 18/26 (69.2)**  |
| Combined               | 4/50 (8)                                 | 8/50 (16)   | 7/50 (14)             | 19/50 (38)**   | 24/50 (48)**    |
| <b>Females</b>         |  |             |                       |                |                 |
| Unscheduled necropsies | 0/12 (0)                                 | 0/13 (0)    | 0/15 (0)              | 0/17 (0)       | 2/23 (8)        |
| Terminal necropsies    | 0/38 (0)                                 | 0/37 (0)    | 3/35 (8.6)            | 8/33 (24.2) ** | 16/27 (59.3) ** |
| Combined               | 0/50 (0)                                 | 0/50 (0)    | 3/50 (6) <sup>a</sup> | 8/50 (16)**    | 18/50 (36)**    |

\*\*p<0.01

<sup>a</sup> outside historical control range (HCD: Males: 0-16.3% (mean 8.2%); Females: 0-2.7% (mean 1.4%))

### 10.9.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), substances are classified for carcinogenicity in **Category 1** (known or presumed human carcinogens) on the basis of epidemiological and/or animal data. Category 1 is subcategorised into 1A if the substance is “*known to have carcinogenic potential for humans*,

*classification is largely based on human evidence” and 1B if “presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.”*

As there is no human data available for thiophanate-methyl that may be relevant for carcinogenicity, criteria for category 1A are not fulfilled.

For classification in category 1B evidence may be derived from “[...] *animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen) [...] In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.*”

Sufficient evidence from animal studies is explained as “*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. [...]*”

The placing of a substance in **Category 2** (suspected human carcinogens) “*is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.*”

Limited evidence from animal studies is explained as “*data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.*”

The significance of tumours observed in the chronic and carcinogenicity studies, *i.e.* thyroid follicular cell adenomas in rats and hepatocellular adenomas in mice is discussed below based on considerations included in the CLP guidance:

### **(a) tumour type and background incidence;**

**Rats:** thyroid follicular cell adenomas. Three cases of adenocarcinoma in the highest dose group. The study report does not include historical control data.

**Mice:** hepatocellular adenomas. According to information stated in the study report, the historical control incidence of hepatocellular adenoma compiled by Charles River Laboratories for their CD-mice on 18-month studies for the time period 1978-1984s are<sup>3</sup>:

Males: 0-16.3% (mean 8.2%)

Females: 0-2.7% (mean 1.4%)

For WIL Research Laboratories, Inc., the incidences recorded in the “most recent” 18-month carcinogenicity study were 16.4% (9/55) and 1.8% (1/55) in males and females respectively. The frequencies observed in the study are outside of the background incidence and the tumours can thus be considered to result from treatment with thiophanate-methyl.

### **(b) multi-site responses;**

**Rats:** The only tumour type observed at higher frequency than in controls was thyroid follicular cell adenoma in males.

**Mice:** The only tumour type observed at higher frequency than in controls was hepatocellular adenoma.

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<sup>3</sup> Spontaneous Neoplastic Lesions in the CrI:CD-1@(ICR)BR Mouse, Charles River Laboratories, Inc.

**(c) progression of lesions to malignancy;**

**Rats:** Follicular cell adenocarcinomas were observed in 3/60 high dose males compared to none in controls. This may indicate a progression into malignancy.

**Mice:** Hepatocarcinoma was observed in one male of the high dose group (7000 ppm) and in one male of the second lowest (640 ppm) dose group. Considering the lack of a dose-response, this is not considered to indicate progression into malignancy.

**(d) reduced tumour latency;**

**Rats:** Follicular cell hyperplasia and hypertrophy were noted in both sexes from the two highest dose groups both at interim sacrifice (12 months) and at terminal sacrifice (24 months). Follicular cell adenocarcinomas were only observed at terminal sacrifice indicating slow tumour development.

**Mice:** At interim sacrifice (9 months), an increase in hepatocellular centrilobular hypertrophy was observed in mice from the two highest treatment groups and mid-dose females. Moreover, a higher severity grade was observed in high dose animals and in the males of the next highest treatment group. Hepatocellular adenomas were only observed at terminal sacrifice indicating slow tumour development.

**(e) whether responses are in single or both sexes;**

**Rats and mice:** The tumour types observed in rats and mice occurred in both sexes but the increase of thyroid follicular cell adenomas was only statistically significant in high dose male rats.

**(f) whether responses are in a single species or several species;**

Follicular cell adenocarcinomas and hepatocellular adenomas were observed in rats and mice, respectively.

**(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;**

No information available.

**(h) routes of exposure;**

Information restricted to studies performed using oral administration (via diet).

**(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;**

A comparative in vitro metabolism study is available (RAR Vol 3, B.6.1.4) and showed no differences in metabolism between humans and rats.

**(j) the possibility of a confounding effect of excessive toxicity at test doses;**

**Rats:** There was an increase in mortality in the top dose group which was partially linked to the thyroid tumours. Although the high rate of mortality could, at least in part, be due to excessive toxicity, the treatment relationship of thyroid tumours was considered real. Follicular cell adenomas of the thyroid were only statistically significant in animals administered the highest dietary concentration (6000 ppm). Adenocarcinomas were also only observed at this dose level. Mortality was high at 6000 ppm as 53 of 55 males died. 8 of these were killed in extremis at weeks 11 and 12 as they showed a fracture of the nasal bone and subsequent dyspnoea (rhinorrhagia) which was attributed to the feeding stations (not test substance related). The main causes of death noted in males of the same dose group were nephropathy (22 rats), thyroid

follicular cell tumours (10 rats) and leukaemia (6 rats). The severe nephropathy was associated with hyperplasia of the parathyroid, demineralization of the bone and metastatic calcification in various organs. It should be noted that leukaemia is common in the F344 rat. At this dose level, the MTD was exceeded.

**Mice:** Hepatocellular adenomas occurred in all treated animals but the frequencies were only statistically significant in the two highest treatment groups (3000 and 7000 ppm). In the high dose group, there was a concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis which is not considered to be test-substance related and the MTD is therefore not considered to have been reached in this study.

**(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.**

The substance induces aneuploidy and clastogenicity in somatic cells and could thus be suspected to have a carcinogenic potential at levels above a threshold level. However, since only a single tumour type was observed in rats and mice there are no clear indications of a link between genetic instability and cancer.

**Rats:** Increased thyroid weight with hypertrophy/hyperplasia and sometimes accompanying effects on thyroid hormones were observed in many of the repeated dose toxicity studies available (including studies on short-term toxicity, long-term toxicity reproduction toxicity and neurotoxicity). In many of the studies there was also effects on the liver which could possibly indicate an up-regulation of the thyroid in response to increased hepatic clearance of T4 by uridine diphospho-glucuronosyltransferase (UDPGT). This is a rodent-specific effect generally considered to lack relevance for humans. Data from the mechanistic study indicate that the hypertrophy of the thyroid and the TSH response is counteracted by T4 supplementation supporting effects being due to a negative feedback mechanism. Data also indicate induction of cytochrome P450 and related drug metabolising enzymes including an increase of UDPGT in the high dose group. However, since T4 supplementation did not influence liver weight and since the pattern differed from phenobarbital, the increased UDPGT does not seem to be the sole explanation for the thyroid effects observed. The results indicated an inhibition of thyroid peroxidase in swine thyroids and this seems to be the principal reason for the T4 depression (see section 10.12-2). Therefore, it is not considered safe to exclude that the thyroid effects observed could be relevant for humans.

**Mice:** Data in the mechanistic study suggest that hepatocellular adenomas result from induction of the cytochrome P450 drug metabolising system. However, the data available to support this claim is limited to an increased liver weight and proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. This is not considered to conclusively link tumours to a phenobarbital-like mode of action lacking human relevance.

**10.9.3 Conclusion on classification and labelling for carcinogenicity**

Life-time exposure to thiophanate-methyl resulted in an increased frequency of thyroid adenomas in rats and hepatocellular adenomas in mice. Human relevance cannot be excluded; however, the tumour types are mainly benign. MTD seems to have been met in the rat study but not in the mouse study. Overall, data is considered as “limited” evidence of carcinogenicity and classification in **Carc. 2 H351** is proposed for thiophanate-methyl. for thiophanate-methyl.

**10.10 Reproductive toxicity**

**10.10.1 Adverse effects on sexual function and fertility**

**Table 43: Summary table of animal studies on adverse effects on sexual function and fertility**

| Test substance<br>Test type<br>Method<br>Guideline | Route of exposure | Species<br>Strain<br>Sex<br>No./group | Exposure Period | Doses | Main findings | Reference |
|--|-------------------|---------------------------------------|-----------------|-------|---------------|-----------|
|--|-------------------|---------------------------------------|-----------------|-------|---------------|-----------|

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|  |                       | Vehicle  |   |  |   |                            |
|--|-----------------------|--|---|--|---|----------------------------|
| <p>Thiophanate-methyl (Topsin M*)</p> <p>2-generation reproduction study</p> <p>OECD 416</p> <p>Acceptable</p>     | <p>Oral (in diet)</p> | <p>Rat Sprague-Dawley CrI:CD (SD)BR M, F 25/sex/dose</p> | <p>P (M, F): 14 weeks prior to mating, during mating and until sacrifice after weaning of the F1 generation. F1 (M, F): 14 weeks maturation period after weaning, during mating and until weaning of the F2a generation. Because of an unusually high pup loss during the weaning period of the F2a pups a second mating was performed. The F1 animals were maintained on test substance/diet for six weeks and then allowed to mate with the same partner as during the first mating to produce an F2b-litter. Exposure was continued until sacrifice after weaning of the F2b generation.</p> | <p>0, 200, 630 and 2000 ppm (equivalent to 0, 14.6, 46.0 and 147.1 mg/kg bw/day in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/day in females)</p>         | <p><u>Parental:</u><br/><u>200 ppm:</u><br/>↑ thyroid weight (F: 18%)<br/>- histopathological changes in the thyroid (hypertrophy, hyperplasia) and liver (hypertrophy)</p> <p><u>Notable effects at higher doses:</u><br/>↑ thyroid and liver weights at 630 and 2000 ppm<br/>- hepatocyte hypertrophy and thyroid hypertrophy and hyperplasia at 630 and 2000 ppm<br/>↑ TSH at 2000 ppm<br/>↓ T<sub>4</sub> at 2000 ppm (M)<br/>↓ bw gain at 2000 ppm</p> <p><u>Offspring:</u><br/><u>200 ppm:</u><br/>↓ bw (-9%)</p> <p><u>Notable effects at higher doses:</u><br/>↓ bw (-10-14% at 630 ppm; -13-16% at 2000 ppm)</p> | <p>RAR Vol 3 B.6.6.1.1</p> |
| <p>Thiophanate-methyl</p> <p>3-generation reproduction study</p> <p>OECD 416 with deviations</p> <p>Supportive</p> | <p>Oral (in diet)</p> | <p>Rat CD 10M, 20F/dose</p>                              | <p>F0 animals received the test substance for 60 days prior to mating and during the mating period. Following weaning of the first litter (Fla), the F0 mated again, thus producing the Flb litter. From the Flb litter 10 males and 20 females were maintained on dietary treatment for 60 days before they were allowed to mate. After weaning of the F2a litter, the animals of the Flb litter remated, resulting in the</p>   | <p>0, 40, 160 and 640 ppm (equivalent to approximately 0, 2.7, 10.6 and 43.3 mg/kg bw/day in males; 0, 3.1, 12.2 and 48.5 mg/kg bw/day in females)</p> | <p>No adverse effects were observed in the study.</p>   | <p>RAR Vol 3 B.6.6.1.2</p> |

|  |  |  |   |  |  |  |
|--|--|--|---|--|--|--|
|  |  |  | F2b litter. With the F2b litter the above described procedure was repeated and thus a third generation (F3a, F3b) was formed. |  |  |  |
|--|--|--|---|--|--|--|

\* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95.93 % in the two batches used in the study.

**Table 44: Summary table of human data on adverse effects on sexual function and fertility**

No data.

**Table 45: Summary table of other studies relevant for toxicity on sexual function and fertility**

No data.

### 10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Two studies on reproduction toxicity are available in rats. The studies are thoroughly presented in Annex I (Volume 3 to the RAR).

The first study was performed in accordance with GLP and mainly followed OECD 416. There were no adverse effects observed on fertility parameters. At 2000 ppm, pup weights were reduced by up to 11% (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16% reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12% during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13%; f: -16%) with no corresponding effect on maternal body weight. It is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The finding are therefore not considered relevant for classification.

The second study was not performed according to GLP and had several deviations compared to OECD 416. The purity of the test substance was not stated and the study is considered to provide supportive information only. No adverse effects on reproduction or parental toxicity were observed in the study and NOAEL for both effects were therefore was 640 ppm (corresponding to approximately 43 mg/kg bw/day in males and 49 mg/kg bw/day in females).

### 10.10.3 Comparison with the CLP criteria

According to CLP Guidance Annex I: 3.7.2.4.3, “Classification is not necessarily the outcome in the case ... when there is only a small reduction in foetal/pup weight ...”.

A slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. Taken together, the reduced pup weight is therefore not considered to justify classification for reproductive toxicity.

### 10.10.4 Adverse effects on development

**Table 46: Summary table of animal studies on adverse effects on development**

| Test substance<br>Test type<br>Method<br>Guideline | Route of exposure | Species<br>Strain<br>Sex<br>No./group<br>Vehicle | Exposure Period | Doses                 | Main findings  | Reference                |
|--|-------------------|--|-----------------|-----------------------|--|--------------------------|
| Thiophanate-methyl                                 | Oral (gavage)     | Rat<br>COBS CD                                   | GD 6-19         | 0, 100, 300 and 1000, | <u>Maternal toxicity:</u><br><u>1000 mg/kg bw/day:</u> | RAR Vol 3<br>B.6.6.2.2.1 |

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| Test substance<br>Test type<br>Method<br>Guideline                                       | Route of exposure   | Species<br>Strain<br>Sex<br>No./group<br>Vehicle  | Exposure Period   | Doses  | Main findings   | Reference             |
|--|---|---|---|--|---|-----------------------|
| (Topsin-M*)<br><br>Developmental toxicity study<br><br>OECD 414<br><br>Acceptable        |   | 25 dams/dose<br><br>5% aqueous Arabic gum   |   | mg/kg bw/day   | ↓ adjusted bw gain (-22%)<br><br><u>Developmental toxicity:</u><br>No adverse effects   |                       |
| Thiophanate-methyl<br><br>Developmental toxicity study<br><br>OECD 414<br><br>Supportive | Oral (gavage)   | Rabbit New Zealand white<br><br><u>Range-finding study:</u><br>4 dams/dose<br><br><u>Main study:</u><br>15 dams/dose<br><br>1% (w/v) aqueous methylcellulose suspension | GD 6-28   | <u>Range-finding study:</u><br>0, 10, 30, 100, 150, 300 and 600 mg/kg<br><br><u>Main study:</u><br>0, 2, 6 and 20 mg/kg bw/day | <b>Range-finding study:</b><br>↓ bw and food consumption at 30 mg/kg bw/day and above<br>↑ mortality at 150 mg/kg bw/day and above (1 at 150, 1 at 300, 2 at 600 mg/kg/bw/day)<br><br><b>Main study:</b><br><u>Maternal toxicity:</u><br><u>6 mg/kg bw/day:</u><br>↓ bw GD 6-12 (-30 g compared to pre-dosing)<br><br><u>20 mg/kg bw/day:</u><br>↓ bw GD 6-12 (-130 g compared to pre-dosing)<br><br><u>Developmental toxicity:</u><br><u>6 and 20 mg/kg bw/day:</u><br>↑ supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis | RAR Vol 3 B.6.6.2.2.2 |
| Thiophanate-methyl<br><br>Developmental toxicity study<br><br>OECD 414<br><br>Acceptable | <u>Range-finding study:</u> Oral (gavage) and dietary<br><br><u>Main study:</u> Oral (gavage) | Rabbit New Zealand white<br><br><u>Range-finding study:</u><br>6 dams/dose<br><br><u>Main study:</u><br>15 dams/dose<br><br>1% (w/v) aqueous methylcellulose suspension | <u>Range-finding study:</u><br>GD 6-18<br><br><u>Main study:</u><br>GD 6-28 | <u>Range-finding study:</u><br>0, 5, 10, 20, 40 and 80 mg/kg/day<br><br><u>Main study:</u><br>0, 5, 10, 20 and 40 mg/kg/day    | <b>Range-finding study:</b><br><u>Maternal toxicity:</u><br><u>40 mg/kg bw/day:</u><br>↑ abortions (3/6 does) (gavage group)<br>↓ bw and food consumption<br><br><u>80 mg/kg bw/day:</u><br>↑ abortions (3/6 does) (gavage group)<br>↓ bw and food consumption<br><br><u>Developmental toxicity:</u><br><u>80 mg/kg bw/day:</u><br>↑ resorptions (gavage group)<br>↑ number of thoracic vertebrae<br>↓ number of lumbar vertebrae<br>↑ number of rib pairs<br><br><b>Main study:</b><br><u>Maternal toxicity:</u><br><u>20 mg/kg bw/day:</u><br>↓ bw gain (-12%) compared to controls<br>↓ food consumption     | RAR Vol 3 B.6.6.2.2.3 |

| Test substance<br>Test type<br>Method<br>Guideline   | Route of exposure | Species<br>Strain<br>Sex<br>No./group<br>Vehicle                            | Exposure Period | Doses                                 | Main findings   | Reference             |
|--|-------------------|---|-----------------|---------------------------------------|---|-----------------------|
|  |                   |   |                 |                                       | <u>40 mg/kg bw/day:</u><br>↓ bw gain (-76%) compared to controls<br>↓ food consumption<br>↓ faecal output<br><br><u>Developmental toxicity:</u><br><u>40 mg/kg bw/day:</u><br>↑ supernumerary thoracic ribs |                       |
| Thiophanate-methyl<br><br>Developmental toxicity study<br><br>OECD 414 with deviations<br><br>Supportive | Oral (gavage)     | Mouse<br>ICR<br><br>20 females/dose<br><br>5% aqueous Arabic gum suspension | GD 1-15         | 0, 40, 200, 500 and 1000 mg/kg bw/day | <u>Maternal toxicity:</u><br>No adverse effects observed<br><br><u>Developmental toxicity:</u><br><u>1000 mg/kg bw/day:</u><br>↓ number of living foetuses  | RAR Vol 3 B.6.6.2.2.5 |

\* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 97.2%.

**Table 47: Summary table of human data on adverse effects on development**

No data.

**Table 48: Summary table of other studies relevant for developmental toxicity**

No data.

**10.10.5 Short summary and overall relevance of the provided information on adverse effects on development**

Studies are available on developmental toxicity in rats, rabbits and mice with the rabbit being the most sensitive species. The studies are thoroughly presented in Annex I (Volume 3 to the RAR).

In the rat study, performed in accordance with GLP and OECD 414, no adverse effects on development were observed at doses up to 1000 mg/kg bw/day. At this dose, there was a 22% reduction in adjusted body weight gain. NOAEL for maternal toxicity was thus found to be 300 mg/kg bw/day.

In the first rabbit study, also performed in accordance with GLP and OECD 414, there was a positive trend for supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis. NOAEL for developmental toxicity was 2 mg/kg bw/day based on these findings. NOAEL for maternal toxicity was 2 mg/kg bw/day based on slight reductions in body weight at 6 mg/kg bw/day. The study is considered to be supportive as it seems some animals may have suffered from infections (please refer to study summary in Annex I).

The second rabbit study was performed in accordance with GLP and OECD 414 and gave higher NOAEL values than the first rabbit study. NOAEL for developmental toxicity was 20 mg/kg bw/day based on supernumerary thoracic ribs at 40 mg/kg bw/day and NOAEL for maternal toxicity was 10 mg/kg bw/day based on minimal, transient but significant reduction in maternal body weight gain and significantly reduced absolute and relative feed consumption values for the entire dosage period at 20 mg/kg bw/day. The 40 mg/kg/day dosage produced adverse clinical observations (reduced faecal output) and significantly reduced body weight gain and absolute and relative feed consumption values for the entire dosage period.

The applicant<sup>4</sup> has submitted a statement discussing the different results obtained in the two rabbit studies. The overall summary and conclusion provided by the applicant was the following:

*“Both studies were critically reviewed to examine the relative strength of each study and to identify potential causes of observations unrelated to the test substance (e.g., intercurrent disease; spontaneous differences in litter sizes; historical experience and techniques of the Testing Facility). Based on my critical review of both studies, it is my opinion that the appropriate maternal NOAEL for thiophanate-methyl in pregnant rabbits is 10 mg/kg/day, and the appropriate developmental NOAEL is greater than 20 mg/kg/day. Transient, significant maternal weight loss, an apparent effect at 20 mg/kg/day in the first study, did not occur at this dosage in the second study, even though a prolonged dosage period was used, indicating that compromised maternal health was a probable confounder in the first study. A dosage of 40 mg/kg/day was required to produce transient significant maternal weight losses followed by a significant reduction in maternal body weight gain in the second study. Transient, significant reductions in feed consumption values were produced by 20 and 40 mg/kg/day dosages in the second study, and the 40 mg/kg/day dosage also reduced faecal output.*

*Reanalysis of the foetal data from the first study indicates that the foetal observations were probably associated with deficient maternal health, technical problems, outlier observations and/or the relatively few animals available for evaluation. No foetal alterations attributable to thiophanate-methyl dosages as high as 20 mg/kg/day occurred in the second study. The results of the second study identify the developmental NOAEL as greater than 20 but less than 40 mg/kg/day. Developmental toxicity at the 40 mg/kg/day dosage consisted of increased incidences of extra thoracic ribs (supernumerary ribs), a reversible variation in ossification. Although this variation was identified as an effect of 6 and 20 mg/kg/day dosages of thiophanate-methyl in the first study, reanalyses of the data identifies that the incidences were within expected normal ranges and not statistically significant. All other variations in skeletal ossification identified in the first study also occurred at incidences that were not statistically significant and were not replicated in the second study, indicating that they were unrelated to the test substance.*

*It is my conclusion that appropriate maternal and developmental NOAELs for the rabbit should be based on the results of the more robust second study, in which larger numbers of maternal rabbits and fetuses were evaluated, a longer treatment period was used, and regarding foetal evaluations, analyses were based on the more appropriate unit, the litter.”*

The dossier submitter agrees that the second study (RAR Vol 3 B.6.6.2.2.3) was of higher reliability and that effects were seen at higher doses. It is unclear how the seemingly compromised maternal health in the first study (RAR Vol 3 B.6.6.2.2.2) affected the outcome of the study. The applicant was asked to recalculate the effects seen in the first study to obtain the grand litter mean. Statistical analyses showed a statistically significant dose-related trend supernumerary ribs, thickened ribs, 27 presacral vertebrae and asymmetric pelvis. The same effect, supernumerary ribs with an associated increase in the average for thoracic vertebrae, were noted in both studies. The first study is therefore considered to support this finding in the more robust second study.

The mouse study is considered to provide supportive information only as it was not performed in accordance with GLP and did not comply with OECD 414. No maternal toxicity was observed at doses up to 1000 mg/kg bw/day. At this dose, the average number of living foetuses was slightly but significantly ( $p < 0.05$ ) lower (9.7%), compared to the control group (10.9%). NOAEL for developmental toxicity is therefore considered to be 300 mg/kg bw/day.

### **10.10.6 Comparison with the CLP criteria**

According to Regulation (EC) No 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on evidence from humans or in 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) largely based on

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<sup>4</sup> The applicant in the EFSA process, *i.e.* applying for renewal of approval as an active substance in plant protection products.

animal data. The animal data required for iB classification “*shall provide clear evidence of an adverse effect on sexual function or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects*”.

Substances are classified in Category 2 when there is “*some evidence from humans or experimental animals... of an adverse effect on sexual function or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.*”

As there is no human data available for thiophanate-methyl, the criteria for category 1A are not fulfilled. The effects noted in the studies that are considered potentially relevant for classification are abortions and skeletal effects.

### **Abortions**

Abortions were noted in both developmental toxicity studies in rabbits. In the first rabbit study, a high level of foetal loss was observed in the range-finding study at 100 mg/kg bw/day and above. This dose level is close to the dose where mortality occurred (starting from 150 mg/kg bw/day) and this effect is therefore not considered relevant for the purpose of classification. One litter was lost by abortion on GD 23 at 30 mg/kg bw/day, also in the range-finding study. This was within the background control range, but involvement with treatment could not be excluded in view of the results obtained at the higher doses. This doe had a body weight on GD 20 that was reduced by 21% compared to the control group. In the main part of the study, one control, one low-dose, one mid-dose and two high-dose animals aborted, although the reasons for these findings remain unclear due to the possibly compromised maternal health status.

Abortions were also seen in the second developmental toxicity study. 3/6 does aborted at both 40 and 80 mg/kg bw/day in the range-finding study. Body weights, body weight gains and feed consumption values for the does that aborted were comparable to other does in the same dosage groups and there were no clinical observations prior to aborting. In the 40 mg/kg bw/day dose group there was a slight reduction in body weight gain compared to controls that was not considered adverse. At 80 mg/kg bw/day there was an adverse reduction in body weight gain compared to controls (-19%). The abortions in the range-finding study were considered treatment-related. However no abortions were observed at 40 mg/kg bw/day (the highest dose) in the main part of the study. It could possibly be discussed whether these findings would support a classification in Repr. 2 but the dossier submitter does not consider it justified as the abortions were not observed in the main part of the most reliable study.

### **Skeletal effects**

Three studies on the developmental toxicity of thiophanate-methyl are available, one in rats and two in rabbits. No signs of developmental toxicity was observed in the rat study. In both rabbit studies there was an increased incidence in supernumerary ribs with an associated increase in thoracic vertebrae. In the first rabbit study, there was also a statistically significant trend observed for thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis. These effects appeared to be dose-related and started at a dose with no apparent maternal toxicity; maternal body weight was reduced by 30 g on GD 6 at 6 mg/kg bw/day where the skeletal effects started and by approximately 180 g on GD 10 at the higher dose of 20 mg/kg bw/day, body weights then recovered and reached levels comparable to the control. The reliability of this study was questioned due to indications of impaired maternal health as several of the animals appeared to have suffered from infections.

In the second study, supernumerary ribs with an associated increase in thoracic vertebrae was also observed but occurred at a dose level which also produced maternal toxicity shown as reduced body weight gain (-76% compared to controls,  $p < 0.01$ ). It therefore seems likely that the effect was secondary to maternal toxicity.

According to CLP Guidance Annex I: 3.7.2.4.3, “*Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup weight or retardation of ossification seen in association with maternal toxicity*”.

**Conclusion on classification for developmental toxicity**

In conclusion, the effects observed in the studies with thiophanate-methyl are not considered to be neither “clear” nor “some” evidence of an adverse effect on development and the criteria for classification are thus not considered to be met.

**10.10.7 Adverse effects on or via lactation**

**Table 49: Summary table of animal studies on effects on or via lactation**

| Test substance<br>Test type<br>Method<br>Guideline   | Route of exposure | Species<br>Strain<br>Sex<br>No./group<br>Vehicle                 | Exposure Period   | Doses   | Main findings  | Reference              |
|--|-------------------|--|---|---|--|------------------------|
| Thiophanate-methyl<br>(Topsin M*)<br><br>2-generation reproduction study<br><br>OECD 416<br><br>Acceptable | Oral (in diet)    | Rat<br>Sprague-Dawley<br>CrI:CD<br>(SD)BR<br>M, F<br>25/sex/dose | P (M, F): 14 weeks prior to mating, during mating and until sacrifice after weaning of the F1 generation.<br>F1 (M, F): 14 weeks maturation period after weaning, during mating and until weaning of the F2a generation. Because of an unusually high pup loss during the weaning period of the F2a pups a second mating was performed. The F1 animals were maintained on test substance/diet for six weeks and then allowed to mate with the same partner as during the first mating to produce an F2b-litter. Exposure was continued until sacrifice after weaning of the F2b generation. | 0, 200, 630 and 2000 ppm (equivalent to 0, 14.6, 46.0 and 147.1 mg/kg bw/day in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/day in females)         | <u>Parental:</u><br><u>200 ppm:</u><br>↑ thyroid weight (F: 18%)<br>- histopathological changes in the thyroid (hypertrophy, hyperplasia) and liver (hypertrophy)<br><br><u>Notable effects at higher doses:</u><br>↑ thyroid and liver weights at 630 and 2000 ppm<br>- hepatocyte hypertrophy and thyroid hypertrophy and hyperplasia at 630 and 2000 ppm<br>↑ TSH at 2000 ppm<br>↓ T <sub>4</sub> at 2000 ppm (M)<br>↓ bw gain at 2000 ppm<br><br><u>Offspring:</u><br><u>200 ppm:</u><br>↓ bw (-9%)<br><br><u>Notable effects at higher doses:</u><br>↓ bw (-10-14% at 630 ppm; -13-16% at 2000 ppm) | RAR Vol 3<br>B.6.6.1.1 |
| Thiophanate-methyl<br><br>3-generation reproduction study<br><br>OECD 416 with deviations                  | Oral (in diet)    | Rat<br>CD<br>10M,<br>20F/dose                                    | F0 animals received the test substance for 60 days prior to mating and during the mating period. Following weaning of the first litter (F1a), the F0 mated  | 0, 40, 160 and 640 ppm (equivalent to approximately 0, 2.7, 10.6 and 43.3 mg/kg bw/day in males; 0, 3.1, 12.2 and 48.5 mg/kg bw/day in females) | No adverse effects were observed in the study.   | RAR Vol 3<br>B.6.6.1.1 |

|            |  |  |   |  |  |  |
|------------|--|--|---|--|--|--|
| Supportive |  |  | again, thus producing the Flb litter. From the Flb litter 10 males and 20 females were maintained on dietary treatment for 60 days before they were allowed to mate. After weaning of the F2a litter, the animals of the Flb litter remated, resulting in the F2b litter. With the F2b litter the above described procedure was repeated and thus a third generation (F3a, F3b) was formed. |  |  |  |
|------------|--|--|---|--|--|--|

\* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95.93 % in the two batches used in the study.

**Table 50: Summary table of human data on effects on or via lactation**

No data.

**Table 51: Summary table of other studies relevant for effects on or via lactation**

No data.

**10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation**

Reduced pup weight was noted in the 2-generation study in rats (RAR Vol 3 B.6.6.1.1). At 2000 ppm, pup weights were reduced by up to 11% (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16% reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12% during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13%; f: -16%) with no corresponding effect on maternal body weight.

**10.10.9 Comparison with the CLP criteria**

According to the Regulation (EC) No 1272/2008 (CLP) Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

- “(a) human evidence indicating a hazard to babies during the lactation period; and/or*
- (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*
- (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.”*

No data is available to address criterias (a) and (c).

A slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus

were exposed directly through the feed. The reduced body weight gain is therefore not considered “provide clear evidence of adverse effect in the offspring due to transfer in the milk”.

### 10.10.10 Conclusion on classification and labelling for reproductive toxicity

No classification is proposed for thiophanate-methyl.

### 10.11 Specific target organ toxicity-single exposure

**Table 52: Summary table of animal studies on STOT SE**

| Test substance/<br>Route   | Vehicle            | Method<br>Guideline                      | Species<br>Strain<br>Sex<br>No./dose | Dose levels   | LD <sub>50</sub> /LC <sub>50</sub>                                     | Reference            |
|--|--------------------|--|--------------------------------------|---|--|----------------------|
| Thiophanate-methyl<br><br>Oral   | Distilled<br>water | EPA OPP 81-1<br>(similar to<br>OECD 401) | Rat<br>Crj:CD(SD)<br>M, F<br>5/sex   | 5000 mg/kg<br>bw  | >5000 mg/kg<br>bw  | RAR Vol 3<br>B.6.2.1 |
| Thiophanate-methyl<br><br>Dermal                                       | Distilled<br>water | EPA OPP 81-2<br>(similar to<br>OECD 402) | Rabbit<br>Kbs:JW<br>5/sex            | 2000 mg/kg<br>bw  | >2000 mg/kg<br>bw  | RAR Vol 3<br>B.6.2.2 |
| Thiophanate-methyl<br><br>Inhalation<br>(4 hr, whole body,<br>aerosol) | -                  | EPA OPP 81-3<br>(similar to<br>OECD 403) | Rat<br>Crj:CD(SD)<br>M, F<br>5/sex   | 0.5 mg/L<br>(females<br>only), 1.0,<br>1.5, 1.6 and<br>1.9 mg/L | LC <sub>50</sub> M: 1.7<br>mg/L<br><br>LC <sub>50</sub> F: 1.9<br>mg/L | RAR Vol 3<br>B.6.2.3 |

M: male; F: female

| Test substance<br>Route<br>Duration of study  | Species<br>Strain<br>Sex<br>No./group<br>Vehicle                                 | Dose levels   | Main findings   | Reference              |
|---|--|---|---|------------------------|
| Thiophanate-methyl<br><br>Acute neurotoxicity<br><br>OECD 424<br><br>Oral<br>(gavage)                 | Rat<br>Sprague-Dawely<br><br>M, F<br><br>10/sex/dose<br><br>0.5% Methylcellulose | 0, 500, 1000 and<br>2000 mg/kg bw<br>(main study)<br><br>0, 50, 125, 500<br>and 2000 mg/kg<br>bw (extension<br>study) | No adverse effects  | RAR Vol 3<br>B.6.7.1.1 |
| Thiophanate-methyl<br><br>Oral<br>(capsule)<br><br>83-1. The study fulfils<br>OECD 409.<br><br>1 year | Dog<br>Beagle<br>M, F<br>4/sex/dose  | 0, 8, 40 and 200<br>mg/kg bw/day  | <u>8 mg/kg bw/day:</u><br>- ↑ Thyroid weight in females<br>(29%)<br><br><u>Notable effects at higher doses:</u><br>- Tremors shortly after dosing at<br>200 mg/kg bw/day<br>- ↓ Bw at 200 mg/kg bw/day (-<br>20%)<br>- Haematological changes (↓ Hct,<br>↓ Hb, ↓ RBC) in males at 200<br>mg/kg bw/day<br>- ↓ T <sub>4</sub> in males at 200 mg/kg<br>bw/day<br>- Histopathological changes in the<br>thyroid (hypertrophy and<br>hyperplasia) | RAR Vol 3<br>B.6.3.2.5 |
| Thiophanate-methyl<br><br>Oral  | Dog<br>Beagle<br>M, F  | 0, 50, 200 and<br>400 mg/kg<br>bw/day (800  | <u>50 mg/kg bw/day:</u><br>- histopathological changes<br>(minimal follicular cell  | RAR Vol 3<br>B.6.3.2.4 |

|  |                   |  |  |  |
|--|-------------------|--|--|--|
| <p>(capsule)<br/>90-day<br/>Acceptable</p> | <p>4/sex/dose</p> | <p>mg/kg bw/day for the first 7 weeks)</p> | <p>hypertrophy) in the thyroid (1m, 1f)<br/><br/> <u>Notable effects at higher doses:</u><br/>                 - One high dose male sacrificed in moribund condition at 800 mg/kg bw/day<br/>                 - ↓ Bw at 200 (-15-24%) and 400 mg/kg bw/day (-26-28%)<br/>                 - ↓ food consumption at 200 and 400 mg/kg bw/day<br/>                 - Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in females at 200 and 400 mg/kg bw/day and males at 400 mg/kg bw/day<br/>                 - Changes in biochemical parameters consistent with malnutrition and dehydration<br/>                 - ↓ T<sub>3</sub> and T<sub>4</sub> in females at 200 and 400 mg/kg bw/day and ↓ T<sub>3</sub> in males at 400 mg/kg bw/day<br/>                 - ↑ Liver and thyroid weights<br/>                 - Histopathological changes in the thyroid. Hypertrophy: incidence (0/8, 2/8, 5/8, 8/8) and severity increased with increased dose. Hyperplasia in 0/8, 0/8, 1/8, 7/8 at the respective dose levels.</p> |  |
|--|-------------------|--|--|--|

**Table 53: Summary table of human data on STOT SE**

No data.

**Table 54: Summary table of other studies relevant for STOT SE**

No data.

**10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure**

In the acute oral a toxicity study no clinical signs were noted. In the dermal toxicity study, the only clinical sign that was noted was erythema which as a local irritancy effect is of no relevance for STOT SE classification (see section 10.1-2). In the acute inhalation toxicity study, several clinical signs were observed and are presented in the table below.

**Table 55: Mortalities and clinical signs observed in acute inhalation toxicity study with thiophanate-methyl**

| Dose (mg/L) | Males   |           | Females   |           |
|-------------|---|-----------|---|-----------|
|             | Clinical signs  | Mortality | Clinical signs  | Mortality |
| 0.5         | Not tested  |           | -   | 0/5       |
| 1.0         | -   | 0/5       | Decreased motor activity (2/5, 1 d)<br>Convulsion (2/5, 1 d)<br>Low sensitivity (2/5, 1 d)  | 1/5       |
| 1.5         | Tremor (5/5, day 1)   | 0/5       | Tremor (4/5, 1 d)   | 0/5       |
| 1.6         | Decreased motor activity (5/5, 1-3 h)<br>Tremor (5/5, 1-3 h)<br>Low sensitivity (5/5, 1-3 h)<br>Ptosis (5/5, 1 h)     | 0/5       | Decreased motor activity (5/5, up to 2 d)<br>Ventral position (2/5, 3 h – 1 d)<br>Convulsion (2/5, 1-2 d)<br>Tremor (5/5, 1 h - 1 d)<br>Low sensitivity (5/5, up to 2 d)<br>Incontinence of urine (2/5, 1 d)<br>Ptosis (2/5, 1-3 h) | 0/5       |
| 1.9         | Decreased motor activity (2/4, 1-3 h)<br>Convulsion (1/4, 1-3 h)<br>Low sensitivity (2/4, 1-3 h)<br>Ptosis (1/4, 3 h) | 5/5       | Decreased motor activity (3/5, 3 h - 1 d)<br>Hypotonia (1/5, 3 h)<br>Ventral position (1/5, 3 h)<br>Convulsion (3/5, 3 h - 1 d)<br>Ataxia (1/5, 3 h)<br>Low sensitivity (3/5, 3 h - 1 d)  | 3/5       |

Two subchronic toxicity studies with thiophanate-methyl are available with dogs (see section 10.12). The only effect observed that could be of relevance to classification in STOT SE is tremors seen in the 1-year study. The same effect was however not observed in the 90-day study.

The 1-year study was performed in accordance with GLP and OECD 409 although following the EPA 83-1 guideline. Thiophanate-methyl was administered to Beagle dogs (Marshall research animals, Inc., North Rose New York). Four animals per sex and dose group received the test substance orally via gelatine capsules daily at dose levels of 0, 8, 40 and 200 mg/kg bw/day. All animals survived throughout the study. Tremors were seen in all high dose animals and one mid-dose animal shortly after dosing on one or more occasions during the initial three weeks of study but were not observed subsequently. The tremors were noted in all high dose animals and one mid-dose animal which indicates a dose-response relationship.

The 90-day study was performed in accordance with GLP and OECD 409 although following the EPA 82-1 guideline. Beagle dogs (Marshall research animals, Inc., North Rose New York) received thiophanate-methyl daily for three months via gelatine capsules at dose levels of 0, 50, 200 and 800 mg/kg bw/day. At study week eight, the high dose (800 mg/kg bw/day) was reduced to 400 mg/kg bw/day and maintained at this dose level until the end of the administration period. Four animals per sex and dose group were used. No tremors were noted in this study.

**Table 56: Incidence of tremors noted in 1 year dog study with thiophanate-methyl**

| Dose (mg/kg bw/day) | Animal no | Sex | Days of study on which observed |                                 |                          |
|---------------------|-----------|-----|---------------------------------|---------------------------------|--------------------------|
|                     |           |     | Slight                          | Moderate or no degree indicated | Severe/tonic convulsions |
| 40                  | 3604      | F   | 13                              |                                 |                          |
| 200                 | 4101      | M   | 7 13                            | 1                               |                          |
| 200                 | 4102      | M   |                                 | 1                               |                          |
| 200                 | 4103      | M   | 7 13                            | 1 4 6                           |                          |
| 200                 | 4104      | M   | 7 12 13                         | 1                               |                          |
| 200                 | 4601      | F   | 7                               | 1                               |                          |
| 200                 | 4602      | F   |                                 | 1                               |                          |
| 200                 | 4603      | F   | 2 13 16 17                      | 1                               | 2 16 17                  |
| 200                 | 4604      | F   |                                 | 4                               |                          |

It is unclear why tremors were seen in the 1-year study but not in the 90-day study in the same species and at the same dose level. The dossier submitter does not consider it a clear indication of a neurotoxic effect as there are no indications of neurotoxicity in other studies and there were no other effects noted in this study that would support such a conclusion. However, it is also not a clearly non-specific effect caused by general toxicity (animals were relatively unaffected by dose at the beginning of the study) or stomach pain (the substance is not corrosive or irritating). But if caused by the administration itself, the tremors should have been observed in all dose groups including controls. In conclusion, the tremors seen are considered to be toxicologically relevant but the significance of the finding is unclear.

#### 10.11.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP), specific target organ toxicity (single exposure) categories 1 and 2 is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture, which are not covered by the other hazard classes. Category 3 covers transient effects, occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE).

##### Categories 1 and 2

In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. After inhalation exposure, clinical signs such as tremors, convulsions, low sensitivity, decreased motor activity, ptosis, ataxia and ventral position were observed at doses close to the LC<sub>50</sub> value. According to the CLP Guidance, care should be taken not to give a “double classification” for the same effect and as these effects occurred close to the lethal doses they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE.

No adverse effects were noted in the acute neurotoxicity study. In the one-year dog study, tremors were seen shortly after dosing in a dose-response related manner during the initial three weeks of study but were not observed subsequently. No tremors were observed in a 90-day study in the same species and at the same dose level. The significance of the finding is unclear and in the dossier submitter’s opinion does not justify classification in STOT SE. There were no relevant early effects noted in the other repeated dose studies.

According to the results of the available studies, thiophanate-methyl thereby does not fulfil the CLP criteria for STOT SE category 1 or 2.

##### Category 3

This evaluation is usually based primarily on human data. No human data is available for thiophanate-methyl. However, appropriate animal data, e.g. clinical signs or histopathology data from acute inhalation studies can also be used if available.

According to the CLP Guidance section 3.8.2.3, clinical signs (e.g. dyspnoea, rhinitis etc) and histopathology (hyperemia, edema, minimal inflammation, thickened mucous layer) observed in inhalation toxicity studies may justify classification for RTI and lethargy, lack of coordination, loss of righting reflex and ataxia

observed in animal studies may justify classification for NE. Such effects were not seen in the studies available with thiophanate-methyl and classification in this category is therefore not justified.

### 10.11.3 Conclusion on classification and labelling for STOT SE

No classification is proposed for thiophanate-methyl.

## 10.12 Specific target organ toxicity-repeated exposure

**Table 57: Summary table of animal studies on STOT RE**

| Test substance<br>Route<br>Duration of study  | Species<br>Strain<br>Sex<br>No./group<br>Vehicle | Dose levels  | Main findings   | Reference              |
|---|--|--|---|------------------------|
| Thiophanate-methyl<br><br>Oral<br>(dietary)<br><br>90-day<br><br>Acceptable   | Rat<br>Fischer-344<br>M, F<br>10/sex/dose        | 0, 200, 2200, 4200, 6200 and 8200 ppm (equivalent to 0, 13.9, 155, 293.2, 426.9 and 564.7 mg/kg bw/day in males; 0, 15.7, 173.4, 323.0, 478.8 and 647.3 mg/kg bw/day in females) | <u>200 ppm:</u><br>No treatment related changes in males.<br>- ↑ Thyroid weights in females (31%)<br><br><u>2200 ppm:</u><br>- ↑ Thyroid weights in males (39%) and females (50%)<br>- ↑ Liver and kidney weights<br>- Histopathological changes in thyroid (M, F), liver (M, F) and kidney (M)<br>- Haematological changes (↓ Hb (-4%),<br>- ↓ MCV, ↓ MCH, ↓ MCHC)<br>- Effects on clinical chemistry (e.g. ↓ plasma Ch-E in females)<br><br><u>Notable effects at higher doses:</u><br>Findings at LOAEL increased in severity and incidence with increased dose.<br>- ↓ Hb (-9%) at 8200 ppm.<br>- ↑ T <sub>3</sub> in females at 8200 ppm<br>- ↓ plasma Ch-E in females from 2200 ppm (>20%)<br>- ↑ plasma Ch-E in males from 4200 ppm (>20%) | RAR Vol 3<br>B.6.3.2.1 |
| Thiophanate-methyl<br><br>Oral<br>(dietary)<br><br>90-day<br><br>Supportive   | Rat<br>Sprague-Dawley<br>M, F<br>12/sex/dose     | 0, 12.8, 64, 320, 1600 and 8000 ppm (equivalent to 0, 1, 4, 20, 95 and 500 mg/kg bw/day in males; 0, 1, 5, 22, 110 and 660 mg/kg bw/day in females)                              | <u>8000 ppm:</u><br>- ↓ Bw (-28-30%)<br>- Haematological changes (↓ Hct, ↓ RBC)<br>- ↑ Relative liver (45-62%) and thyroid weights (51-62%)<br>- Histopathological changes in thyroid   | RAR Vol 3<br>B.6.3.2.2 |
| Thiophanate-methyl<br><br>Subchronic neurotoxicity OECD 424<br><br>Oral<br>(diet)<br><br>13 weeks<br><br>Acceptable | Rat<br>CD IGS<br><br>M, F<br><br>10/sex/dose     | 0, 100, 500, and 2500 ppm (males: 6.2, 30.3 and 149.6 mg/kg bw/day, females: 6.8, 34.9 and 166.3 mg/kg bw/day)   | <u>2500 ppm:</u><br>↑ absolute liver weight (m: 20%)<br>↑ absolute thyroid weight (m: 100%)<br>↓ bw (f: -10%)<br>↓ food consumption (f)   | RAR Vol 3<br>B.6.3.2.3 |

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|  |                                     |   |   |                        |
|--|-------------------------------------|---|---|------------------------|
| Thiophanate-methyl<br>Oral (dietary)<br>90-day<br>Supportive | Mouse<br>ICR<br>M, F<br>12/sex/dose | 0, 12.8, 64, 320, 1600 and 8000 ppm (equivalent to 0, 2, 10, 50, 250 and 1240 mg/kg bw/day in males; 0, 2, 11, 52, 231, 1630 mg/kg bw/day in females) | <u>8000 ppm:</u><br>- ↓ Bw (-11-17%)<br>- Haematological changes (↓ Hct, ↓ RBC)<br>- ↑ Liver weight (17-18%)<br>- Histopathological changes in liver  | RAR Vol 3<br>B.6.3.2.5 |
| Thiophanate-methyl<br>Oral (capsule)<br>90-day<br>Acceptable | Dog<br>Beagle<br>M, F<br>4/sex/dose | 0, 50, 200 and 400 mg/kg bw/day (800 mg/kg bw/day for the first 7 weeks)  | <u>50 mg/kg bw/day:</u><br>- histopathological changes (minimal follicular cell hypertrophy) in the thyroid (1m, 1f)<br><br><u>Notable effects at higher doses:</u><br>- One high dose male sacrificed in moribund condition at 800 mg/kg bw/day<br>- ↓ Bw at 200 (-15-24%) and 400 mg/kg bw/day (-26-28%)<br>- ↓ food consumption at 200 and 400 mg/kg bw/day<br>- Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in females at 200 and 400 mg/kg bw/day and males at 400 mg/kg bw/day<br>- Changes in biochemical parameters consistent with malnutrition and dehydration<br>- ↓ T <sub>3</sub> and T <sub>4</sub> in females at 200 and 400 mg/kg bw/day and ↓ T <sub>3</sub> in males at 400 mg/kg bw/day<br>- ↑ Liver and thyroid weights<br>- Histopathological changes in the thyroid. Hypertrophy: incidence (0/8, 2/8, 5/8, 8/8) and severity increased with increased dose. Hyperplasia in 0/8, 0/8, 1/8, 7/8 at the respective dose levels. | RAR Vol 3<br>B.6.3.2.4 |
| Thiophanate-methyl<br>Oral (capsule)<br>1 year<br>Acceptable | Dog<br>Beagle<br>M, F<br>4/sex/dose | 0, 8, 40 and 200 mg/kg bw/day   | <u>8 mg/kg bw/day:</u><br>- ↑ Thyroid weight in females (29%)<br><br><u>Notable effects at higher doses:</u><br>- Tremors shortly after dosing at 200 mg/kg bw/day<br>- ↓ Bw at 200 mg/kg bw/day (-20%)<br>- Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in males at 200 mg/kg bw/day<br>- ↓ T <sub>4</sub> in males at 200 mg/kg bw/day<br>- Histopathological changes in the thyroid (hypertrophy and hyperplasia)  | RAR Vol 3<br>B.6.3.2.5 |

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|  |  |  |   |                        |
|--|--|--|---|------------------------|
| Thiophanate-methyl<br>Short-term<br>Dermal<br>21 days<br>Acceptable  | Rabbit<br>New-Zeeland<br>White<br>M, F<br>5/sex/dose   | 0, 100, 300 and 1000<br>mg/kg bw/day in males  | No adverse effects were noted at any dose level.  | RAR Vol 3<br>B.6.3.3.1 |
| Thiophanate-methyl<br>Developmental<br>GD 6-19<br>Oral (gavage)<br>Acceptable  | Rat<br>COBS CD<br><br>25 dams/dose   | 0, 100, 300 and 1000,<br>mg/kg bw/day  | <u>Maternal toxicity:</u><br><u>1000 mg/kg bw/day:</u><br>↓ adjusted bw gain (-22%)<br><br><u>Developmental toxicity:</u><br>No adverse effects   | RAR Vol 3<br>B.6.6.2.1 |
| Thiophanate-methyl<br>Developmental<br>GD 6-28<br>Oral (gavage)<br>Supportive  | Rabbit<br>New Zealand<br>white<br><br><u>Range-finding study:</u><br>4 dams/dose<br><br><u>Main study:</u><br>15 dams/dose | <u>Range-finding study:</u><br>0, 10, 30, 100, 150, 300 and 600 mg/kg<br><br><u>Main study:</u><br>0, 2, 6 and 20 mg/kg bw/day | <b>Range-finding study:</b><br>↓ bw and food consumption at 30 mg/kg bw/day and above<br>↑ mortality at 150 mg/kg bw/day and above (1 at 150, 1 at 300, 2 at 600 mg/kg/bw/day)<br><br><b>Main study:</b><br><u>Maternal toxicity:</u><br><u>6 mg/kg bw/day:</u><br>↓ bw GD 6-12 (-30 g compared to pre-dosing)<br><br><u>20 mg/kg bw/day:</u><br>↓ bw GD 6-12 (-130 g compared to pre-dosing)<br><br><u>Developmental toxicity:</u><br><u>6 and 20 mg/kg bw/day:</u><br>↑ supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis | RAR Vol 3<br>B.6.6.2.2 |
| Thiophanate-methyl<br>Developmental<br><u>Range-finding study:</u><br>GD 6-18<br><u>Main study:</u><br>GD 6-28<br><br><u>Range-finding study:</u> Oral (gavage) and dietary<br><br><u>Main study:</u><br>Oral (gavage)<br>Acceptable | Rabbit<br>New Zealand<br>white<br><br><u>Range-finding study:</u><br>6 dams/dose<br><br><u>Main study:</u><br>15 dams/dose | <u>Range-finding study:</u><br>0, 5, 10, 20, 40 and 80 mg/kg/day<br><br><u>Main study:</u><br>0, 5, 10, 20 and 40 mg/kg/day    | <b>Range-finding study:</b><br><u>Maternal toxicity:</u><br><u>40 mg/kg bw/day:</u><br>↑ abortions (3/6 does) (gavage group)<br>↓ bw and food consumption<br><br><u>80 mg/kg bw/day:</u><br>↑ abortions (3/6 does) (gavage group)<br>↓ bw and food consumption<br><br><u>Developmental toxicity:</u><br><u>80 mg/kg bw/day:</u><br>↑ resorptions (gavage group)<br>↑ number of thoracic vertebrae<br>↓ number of lumbar vertebrae<br>↑ number of rib pairs<br><br><b>Main study:</b><br><u>Maternal toxicity:</u><br><u>20 mg/kg bw/day:</u><br>↓ bw gain (-12%) compared to controls<br>↓ food consumption     | RAR Vol 3<br>B.6.6.2.3 |

|  |  |  |  |  |
|--|--|--|--|--|
|  |  |  | <p><u>40 mg/kg bw/day:</u><br/>                 ↓ bw gain (-76%) compared to controls<br/>                 ↓ food consumption<br/>                 ↓ faecal output</p> <p><u>Developmental toxicity:</u><br/> <u>40 mg/kg bw/day:</u><br/>                 ↑ supernumerary thoracic ribs</p> |  |
|--|--|--|--|--|

**Table 58: Summary table of human data on STOT RE**

No data.

**Table 59: Summary table of other studies relevant for STOT RE**

No data.

**10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure**

All studies are thoroughly presented in the Annex I (Volume 3 to the RAR).

Two short-term studies with thiophanate-methyl are available with rats. In the first study, considered as acceptable, haematology effects (reduced Hb (-4%), MCV, MCH and MCHC) and dose-related histopathological effects, accompanied by weight changes of the affected organs, were observed in the thyroid, liver, adrenal and kidney at 2200 ppm and above. There was also an increase in total cholesterol, total protein, albumin and calcium in animals at 2200 ppm and above as well as increased T3 levels noted in males at 8200 ppm. There was also a statistically significant (p<0.01) and dose-related decrease in plasma cholinesterase starting from 2200 ppm in females and increase from 4200 ppm in males.

The second rat study was limited in scope and reporting and is considered to provide supportive information only. Effects were seen at the highest dose of 8000 ppm (500-660 mg/kg bw/day in males-females) and consisted of reduced body weights (28-30%), slight effects on haematology (reduced Hct by 4-10% and RBC count by 10-14%) and increased thyroid weights (absolute: 12% in males and 18% in females) accompanied by histopathological changes (follicles, cubic epithelium cells and a decrease of colloidal substance).

The neurotoxicity potential of thiophanate-methyl was investigated after subchronic exposure to rats. No neurotoxic effect was observed. However, in line with the results obtained in previous studies, reduced body weight/body weight gain and food consumption as well as increased liver (m: 20%) and thyroid weights (m: 100%) were noted at 2500 ppm.

In a mouse study, also considered to provide supportive information, treatment with thiophanate-methyl was associated with reduced bodyweights (-11-17%), slight effects on haematology (reduced Hct by 8-13% and RBC count by 7-12%) and increased liver weights accompanied by hepatocyte swelling and enlargement (hypertrophy) at 8000 ppm (1240-1630 mg/kg bw/day in males-females).

Two studies with thiophanate-methyl are available with dogs. In the 90-day study, treatment with thiophanate-methyl was associated with severe weight loss leading to the sacrifice of one dog at the highest dose (800 mg/kg bw/day) and lowering of the dose to 400 mg/kg bw/day from week 8 onwards. Evaluation of haematology and biochemistry values revealed changes consistent with malnutrition/dehydration in mid- and high-dose dogs. Reductions in Hb (-12-25%) and Hct (-10-24%) together with increases in platelet count and Activated Partial Thromboplastin Time were noted at 400 mg/kg bw/day with Hb also reduced by 11% in females at 200 mg/kg bw/day (all statistically significant). T3 levels were reduced in females at both 200 and 400 mg/kg bw/day. In spite of marked decreases in terminal body weights, absolute liver (6-11%) and thyroid weights (11-49%) were increased compared to concurrent controls. Statistical significance was however reached for relative weights only. Hypertrophy of the follicular epithelial cells of the thyroid was

seen in each treated group, but not in the control group. Minimal to marked hyperplasia of the follicular epithelium was also seen starting from 200 mg/kg bw/day.

In the one-year dog study, tremors were seen shortly after dosing on one or more occasions during the initial three weeks of study but were not observed subsequently. Reduced body weights (-20%) and slight decreases in Hb, Hct and RBC (in males only) were also observed as well as alterations in biochemistry. There was a reduction in T4 levels in males but no effects on T3 or TSH in any sex at any dose level. As in the subchronic dog study, absolute liver and thyroid weights were increased compared to concurrent controls but statistical significance was reached for relative weights only. Minimal to moderate hypertrophy and slight hyperplasia was also noted in the thyroid.

In the 90-day dermal toxicity study in rabbits, the only effect observed was a reduction in food consumption at the highest dose of 1000 mg/kg bw/day.

Studies relevant for STOT RE classification also include chronic toxicity studies and reproductive toxicity studies (section 10.10).

The findings in the long-term toxicity studies (see section 10.9) were consistent with results of subchronic studies identifying the liver, kidney and the thyroid as target organs also following long-term exposure. In addition, thiophanate-methyl was shown to induce anaemia in the rat.

The findings in the reproductive toxicity studies (see section 10.10) were also consistent with results of subchronic studies identifying the liver and thyroid as target organs. Mortality was noted in one developmental toxicity study (RAR Vol 3 B.6.2.2.2), see below.

### 10.12.2 Comparison with the CLP criteria

Regulation (EC) No 1272/2008 (CLP), 3.9.2.7.3, states for STOT RE:

*“All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:*

*(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;*

*(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);*

*(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;*

*(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;*

*(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;*

*(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);*

*(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.*

Effects noted in studies with repeated exposure to thiophanate-methyl considered to be potentially relevant in terms of classification include mortality, haematological effects and effects on thyroid.

### Mortality

Mortality was noted in dogs and rabbits. In dogs, mortality was seen in a 90-day study (RAR Vol 3, B.6.3.4.2) at 800 mg/kg bw/day. This is above the Guidance Value for STOT RE 2 of 100 mg/kg bw/day for a 90-day study and not considered relevant for classification.

In rabbits, mortality was observed at 150 (1 animal), 300 (1 animal) and 600 (2 animals) mg/kg bw/day in a developmental toxicity study (RAR Vol 3 B.6.2.2.2). Exposure in this study was for 14 days (GDs 6-19) and mortality occurred on days 19 (150 mg/kg bw/day), 24 (300 mg/kg bw/day), 20 and 23 (600 mg/kg bw/day). It is therefore considered an effect of repeated administration of the test substance rather than an acute effect. As exposure was for 14 days *i.e.* 1/6 of 90 days, it is proposed to multiply the Guidance Values of 10 for Cat 1 and 100 for Cat 2 classification of 10 and 100 mg/kg bw/day, respectively, by a factor 6, giving adjusted Guidance Values for 14 days of 60 and 600 mg/kg bw/day for STOT RE 1 and 2, respectively. The mortalities occurred above 60 mg/kg bw/day but below 600 mg/kg bw/day and therefore justify classification in STOT RE 2.

### Haematological effects

Haematological effects were seen in both rats and dogs. In the 90-day study in dogs, Hb was reduced by 25% in males and 12% in females at 400 mg/kg bw/day and by 11% in females at 200 mg/kg bw/day, which is above the Guidance Value of 100 mg/kg bw/day for a 90-day study. There were also reductions in Htc, APTT and platelet count at the higher dose level but no other signs of anaemia observed in the study.

The effect was less pronounced in the 1-year dog study with Hb reduced by 14% in males at 12 months at 200 mg/kg bw/day but no reduction observed in females, and again above the Guidance Value.

Reductions in Hb, MCV, MCH, MCHC and PCV were also observed in the 90-day study in rats, but these reductions were of low magnitude (<10%) and were not accompanied of other effects indicative of anaemia, thus not justifying classification. In the 2-year study in rats, treatment-related anaemia (decreases of red blood cell count, haemoglobin, haematocrit, MCV, MCH and MCHC) was noted in both sexes at 300 mg/kg bw/day but not to any significant degree at lower dose levels. As these findings were above the Guidance Value, they also do not justify classification.

### Thyroid

Effects on thyroid were seen in rats and dogs in studies with repeated administration of thiophanate-methyl. Slight effects were also seen in the 2-year mouse study. The effects are usually first seen as hypertrophy which then progresses to hyperplasia and in the 2-year study in rats then to adenoma and in a few animals to carcinoma (albeit at doses exceeding the MTD). Effects on T4, T3 and TSH were also seen, see the table below.

**Table 60: Summary of *in vivo* data referring to findings in liver and thyroid (effects marked with bold indicate doses within the Guidance value for classification in STOT RE 2)**

| Study   | Species                     | Dose   | Liver effects                       | Thyroid effects                                     |
|---|-----------------------------|--|-------------------------------------|---|
| 90-day study  | Rat<br>RAR Vol 3, B.6.3.2.1 | <i>Guidance value STOT RE 2: 100 mg/kg bw/day</i>            |                                     |   |
|   |                             | 200 ppm<br>(M: 13.9 mg/kg bw/d;<br>F: 15.7 mg/kg bw/d)       | No effects                          | <b>F: Weight↑</b>                                   |
|   |                             | 2200 ppm<br>(M: 155.0 mg/kg bw/d;<br>F: 173.4 mg/kg bw/d)    | F: Weight↑<br>M, F: hypertrophy     | M, F: Weight ↑,<br>M, F:<br>Hypertrophy/hyperplasia |
|   |                             | 4200 ppm<br>(M: 293.2 mg/kg<br>bw/d;<br>F: 323.0 mg/kg bw/d) | M, F: Weight ↑<br>M, F: hypertrophy |   |
| 6200 ppm<br>(M: 426.9 mg/kg bw/d;<br>F: 478.8 mg/kg bw/d) |                             |  |                                     |   |

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|  |                                    |   |  |   |
|--|------------------------------------|---|--|---|
|  |                                    | 8200 ppm<br>(M: 564.7 mg/kg bw/d;<br>F: 647.3 mg/kg bw/d) |  |   |
|  | Dog<br>RAR Vol 3, B.6.3.2.4        | <i>Guidance value STOT RE 2: 100 mg/kg bw/day</i>         |  |   |
|  |                                    | 50 mg/kg bw/d   | No effects   | <b>M, F: hypertrophy;<br/>hyperplasia<br/>2 animals – minimal<br/>severity</b>                      |
|  |                                    | 200 mg/kg bw/d  | M, F: Weight↑  | F: T3, T4↓<br>M: Weight↑<br>M, F: hypertrophy or<br>hyperplasia                                     |
|  |                                    | 800/400 mg/kg bw/d  |  | M: T3↓, F: T3, T4↓<br>M: Weight↑<br>M, F: hypertrophy or<br>hyperplasia                             |
| Long-term toxicity<br>study                                    | Rat 2 year<br>RAR Vol 3, B.6.5.1   | <i>Guidance value STOT RE 2: 12.5 mg/kg bw/day</i>        |  |   |
|  |                                    | 75 ppm<br>(M: 3.3 mg/kg bw/d;<br>F: 3.8 mg/kg bw/d)       | No effects   | <b>No effects</b>   |
|  |                                    | 200 ppm<br>(M: 8.8 mg/kg bw/d;<br>F: 10.2 mg/kg bw/d)     | No effects   | <b>No effects</b>   |
|  |                                    | 1200 ppm<br>(M: 54.4 mg/kg bw/d;<br>F: 63.5 mg/kg bw/d)   | M, F: Weight ↑<br>M, F: hypertrophy  | M, F: Weight ↑<br>M, F:<br>hypertrophy/hyperplasia  |
|  |                                    | 6000 ppm<br>(M: 280.6 mg/kg bw/d;<br>F: 334.7 mg/kg bw/d) |  | M, F: Weight ↑<br>M, F:<br>hypertrophy/hyperplasia,<br>adenoma                                      |
|  | Dog 1 year<br>RAR Vol 3, B.6.3.2.5 | <i>Guidance value STOT RE 2: 25 mg/kg bw/day</i>          |  |   |
|  |                                    | 8 mg/kg bw/d  | No effects   | <b>F: Weight↑</b>   |
|  |                                    | 40 mg/kg bw/d   | No effects   | M, F: Weight↑<br>F: Hypertrophy, (M: T4↓,<br>interim: 6 m)  |
|  |                                    | 200 mg/kg bw/d  | M, F: Weight ↑   | M, F: Weight↑<br>M, F: Hypertrophy;<br>hyperplasia (2 animals)<br>(M: T4↓, interim 6 m and<br>12 m) |
|  | Mouse 18-m<br>RAR Vol 3, B.6.5.3   | <i>Guidance value STOT RE 2: 16.7 mg/kg bw/day</i>        |  |   |
|  |                                    | 150 ppm<br>(M: 23.7 mg/kg bw/d,<br>F: 28.7 mg/kg bw/d)    | No effects   | No effects  |
|  |                                    | 640 ppm<br>(M: 98.6 mg/kg bw/d,<br>F: 123.3 mg/kg bw/d)   | F: hypertrophy   | No effects  |
|  |                                    | 3000 ppm<br>(M: 467.6 mg/kg bw/d,<br>F: 557.9 mg/kg bw/d) | M,F: Weight ↑<br>M, F hypertrophy,<br>adenoma  | M: Weight ↑<br>TSH ↑ at 9 m but not at 18<br>m  |
| 7000 ppm<br>(M: 1078.8 mg/kg<br>bw/d, F: 1329.4 mg/kg<br>bw/d) |                                    | M, F: Weight ↑<br>M, F hypertrophy,<br>adenoma            | M, F: Weight ↑<br>TSH ↑ at 9 m but not at 18<br>m<br>F: T4 ↓ at 9 m but not at<br>18 m |   |
| Mechanism study<br>(in vivo)                                   | Rat<br>RAR Vol 3, B.6.5.1          | 6000 ppm<br>Male  | Weight↑<br>T-Cho↑<br>P-450, UDP-GT↑<br>PCNA↑, TPO ↓                                    | Weight ↑<br>T3, T4 ↓ , TSH ↑  |
| 2-gen study  | Rat                                | <i>Guidance value STOT RE 2: 40 mg/kg bw/day</i>          |  |   |

|                            |                             |   |   |  |
|----------------------------|-----------------------------|---|---|--|
|                            | RAR Vol 3, B.6.6.1.1        | 200 ppm<br>(M: 14.6 mg/kg bw/d,<br>F: 18.0 mg/kg bw/d)    | M, F: hypertrophy                               | <b>(F: Weight )<br/>M : hypertrophy<br/>/hyperplasia</b>                     |
|                            |                             | 630 ppm<br>(M: 46.0 mg/kg bw/d,<br>F: 55.4 mg/kg bw/d)    | M, F: Weight↑<br>M, F: hypertrophy              | M, F: Weight↑<br>M, F:<br>hypertrophy/hyperplasia                            |
|                            |                             | 2000 ppm<br>(M: 147.1 mg/kg bw/d,<br>F: 172.9 mg/kg bw/d) | M, F: Weight↑<br>M, F: hypertrophy              | M, F: Weight↑<br>M, F:<br>hypertrophy/hyperplasia<br>M, F: TSH ↑ , M: T4 ↓   |
| 90-day neurotoxicity study | Rat<br>RAR Vol 3, B.6.7.1.2 | <i>Guidance value STOT RE 2: 100 mg/kg bw/day</i>         |   |  |
|                            |                             | 100 ppm<br>(M: 6.2 mg/kg bw/d,<br>F: 6.8 mg/kg bw/d)      | No effects<br>(histopathology not investigated) | <b>No effects<br/>(histopathology and<br/>hormones not<br/>investigated)</b> |
|                            |                             | 500 ppm<br>(M: 30.3 mg/kg bw/d,<br>F: 34.9 mg/kg bw/d)    |   |  |
|                            |                             | 2500 ppm<br>(M: 149.6 mg/kg bw/d,<br>F: 166.3 mg/kg bw/d) | M: Weight↑<br>(histopathology not investigated) | M, F: Weight↑<br>(histopathology and hormones not investigated)              |

In addition to the thyroid effects there was also an effect on liver, as shown in the table. The mechanistic study (RAR Vol 3, B.6.5.1) showed that there was an upregulation of uridine diphosphoglucuronosyltransferase (UDPGT), indicating that the thyroid effects could be a result of increased hepatic clearance of T4. Rodents (especially the rat) are generally considered more sensitive to this effect than humans.

However, the mechanistic study also showed that thiophanate-methyl inhibited thyroid peroxidase (TPO) which oxidizes iodide ions in the thyroid to form iodine atoms for addition onto tyrosine residues on thyroglobulin for the production of T4 or T3. The study also showed that T4 supplementation had no influence on the increased liver weight and that phenobarbital, which was used as a reference substance, also induced liver cell proliferation and the drug metabolizing enzymes but caused only a very slight increase of TSH without thyroid hypertrophy, further indicating that the increased UDPGT was not the only mechanism behind the thyroid effects. Thus, it was concluded in the study that the inhibition of hormone synthesis in the thyroid due to an inhibition of TPO could be the main cause of the T4 depression.

In March 2017 a workshop was held at ANSES (FR) and the draft report from the workshop<sup>5</sup> provides in-depth information about the thyroid system, including differences between species and relevance of animal models to humans, case studies of thyroid-disrupting substances and recommendations on data interpretation and evaluation. It is concluded in the draft report is that a mode of action that involves upregulation of UDPGT is likely to be more relevant to humans than previously thought. There is also a case study of mancozeb where the mode of action was TPO inhibition. Thyroid effects were seen in all tested species (rat, mouse, dog and monkey) and it was concluded that this fact indicated that “the mechanism of TPO inhibition is probably also a relevant molecular initiating event in humans”.

The thyroid effects seen in the studies are therefore considered relevant for the purpose of classification.

The endocrine properties of thiophanate-methyl were discussed at EFSA’s PPR 162 meeting. All experts were of the opinion that thiophanate-methyl should be considered to be an endocrine disruptor (however not according to the interim criteria as laid out in Regulation (EC) No 1107/2009 Annex II point 3.6.5) and that the effects in the thyroid should be regarded as relevant for humans.

<sup>5</sup> Supporting the organisation of a workshop on thyroid disruption – Draft Final Report. December 2016

Comparison with Guidance values for classification in STOT RE 2 In the 90-day rat study, thyroid weights were increased in females by 31% compared to controls (statistically significant) at 16 mg/kg bw/day. At the next dose of 155 mg/kg bw/day and above, thyroid weights were increased in both sexes in a dose-related manner and the finding was accompanied by follicular hyperplasia and hypertrophy. Only the 31% weight increase in females was within the Guidance Value of 100 mg/kg bw/day for a 90-day study.

In the 2-generation study in rats (RAR Vol 3, B.6.6.1.1), the test substance was given continuously from the start of treatment until necropsy. Based on these data, information on exposure time for the P and F1 generation is given in the table below.

|         | Days of study |                              |                               |
|---------|---------------|------------------------------|-------------------------------|
|         | P generation  | F1 generation (first mating) | F1 generation (second mating) |
| Males   | 227 – 240     | 165                          | 143 – 157                     |
| Females | 241 – 249     | 165                          | 163 – 165                     |

Exposure was for maximum 240 days in males (the sex where most effects were seen), and a Guidance Value of approximately 40 mg/kg bw/day can then be calculated ( $90 / 240 = 0.375$ .  $100 \text{ mg/kg bw/day} \times 0.375 = 37.5 \text{ mg/kg bw/day}$ ). The following effects were observed below this dose level (200 ppm or 15-18 mg/kg bw/day): Thyroid hypertrophy was observed in 9/25 males at in the P generation and 3/25 males of the F1 generation. Incidences were lower in females with 1/24 females of the P generation and 0/24 in the F1 generation being affected. Thyroid hyperplasia in males was observed in 4-6/25 (study author-peer reviewer) animals the P generation and 4-1/25 (study author-peer reviewer) animals in the F1 generation at the same dose level. There was no hyperplasia noted in females at this dose, however thyroid weights were statistically significantly increased (9%). At doses above the Guidance Value, higher incidences of thyroid hypertrophy and hyperplasia were observed.

In a 2-year study in rats (RAR Vol 3, B.6.5.1), increased thyroid weights (m: 24%, f: 27%), morphological and functional changes in the thyroid and follicular cell hyperplasia and hypertrophy (m: 38%, f: 47%) were observed at 1200 ppm (54.4 and 63.5 mg/kg bw/day in male and female rats, respectively). This dose level is above the Guidance Value for a 2-year rat study of 12.5 mg/kg bw/day do therefore not justify classification in STOT RE 2.

In the 90-day dog study (RAR Vol 3, B.6.3.2.4), histopathological changes (minimal follicular cell hypertrophy) in the thyroid (1m, 1f) were observed at 50 mg/kg bw/day. At the next dose of 200 mg/kg bw/day (above the Guidance Value for a 90-day study) this finding increased in severity and incidence and hyperplasia and increased organ weight was observed as well as a reduction in T3 and T4.

In the 1-year dog study, thyroid weights were increased by 29% in females at 8 mg/kg bw/day (within the Guidance Value). Microscopic alterations attributed to test material administration were limited to the follicular epithelium of the thyroid gland with minimal to moderate hypertrophy in males (0/0, 0/0, 0/0, 4/4) and females (0/0, 0/0, 2/4, 3/4) and slight hyperplasia (group IV, one male and one female). These effects were noted above the Guidance Value.

**Table 61: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days**

| Study reference      | Effective dose (mg/kg/d) | Length of exposure | Extrapolated effective dose when extrapolated to 90-day exposure | Classification supported by the study |
|----------------------|--------------------------|--------------------|--|---------------------------------------|
| RAR Vol 3B.6.2.2.2   | 150-600                  | 14 d               | 25-100   | Yes                                   |
| RAR Vol 3, B.6.6.1.1 | 15-18                    | 240 d              | 40-48  | Yes                                   |
| RAR Vol 3, B.6.5.1   | 54.4 - 63.5              | 2 years            | Approx. 440  | No                                    |

### **10.12.3 Conclusion on classification and labelling for STOT RE**

Classification in **STOT RE 2** is proposed based on the mortalities seen in rabbits at 125, 300 and 600 mg/kg bw/day in a developmental toxicity study with 14 days exposure to thiophanate-methyl, as well as on thyroid effects (hypertrophy, hyperplasia and increased organ weight) in rats, supported by similar findings in dogs. The following hazard statement is proposed: **H373 – May cause damage to organs through prolonged or repeated exposure.**

## **10.13 Aspiration hazard**

### **10.13.1 Short summary and overall relevance of the provided information on aspiration hazard**

No data is available.

### **10.13.2 Comparison with the CLP criteria**

Not relevant as no data is available.

### **10.13.3 Conclusion on classification and labelling for aspiration hazard**

No classification is proposed for thiophanate-methyl.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

### 11.1 Rapid degradability of organic substances

All the information on rapid biodegradability are taken from the RAR for thiophanate-methyl, October 2017. In the sections further below study summaries have been copied from the RAR (and in a few cases from the DRAR on carbendazim).

The data on thiophanate-methyl and the major metabolite carbendazim (more toxic than the parent compound, therefore relevant for the environmental classification) that was considered reliable and relevant for C&L are summarised in the table below. For completeness, data on all metabolites and transformation products observed as >5% of the applied radioactivity are included in the table.

Where half-lives were determined by SFO (single first order kinetic equation) only the DT<sub>50</sub> is stated in the table (since the SFO DT<sub>90</sub> is always 3.32 x DT<sub>50</sub>). Where the quoted DT<sub>50</sub> was derived by other kinetic models, also the DT<sub>90</sub> is mentioned.

Reliable half-lives could not be determined for all metabolites/transformation products in all test systems. There may be various natural reasons for this, such as presence of metabolite only at low levels and/or only at few sampling points, variability between replicates and therefore failure to fulfil statistical requirements, no clear degradation pattern observed over the course of the study etc. Hence, the studies as such may very well be considered reliable despite a lack of reliable half-lives for all metabolites/transformation products observed.

Some of the metabolites not identified in the original studies were later identified. Molecular structures of the main metabolites/transformation products are shown in the next table.

**Table 62: Summary of relevant information on rapid degradability.**

| Method  | Results   | Remarks  | Reference  |
|---|---|--|--|
| OECD TG No 301 C                              | Study on ready biodegradability of Thiophanate-methyl.<br>The substance was not ready biodegradable (4% biodegradation). Primary degradation was observed, probably to the largest extent caused by hydrolysis.<br><br>The main metabolite was Carbendazim (18% after 28 days). Another metabolite observed above 5% was AV-1951 (7% after 28 days).  | Reliable   | Tsushima S. (2013)<br><br>(kinetic re-evaluation elsewhere)          |
| German guideline, conforms to US EPA TG 161-1 | Hydrolysis study on Thiophanate-methyl. Results at 22°C:<br><br>pH 5: Stable over 35 days.<br><br>pH 7: DT <sub>50</sub> 46.8 days (SFO).<br>Transformation products observed:<br>Carbendazim (28.6% after 33 days). Carbendazim was stable.<br>AV-1951 (8.1% after 33 days), half-life not available.<br><br>pH 9: DT <sub>50</sub> 1.0 day (SFO).<br>Transformation products observed:<br>Carbendazim (58.7% after 4 days). Carbendazim was stable. | Reliable<br>(though kinetic results for AV-1951 uncertain) | Soeda Y. & Nomura O. (1986)<br><br>(kinetic re-evaluation elsewhere) |

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| Method                               | Results  | Remarks  | Reference   |
|--------------------------------------|--|--|---|
|                                      | AV-1951 (24.9% after 4 days). DT <sub>50</sub> 5.4 days (SFO-SFO) but this DT <sub>50</sub> was considered uncertain.  |  |   |
| OECD TG No 111                       | Hydrolysis study on Carbendazim.<br>Degradation of Carbendazim was <10% after 5 days at pH 4 and 7 and 50°C. Therefore the main test was carried out at pH 9 at 45°C, 55°C and 65°C. The interpolated DT <sub>50</sub> at 20°C was 435 days (SFO).<br><br>Transformation product observed was 2-AB (not quantified).   | Reliable   | Shiotani H. (2003)<br><br>(kinetic re-evaluation elsewhere) |
| OECD TG No 309                       | Study on degradation of Thiophanate-methyl in lake water (pelagic system) at 20°C, at low and high dose. pH varied 7.6-9.0 over the test. Mineralisation was ≤0.5% (by study end at 30 days).<br>Thiophanate-methyl DT <sub>50</sub> 0.64 days (low dose, DFOP, DT <sub>90</sub> 5.0 days) and 2.2 days (high dose, SFO).<br><br>Observed metabolites at low dose:<br>Carbendazim, max 82.8% (14 days), DT <sub>50</sub> 64.8 days (DFOP-SFO),<br>FH-432, max 5.6% (1 day), half-life not available,<br>UM 1, max 11.4% (30 days), half-life not reliable,<br>UM 2, max 8.0% (30 days), half-life not available,<br>2-AB, max 9.5% (30 days), half-life not available.<br><br>Observed metabolites at high dose:<br>Carbendazim, max 73.4% (14 days), half-life not reliable<br>FH-432, max 11.3% (4 days), DT <sub>50</sub> 5.2 days (SFO-SFO)<br>UM 1, max 8.7% (30 days), half-life not available,<br>UM 2, max 5.3% (30 days), half-life not available,<br>2-AB, max 6.4% (30 days), half-life not available.  | Reliable<br>(but the duration of the study did not allow reliable estimation of degradation rates for all metabolites) | Hurst (2015)<br><br>(kinetic re-evaluation elsewhere)       |
| SETAC guideline and German guideline | Water/sediment study on Thiophanate-methyl, in pond and river systems, at 20°C. pH of waters 7.7-8.4, pH of sediments 7.6-7.8.<br>Mineralisation ( <sup>14</sup> CO <sub>2</sub> ) was 1.3-1.6% (after 100 days).<br>Thiophanate-methyl DT <sub>50</sub> 3.5 days (pond system, SFO) and 1.6 days (river system, SFO).<br><br>Observed metabolites in pond system (samples taken until 301 days):<br>Carbendazim, max 75.1% (16 days), DT <sub>50</sub> 76.2 days (SFO-SFO),<br>4-OH-TM, max 8.6% (30 days), DT <sub>50</sub> 32.4 days (SFO, calculated over decline from peak),<br>AV-1951, max 6.1% (2 days), half-life not available,<br>2-AB, max 7.5% (58 days), DT <sub>50</sub> 189 days (SFO, calculated over decline from peak),<br>M10, max 9.3% (140 days), half-life not reliable.<br><br>Observed metabolites in river system (samples taken until 100 days):<br>Carbendazim, max 81.6% (8 days), DT <sub>50</sub> 91.6 days (SFO-SFO),<br>4-OH-TM, max 9.5% (8 days), DT <sub>50</sub> 26.2 days (SFO, calculated over decline from peak),<br>AV-1951, max 6.3% (2 days), half-life not available<br>2-AB, max 6.5% (16 days), half-life not available. | Reliable   | Voelkl S. (2001)<br><br>(kinetic re-evaluation elsewhere)   |
|                                      | Water/sediment study on Carbendazim in two systems (Bickenbach   | Reliable   | Knoch E.  |

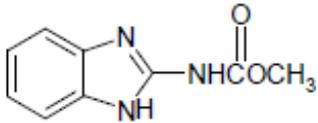
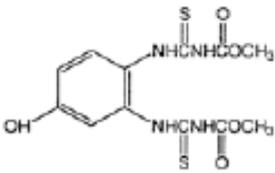
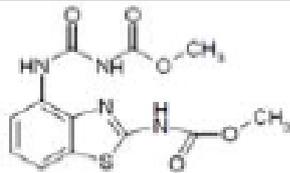
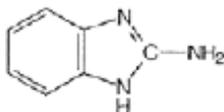
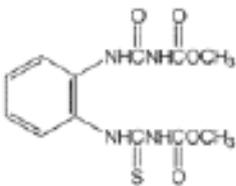
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| Method                                 | Results  | Remarks  | Reference  |
|--|--|--|--|
| SETAC Europe, Dutch guideline          | and Unter Widdersheim), at 20°C. pH of waters 8.1-8.5, pH of sediments 7.5-8.0.<br>Mineralisation ( <sup>14</sup> CO <sub>2</sub> ) was 4.7-20.4% (by study end, at 120 days).<br>Carbendazim DT <sub>50</sub> 15.1 days (SFO, Bickenbach system) and 75.2 days (SFO, Unter Widdersheim system).<br><br>Observed metabolites:<br>2-AB, max 6.3% (76 days), half-life not available.  |  | (2001) *, cited in EFSA (2010)<br><br>(kinetic re-evaluation elsewhere)  |
| Draft OECD TG No 307; USA EPA TG 162-1 | Study on degradation of Thiophanate-methyl in aerobic soil at 20°C, three soils with pH 5.8-7.5. Mineralisation ( <sup>14</sup> CO <sub>2</sub> ) reached 7.3-25.7% (by study end, day 120).<br>DT <sub>50</sub> 0.44-0.70 days (n=3, SFO).<br><br>Metabolites observed:<br>Carbendazim, max 62.8-75.8% (3-7 days), DT <sub>50</sub> 22.0-63.2 days (n=3, SFO-SFO),<br>CM-0237, max 4.1-9.8% (1-7 days), DT <sub>50</sub> 2.8-86.5 days (n=3, SFO-SFO)<br>2-AB, max 2.3-6.1% (14 days), DT <sub>50</sub> 11.5-13.5 (n=2, SFO-SFO). | Reliable   | Voelkl S. (2002)<br><br>(kinetic re-evaluation elsewhere)  |
| OECD TG No 307                         | Study on degradation of Thiophanate-methyl in one aerobic soil at 20.9°C, pH 7.9. Mineralisation ( <sup>14</sup> CO <sub>2</sub> ) reached 7.6% (by study end, day 120).<br>DT <sub>50</sub> 0.27 day (SFO).<br><br>Metabolites observed:<br>Carbendazim, max 48.3% (3 days), DT <sub>50</sub> 40.1 days (SFO-SFO),<br>CM-0237, max 4.4% (28 days), half-life not available,<br>2-AB, max 3.8% (14 days), DT <sub>50</sub> 5.3 days (SFO-SFO)  | Reliable   | Adam D. (2014, 2016)   |
| BBA leaflet no. 36                     | Study on degradation of Carbendazim in soil at 22°C (two soils), pH 6.8 and 5.2. A complete mass balance was not provided.<br>DT <sub>50</sub> 37 and 44 days (n=2, SFO).<br><br>Metabolites observed:<br>No details provided, the RMS (DE) stated that 2-AB or other extractable residues each accounted for <5% of the applied radioactivity throughout the experiment.  | Reliable, but the study was poorly described and no mass balance was provided. | Otto S. (1975) *, cited in EFSA (2010)<br><br>(kinetic evaluation elsewhere)                                   |
| BBA-leaflet No. 36                     | Study on degradation of Carbendazim in soil at 15°C, 20°C and 25°C (one aerobic soil), pH 4.7. A complete mass balance was not provided.<br>DT <sub>50</sub> 34 days (15°C), 31 days (20°C), 26 days (25°C) (n=1, SFO).<br><br>Metabolites observed:<br>No details provided, the RMS (DE) stated that 2-AB or other extractable residues each accounted for <5% of the applied radioactivity throughout the experiment.  | Reliable, but the study was poorly described and no mass balance was provided. | Gilde-meister H., Jordan H.J., Remmert U. (1981) *, cited in EFSA (2010)<br><br>(kinetic evaluation elsewhere) |
|  |  |  |  |

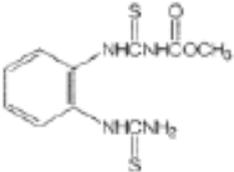
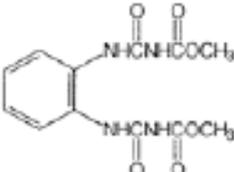
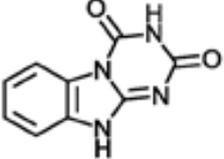
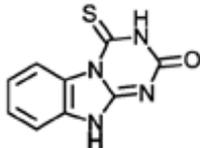
| Method          | Results  | Remarks  | Reference  |
|-----------------|--|----------|--|
| US EPA TG 161-2 | <p>Study on photochemical transformation of Thiophanate-methyl in sterile buffer (pH 5) exposed to natural sunshine outdoors in Japan (latitude 35°, longitude 139°) in mid-December at ambient temperatures (2.9–16.3°C).</p> <p>DT<sub>50</sub> 2.3 days (SFO).</p> <p>Additionally, the quantum yield was determined to 3.84 x 10<sup>-3</sup> and from this results DT<sub>50s</sub> for water bodies under clear sky conditions at 40° latitude were estimated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter).</p> <p>Observed transformation products:</p> <p>Carbendazim, max 49.7% (by study end, 5.5 days), no half-life available,</p> <p>DX-105, max 14.3% (5.5 days), no half-life available.</p> <p>Un-identified product, max 5.6% (4.0 days), not expected to be formed in significant amounts in microbially viable systems.</p> | Reliable | Shiotani (2003)<br><br>(kinetic re-evaluation elsewhere) |

\* The study summaries provided below on Carbendazim are derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not re-evaluated by Sweden at this stage.

**Table 63: Molecular structures, chemical names (or SMILES codes) and codes of metabolites/transformation products mentioned in the above summary table.**

|                                  |  |  |
|----------------------------------|--|--|
| <b>Carbendazim</b><br><b>MBC</b> | Methyl 1H-benzimidazol-2-ylcarbamate                           |  |
| <b>4-OH-TM</b>                   | Dimethyl 4,4'-(4-hydroxy-o-phenylene)bis(3-thioallophanate)    |  |
| <b>CM-0237</b>                   | Methyl 4-[2-(methoxycarbonylamino)benzothiazolyl]allophanate   |  |
| <b>2-AB</b>                      | 2-amino-1H-benzimidazole                                       |  |
| <b>DX-105</b>                    | Methyl 4-[2-(3-methoxycarbonyl-2-thioureido)phenyl]allophanate |  |

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|                                  |  |   |
|----------------------------------|--|---|
| <p><b>AV-1951</b></p>            | <p>Methyl 4-[2-(2-thioureido)phenyl]-3-thioallophanate</p> |  |
| <p><b>FH-432</b></p>             | <p>Dimethyl 4,4'-(o-phenylene)bis(allophanate))</p>        |  |
| <p><b>M10</b><br/><b>UM2</b></p> | <p>Smiles code:<br/>O=C3N=C2Nc1cccc1N2C(=O)N3</p>          |   |
| <p><b>UM1</b></p>                | <p>Smiles code:<br/>O=C3N=C2Nc1cccc1N2C(=O)N3</p>          |   |

### 11.1.1 Ready biodegradability

Based on the available data (testing according to OECD TG No 301 C; MITI (I) (Ministry of International Trade and Industry, Japan), summarised in RAR Volume 3, B.8.2.2.1, from which the study summary has been copied below), it was concluded that thiophanate-methyl is not ready biodegradable. Primary degradation was observed, but probably to the largest extent caused by hydrolysis. The main transformation product was MBC (carbendazim). Percentage biodegradation was on average 4% in 28 days. The study was considered as reliable and can be regarded as the key study for classification on biodegradability.

|                              |   |
|------------------------------|---|
| <b>Reference:</b>            | <b>Tsushima, S (2013)</b> Ready biodegradability Test of Thiophanate-methyl   |
| Report No.:                  | RD-02537  |
| Document No.:                | 713-003   |
| Guideline:                   | OECD TG No 301 C  |
| GLP:                         | Yes   |
| <b>Previous evaluation:</b>  | Submitted for the purpose of renewal.   |
| <b>Material and methods:</b> |   |
| Test material:               | Thiophanate-methyl  |
| Lot/Batch No:                | YS-0175   |
| Purity:                      | 99.3%   |
| Activity control:            | Aniline (purity 100%)   |
| Activated sludge:            | Standard activated sludge was obtained from the Chemicals Evaluation and Research Institute, Japan.   |
| Test concentration:          | Test material and positive control: 100 mg/L<br>Activated sludge: 30 mg/l   |
| Mineral medium:              | Prepared from stock solutions A-D as recommended in OECD TG No 301 C  |
| Test description:            | Six bottles were prepared for the measuring of biological oxygen demand (BOD). The total volume of test solution in in each bottle was 300 ml. <ul style="list-style-type: none"> <li>• Bottle No. 1 (abiotic control): water and test material.</li> <li>• Bottles No. 2-4 (test suspensions): mineral medium, activated sludge and test material.</li> <li>• Bottle No. 5 (activity control): mineral medium, activated sludge and aniline</li> <li>• Bottle No. 6 (inoculum blank): mineral medium and standard activated sludge</li> </ul> <p>To measure nitrification another six bottles prepared as described above was set up.</p> <p>The bottles were incubated under stirring at 25.3 - 25.8 °C for 28 days. The BOD was measured with an enclosed respirometer. The contents of the second set of bottles were filtered and the concentration of ammonium ion in the solution of each bottle was determined by ion electrometry.</p> |
| Method of analysis:          | The pH of test systems was measured at day 0 and day 28.<br>HPLC and LC/MS were used to identify and quantify test material and transformation products.<br>Reference items: MBC (carbendazim), 2-AB, AV-1951, DX-105 and 4-OH-TM   |

## Results

### Biological oxygen demand

The amount of oxygen uptake of the inoculum blank system (bottle 6) after 28 days was 2.359 mg and the amount of oxygen uptake of the abiotic control (bottle 1) was 0 mg. The amount of oxygen uptake of the test suspensions after 28 days was 4.134, 4.777 and 4.667 mg for bottle No. 2, 3 and 4, respectively corresponding to an average biodegradation of 4%. The percentage biodegradation of the activity control (aniline) was 87% after 7 days and 100% after 28 days confirming that the inoculum was viable. The results of the BOD measurements in the set of bottles used for the confirmation of nitrification were similar.

### Confirmation of Nitrification

The production rate of ammonia in the test suspensions, was nearly zero indicating no biodegradation. For the activity control (aniline) the production rate of ammonia was 95.7% (average of triplicate measures).

### Primary degradation

The mean residual amount of thiophanate-methyl in the test suspensions was 19.5 mg corresponding to an average disappearance rate of 35%. Three degradation products were identified and quantified with HPLC and LC/MS: Carbendazim (MBC), methyl N-[2-(thioureido)phenylaminocarbonothioyl]carbamate (AV-1951) and 2-aminobenzimidazole (2-AB). One peak in the chromatogram could not be identified but this peak was less than 1% of the area of thiophanate-methyl. Carbendazim (MBC) was the main transformation product accounting for approximately 18% of the total recovery after 28 days while AV-1951 accounted for 7% and 2-AB for 0.3%.

The disappearance rate of thiophanate-methyl in the abiotic control was 32%. The same transformation products were identified but at a lower rate. However, the total recovery was ca 12% lower than for the test suspensions. The results indicate that hydrolysis was the main mechanism for the transformation of thiophanate-methyl.

**Table 64: Thiophanate-methyl – Biological oxygen demand and % degradation after 28 days of incubation at 25°C in a ready biodegradability test according to OECD TG No 301 C.**

| Bottle No. | Test system                | O <sub>2</sub> uptake day 28 (mg) | degradation (%) | Average degradation (%) |
|------------|----------------------------|-----------------------------------|-----------------|-------------------------|
| 1          | Abiotic control            | 0.000                             | -               | -                       |
| 2          | Sludge + test subst.       | 4.134                             | 3               | 4                       |
| 3          |                            | 4.777                             | 4               |                         |
| 4          |                            | 4.667                             | 4               |                         |
| 5          | Positive control (Aniline) | 74.23                             | 100             | -                       |
| 6          | Inoculum blank             | 2.359                             | -               | -                       |

**Table 65: Thiophanate-methyl remaining and transformation products formed after 28 days of incubation at 25°C in a ready biodegradability test according to OECD TG No 301 C.**

| Bottle No | Test system             | Thiophanate-methyl  |                             |                          |                        | MBC (Carbendazim)          | AV-1951                    | 2-AB                       | Total recovery (%) |
|-----------|-------------------------|---------------------|-----------------------------|--------------------------|------------------------|----------------------------|----------------------------|----------------------------|--------------------|
|           |                         | Initial amount (mg) | Residual amount day 28 (mg) | Residual rate day 28 (%) | Disappearance rate (%) | Conversion rate day 28 (%) | Conversion rate day 28 (%) | Conversion rate day 28 (%) |                    |
| 1         | Abiotic control         | 30.02               | 20.53                       | 68.4                     | 32                     | 6.5                        | 3.4                        | 0.0                        | 78.3               |
| 2-4       | Sludge + Test substance | 30.02 *             | 19.5 *                      | 65.1 *                   | 35 *                   | 17.9 *                     | 6.9 *                      | 0.3 *                      | 90.1 *             |

\* Average of 3 replicates

### RMS comments and conclusion

The study seems to have been well performed and the validity criteria of the guideline were fulfilled.

It can be concluded that thiophanate-methyl is not ready biodegradable. Primary degradation was observed, probably to the largest extent caused by hydrolysis. The main transformation product was MBC (carbendazim).

#### 11.1.2 BOD<sub>5</sub>/COD

Not relevant, no data available.

#### 11.1.3 Hydrolysis

The available data were summarised in RAR Volume 3, B.8.2.1.1 (study summaries copied below). The RMS concluded that thiophanate-methyl is stable to hydrolysis at ambient temperature (22°C) and pH-values (5–7) but hydrolytically degraded at higher pH. The major products resulting from hydrolysis are carbendazim (MBC) and AV-1951 at pH conditions in which hydrolysis occurred. Carbendazim is considered as stable under environmentally relevant conditions (pH, temperature).

|                             |  |
|-----------------------------|--|
| <b>Reference:</b>           | <ol style="list-style-type: none"> <li>1. Soeda, Y. and Nomura, O. (1986) Thiophanate-methyl – Hydrolysis.</li> <li>2. Kiesel, A. and Geibel, E. (2015c) Assessment of degradation kinetics of thiophanate-methyl in a hydrolysis study according to the recommendations of the FOCUS report on degradation kinetics (2006, 2014).</li> <li>3. Kiesel, A. and Geibel, E. (2015d) Raw data to the assessment of degradation kinetics for thiophanate-methyl based on data of a laboratory hydrolysis study according to FOCUS degradation kinetics (2006, 2014) related to the supplementary dossier - M-CA Section 7.</li> </ol> |
| Report No.:                 | <ol style="list-style-type: none"> <li>1. EC-67 (RD-8679)</li> <li>2. RD-03328</li> <li>3. RD-03370</li> </ol>   |
| Document No.:               | <ol style="list-style-type: none"> <li>1. 711-001</li> <li>2. 782-037</li> <li>3. 782-043</li> </ol>   |
| Guideline:                  | <ol style="list-style-type: none"> <li>1. BBA Merkblatt No. 55 Part 1 (also conforms to later US EPA Guideline 161-1)</li> <li>2., 3. FOCUS degradation kinetics (2006, 2014)</li> </ol>   |
| GLP:                        | <ol style="list-style-type: none"> <li>1. No</li> <li>2. and 3. not applicable</li> </ol>  |
| <b>Previous evaluation:</b> | <ol style="list-style-type: none"> <li>1. In DAR (1997)</li> <li>2. and 3. submitted for the purpose of renewal.</li> </ol>  |

**Material and methods:**

Test material: Thiophanate-methyl, unlabelled

Lot Batch No: GP-73

Purity: 99.6%

Test concentration: 10 mg/L thiophanate-methyl

Test system and conditions: Stock solution of 250 mg thiophanate-methyl in 100 ml acetone was prepared and diluted in buffers (2 ml stock solution in 500 ml buffer). 30 mL buffered test solution in each 50 mL stoppered sterilised Erlenmeyer flask was monitored in a dark incubator. Experimental temperatures were 22, 45 and 65°C. Buffers: Clark-Lubs solutions (potassium hydrogen phthalate and sodium hydroxide; pH 5), Sorensen solution (potassium dihydrogen phosphate; pH 7), and Clark-Lubs (boric acid + potassium chloride and sodium hydroxide; pH 9). De-ionised water was used and all buffers were filtered through 0.2 µm membrane filter for sterilisation. Tests were conducted during 35 days. The test was performed with duplicate samples for each temperature and for each pH-value.

Method of analysis: HPLC (UV detector-280 nm), thin-layer chromatography, and mass spectroscopy (EI-DI) were used to identify products formed.

A first preliminary test was carried out at 65°C for a short period of time to identify major hydrolysis products. The second part of the study quantified the relative amounts of parent and hydrolysis products formed over time at different pH and temperatures as above. Quantification was done by comparison of HPLC-MS diagrams with standard solutions of available reference substances.

Used standards: The following reference standards were used: Carbendazim (MBC), DX-105, AV-1951, FH-432 and 2-AB.

Kinetic evaluation: In the original report, hydrolysis rate constants and half-lives were calculated with SFO equation, and interpolated with Arrhenius equation for temperature adjustment to 25°C. Degradation rates were determined later by Kiesel and Geibel (2015c,d), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.

**Results**

No contamination of incubation samples by bacteria/fungi was observed over the experiment, and the pH remained stable throughout the experiment. In the study report results over all sampling days were presented as mg/l parent equivalents. The RMS has expressed this as % AR (parent equivalents) for the last sampling interval (see table below). Hydrolysis occurred over the pH range 5-9 and was most rapid for the parent substance at the highest pH. The hydrolysis rate also substantially increased with temperature (22–65°C).

**Table 66: Recovery in % of initial amount of thiophanate-methyl for the parent and its hydrolysis products over the course of the experiment in Soeda and Nomura (1986), calculated by the RMS.**

| pH, temperature and final sampling time | thiophanate-methyl | MBC  | DX-105 | AV-1951 | 2-AB | Total |
|---|--------------------|------|--------|---------|------|-------|
| pH 5, 22°C, 35 d                        | 98.1               | 0    | 0      | 0       | 0    | 98.1  |
| pH 5, 45°C, 35 d                        | 61.3               | 29.2 | 0      | 2.7     | 0    | 93.2  |
| pH 5, 65°C, 168 h                       | 14.6               | 68.2 | 0      | 2.4     | 12.4 | 97.6  |
| pH 7, 22°C, 33 d                        | 59.6               | 28.6 | 0      | 8.1     | 0    | 96.3  |
| pH 7, 45°C, 137 h                       | 16.7               | 67.2 | 0      | 11.2    | 0    | 95.1  |
| pH 7, 65°C, 3.5 h                       | 21.8               | 66.7 | 0      | 8.1     | 0    | 96.6  |
| pH 9, 22°C, 96 h                        | 6.5                | 58.7 | 0      | 24.9    | 0    | 90.1  |

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| pH, temperature and final sampling time | thiophanate-methyl | MBC  | DX-105 | AV-1951 | 2-AB | Total |
|---|--------------------|------|--------|---------|------|-------|
| pH 9, 45°C, 2 h                         | 21.2               | 59.8 | 0      | 15.8    | 0    | 98.8  |
| pH 9, 65°C, 8 minutes                   | 22.5               | 61.5 | 0      | 11.4    | 0    | 95.4  |

Hydrolysis products were identified as carbendazim (MBC), AV-1951 and 2-AB. At 22°C MBC was found as maximum 58.7% (pH 9, last sampling point) and AV-1951 was found as max 26.4% (pH 9, sampling point at 75 hours). 2-AB was only found in the experiment at pH 5 and 65°C, at concentration that increased over the incubation period, to 12.4%. No other compounds were found in the test solutions in the definitive test.

In the original report half-lives of thiophanate-methyl (at 25°C) were estimated to 867 days (pH 5), 36 days (pH 7) and 0.7 days (pH 9). These results are superseded by the kinetic re-analyses in Kiesel & Geibel (2015c,d). Data from the experiments at 22°C, pH 7 and 9 were included in the analyses (in mg/l parent equivalents). Data from both replicates were used. For the experiment at pH 7 thiophanate-methyl was assumed to degrade into MBC in a second step of a pathway fit. For the experiment at pH 9 thiophanate-methyl was assumed to degrade into MBC and AV-1951 in step 2, and in a 3<sup>rd</sup> step formation of MBC from AV-1951 was added.

SFO was selected as the best fit model for the parent (for data from experiment at pH 7 the Chi2 error-% was lowest in SFO, and for the experiment at pH 9 the confidence interval of both  $\alpha$  and  $\beta$  included zero). The SFO (and SFO-SFO) results are summarised in the tables below. Visual assessments are not shown but were good in all cases. Carbendazim increased over the duration of the test and no reliable parameters could be determined as demonstrated by the p-value 0.5.

**Table 67: Summary of kinetic re-analysis by Kiesel and Geibel (2015c,d) of the hydrolysis data from Soeda and Nomura (1986).**

| Substance              | Test condition | Kinetic model | Mo   | Parameter               | $\chi^2$ , %-error | Prob>t                | Lower CI | Upper CI | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|------------------------|----------------|---------------|------|-------------------------|--------------------|-----------------------|----------|----------|-------------------------|-------------------------|
| <b>Parent only fit</b> |                |               |      |                         |                    |                       |          |          |                         |                         |
| Thiophanate-methyl     | pH 7 22°       | SFO           | 9.93 | k=0.01481               | 1.4                | 2.4x10 <sup>-10</sup> | 0.01     | 0.02     | 46.8                    | 155.5                   |
|                        | pH 9 22°       | SFO           | 9.97 | k=0.6881                | 0.6                | 1.5x10 <sup>-15</sup> | 0.7      |          | 1.0                     | 3.3                     |
| <b>Pathway fits</b>    |                |               |      |                         |                    |                       |          |          |                         |                         |
| Carbendazim            | pH 7 22°       | SFO-SFO       | n.r. | k=2.3x10 <sup>-14</sup> | 2.8                | 0.5                   | -0.01    | 0.01     | >1000                   | >1000                   |
|                        | pH 9 22°       | SFO-SFO       | n.r. | k=2.2x10 <sup>-14</sup> | 0.9                | 0.5                   | -0.2     | 0.2      | >1000                   | >1000                   |
| AV-1951                | pH 9 22°       | SFO-SFO       | n.r. | k=0.1273                | 0.8                | 2x10 <sup>-16</sup>   | 0.11     | 0.13     | 5.4                     | 18.1                    |

n.r. Not relevant.

**Table 68: Formation fractions of carbendazim and AV-1951, from kinetic re-analyses by Kiesel and Geibel (2015c,d) of the data from Soeda and Nomura (1986).**

| Formation                        | Test condition | Formation fraction | St. dev.         |
|----------------------------------|----------------|--------------------|------------------|
| Thiophanate-methyl → Carbendazim | pH 7 22°       | 0.69               | 0.08             |
|                                  | pH 9 22°       | 0.62               | 0.02             |
| Thiophanate-methyl → AV-1951     | pH 9 22°       | 0.38               | 0.005            |
| AV-1951 → Carbendazim            | pH 9 22°       | 0.057              | 1.6 <sup>a</sup> |

a RMS comment: The large standard deviation demonstrate that the formation fraction was unreliable.

### **RMS comments and conclusion**

The study was considered acceptable in the DAR (1997) and is considered acceptable also for the purpose of renewal. No mass balance was reported (non-radiolabelled test substance use) but the total recovery was always  $\geq 9$  mg/l parent equivalents.

The analytical techniques were acceptable for the identification of parent and transformation products although the method for quantification (HPLC peak height) was relatively un-precise. Test concentrations were above detection limits and limits of quantification for all reference compounds but 2-AB. This was a non-GLP study but the experiments were scientifically sound. It is not considered likely that repetition of the study would alter the conclusion substantially.

The data showed that thiophanate-methyl is stable to hydrolysis at ambient temperature (22°C) and pH-values (5–7). The RMS concludes that the major products resulting from hydrolysis are carbendazim (MBC) and AV-1951 at pH conditions in which hydrolysis occurred.

The kinetic re-analyses are accepted by the RMS. However, for the experiment at pH 9 Kiesel & Geibel (2015c,d) mixed up hours and days and the results were erroneously presented in hours instead of days (e.g.,  $DT_{50}$  for parent was given as 1.0 hour but should have been 1.0 day, for AV 1951  $DT_{50}$  was given as 0.23 days but should be 5.4 days). For AV-1951 statistically and visually acceptable results were obtained however the RMS notes that the  $DT_{50}$  of 5.4 days was extrapolated beyond the study duration of 4 days (96 hours) and therefore uncertain. Additionally, it is questionable if results can be accepted when the parameters for the other product (MBC) simultaneously optimised were unreliable.

|                             |   |
|-----------------------------|---|
| <b>Reference:</b>           | <ol style="list-style-type: none"> <li>1. <b>Shiotani, H. (2003b)</b> Hydrolysis Study of MBC (ID No. 2-9-13)</li> <li>2. <b>Kiesel, A., Geibel, E. (2015e)</b> Assessment of degradation kinetics of MBC in a hydrolysis study according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)</li> <li>3. <b>Kiesel, A., Geibel, E. (2015f)</b> Raw data to the assessment of degradation kinetics for MBC based on data of a laboratory hydrolysis study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7</li> </ol> |
| Report No.:                 | <ol style="list-style-type: none"> <li>1. RD-02326</li> <li>2. RD-03327</li> <li>3. RD-03371</li> </ol>   |
| Document No.:               | <ol style="list-style-type: none"> <li>1. 711-002</li> <li>2. 782-038</li> <li>3. 782-044</li> </ol>  |
| Guideline:                  | <ol style="list-style-type: none"> <li>1. OECD TG No. 111 (1981)</li> <li>2. and 3. FOCUS degradation kinetics (2006, 2014)</li> </ol>  |
| GLP:                        | <ol style="list-style-type: none"> <li>1. Yes (RMS comment: not signed)</li> <li>2. and 3. not applicable</li> </ol>  |
| <b>Previous evaluation:</b> | All three studies submitted for the purpose of renewal.   |

|                              |   |
|------------------------------|---|
| <b>Material and methods:</b> |   |
| Test material:               | Carbendazim (Methyl benzimidazol-2-ylcarbamate, MBC) unlabelled   |
| Lot/Batch No.:               | 31-8214-C   |
| Purity:                      | 99.8%, HPLC determined  |
| Test concentration:          | 3.0 mg/L.   |
| Test system and conditions:  | <p>Stock solutions of ca 3.0 mg MBC in 10 ml methanol:acetonitrile (2:8, v/v) were prepared and 2.0 ml diluted in 200 ml buffer. Buffers: pH 4.0 (AcOH and AcONa), pH 7.0 (NaOH and KH<sub>2</sub>PO<sub>4</sub>), pH 9.0 (NaOH and H<sub>3</sub>BO<sub>3</sub>).</p> <p>The initial test to determine stability was done at 50°C and pH 4, 7 and 9 during 5 days in the dark. The main test was done only at pH 9 during 0–21.8 days at 45°C, 0–4.96 days at 55°C, and during 0–2.04 days at 65°C tests, in the dark. All test solutions were sterile. (The intention was to carry out the main test at 25 and 35°C but the OECD TG requirement of 6 data points in the range 20–70% degradation was not met and therefore higher temperatures were finally used.)</p> |
| Method of analysis:          | <p>HPLC (UV detector 282 nm) and mass spectroscopy (EI-DI) were used to identify products formed. The test was performed with duplicate samples for each temperature and for each pH-value.</p> <p>The main study quantified the relative amounts of MBC and hydrolysis products formed over time at pH 9 and temperatures as above. Quantification was done by comparison of HPLC-MS diagrams with standard solutions of available reference substances.</p>   |
| Used standards:              | MBC and 2-AB (2-Aminobenzimidazole).  |
| Kinetic evaluation:          | <p>Hydrolysis constants and half-lives were calculated with SFO equation and interpolated with Arrhenius equation for temperature adjustment to 25°C in the study report. Degradation rates were re-assessed by Kiesel and Geibel (2015e,f), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.</p>   |

## Results

Sterile conditions were maintained and pH remained stable throughout the experiment.

Degradation of carbendazim (MBC) at pH 4 and 7 was less than 10% in all samples after 5 days at 50°C, but exceeded 10% in pH 9 samples after 5 days. Therefore, the main test was conducted only at pH 9.

Carbendazim decreased from the initial concentration of 3.0 mg/l to 0.85-1.05 mg/l after 22 days at 45°C, after 5 days at 55°C and after 2 days at 65°C. HPLC chromatograms provided showed only presence of carbendazim and 2-AB in all solutions. The amounts of 2-AB were not quantified.

The estimated hydrolysis DT<sub>50</sub> at pH 9 and 25°C was calculated in the study report to 200 days.

The results from Shiotani (2003b) were superseded by the kinetic re-analyses by Kiesel and Geibel (2015e,f). Data from the experiments at pH 9 and 45, 55 and 65°C were included in the analyses (in mg/l). Data from both replicates were used. For the results at 45 and 55°C the confidence intervals of  $\alpha$  and  $\beta$  included zero and therefore the SFO results were more reliable than the FOMC results (Chi2 error-% was also marginally lower in SFO). At 65°C both kinetic models provided acceptable and very similar results, and the results from SFO was selected as endpoints. All visual assessments were good. The results are summarised in the table below.

As the tests were conducted only at 45, 55 and 65°C and as these temperatures do not represent realistic environmental conditions, the DT<sub>50</sub> and DT<sub>90</sub> values derived by SFO for MBC were extrapolated to 20°C by the Arrhenius equation. The interpolated (20°C) DT<sub>50</sub> value was 435 days and the corresponding DT<sub>90</sub> 1444 days.

**Table 69: Summary of kinetic re-analysis by Kiesel and Geibel (2015e,f) of the hydrolysis data from Shiotani (2003b).**

| Substance   | Test condition | Kinetic model | Mo   | Parameter                         | $\chi^2$ , %-error | Prob>t               | Lower CI   | Upper CI     | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|-------------|----------------|---------------|------|-----------------------------------|--------------------|----------------------|------------|--------------|-------------------------|-------------------------|
| Carbendazim | pH 9 45°       | SFO           | 3.02 | k=0.05484                         | 1.2                | <2x10 <sup>-16</sup> | 0.05       | 0.06         | 12.6                    | 42.0                    |
|             | pH 9 55°       | SFO           | 2.98 | k=0.2019                          | 1.4                | <2x10 <sup>-16</sup> | 0.19       | 0.21         | 3.4                     | 11.4                    |
|             | pH 9 65°       | SFO           | 3.02 | k=0.6345                          | 1.1                | <2x10 <sup>-16</sup> | 0.62       | 0.65         | 1.1                     | 3.6                     |
|             |                | FOMC          | 3.04 | $\alpha$ =5.815<br>$\beta$ =2.194 | 0.85               | n.r.                 | 1.5<br>1.7 | 10.1<br>15.2 | 1.1                     | 4.1                     |

n.r. Not relevant.

**RMS comments and conclusion**

The study is considered acceptable. The OECD guideline was followed and the presented data is acceptable for the purpose of determining the half-life for hydrolysis of MBC. Amounts of 2-AB were not quantified but since the precursor carbendazim can be considered as hydrolytically stable at environmentally realistic pH values and temperatures this is not considered as a deficiency of the study.

**11.1.4 Other convincing scientific evidence**

**11.1.4.1 Field investigations and monitoring data (if relevant for C&L)**

Field dissipation and monitoring data were available but not considered relevant for C&L.

Just for information: The field dissipation DT<sub>50</sub>s for thiophanate-methyl were 0.99-1.8 days in two Italian soils (pH 7.7-8.0) and 1.8-3.3 days in two German soils (pH 5.8-6.2) (all SFO). For the same trials field dissipation DT<sub>50</sub>s for carbendazim were estimated to 13.6-18.9 days (Italian sites) and to 22.1-24.8 days (German sites) (all SFO-SFO). When the data from these field trials were normalised to 20°C and field capacity moisture conditions, the DT<sub>50</sub>s for thiophanate-methyl were 1.1-6.5 days (Italian sites) and 0.8-1.7

days (German sites), and the DT<sub>50</sub>s for carbendazim were 23.1-33.3 days (Italian sites) and 13.6-19.2 days (German sites).

#### 11.1.4.2 Inherent and enhanced ready biodegradability tests

No relevant data available.

#### 11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Individual studies were summarised in the RAR Volume 3 B.8.2.2 (water and water/sediment) and B.8.1.1 (soil), respectively, and the below study summaries have been copied from the RAR.

In the study on mineralisation in surface water (Hurst, 2015) as well as in the water/sediment study (Voelkl, 2001) carbendazim was formed as >80% of the applied radioactivity. Thiophanate-methyl had a relatively short half-life in the water-only system (DT<sub>50</sub> 0.64-2.2 days) as well as in the water/sediment systems (DT<sub>50</sub> 1.6-3.5 days), whereas degradation of carbendazim was considerably more slow (DT<sub>50</sub> 64.6 days in the water-only system and 76.2-91.6 days in water/sediment systems). Additional DT<sub>50</sub>s of 15.1-75.2 days in water/sediment systems were presented in study with carbendazim as test substance.

The same conclusion could be drawn from studies on degradation in soil; carbendazim was the major metabolite formed (max 75.8%), and while the half-lives for thiophanate-methyl were short (< 1 day) the half-lives for carbendazim were considerably longer (22-63.2 days). Additional DT<sub>50</sub> presented in studies with carbendazim as test substance were in the same range.

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|                   |  |
|-------------------|--|
| <b>Reference:</b> | <ol style="list-style-type: none"> <li>1. <b>Hurst, L. (2015)</b> Thiophanate-methyl: Aerobic Mineralisation of <sup>14</sup>C-Thiophanate-methyl in Surface Water</li> <li>2. <b>Geibel, E. and Lobe, I. (2015)</b> Assessment of degradation kinetics of Thiophanate-methyl in pelagic water under aerobic laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)</li> <li>3. <b>Geibel, E. (2015)</b> Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a pelagic water study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7</li> <li>4. <b>Geibel, E. and Lobe, I. (2016)</b> Assessment of degradation kinetics of Thiophanate-methyl in pelagic water under aerobic laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)</li> <li>5. <b>Geibel, E. and Moshenberg, K. (2016)</b> Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a pelagic water study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7</li> </ol> |
| Report No.:       | <ol style="list-style-type: none"> <li>1. RD-03335</li> <li>2. RD-03402</li> <li>3. RD-03403</li> <li>4. RD-03402N</li> <li>5. RD-03403N</li> </ol>  |
| Document No.:     | <ol style="list-style-type: none"> <li>1. 714-002</li> <li>2. 782-036</li> <li>3. 782-042</li> <li>4. 782-090</li> <li>5. 782-091</li> </ol>   |
| Guideline:        | <ol style="list-style-type: none"> <li>1. OECD TG No 309</li> <li>2. - 5. FOCUS Degradation Kinetics report (2006, 2014)</li> </ol>  |

# CLH REPORT FOR THIOPHANATE-METHYL

GLP: 1. Yes  
2. - 5. Not applicable

**Previous evaluation:** Submitted for the purpose of renewal.

**Material and methods:**

Test material: [Ring-U-<sup>14</sup>C] labelled -Thiophanate-methyl

Lot/Batch No: XX/41/2

Radiochemical Purity: 98.46% (as quoted on the Certificate of Analysis), 95.7% within the application solution, as determined in the study

Test concentration: Low dose: 10 µg/l  
High dose: 95 µg/l  
The stock solution was prepared in acetone. Concentration of acetone in final test solutions was <1%.

Test system: Route and rate of degradation of thiophanate-methyl was studied in natural water from a lake (pelagic system) at a high (95 µg/l) and a low concentration (10 µg/l). The test system consisted of glass flasks, connected to two 2M NaOH, traps to capture CO<sub>2</sub>. Each flask was aerated with moistened air. Air passing through sterilised samples was passed through bacterial air filters positioned at the inlet and outlet of each flask to maintain sterility. Samples (100 mL) of natural water (100 µm sieved) were used in the tests. The water used had a suspended solids concentration of 2.94 mg/L. Thiophanate-methyl dissolved in acetone was applied onto the water surface using a syringe.

There were seven incubation groups for the main test:

| Group                           | Condition *  | No. of flasks |
|---------------------------------|--|---------------|
| A: Low test conc.               | Thiophanate-methyl (10 µg/l) + acetone (712 mg/l)                | 18 **         |
| B: High test conc.              | Thiophanate-methyl (95 µg/l) + acetone (759 mg/l)                | 18            |
| C: Abiotic control (sterilised) | Thiophanate-methyl (95 µg/l) + acetone (759 mg/l)                | 2             |
| D: Activity control             | Sodium [ <sup>14</sup> C]benzoate (10 µg/l)                      | 2             |
| E1: Blank control               | Acetone (759 mg/l)   | 1             |
| E2: Blank control               | Untreated  | 1             |
| F: Activity/solvent control     | Sodium [ <sup>14</sup> C]benzoate (10 µg/l) + acetone (759 mg/l) | 2             |

\* RMS comment: In Hurst (2015) the concentration of acetone was apparently given in an erroneous unit (µg/l). The correct unit should be mg/l, as stated above.

Example: Blank control E1:

96 µL acetone = 0.096 mL acetone

0.096 mL acetone x density 0.791 g/mL = 0.0759 g acetone

which was added to test solution of 100 mL:

0.0759 g acetone / 0.1 L water = 0.759 g/L = 759 mg/L.

\*\*14 + 4 spares

Test conditions: Samples were incubated under dark conditions at 20 ± 2°C under continuous stirring.

Sampling time points: High and low dose: Day 0, 1, 2, 4, 7, 14 and 30 (duplicate samples).  
Sterile control: Day 0 and 2 (duplicate samples).  
Activity control: Day 2, 7, 14 and 30 (single samples).  
CO<sub>2</sub>-traps were assayed whenever the associated samples were removed for analysis. Traps associated with the activity controls were assayed at days 0, 3, 7, 14 and 30. Oxygen and pH in water were measured in the blank controls at each sampling interval.

Method of analysis: At the appropriate sampling interval, water was aspirated from the flask and radioactivity was quantified by LSC. Acetone (25 mL) was used to rinse the vessel to ensure all radioactivity had been transferred. No organic wash sample contained >3% AR thus none were analysed chromatographically. The water phase was analysed by HPLC. Confirmation of degradation products in selected samples was done by TLC

and structural elucidation of two unknown metabolites was performed by LC-MS of a single sample.

Reference items: FH-432, DX-105, Carbendazim (MBC), 2-AB, CM-0237, CM-0238, 4-OH-TM and AV-1951.

Kinetic analysis:

Degradation rates were determined in the study. These results were superseded by kinetic re-evaluations performed in accordance with FOCUS (2006, 2014) and using KinGUI 2.1 software. The first kinetic re-evaluation (Giebel & Lobe, 2015, and Geibel, 2015) was not accepted by the RMS since erroneous input data were used for the optimisations and since the KinGUI results were not presented in Word or pdf format. During the evaluation the applicant therefore submitted a revised kinetic re-evaluation (Giebel & Lobe, 2015, and Geibel & Moshenberg, 2016). The results presented below are all from the revised kinetic re-evaluation.

**Table 70: Water characteristics.**

|  |                           | <b>Lake water</b>  |
|--|---------------------------|--|
| Source                                   |                           | Fountains Abbey, Studley Royal, Ripon, UK<br>The lake is surrounded by parkland and no pesticides had been used in the vicinity 10 years prior to sampling |
| Storage and handling                     |                           | The water was collected into plastic containers via a 100 µm sieve   |
| Temperature                              | [°C]                      | 6.4 (at sampling) Prior to use the water was stored in the dark 4 ± 2°C, with free access to air.  |
| pH                                       |                           | 8.73   |
| Conductivity                             | [µS/cm]                   | 236  |
| Redox potential                          | [mV]                      | 189.2  |
| Oxygen content                           | [mg/l]                    | 11.83  |
| Colour                                   |                           | Clear, slightly yellow   |
| Alkalinity                               | [mg CaCO <sub>3</sub> /l] | 104  |
| Hardness                                 | [mg CaCO <sub>3</sub> /l] | 146  |
| Total organic carbon (TOC)               | [mg C/l]                  | 4.55   |
| Dissolved organic carbon (DOC)           | [mg C/l]                  | 4.51   |
| Total Phosphorous                        | [mg/l]                    | 0.19   |
| Total nitrogen                           | [%]                       | 0.00938  |
| Total nitrate                            | [mg/l]                    | 1.7  |
| Total nitrite                            | [mg/l]                    | 0.021  |
| Ammonium (NH <sub>4</sub> <sup>+</sup> ) | [mg/l]                    | 0.152  |
| Suspended solids                         | [mg/l]                    | 2.94   |
| BOD <sub>5</sub>                         | [mgO <sub>2</sub> /l]     | 11.99  |

**Results**

The pH during the test was between 7.6 and 9.0 and the water was maintained under aerobic conditions (oxygen concentrations 6.1 to 9.4 mg/L). Comparison of the activity control treated with acetone and that not treated with acetone showed similar results. In both units, >80% of the applied sodium benzoate had mineralised within 14 days, showing that the water was microbially active with or without the addition of acetone. Comparison of the blank control unit treated with acetone and that not treated with acetone showed similar water quality results.

There was almost no mineralisation (≤ 0.5% AR) and most of the radioactivity remained in the water. Only small amounts of AR were detected in the organic vessel wash. Total recovery of radioactivity (mass balance) was ≥ 94% AR in all units sampled. An overview of the distribution at start and end of the test is given in the table below.

**Table 71: Material balance in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) over 30 days of incubation. As % of applied radioactivity, mean of duplicates (single samples for high dose, sterile system).**

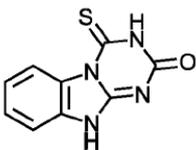
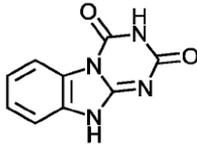
## CLH REPORT FOR THIOPHANATE-METHYL

| Incubation group       | Treatment rate (µg/l) | Time (DAT) | Recovery of radioactivity (% AR) |              |       |              |
|------------------------|-----------------------|------------|----------------------------------|--------------|-------|--------------|
|                        |                       |            | Aqueous                          | Organic wash | Traps | Mass balance |
| A (low dose)           | 10                    | 0          | 97.7                             | 1.0          | NA    | 98.7         |
|                        |                       | 30         | 96.4                             | 1.4          | 0.5   | 98.3         |
| B (high dose)          | 95                    | 0          | 94.2                             | 1.0          | NA    | 95.1         |
|                        |                       | 30         | 93.2                             | 1.5          | 0.5   | 95.1         |
| C (high dose, sterile) | 95                    | 0          | 98.3                             | 0.6          | NA    | 98.9         |
|                        |                       | 2          | 99.3                             | 0.9          | ND    | 100.2        |

NA Not applicable/Not analysed.

ND Not detected

Thiophanate-methyl degraded to several known metabolites (including MBC, FH-432, 2-AB, CM-0237 and DX-105) and two unknown metabolites (UM1 and UM2). Following LC-MS analysis structures were postulated for the two unknown metabolites, as shown below:

|                                 |  |   |
|---------------------------------|--|---|
| Name as referenced in the study | UM1  | UM2   |
| Molecular formula               | C <sub>9</sub> H <sub>7</sub> ON <sub>4</sub> S                                    | C <sub>9</sub> H <sub>7</sub> O <sub>2</sub> N <sub>4</sub>                         |
| IUPAC name                      | 4-Thioxo-1,3,4a,9-tetraza-<br>1,3,9,9atetrahydrofluoren-2-one                      | 1,3,4a,9-Tetraza-<br>1,3,9,9atetrahydrofluorene-2,4-dione                           |
| Postulated structure            |  |  |

The levels of thiophanate-methyl rapidly decreased, and by day 14 none remained. The main metabolite was carbendazim (MBC) which accounted for around 70% of the AR already after 4 -7 days of incubation depending on test concentration (see below table). Metabolite FH-432 appeared early (day 0 in the low and day 1 in the high test concentration) and reached ca 11% AR by day 14 in the high test concentration. It was however not detected at the end of incubation. UM 1 was detected at all sampling occasions in the lower test concentration and seemed to increase throughout the study, to 11.4% at the final day. At the higher test concentration UM1 appeared on day 4 and increased from 2.5% AR to 8.7% AR. The sterile units showed a similar profile, with thiophanate-methyl degrading rapidly to carbendazim (MBC) from day 0 and to FH-432 by day 2.

**Table 72: Thiophanate-methyl and major metabolites in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) and over 30 days of incubation. As % of applied radioactivity, individual replicates and mean of duplicates (single samples for high dose, sterile system).**

| Day                                 | Thiophanate-methyl |      |             | MBC       |            |             | FH-432    |      |             | UM1       |           |             |
|-------------------------------------|--------------------|------|-------------|-----------|------------|-------------|-----------|------|-------------|-----------|-----------|-------------|
|                                     | Replicate          |      | Mean        | Replicate |            | Mean        | Replicate |      | Mean        | Replicate |           | Mean        |
|                                     | 1                  | 2    |             | 1         | 2          |             | 1         | 2    |             | 1         | 2         |             |
| <b>Low dose 10 µg/l</b>             |                    |      |             |           |            |             |           |      |             |           |           |             |
| 0                                   | 65.3               | 56.8 | <b>61.1</b> | 30.1      | 32.5       | <b>31.3</b> | ND        | 5.9  | <b>2.9</b>  | ND        | 1.4       | <b>0.7</b>  |
| 1                                   | 32.1               | 39.7 | <b>35.9</b> | 46.7      | 44.7       | <b>45.7</b> | 4.6       | 6.7  | <b>5.6</b>  | 6.8       | 4.6       | <b>5.7</b>  |
| 2                                   | 21.6               | 22.5 | <b>22.1</b> | 60.9      | 59.6       | <b>60.2</b> | 4.6       | 3.1  | <b>3.9</b>  | 7.6       | 9.1       | <b>8.3</b>  |
| 4                                   | 11.5               | 11.2 | <b>11.4</b> | 69.1      | 77.1       | <b>73.1</b> | 4.5       | ND   | <b>2.3</b>  | 7.8       | 8.9       | <b>8.3</b>  |
| 7                                   | 6.7                | 7.2  | <b>6.9</b>  | 79.9      | 76.3       | <b>78.1</b> | ND        | ND   | <b>ND</b>   | 10.5      | 8.0       | <b>9.2</b>  |
| 14                                  | ND                 | ND   | <b>ND</b>   | 86.2      | 79.4       | <b>82.8</b> | ND        | ND   | <b>ND</b>   | 4.8       | 7.3       | <b>6.0</b>  |
| 30                                  | ND                 | ND   | <b>ND</b>   | 71.2      | 47.5       | <b>59.3</b> | ND        | ND   | <b>ND</b>   | 7.6       | 15.1      | <b>11.4</b> |
| <b>High dose 95 µg/l</b>            |                    |      |             |           |            |             |           |      |             |           |           |             |
| 0                                   | 89.8               | 88.4 | <b>89.1</b> | 3.2       | 1.4        | <b>2.3</b>  | ND        | ND   | <b>ND</b>   | ND        | ND        | <b>ND</b>   |
| 1                                   | 72.9               | 60.0 | <b>66.4</b> | 16.5      | 28.7       | <b>22.6</b> | 5.8       | 7.6  | <b>6.7</b>  | ND        | ND        | <b>ND</b>   |
| 2                                   | 44.8               | 48.7 | <b>46.8</b> | 40.4      | 37.6       | <b>39.0</b> | 11.2      | 10.8 | <b>11.0</b> | ND        | ND        | <b>ND</b>   |
| 4                                   | 32.3               | 23.8 | <b>28.0</b> | 49.9      | 61.7       | <b>55.8</b> | 11.3      | 11.2 | <b>11.3</b> | 5.1       | ND        | <b>2.5</b>  |
| 7                                   | 8.5                | 13.1 | <b>10.8</b> | 71.6      | 65.7       | <b>68.7</b> | 11.5      | 10.6 | <b>11.1</b> | 5.8       | 6.3       | <b>6.0</b>  |
| 14                                  | ND                 | ND   | <b>ND</b>   | 76.2      | 70.6       | <b>73.4</b> | 4.8       | 6.4  | <b>5.6</b>  | 9.4       | 4.9       | <b>7.2</b>  |
| 30                                  | ND                 | ND   | <b>ND</b>   | 72.3      | 71.8       | <b>72.0</b> | ND        | ND   | <b>ND</b>   | 9.3       | 8.1       | <b>8.7</b>  |
| <b>High dose 95 µg/l, sterile *</b> |                    |      |             |           |            |             |           |      |             |           |           |             |
| 0                                   | <b>92.1</b>        |      | <b>4.2</b>  |           | <b>ND</b>  |             | <b>ND</b> |      | <b>ND</b>   |           | <b>ND</b> |             |
| 2                                   | <b>67.1</b>        |      | <b>23.4</b> |           | <b>6.9</b> |             | <b>ND</b> |      | <b>ND</b>   |           | <b>ND</b> |             |

ND Not detected

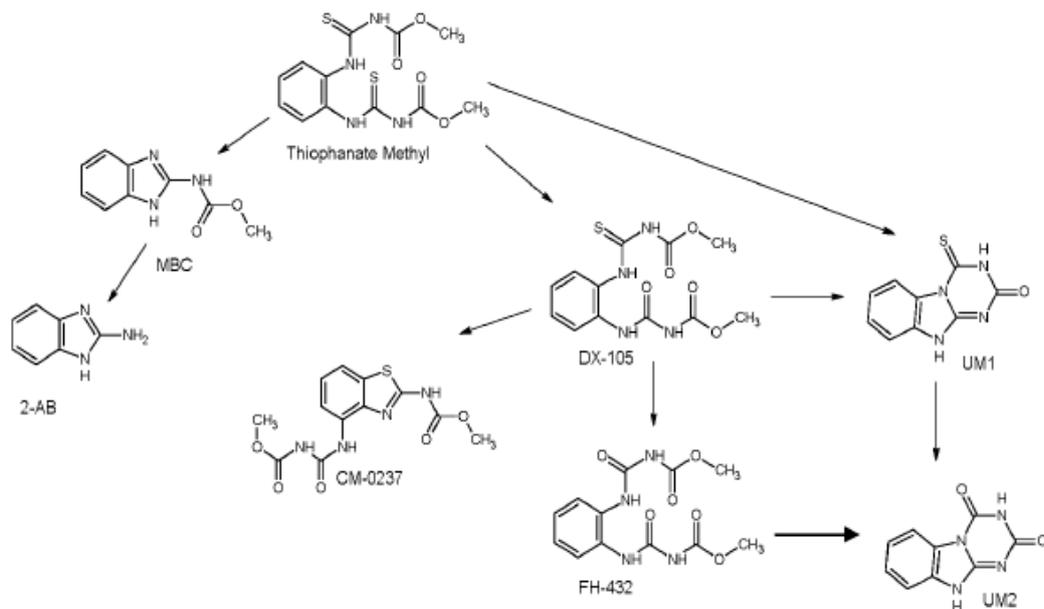
\* Single samples

In addition to the metabolites listed in the table above, the following metabolites were detected: DX-105, 2-AB, CM-0237 and UM2 (see below table). UM2 was detected at levels of 8.0% AR and 5.3% AR at study end in the low and high dose, respectively. 2-AB was detected at the two last sampling in both test concentrations, at max 9.5 and 6.4% AR. DX-105 and CM-0237 were considered as transient products as they were only detected in low concentrations at single occasions. Also other metabolites were detected (in total 6.9% AR by study end in the lower dose), but they were not further characterised. The potential transformation products 4-OH-TM, AV-1951 and CM-0238 were not detected. A tentative degradation scheme is given in the figure below.

**Table 73: Additional metabolites in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) over 30 days of incubation. Sampling days with only non-detects are not included. As % of applied radioactivity, mean of duplicates.**

| Day                      | DX-105     | 2-AB       | CM-0237    | UM2        | Other      |
|--------------------------|------------|------------|------------|------------|------------|
| <b>Low dose 10 µg/l</b>  |            |            |            |            |            |
| 1                        | <b>1.5</b> | ND         | ND         | ND         | <b>1.5</b> |
| 7                        | ND         | ND         | <b>2.4</b> | ND         | ND         |
| 14                       | ND         | <b>8.0</b> | ND         | ND         | ND         |
| 30                       | ND         | <b>9.5</b> | ND         | <b>8.0</b> | <b>6.9</b> |
| <b>High dose 95 µg/l</b> |            |            |            |            |            |
| 0                        | <b>1.2</b> | ND         | ND         | ND         | ND         |
| 14                       | ND         | <b>5.9</b> | ND         | ND         | <b>2.7</b> |
| 30                       | ND         | <b>6.4</b> | ND         | <b>5.3</b> | ND         |

ND Not detected



**Figure 8.2.2.2-1. Proposed pathway for degradation in lake water (from Hurst, 2015).**

A step-wise assessment of the degradation kinetics was conducted in accordance with FOCUS degradation kinetics guidance (Geibel & Lobe, 2016, and Geibel & Moshenberg, 2016). For the low dose test system thiophanate-methyl and degradation products carbendazim (MBC) and UM1 were considered; for the high dose test system thiophanate-methyl, carbendazim (MBC) and FH-432. FH-432 was not considered for the low dose system and UM1 not for the high dose system due to too few data points. Conceptually, all three metabolites were treated as primary metabolites. 2-AB and UM2 could not be included in the analyses as they were only detected occasionally. Data from both replicates were used and time zero amounts of metabolites were added to the parent compartment. The results are presented in tables and figures below (graphs not shown for statistically not reliable results).

At the low concentration (10  $\mu\text{g/l}$ ) FOMC provided a lower Chi2 error-% than SFO and additional biphasic models were therefore used. DFOP was selected as the best fit model for thiophanate-methyl. At the high test concentration (95  $\mu\text{g/l}$ ), the SFO model provided a good fit to the data for the parent whereas FOMC parameters were not reliable.

For the metabolites acceptable results were obtained for carbendazim (MBC) in the low dose system and for FH-432 (high dose system). The authors apparently accepted also the results for UM1 (low dose) and carbendazim (MBC) (high dose) but since the rate constants did not pass the t-test the RMS did not consider these results reliable.

**Table 74: Kinetic evaluation for thiophanate-methyl; lake water treated at low (10 µg/l) dose. Results for thiophanate-methyl are from parent only fit. From Geibel & Lobe (2016) and Geibel & Moshenberg (2016) based on data from Hurst (2015).**

| Substance          | Dose rate | kinetic model | Mo    | $\chi^2$ , % error | parameter                             | Lower Confidence interval | prob > t   | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|--------------------|-----------|---------------|-------|--------------------|---------------------------------------|---------------------------|--|-------------------------|-------------------------|
| Thiophanate-methyl | Low       | SFO           | 94.29 | 14.0               | k=0.8096                              | 0.66                      | $5.4 \times 10^{-7}$   | 0.86                    | 2.8                     |
|                    |           | FOMC          | 95.95 | 3.0                | $\alpha= 1.223$<br>$\beta= 0.829$     | 0.87<br>0.42              | n.r.   | 0.63                    | 4.6                     |
|                    |           | DFOP          | 95.99 | 2.1                | k1= 1.7342<br>k2= 0.2332<br>g= 0.6763 | 1.1<br>0.13<br>0.53       | $4.2 \times 10^{-4}$<br>$1.4 \times 10^{-3}$<br>$8.4 \times 10^{-6}$ | 0.64                    | 5.0                     |
|                    |           | HS            | 96.00 | 2.7                | k1= 0.9836<br>k2= 0.2663<br>tb= 1.34  | 0.89<br>0.18<br>1.0       | $1.1 \times 10^{-8}$<br>$1.5 \times 10^{-4}$<br>$7.3 \times 10^{-6}$ | 0.70                    | 5.0                     |
| MBC                | Low       | DFOP-SFO      | n.r.  | 6.4                | k= 0.01070                            | 0.0034                    | 0.0035   | 64.8                    | 215                     |
| UM1                | Low       | DFOP-SFO      | n.r.  | 15.8               | k= $2.33 \times 10^{-14}$             | -0.017                    | 0.50   | >1000                   | >1000                   |

n.r. Not relevant

**Table 75: Kinetic evaluation for thiophanate-methyl; lake water treated at high (95 µg/l) dose. Results for thiophanate-methyl are from parent only fit. From Geibel & Lobe (2016) and Geibel & Moshenberg (2016) based on data from Hurst (2015).**

| Substance          | Dose rate | kinetic model | Mo    | $\chi^2$ , % error | parameter                         | Lower Confidence interval | prob > t              | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|--------------------|-----------|---------------|-------|--------------------|-----------------------------------|---------------------------|-----------------------|-------------------------|-------------------------|
| Thiophanate-methyl | High      | SFO           | 91.66 | 2.5                | k=0.3142                          | 0.28                      | $1.0 \times 10^{-8}$  | 2.2                     | 7.3                     |
|                    |           | FOMC          | 92.28 | 2.4                | $\alpha= 10.65$<br>$\beta= 31.86$ | -26.6<br>-86.5            | n.r.                  | - <sup>a</sup>          | - <sup>a</sup>          |
| MBC                | High      | SFO-SFO       | n.r.  | 1.2                | k= $6.419 \times 10^{-4}$         | -0.0038                   | 0.39                  | >1000                   | >1000                   |
| FH-432             | High      | SFO-SFO       | n.r.  | 7.1                | k= 0.1334                         | 0.10                      | $3.6 \times 10^{-11}$ | 5.2                     | 17.3                    |

n.r. Not relevant

<sup>a</sup> Not considered acceptable.

**Table 76: Formation fractions of carbendazim (MBC), FH-432 and UM1, according to kinetic re-analyses by Geibel & Lobe (2016) and Geibel & Moshenberg (2016) of the data of from Hurst (2015).**

| Degradation pathway         | Dose rate | Formation fraction | Standard deviation |
|-----------------------------|-----------|--------------------|--------------------|
| Thiophanate-methyl → MBC    | Low       | 0.901              | 0.045              |
|                             | High      | 0.768 <sup>a</sup> | 0.044              |
| Thiophanate-methyl → UM1    | Low       | 0.099 <sup>a</sup> | 0.070              |
| Thiophanate-methyl → FH-432 | High      | 0.232              | 0.074              |

<sup>a</sup> Result not considered reliable by the RMS since the corresponding rate constant was not reliable (p-values > 0.10).

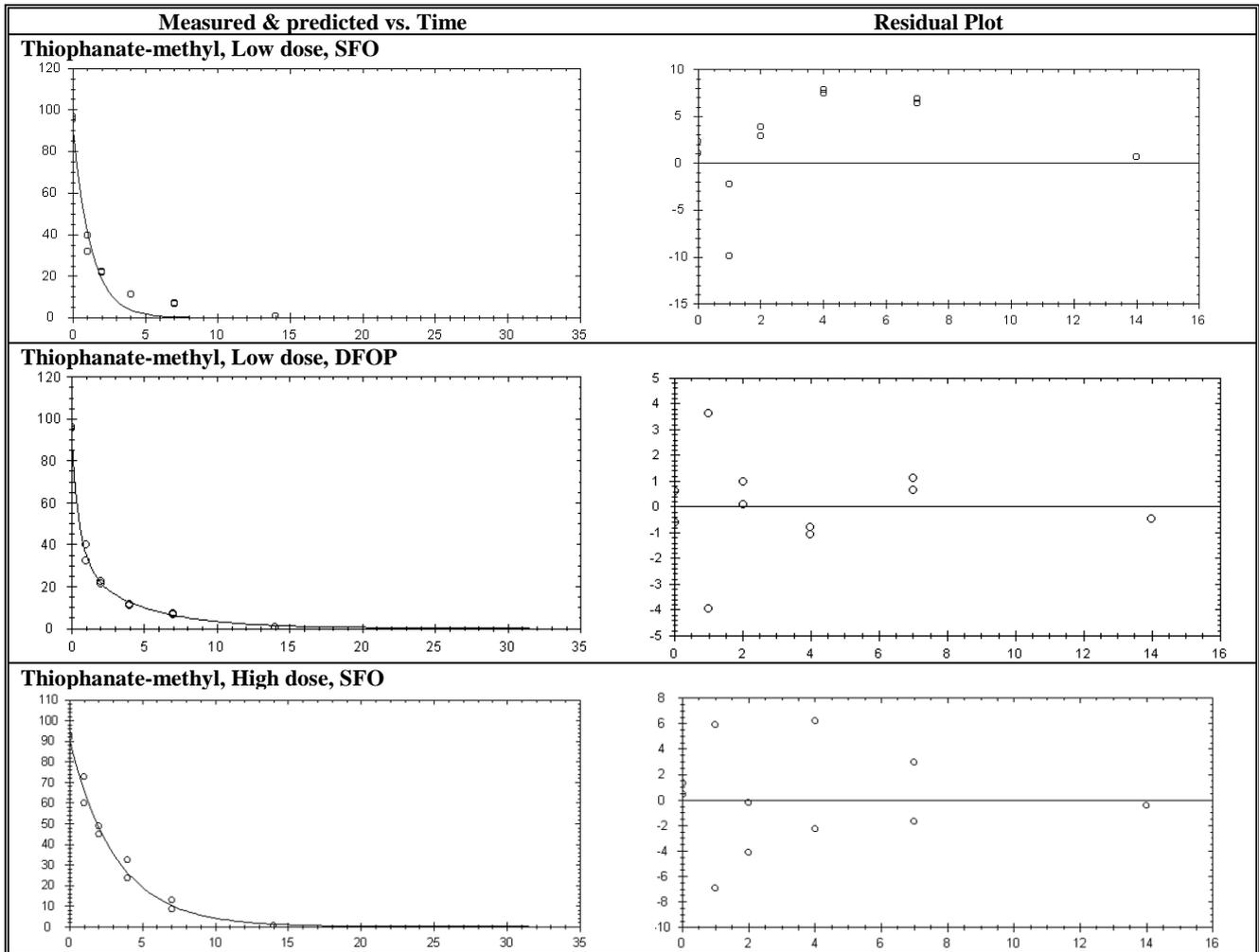


Figure 8.2.2-2 Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl from a surface water simulation study. Low dose (10  $\mu\text{g/l}$ ) and high dose (95  $\mu\text{g/l}$ ) (from Geibel & Moshenberg, 2016).

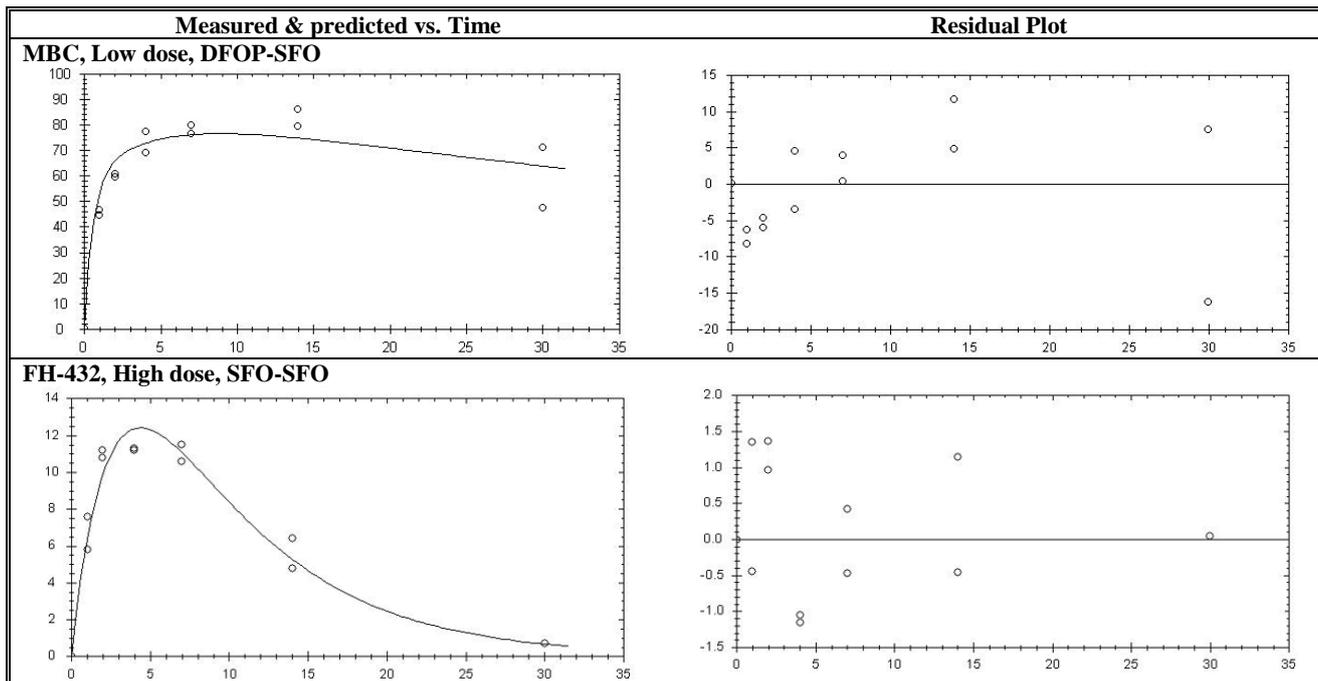


Figure 8.2.2.2-3 Visual fit and plot of residuals for kinetic fitting of data for metabolites of thiophanate-methyl in a surface water simulation study (from Geibel & Moshenberg, 2016). Only results accepted by the RMS.

**RMS comments and conclusion**

The observed degradation of the reference compound sodium benzoate demonstrated that the validity criteria for the test was fulfilled. The simulation study is therefore considered acceptable. However, a prolongation of the test would have enabled a more reliable estimation of the degradability of the observed transformation products.

For UM1 (low dose) and carbendazim (high dose) the rate constant did not pass the t-test and the results should not be further used.

|                              |   |
|------------------------------|---|
| <b>Reference:</b>            | <ol style="list-style-type: none"> <li>1. <b>Völkl, S. (2001)</b> <sup>14</sup>C-Thiophanate-methyl: Route and rate of degradation in aerobic aquatic systems</li> <li>2. <b>Kiesel, A. and Geibel, E. (2015i)</b> Assessment of degradation kinetics of Thiophanate-methyl in water/sediment systems under laboratory conditions according to recommendations of the FOCUS Report on Degradation kinetics (2006, 2014)</li> <li>3. <b>Kiesel, A. and Geibel, E. (2015j)</b> Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a laboratory water/sediment study according to FOCUS Degradation Kinetics (2006, 2014) related to the supplementary dossier – M-CA Section 7</li> <li>4. <b>Moshenberg, K. and Drechsler, S. (2017a)</b> Assessment of degradation kinetics of 4-OH-TM and 2-AB in water/sediment systems under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)</li> <li>5. <b>Moshenberg, K. and Drechsler, S. (2017b)</b> Raw data to the assessment of degradation kinetics of 4-OH-TM and 2-AB based on data of a laboratory water/sediment study according to FOCUS Degradation Kinetics (2006, 2014)</li> </ol>                                   |
| Report No.:                  | <ol style="list-style-type: none"> <li>1. 735063</li> <li>2. RD-03329</li> <li>3. RD-03373</li> <li>4. RD-10140</li> <li>5. RD-10141</li> </ol>   |
| Document No.:                | <ol style="list-style-type: none"> <li>1. 714-001</li> <li>2. 782-040</li> <li>3. 782-046</li> <li>4. 782-092</li> <li>5. 782-093</li> </ol>  |
| Guideline:                   | <ol style="list-style-type: none"> <li>1. SETAC guideline (1995).<br/>BBA Guideline Part IV:5-1 (1990)</li> <li>2. and 3. FOCUS Degradation kinetics report (2006, 2014)</li> </ol>   |
| GLP:                         | <ol style="list-style-type: none"> <li>1. Yes</li> <li>2. – 5. Not applicable</li> </ol>  |
| <b>Previous evaluation:</b>  | <ol style="list-style-type: none"> <li>1. In Addendum (2003) to the DAR (1997). The test description has been copied from the addendum (A brief description of the method of analysis has been added by the RMS SE).</li> <li>2. – 5. Submitted for the purpose of renewal.</li> </ol>  |
| <b>Material and methods:</b> |   |
| Test material:               | <sup>14</sup> C-Thiophanate-methyl  |
| Lot/Batch No:                | EC-01-02  |
| Radiochemical purity:        | 98.0%   |
| Test concentration:          | 0.2 mg/l  |
| Test system and conditions:  | <p>The route and rate of degradation of <sup>14</sup>C-thiophanate-methyl was investigated in two equilibrated water/sediment-systems. The water/sediment systems sampled from a river (Rhein river/Rheinsulz AG/Switzerland) and from a pond (Ormalingen BL/Switzerland), consisted of natural water filtered through a 0.2 mm sieve and the uppermost 5-10 cm of the sediment sieved through a 2 mm mesh.</p> <p>The experiment was performed in a gas flow-system in 1000 ml glass metabolism flasks (inner diameter: about 10.6 cm, area about 88.2 cm<sup>2</sup>). Sediment was added to a depth of about 2.5 – 3.5 cm, corresponding to a wet weight of 250 g for both river and pond sediment. The respective water (550 ml) was added to the sediments to reach a depth of about 6 cm. The flasks were connected to a trap containing only water during the equilibration (21 d) to moisten incoming air. After treatment with the test item (0.205 mg a.s./l) the flasks were additionally connected to a series of traps, the first trap contained 50 ml ethylene glycol and the second trap 50 ml 2N NaOH in order to trap volatile compounds. The samples were incubated at 20°C in the dark. The radiolabelled test item was added to the water surface. This</p> |

concentration corresponded to an application rate of 600 g a.s./ha, assuming uniform distribution within a 30 cm water layer. Single samples were taken for analysis at days 0, 1, 2, 8, 16, 30, 58 and 100 for both systems. Additional samples were taken from the pond system after 140, 202, and 301 days of incubation.

**Method of analysis:** The water phase was separated from the sediment using a pipette. The radioactivity content was measured with LSC. The samples were further analysed with HPLC. If the radioactivity level was too low the samples were concentrated under reduced pressure and analysed by HPLC and TLC. The sediments including the water that was left after pipetting were extracted with:  
a) Acetonitrile:water (8:2; v/v) up to three times  
b) Water, once (if necessary)  
c) Soxhlet extraction with acetone:water (9:1; v/v) except samples on day 0, 1 and 2.  
The radioactivity in the individual extracts was quantified by LSC. The extracts (a-c) were combined and concentrated under reduced pressure and thereafter analysed by HPLC. The radiocarbon content of the sediments was determined by LSC after combustion.  
Reference compounds: DX-105, FH-432, MBC (carbendazim), AV-1951, 2-AB, 3-OH-TM, 4-OH-TM and 3-OH-TM-S.

**Kinetic evaluation:** Degradation rates were determined in the study. These results are now superseded by the kinetic re-evaluation performed in accordance with FOCUS (2006, 2014) (Kiesel and Geibel, 2015i and j). During peer review the applicant was requested to submit degradation rates for the water/sediment systems for metabolites 4-OH-TM and 2-AB considering only the data from maximum occurrence onwards (Evaluation table, Data requirement 4.8). These data were provided in Moshenberg and Drechsler (2017a,b).

**Table 77: Water and sediment characteristics.**

| Parameters                                      | River sediment | Pond sediment |
|---|----------------|---------------|
| -   | Sandy loam     | Clay loam     |
| sand [%]  | 61.05          | 38.35         |
| silt [%]  | 27.65          | 34.50         |
| clay [%]  | 11.30          | 27.15         |
| org. C [%]                                      | 0.97           | 6.13          |
| pH (CaCl <sub>2</sub> )                         | 7.83           | 7.61          |
| CEC [mval/100 g dry sedim.]                     | 7.59           | 21.52         |
| Microbial Biomass [g microb. C/kg dry sediment] | 0.16           | 0.66          |

**Results** (tables copied from Addendum 7 to the Monograph of 13 November 1997 (2003).

During incubation, the oxygen content in water samples varied from 4.2 to 8.3 mg/l in the pond system and from 4.5 to 8.2 mg/l in the river system. The redox potential in water ranged from 115 to 210 mV in the pond system and from 140 to 203 mV in the river system. In the sediment the redox potential varied between – 77 and -161 mV in the pond system and between -108 and -205 mV in the river system, confirming reductive conditions. The pH in the pond system varied between 7.67 and 8.38 and between 7.86 and 8.42 in the river system.

The material balance for the pond and river test systems after treatment with <sup>14</sup>C-thiophanate-methyl is given in the tables below.

**Table 78: Mass balance for Pond water/sediment system. As % of applied radioactivity, single replicates.**

| Fraction                      | Incubation time (d) |       |       |      |      |      |      |      |
|-------------------------------|---------------------|-------|-------|------|------|------|------|------|
|                               | 0                   | 1     | 2     | 8    | 16   | 30   | 58   | 100  |
| Water                         | 105.9               | 87.0  | 80.0  | 43.4 | 30.9 | 20.1 | 10.2 | 3.3  |
| Sediment                      | 1.2                 | 15.8  | 25.3  | 55.9 | 65.7 | 77.1 | 87.5 | 89.4 |
| <sup>14</sup> CO <sub>2</sub> | -                   | <0.1  | <0.1  | <0.1 | 0.1  | 0.2  | 0.7  | 1.3  |
| volatiles                     | -                   | <0.1  | <0.1  | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Total                         | 107.1               | 102.8 | 105.2 | 99.3 | 96.7 | 97.5 | 98.5 | 94.0 |

**Table 79: Mass balance for Pond water/sediment system. As % of applied radioactivity, single replicates.**

| Fraction                      | Incubation time (d) |       |       |       |       |      |      |      |
|-------------------------------|---------------------|-------|-------|-------|-------|------|------|------|
|                               | 0                   | 1     | 2     | 8     | 16    | 30   | 58   | 100  |
| Water                         | 101.6               | 94.9  | 86.9  | 51.1  | 42.5  | 32.0 | 10.1 | 5.5  |
| Sediment                      | 0.8                 | 6.8   | 14.6  | 49.0  | 58.5  | 66.7 | 84.1 | 84.2 |
| <sup>14</sup> CO <sub>2</sub> | -                   | <0.1  | <0.1  | 0.2   | 0.4   | 0.9  | 1.0  | 1.6  |
| volatiles                     | -                   | <0.1  | <0.1  | <0.1  | <0.1  | <0.1 | <0.1 | <0.1 |
| Total                         | 102.5               | 101.6 | 101.5 | 100.3 | 101.4 | 99.6 | 95.3 | 91.3 |

Carbendazim (MBC) was the major metabolite in both systems. Other metabolites detected were: 4-OH-TM, 2-AB, M10 and AV-1951. 4-OH-TM had a max concentration of 8.6% AR in the pond system and 9.5% AR in the river system; 2-AB, which appeared later (day 30 in the pond and day 16 in the river system) with a max conc. of 7.5% AR in the pond system and 6.5% in the river system. The max concentration of M10 was 9.3% AR in the pond system while the maximum concentration in the river system was only 3.2% AR. AV-1951 seemed to be of more transient nature and had a max conc. of around 6% AR in both systems. The tentative degradation products DX-105, FH-432, 3-OH-TM, and 3-OH-TM-S were not detected.

The non-extractable residues increased continuously in both systems and reached 70% AR after 301 days in the pond system and 48.1% after 100 days in the river system. The distribution of thiophanate-methyl and its degradation products in water, sediment (extract) and the total system, expressed as % of applied radioactivity is given in the tables below. The degradation pathway proposed by the author is shown in Figure 8.2.2.3-1.

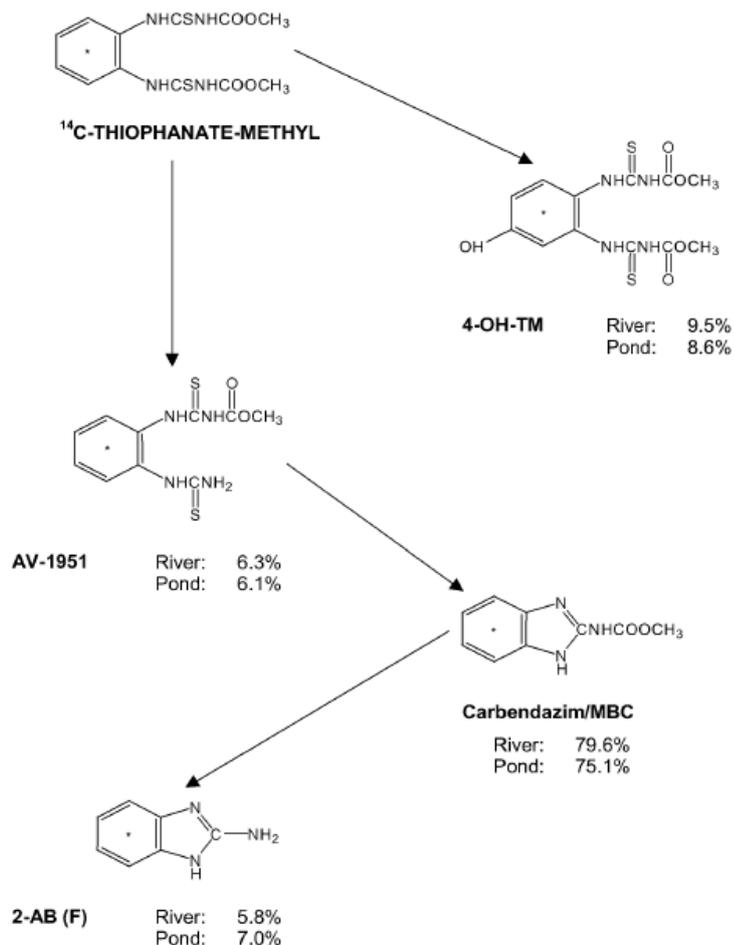
**Table 80: Characterisation of radioactivity in Pond water/sediment system. As % of applied radioactivity. Single samples.**

| Fraction   | Sample | Incubation time (d) |       |       |       |       |      |      |      |      |      |      |
|--|--------|---------------------|-------|-------|-------|-------|------|------|------|------|------|------|
|  |        | 0                   | 1     | 2     | 8     | 16    | 30   | 58   | 100  | 140* | 202* | 301* |
| Parent   | Water  | 98.4                | 85.9  | 66.5  | 9.7   | -     | 0.9  | -    | -    | -    | -    | -    |
|  | Sedim. | -                   | 2.0   | 6.0   | 8.1   | 6.1   | 7.4  | -    | -    | -    | -    | -    |
|  | Total  | 98.4                | 87.8  | 72.5  | 17.9  | 6.1   | 8.3  | -    | -    | -    | -    | -    |
| MBC  | Water  | 2.7                 | 6.5   | 14.8  | 34.3  | 38.2  | 26.8 | 8.0  | 2.6  | 1.7  | 1.9  | 0.4  |
|  | Sedim. | -                   | 2.4   | 5.5   | 28.4  | 36.9  | 36.7 | 46.5 | 30.2 | 18.3 | 18.7 | 4.7  |
|  | Total  | 2.7                 | 8.9   | 20.3  | 67.2  | 75.1  | 63.5 | 54.5 | 32.8 | 20.0 | 20.5 | 5.1  |
| 4-OH-TM  | Water  | -                   | -     | -     | 2.8   | 2.3   | 2.9  | -    | -    | -    | -    | -    |
|  | Sedim. | -                   | -     | 0.2   | 2.3   | 5.0   | 5.7  | 4.6  | 1.4  | 1.5  | 0.6  | 0.5  |
|  | Total  | -                   | -     | 0.2   | 5.1   | 7.3   | 8.6  | 4.6  | 1.4  | 1.5  | 0.6  | 0.5  |
| AV-1951  | Water  | 0.5                 | 2.6   | 5.6   | 2.3   | -     | -    | -    | -    | -    | -    | -    |
|  | Sedim. | -                   | 0.4   | 0.6   | 1.5   | -     | 1.8  | -    | -    | -    | -    | -    |
|  | Total  | 0.5                 | 2.9   | 6.1   | 3.8   | -     | 1.8  | -    | -    | -    | -    | -    |
| 2-AB   | Water  | -                   | -     | -     | -     | -     | -    | 0.5  | -    | -    | -    | 0.3  |
|  | Sedim. | -                   | -     | -     | -     | -     | 2.3  | 7.0  | 6.0  | 6.2  | 3.9  | 2.9  |
|  | Total  | -                   | -     | -     | -     | -     | 2.3  | 7.5  | 6.0  | 6.2  | 3.9  | 3.2  |
| M10  | Water  | -                   | -     | -     | -     | 2.1   | 0.9  | 1.4  | 2.2  | 1.7  | 0.8  | 1.0  |
|  | Sedim. | -                   | -     | -     | -     | 2.4   | 2.9  | 5.3  | 6.1  | 7.7  | 7.7  | 6.1  |
|  | Total  | -                   | -     | -     | -     | 4.5   | 3.8  | 6.7  | 8.3  | 9.3  | 8.5  | 7.1  |
| <sup>14</sup> CO <sub>2</sub><br>non extractable |        | -                   | <0.1  | <0.1  | 0.2   | 0.4   | 0.9  | 1.0  | 1.6  | 4.7  | 2.5  | 5.2  |
|  |        | 0.2                 | 2.0   | 1.9   | 5.1   | 6.2   | 8.9  | 20.7 | 40.6 | 48.5 | 52.1 | 70.0 |
| <b>Total</b>                                     |        | 101.8               | 101.6 | 101.5 | 100.3 | 101.4 | 99.6 | 95.3 | 91.3 | 92.1 | 90.9 | 95.2 |

\* Data from these sampling days were not presented in Addendum 7 to the Monograph of 13 November 1997 (2003).

**Table 81: Characterisation of radioactivity in River water/sediment system. As % of applied radioactivity. Single samples.**

| Fraction   | Sample | Incubation time (d) |       |       |      |      |      |      |      |
|--|--------|---------------------|-------|-------|------|------|------|------|------|
|  |        | 0                   | 1     | 2     | 8    | 16   | 30   | 58   | 100  |
| Parent   | Water  | 103.2               | 69.2  | 48.4  | -    | -    | -    | -    | -    |
|  | Sedim. | -                   | -     | -     | 0.4  | -    | -    | -    | -    |
|  | Total  | 103.2               | 69.2  | 48.4  | 0.4  | -    | -    | -    | -    |
| MBC  | Water  | 2.8                 | 14.4  | 25.3  | 39.0 | 27.3 | 17.3 | 9.4  | 2.3  |
|  | Sedim. | -                   | 9.0   | 16.7  | 42.6 | 42.8 | 48.7 | 50.4 | 33.0 |
|  | Total  | 2.8                 | 23.5  | 42.0  | 81.6 | 70.1 | 66.1 | 59.8 | 35.3 |
| 4-OH-TM  | Water  | -                   | -     | -     | 4.4  | 1.3  | 1.4  | -    | -    |
|  | Sedim. | -                   | 0.6   | 1.4   | 5.1  | 4.7  | 3.8  | 3.1  | -    |
|  | Total  | -                   | 0.6   | 1.4   | 9.5  | 6.0  | 5.2  | 3.1  | -    |
| AV-1951  | Water  | -                   | 3.4   | 6.3   | -    | -    | -    | -    | -    |
|  | Sedim. | -                   | -     | -     | -    | -    | -    | -    | -    |
|  | Total  | -                   | 3.4   | 6.3   | -    | -    | -    | -    | -    |
| 2-AB   | Water  | -                   | -     | -     | -    | 0.7  | -    | -    | -    |
|  | Sedim. | -                   | -     | -     | -    | 5.8  | 4.8  | 4.7  | 4.8  |
|  | Total  | -                   | -     | -     | -    | 6.5  | 4.8  | 4.7  | 4.8  |
| M10<br>Unknown                                   | Water  | -                   | -     | -     | -    | 1.6  | 1.3  | 0.8  | 0.7  |
|  | Sedim. | -                   | -     | -     | -    | -    | -    | 2.2  | 2.4  |
|  | Total  | -                   | -     | -     | -    | 1.6  | 1.3  | 3.1  | 3.2  |
| <sup>14</sup> CO <sub>2</sub><br>non extractable |        | -                   | <0.1  | <0.1  | <0.1 | 0.1  | 0.2  | 0.7  | 1.3  |
|  |        | 0.4                 | 5.5   | 6.3   | 7.3  | 11.3 | 19.9 | 27.1 | 48.1 |
| <b>Total</b>                                     |        | 106.3               | 102.8 | 105.2 | 99.3 | 96.7 | 97.5 | 98.5 | 94.0 |



**Figure 8.2.2.3-1. Proposed pathway for degradation in water/sediment systems (from Völkl, 2001). Note that some of the percentages of individual metabolites were not correct.**

The degradation kinetics of thiophanate-methyl was re-evaluated by Kiesel and Geibel (2015i,j) based on the data from Völkl (2001). The assessment was conducted according to the FOCUS degradation kinetics guidance (2006, 2014) using the model KinGUI 2.1. The outcome is summarised in the table and figures below (though graphs are only shown for results that are discussed further, and not for dissipation in sediment since first of all the kinetic assessment gave unreliable results and secondly, these results are seldom used).

Degradation kinetics for thiophanate-methyl was calculated at the PI level for the total system for both the pond and river systems as was dissipation from the water phase. Dissipation from the sediment was only calculated for the pond system as not enough data points were observed from the maximum occurrence onwards in the river system.

The best fit for data from both the pond and the river water/sediment system was achieved with the SFO model for the total system.

Formation and degradation of the metabolites MBC, 4-OH-TM, 2-AB and M10 were calculated for the total systems at level MI. Metabolite M10 was at first considered as a primary metabolite but the estimated parameters did not reflect the measured values adequately. The author suggested that this may have been caused by the fact that most of parent

had disappeared before the maximum occurrence of M10 was measured. In a second optimisation, a dummy compound was therefore introduced as a primary metabolite and M10 added as a secondary metabolite. The results for 4-OH-TM were used as input data for the dummy compound since the maximum occurrences of M10 and 4-OH-TM were similar and the max occurrence of 4-OH-TM was earlier than the one for M10. The Chi2 error-% for the dummy compound in this pathway fit (parent → dummy → M10) was 13.8% and p-value was 0.001. For 4-OH-TM (river system) the lower CI was marginally below zero but the p-value was acceptable, however due to high Chi2 error-% and poor visual fit the RMS did not accept the result. The Chi2 error-% was high for 4-OH-TM also in the pond system but here the visual fit was better so the result was considered acceptable by the RMS. For 2-AB and M10 the half-lives were estimated only for the pond system, as it was not possible to calculate half-lives for these metabolites in the river system. The results for 4-OH-TM and 2-AB are discussed further below since additional data were submitted during peer review.

**Table 82: Kinetic parameters and the resulting DT50/90 values for thiophanate-methyl and its metabolites carbendazim (MBC), 4-OH-TM, 2-AB and M10 for the total water/sediment systems. Results for thiophanate-methyl are from parent only fits, other results are from pathway fits. From kinetic evaluation in Kiesel and Geibel (2015i,j) based on data in Völkl (2001).**

| Substance          | System | kinetic model | Mo     | $\chi^2$ , % error | parameter                 | Confidence interval     |       | Prob. > t               | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|--------------------|--------|---------------|--------|--------------------|---------------------------|-------------------------|-------|-------------------------|-------------------------|-------------------------|
|                    |        |               |        |                    |                           | lower                   | upper |                         |                         |                         |
| Thiophanate-methyl | Pond   | SFO           | 105.18 | 6.7                | k=0.199                   | 0.16                    | 0.24  | 10 x 10 <sup>-5</sup>   | 3.5                     | 11.6                    |
| MBC                |        | SFO-SFO       | 0      | 8.5                | k=9.09 x 10 <sup>-3</sup> | 7.4 x 10 <sup>-3</sup>  | 0.011 | 9.8 x 10 <sup>-13</sup> | 76.2                    | 253.2                   |
| 4-OH-TM            |        | SFO-SFO       | 0      | 33                 | k=1.22 x 10 <sup>-3</sup> | 5.2 x 10 <sup>-3</sup>  | 0.019 | 0.0008                  | 56.7                    | 188.4                   |
| 2-AB               |        | SFO-SFO       | 0      | 22                 | k=1.27 x 10 <sup>-3</sup> | 5.1 x 10 <sup>-3</sup>  | 0.020 | 0.001                   | 54.7                    | 181.8                   |
| M10 *              |        | SFO-SFO       | 0      | 12                 | k=1.45 x 10 <sup>-3</sup> | -7.0 x 10 <sup>-4</sup> | 0.004 | 0.10                    | 478.5 <sup>a</sup>      | >1000                   |
| Thiophanate-methyl | River  | SFO           | 108.39 | 3.3                | k=0.424                   | 0.38                    | 0.47  | 2.1 x 10 <sup>-4</sup>  | 1.6                     | 5.4                     |
| MBC                |        | SFO-SFO       | 0      | 6.4                | k=7.6 x 10 <sup>-3</sup>  | 0.0051                  | 0.010 | 1.2 x 10 <sup>-5</sup>  | 91.6                    | 304.2                   |
| 4-OH-TM            |        | SFO-SFO       | 0      | 38                 | k=0.0182                  | -1.3 x 10 <sup>-3</sup> | 0.038 | 0.043                   | 38.2 <sup>a</sup>       | 126.8                   |

\* The results for M10 are from separate pathway fit parent → dummy → M10.

<sup>a</sup> Results not considered acceptable by the RMS.

**Table 83: Summary of formation fractions optimised in Kiesel and Geibel (2015i,j) based on data in Völkl (2001), for the total systems pathway fits.**

| Degradation pathway          | Kinetic model | System            | Formation fraction | Standard deviation |
|------------------------------|---------------|-------------------|--------------------|--------------------|
| Thiophanate-methyl → MBC     | SFO-SFO       | Pond              | 0.81               | 0.061              |
|                              |               | River             | 0.76               | 0.044              |
| MBC → 2-AB                   |               | Pond              | 0.25               | 0.063              |
| Thiophanate-methyl → 4-OH-TM |               | Pond              | 0.08               | 0.012              |
|                              |               | River             | 0.07 <sup>a</sup>  | 0.016              |
| Thiophanate-methyl → Dummy   |               | Pond              | 0.10               | 0.017              |
| Dummy → M10 *                | Pond          | 1.00 <sup>a</sup> | 0.24               |                    |

\* The results for M10 are from separate pathway fit parent → dummy → M10.

<sup>a</sup> Results not considered acceptable by the RMS.

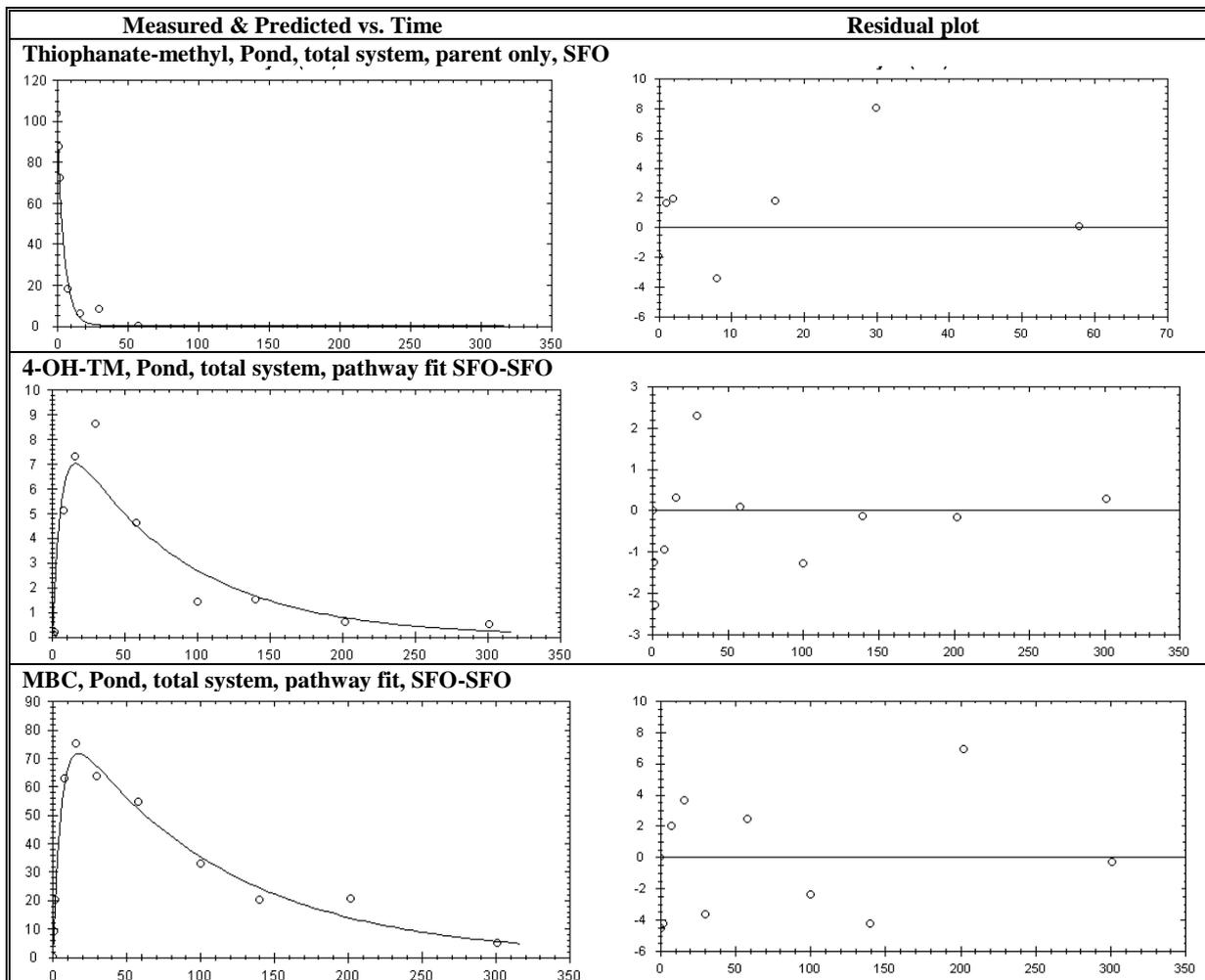
**Table 84: Water dissipation rates, summary of kinetic re-evaluation, from Kiesel and Geibel (2015i,j).**

| Substance          | System | kinetic model | Mo    | $\chi^2$ , % error | parameter | Confidence interval |       | prob > t               | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|--------------------|--------|---------------|-------|--------------------|-----------|---------------------|-------|------------------------|-------------------------|-------------------------|
|                    |        |               |       |                    |           | lower               | upper |                        |                         |                         |
| Thiophanate-methyl | Pond   | SFO           | 106.3 | 5.0                | k=0.250   | 0.21                | 0.29  | 1.7 x 10 <sup>-4</sup> | 2.8                     | 9.2                     |
| Thiophanate-methyl | River  | SFO           | 108.1 | 3.2                | k=0.425   | 0.36                | 0.49  | 0.0027                 | 1.6                     | 5.4                     |

**Table 85: Sediment dissipation rate, summary of kinetic re-evaluation, from Kiesel and Geibel (2015i,j).**

| Substance          | System | kinetic model | Mo   | $\chi^2$ , % error | parameter | Confidence interval |       | prob > t | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|--------------------|--------|---------------|------|--------------------|-----------|---------------------|-------|----------|-------------------------|-------------------------|
|                    |        |               |      |                    |           | lower               | upper |          |                         |                         |
| Thiophanate-methyl | Pond   | SFO           | 8.45 | 26.6               | k=0.027   | -0.010              | 0.063 | 0.15     | 26.1 <sup>a</sup>       | 86.7                    |

a Result not considered acceptable by the RMS.



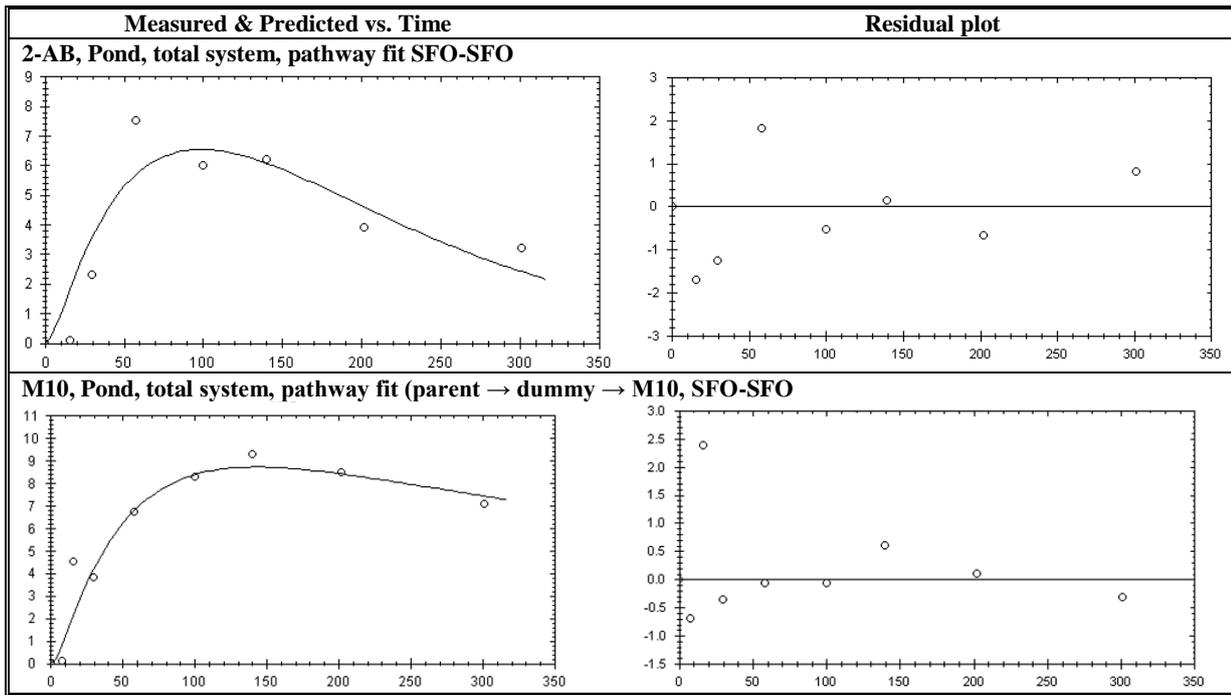


Figure 8.2.2.3-2. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl and metabolites in Pond total system (from Kiesel and Geibel, 2015i,j).

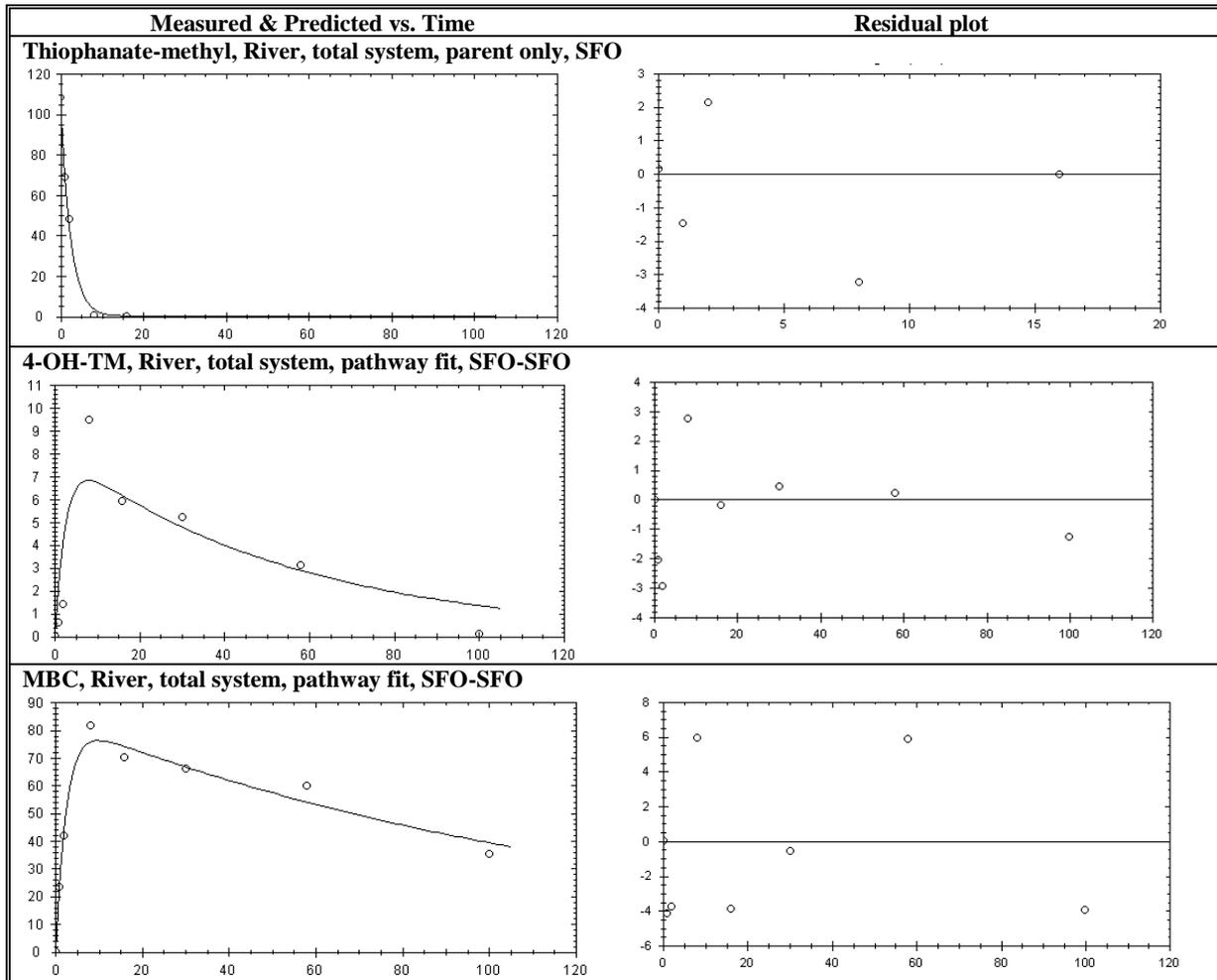


Figure 8.2.2.3-3. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl and metabolites in River total system (from Kiesel and Geibel, 2015i,j).

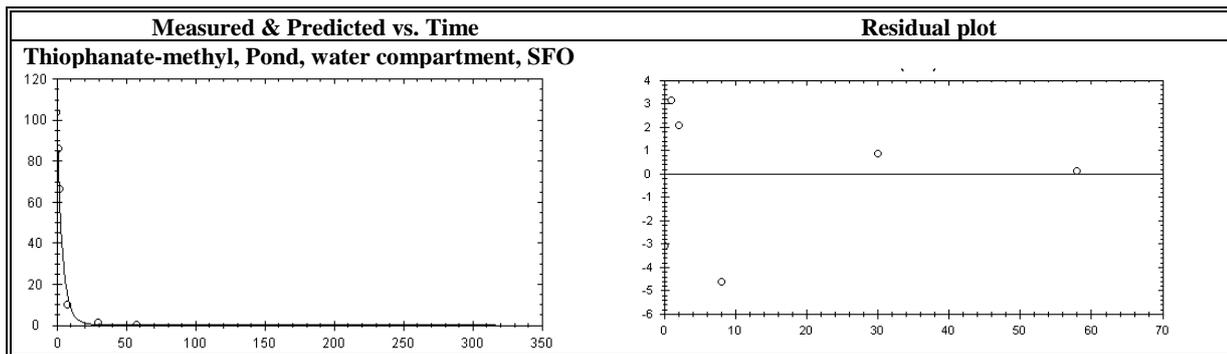


Figure 8.2.2.3-4. Visual fit and plot of residuals for kinetic fitting of data for Thiophanate-methyl in Pond water compartment (from Kiesel and Geibel 2015i,j)

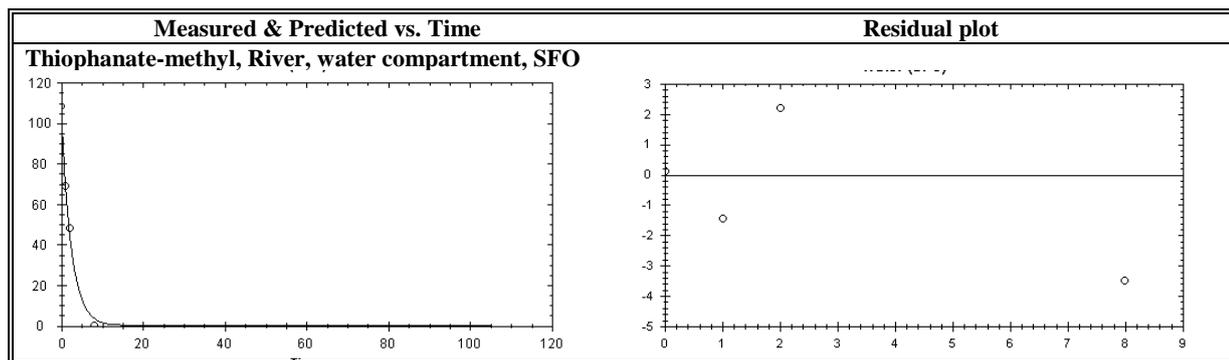


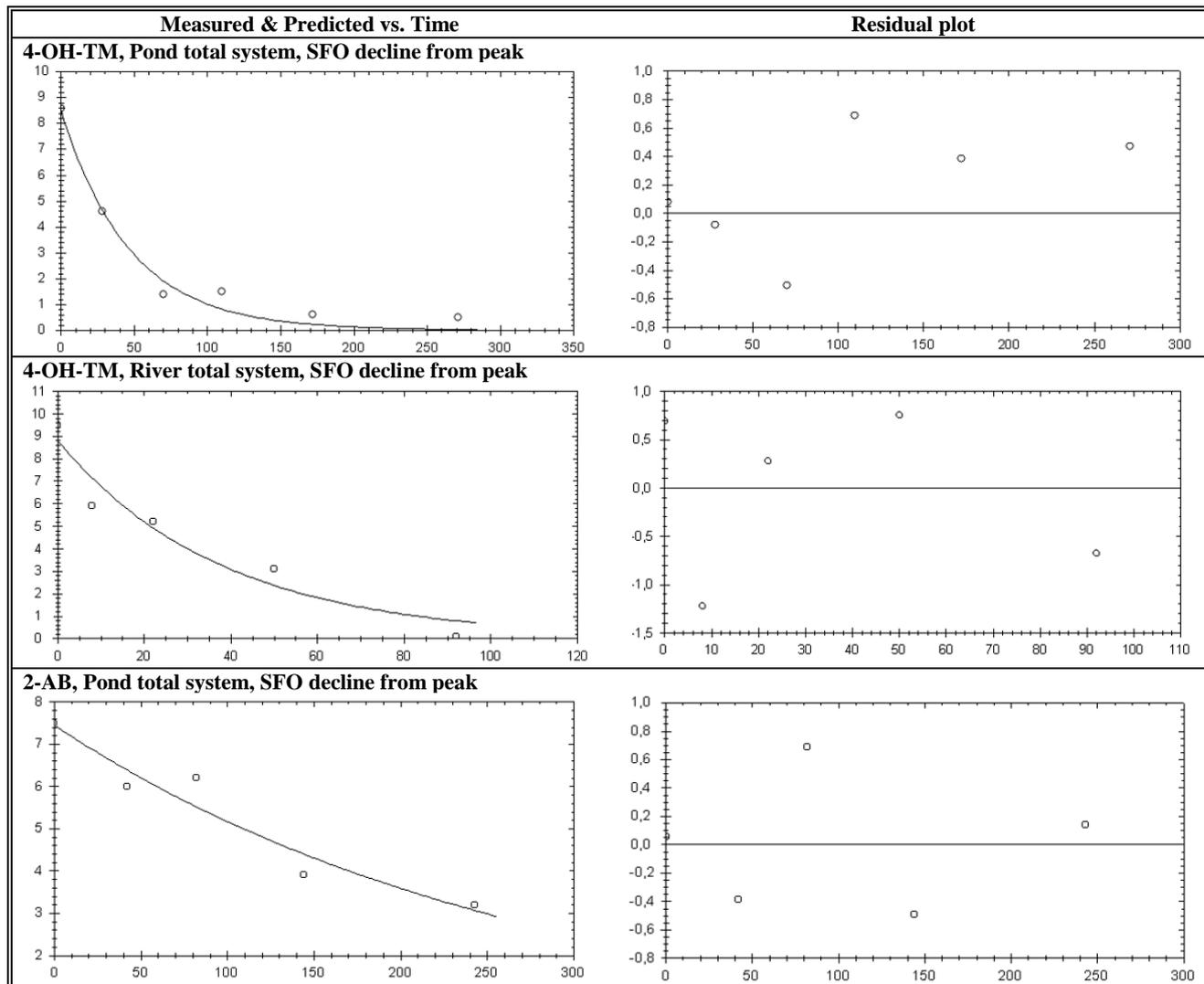
Figure 8.2.2.3-5. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl in River water compartment (from Kiesel and Geibel, 2015i,j).

During peer review the above results for 4-OH-TM and 2-AB in the pond system were questioned since Chi2 error-% were relatively high and since the peak was not covered by the fit. Kinetic evaluation using only data from the maximum observed onwards was therefore requested (Evaluation table, Data requirement 4.8). In response, Moshenberg and Drechsler (2017a,b) submitted such optimisations for 4-OH-TM for both water/sediment systems, and for 2-AB for the pond system (no data were presented for 2-AB in the river system presumably since only 4 data points were available from the peak observation). SFO and FOMC kinetic models were run in KinGUI 2.1. The first data point with no residues was set to ½LOQ (0.1% AR). The SFO endpoints were considered acceptable, and the authors suggested that these could be used for modeling as well as trigger endpoints. The RMS agrees to the conclusions.

**Table 86: Kinetic parameters and the resulting DT50/90 values for metabolites 4-OH-TM and 2-AB using only data from max occurrence onwards (decline from peak). Total water/sediment systems. From kinetic evaluation in Moshenberg and Drechsler (2017a,b) based on data in Völkl (2001).**

| Substance | System | kinetic model | Mo   | χ <sup>2</sup> , % error | parameter          | Confidence interval |              | Prob. > t    | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) |
|-----------|--------|---------------|------|--------------------------|--------------------|---------------------|--------------|--------------|-------------------------|-------------------------|
|           |        |               |      |                          |                    | lower               | upper        |              |                         |                         |
| 4-OH-TM   | Pond   | SFO           | 8.52 | 12.0                     | k=0.02136          | 0.02                | 0.03         | 8.1E-04      | 32.4                    | 108                     |
|           |        | FOMC          | 8.65 | 9.8                      | α= 2.32<br>β= 80.5 | -0.8<br>-63         | 5.4<br>225   | n.r.<br>n.r. | not reliable            | not reliable            |
| SFO       |        | 7.45          | 6.3  | k=0.003662               | 0.002              | 0.005               | 0.006        | 189          | 629                     |                         |
| FOMC      |        | 7.46          | 7.2  | α= 19.5<br>β= 5220       | -1098<br>-3.0E+05  | 1137<br>3.1E+05     | n.r.<br>n.r. | not reliable | not reliable            |                         |
| 4-OH-TM   | River  | SFO           | 8.80 | 13.2                     | k=0.02641          | 0.01                | 0.04         | 0.01         | 26.2                    | 87.2                    |
|           |        | FOMC          |      |                          |                    |                     |              |              |                         |                         |

n.r. Not relevant.



**Figure 8.2.2.3-6. Visual fit and plot of residuals for kinetic fitting of data for 4-OH-TM in Pond and River total systems, and for 2-AB in Pond total system. As decline from peak observation. (from Moshenberg and Drechsler (2017b))**

#### RMS comments and conclusion

The water/sediment study was accepted in the Addendum (2003) to the DAR (1997) and it is considered acceptable also for the purpose of renewal. Most of the kinetic evaluation is accepted. The derived endpoints for metabolite M10 is however not accepted due to the fact that the derived degradation rate constant was not statistically significant different from zero. Also the results for 4-OH-TM from the river system presented in Kiesel and Geibel (2015i,j) are considered as unreliable, since the fit was poor visually and since the Chi2 error-% was high (the lower confidence interval was below zero however the t-test indicated that the result was acceptable so this is not considered as a reason for omitting the endpoints). The DisT<sub>50</sub> in sediment calculated for the parent for the pond system is not considered reliable (t-test failed). Besides this a few errors in the kinetic report has been noted. Kiesel and Geibel (2015i,j) stated that metabolite M10 was only detected in the pond system. This was not correct but the RMS accepts that no kinetic evaluation was submitted for M10 for the river system, since it is not considered likely that useful results would have been obtained. Furthermore, Kiesel and Geibel (2015i,j) claimed that the half-lives for 2-AB and M10 are geometric means when in fact they were single values. Finally, the day 0 value entered for thiophanate-methyl was higher than the total mass balance for each system. However, the deviations were not large (+1.4 and +1.9% AR for the pond and river system,

respectively). Finally, the endpoints presented for 4-OH-TM and 2-AB by Moshenberg and Drechsler (2017a,b) are considered acceptable and the RMS suggests that those results should replace the corresponding results presented by Kiesel and Geibel (2015i,j). The requirement in Evaluation table, Data requirement 4.8 is considered as fulfilled.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

|                   |  |
|-------------------|--|
| <b>Reference:</b> | <p><b>1. Knoch, E. (2001)</b> Degradability and fate of (U-<sup>14</sup>C-phenyl) carbendazim in the aquatic environment (water/sediment system)</p> <p><b>2. Zillgens, B. (2007c)</b> Predicted environmental concentrations of carbendazim in surface water and sediment associated with uses in arable crops: Modeling for the European Union</p> |
| Report No.:       | <p>1. C017201, WAS2002-167</p> <p>2. DuPont-23452</p>  |
| Guideline:        | SETAC Europe, Dutch guideline  |
| GLP:              | 1. Yes   |
| Validity          | 1. Valid   |

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**Material and methods:**

Two test systems were chosen according to the recommendations of the OECD Belgrade Workshop that differed significantly in their characteristics. The data are reported in the table below.

The metabolism flasks were filled with a layer of water-saturated sediment (1 cm for sediment Bickenbach, 2 cm for sediment Unter Widdersheim). The height of the sediment layers corresponded to approximately 95 g (Bickenbach) and 120 g (Widdersheim), respectively. The water column height corresponded to about 6 cm (approximately 300 ml of water). The sediment dry weight to water ratio was approx. 1:4 (w:w) for both test systems. Air entering the system by pump suction was passed through a washing bottle filled with water. Water/sediments were aerated and slightly agitated from the top by means of suspended magnetic stirrer. Air leaving the test system was successively passed through sodium hydroxide and 2-methoxy ethanol traps to collect <sup>14</sup>CO<sub>2</sub> and other organic volatiles, respectively. [U-<sup>14</sup>C-phenyl] Carbendazim was applied onto the water surface of each sample. Each water phase was treated at 0.2 ppm level (equivalent to approximately 60 µg [U-<sup>14</sup>C-phenyl] carbendazim/300 ml water). A further exaggerated rate was applied at 2.0 mg/l <sup>14</sup>C-carbendazim, installed for possible metabolite identification. The experiment was carried out in the dark with single samples at each sampling point and radioactive treated water/sediment system at 20°C. Water and sediment were analysed separately at the following times: zero time, 3, 7, 14, 21, 28, 42, 62, 76, 98, 120 and 149 days after application. Sediment samples were extracted several times by shaking with 50 to 100 ml of acetonitrile:water (4:1; w:w). The remaining residual radioactivity in sediments were assayed by combustion. The total recoveries of radioactivity ranged from 93.3 % to 105.9 % of the total applied radioactivity.

Kinetic analyses: DT<sub>50</sub>/DT<sub>90</sub> were presented in the original report (Knoch, 2001). The results were also re-calculated using TopFit model (Kley, 2002) but those results are not reproduced here because the DT<sub>50</sub>/DT<sub>90</sub> were also re-calculated in accordance with FOCUS guidance (in Zillgens, 2007c) and those results were the final ones.

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**Table 87: Physico-chemical characterisation of water and sediment systems before incubation with [U-<sup>14</sup>C-phenyl]-carbendazim.**

| Parameters                          | Bickenbach-System |          | Unter Widdersheim-System |          |
|-------------------------------------|-------------------|----------|--------------------------|----------|
|                                     | Water             | Sediment | Water                    | Sediment |
| pH                                  | 8.5               | 8.0      | 8.1                      | 7.5      |
| Redox pot. [mV]                     | 167               | 136      | 157                      | 184      |
| CEC [meq/100 g]                     |                   | 11.1     |                          | 127      |
| Org. C [%]                          |                   | 0.41     |                          | 2.28     |
| Microbial act. (start) [mg C/100 g] |                   | 18       |                          | 22       |
| Dry weight [%]                      |                   | 80.1     |                          | 63.9     |
| Sediment texture (USDA)             |                   | sand     |                          | loam     |
| Sand [%]                            |                   | 93.1     |                          | 41.7     |
| Silt [%]                            |                   | 2.0      |                          | 37.0     |
| Clay [%]                            |                   | 4.6      |                          | 21.3     |

## Results

The formation of <sup>14</sup>CO<sub>2</sub> accounted for 6.0 to 23.0% AR by the end of the study. Other volatile degradation products were observed at equal or less than 0.1% of applied dose during the experiment.

The balance and distribution of the applied radioactivity (% applied) is given in the tables below.

**Table 88: Distribution of applied radioactivity in Bickenbach water/sediment-system during incubation with [U-<sup>14</sup>C-phenyl]-carbendazim (% AR).**

| Fraktion                 | Incubation time (days) |       |       |       |      |      |      |
|--------------------------|------------------------|-------|-------|-------|------|------|------|
|                          | 0                      | 3     | 14    | 28    | 62   | 98   | 120  |
| Water                    | 99.0                   | 85.5  | 44.4  | 24.0  | 11.0 | 8.4  | 9.1  |
| Sediment (extractable)   | 4.2                    | 13.7  | 17.2  | 14.9  | 8.1  | 5.8  | 9.0  |
| Sediment (unextractable) | 0.3                    | 4.4   | 39.5  | 62.5  | 63.4 | 61.0 | 55.2 |
| Sediment total           | 4.5                    | 18.1  | 56.7  | 77.4  | 71.5 | 66.8 | 64.2 |
| CO <sub>2</sub>          | -                      | <0.1  | 0.5   | 2.7   | 13.0 | 18.1 | 20.4 |
| Volatiles                | -                      | <0.1  | <0.1  | <0.1  | <0.1 | <0.1 | <0.1 |
| Mass balance             | 103.5                  | 103.6 | 101.6 | 104.1 | 95.5 | 93.3 | 93.7 |

**Table 89: Distribution of applied radioactivity in Unter Widdersheim water/ sediment-system during incubation with [U-<sup>14</sup>C-phenyl]-carbendazim (% AR).**

| Fraktion                 | Incubation time (days) |       |       |       |      |      |      |
|--------------------------|------------------------|-------|-------|-------|------|------|------|
|                          | 0                      | 3     | 14    | 28    | 62   | 98   | 120  |
| Water                    | 103.2                  | 55.6  | 30.4  | 9.7   | 4.7  | 3.8  | 3.4  |
| Sediment (extractable)   | 2.0                    | 37.8  | 64.7  | 73.2  | 55.6 | 42.5 | 28.5 |
| Sediment (unextractable) | 0.7                    | 10.4  | 9.3   | 19.1  | 33.8 | 46.4 | 59.4 |
| Sediment total           | 2.7                    | 48.2  | 74.0  | 92.3  | 89.4 | 88.9 | 87.9 |
| CO <sub>2</sub>          | -                      | <0.1  | 0.2   | .6    | 2.0  | 3.7  | 4.7  |
| Volatiles                | -                      | <0.1  | <0.1  | <0.1  | <0.1 | <0.1 | 0.1  |
| Mass balance             | 105.9                  | 103.8 | 104.6 | 102.6 | 96.2 | 96.5 | 96.1 |

The test substance carbendazim was degraded in both test systems. The initial step of carbendazim degradation resulted in the formation of 2-aminobenzimidazole (AEF033008). 1,2-Phenylendiamine (AE F037197), a possible next step in degradation, was not found in any compartment of the aerobic aquatic model systems. The overall conversion was accompanied by the formation of non-extractable residues and mineralisation to <sup>14</sup>CO<sub>2</sub>.

**Table 90: Distribution of parent and metabolites in Bickenbach and Unter Widdersheim system during incubation with carbendazim (% AR).**

| Component             | Analysis time (days) |       |       |        |        |                                      |                                      |  |
|-----------------------|----------------------|-------|-------|--------|--------|--------------------------------------|--------------------------------------|--|
|                       | 0                    | 3     | 7     | 14     | 28     | 62                                   | 98                                   | 120  |
| Bickenbach            |                      |       |       |        |        |                                      |                                      |  |
| Water                 |                      |       |       |        |        |                                      |                                      |  |
| Carbendazim           | 99.0                 | 97.1  | 67.0  | 35.9   | 13.3   | 0.8                                  | 0.4                                  | 0.2  |
| 1-Amino benzimidazole | <LOD                 | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | <LOD   |
| 1.2-Phenylendiamine   | <LOD                 | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | <LOD   |
| Unknown fractions     | <LOD                 | <LOD  | <LOD  | U1:6.9 | U1:9.8 | U1:7.8<br>U3:0.7<br>U4:1.0<br>U6:0.9 | U1:6.2<br>U3:0.7<br>U4:0.5<br>U6:0.3 | U1:2.4<br>U2:2.6<br>U4:4.3<br>U5:3.0<br>U6:5.5 |
| Sediment extracts     |                      |       |       |        |        |                                      |                                      |  |
| Carbendazim           | n.a.                 | 13.0  | 14.3  | 14.9   | 9.4    | 5.3                                  | 3.7                                  | 3.8  |
| 1-Amino benzimidazole |                      | <LOD  | <LOD  | <LOD   | <LOD   | 0.9                                  | 0.4                                  | 1.1  |
| 1.2-Phenylendiamine   |                      | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | <LOD   |
| Unknown fractions     |                      | <LOD  | <LOD  | <LOD   | U1:2.5 | U1: 1.1                              | <LOD                                 | <LOD   |
| Unter Widdersheim     |                      |       |       |        |        |                                      |                                      |  |
| Water                 |                      |       |       |        |        |                                      |                                      |  |
| Carbendazim           | 103.2                | 54.7  | 36.8  | 28.7   | 7.9    | 3.5                                  | 1.1                                  | n.a.   |
| 1-Amino benzimidazole | <LOD                 | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | n.a.   |
| 1.2-Phenylendiamine   | <LOD                 | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | n.a.   |
| Unknown fractions     | <LOD                 | <LOD  | <LOD  | <LOD   | <LOD   | U1:0.7<br>U4:0.5                     | U1:2.4                               | n.a.   |
| Sediment extracts     |                      |       |       |        |        |                                      |                                      |  |
| Carbendazim           | n.a.                 | 36.7  | 51.7  | 61.8   | 68.0   | 50.9                                 | 37.9                                 | 24.8   |
| 1-Amino benzimidazole |                      | <LOD* | <LOD* | <LOD*  | <LOD*  | 3.3<br>6.3(76 d)                     | 4.8                                  | 3.1  |
| 1.2-Phenylendiamine   |                      | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | <LOD   |
| Unknown fractions     |                      | <LOD  | <LOD  | <LOD   | <LOD   | <LOD                                 | <LOD                                 | <LOD   |

LOD between 0.2% AR and 5% AR

\*LOD of 2.5% AR

At zero-time, <sup>14</sup>C-carbendazim was observed in whole test systems at 99.0 and 103.2% of the applied dose decreasing to <1.0 and 33.4% AR (Bickenbach/Widdersheim respectively) at the end of the study, day 149. The metabolite 2-aminobenzimidazole was detected at a maximum peak value of 6.3% AR in the whole system of Unter Widdersheim after 76 days of incubation. A maximum of six unknown metabolites were assigned in the chromatographed radioactivity.

Radiolabeled carbendazim was present in all water phases analyzed. The initial values of the test substance (99.0 to 103.2% AR) decreased to a minimum value of 0.5% AR on day 149.

The presence of 2-aminobenzimidazole in the water phases of both test systems was reflected by peak values below 2.5% of the applied dose. A maximum of six unknown metabolites were assigned in the chromatographed radioactivity. After 3 days of incubation radiolabeled carbendazim was found with 13.0 and 36.7% AR in the sediment extractable radioactivity. A maximum peak value of 68% AR was observed in the sediment on day 28. After 5 months of incubation <sup>14</sup>C-carbendazim decreased to <5 % and 33.4% AR in the sediment extractable radioactivity of "Bickenbach" and "Unter Widdersheim" location, respectively. In the sediment extracts unknown metabolites reached

radioactivity levels clearly below 5% of the applied radioactivity. 2-Aminobenzimidazole yielded for a peak maximum of 6.3% AR in the sediment extractables on day 76 not exceeding 5% in two consecutive time points.

The non-extractable residues were subjected to an organic matter fraction of the sediments. With a value of 32.1 to 40.8% AR, most of the residual radioactivity was found in the humin fraction after 98 to 120 days of incubation.

Carbendazim was degraded in both aquatic test systems. The half-life for parent compound along with their disappearance times (DT-90) and their errors in the water phases and the total systems are summarised in the below table. The calculations were carried out by using the software EXCEL and based on a single-exponential first-order kinetics as the mathematical model.

**Table 91: DT<sub>50</sub> and DT<sub>90</sub> for carbendazim in the two water/sediment systems.**

| System      | Disappearance time |           |                |            |           |                |
|-------------|--------------------|-----------|----------------|------------|-----------|----------------|
|             | From total systems |           |                | From water |           |                |
|             | DT-50 (d)          | DT-90 (d) | r <sup>2</sup> | DT-50 (d)  | DT-90 (d) | r <sup>2</sup> |
| Bickenbach  | 16.1               | 53.5      | 0.9893         | 10.8       | 36.0      | 0.9947         |
| Widdersheim | 73.6               | 244.4     | 0.9940         | 5.8        | 19.2      | 0.9651         |

The DT<sub>50</sub> for both systems were recalculated regarding FOCUS Kinetics Guideline (2006) and documented as in the below table.

**Table 92: Re-calculation according FOCUS Kinetics Guideline (2006) (Zillgens, 2007c).**

| System      | Disappearance time from total systems |          |                      |       |
|-------------|---------------------------------------|----------|----------------------|-------|
|             | DT50 (d)                              | DT90 (d) | χ <sup>2</sup> error | Model |
| Bickenbach  | 15.1                                  | 50       | 11                   | SFO   |
| Widdersheim | 75.2                                  | 249.7    | 12                   | SFO   |

**Conclusion**

The study is valid and plausible.

|                             |   |
|-----------------------------|---|
| <b>Reference:</b>           | <ol style="list-style-type: none"> <li>1. <b>Völkl, S. (2002)</b> <sup>14</sup>C-Thiophanate-methyl: Degradation and Metabolism in Three Soils Incubated under Aerobic Conditions.</li> <li>2. <b>Kiesel, A. and Geibel, E. (2015a)</b> Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014).</li> <li>3. <b>Kiesel, A. and Geibel, E. (2015b)</b>. Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on a laboratory study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier – M-CA Section 7.</li> <li>4. <b>Kiesel, A. and Geibel, E. (2016a)</b> Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014).</li> <li>5. <b>Kiesel, A. and Geibel, E. (2016b)</b>. Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on a laboratory study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier – M-CA Section 7.</li> <li>6. <b>Kiesel, A., Drechsler, S., Geibel, E. (2017a)</b> Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)</li> <li>7. <b>Kiesel, A., Drechsler, S., Geibel, E. (2017b)</b> Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a laboratory soil study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7</li> </ol> |
| Report No.:                 | <ol style="list-style-type: none"> <li>1. 815051</li> <li>2. RD-03326</li> <li>3. RD-03369</li> <li>4. RD-03326N</li> <li>5. RD-03369N</li> <li>6. RD-03326N2</li> <li>7. RD-03369N2</li> </ol>   |
| Document No.:               | <ol style="list-style-type: none"> <li>1. 721-004</li> <li>2. 782-035</li> <li>3. 782-041</li> <li>4. 782-088</li> <li>5. 782-089</li> <li>6. 782-097</li> <li>7. 782-098</li> </ol>  |
| Guideline:                  | <ol style="list-style-type: none"> <li>1. OECD TG No. 307 (draft August 2000); US EPA 162-1</li> <li>2. - 7. FOCUS (2006, 2014)</li> </ol>  |
| GLP:                        | <ol style="list-style-type: none"> <li>1. Yes</li> <li>2. - 7. Not applicable</li> </ol>  |
| <b>Previous evaluation:</b> | <ol style="list-style-type: none"> <li>1. In Addendum (2003) to DAR (1997)</li> <li>2. - 7. Submitted for the purpose of renewal</li> </ol>   |

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**Material and methods:**

|                              |   |
|------------------------------|---|
| Test material:               | Phenyl-UL- <sup>14</sup> C-labelled thiophanate-methyl  |
| Lot No:                      | EC-01-01 (RCC Ltd Code: 115065/A, 116973/A)   |
| Radiochemical purity:        | 98.9% (determined by HPLC)  |
| Test system/test conditions: | <p>In Völkl (2002), the route and rate of degradation of thiophanate-methyl under aerobic conditions was studied in three soils.</p> <p>100 g dry soil were filled into all-glass metabolism flasks. Soil samples were treated with <sup>14</sup>C-thiophanate-methyl dissolved in acetone, at nominally 1.48 mg a.s./kg dry soil, which corresponded to 1.54–1.86 kg a.s./ha assuming 10 cm mixing depth and the soil's dry bulk densities.</p> <p>The soils were incubated in the dark at 20±2°C at 46% MWHC. Flow-through systems with absorption traps with ethylene glycol and 2N NaOH for VOC and <sup>14</sup>CO<sub>2</sub>, respectively, were used.</p> |

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Duplicated samples were taken after 0, 1, 3, 7, 14, 28, 56, 120 days of incubation. For day 0, no adsorption traps were used and only one extraction with acetonitrile at room temperature was performed.

For all other sampling intervals, the samples were subject to extraction with acetonitrile:water (8:2; v/v) at room temperature up to four times and one Soxhlet extraction with acetonitrile:water (9:1; v/v).

On day 120, an additional reflux extraction was performed for 4 hours with acetonitrile:2M hydrochloric acid (8:2; v/v). The radioactivity in all extracts was measured with LSC. Non-extractable radioactivity was quantified by LSC after combustion. The LOQ of the LSC counter was 0.06% AR.

An additional test with Soxhlet extraction was performed to proof that radioactive fractions M1 and M2 were not formed during the extraction step. Therefore, one soil sample of soil III (Speyer 2.3) was fortified with  $^{14}\text{C}$ -thiophanate-methyl and extracted by Soxhlet extraction as described above and analysed by HPLC.

The extracts were pooled, concentrated and then chromatographically profiled with HPLC to determine parent (thiophanate-methyl) and its main metabolites with a LOQ of 0.3% AR. For the purpose of metabolite identification, the following reference standards were used: DX-105, FH-432, CF-27 (Carbendazim, MBC), AV-1951, 2-AB, 3-OH-TM, 4-OH-TM, 3-OH-TM-S. TLC was used as confirmatory method on selected soil samples.

Soil organic matter fractionation was carried out on a sample from day 120 (after the additional harsh extraction).

The presence of  $^{14}\text{CO}_2$  in the NaOH-traps was confirmed by  $\text{BaCO}_3$ -precipitation. Microbial biomass was determined before treatment and after about 127 days of incubation. It corresponded to at least 1% of the organic carbon content of the soils.

In Völkl (2002),  $\text{DT}_{50\text{s}}$  were calculated for thiophanate-methyl and the metabolites carbendazim and the unknown M2 using first-order kinetic model (non-linear curve fitting). These results were superseded by the re-evaluation by Kiesel and Geibel (2015a,b) which during the evaluation was replaced by Kiesel and Geibel (2016a,b). The kinetic re-evaluation was performed according to FOCUS Degradation kinetics report (2006, 2014) with KinGUI version 2.1. Parent only data were fitted to SFO and FOMC kinetic models. Degradation pathway-fits were performed for thiophanate-methyl and the metabolites carbendazim, CM-0237 and 2-AB in a sequential fitting approach, using SFO-SFO. The optimization method IRLS and the optimization algorithm LM were used. The results were normalised to standard conditions (20°C and pF 2) to fulfil requirements for modelling endpoints according to FOCUS Groundwater (2000, 2014). During peer review degradation rate for one additional soil was requested for the metabolite CM-0237 (Evaluation table, Data requirement 4.2). In response to the requirement, Kiesel, Drechsler & Geibel (2017a,b) estimated parameters for CM-0237 for the Bretagne soil (soil I). In the previous kinetic evaluations parameters were not estimated for CM-0237 in the Bretagne soil (soil I), since CM-0237 was only found in minor amounts in that soil.

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**Table 93: Soil characteristics.**

|                                |                      | Bretagne (soil I)   | Mussig (soil II) | Speyer 2.3 (soil III)  |
|--------------------------------|----------------------|---|------------------|--|
| Origin                         |                      | France  | France           | Germany  |
| Soil plot history              |                      | No pesticide treatments for at least 5 years prior to soil sampling. No cultivation in these fields since 1995.                 |                  | No pesticides between 1997 and 2001.   |
| Collection and storage         |                      | Collected in September 2001. Stored for about 3 weeks at 4°C. Sieved (2 mm). Acclimation at 20°C for about 5 days before study. |                  | Collected on July 20, 2001. Sieved (2 mm). Stored for about 8 weeks at 4°C. Acclimation at 20°C for about 5 days before study. |
| Soil type (USDA)               |                      | Silt loam   | Clay loam        | Sandy loam   |
| Clay <2µm                      | [%]                  | 19.5  | 34.2             | 9.0  |
| Silt 2–50 µm                   | [%]                  | 61.2  | 44.3             | 33.3   |
| Sand >50µm                     | [%]                  | 19.3  | 21.5             | 57.8   |
| Dry bulk density               | [g/cm <sup>3</sup> ] | 1.04  | 1.04             | 1.26   |
| Water content at pF 1.0 (MWHC) | [g/100 g soil]       | 60.5  | 66.0             | 38.0   |
| 40–60% MWHC                    | [g/100 g soil]       | 24.2-36.3   | 26.4-39.6        | 15.2-22.8  |
| Water content at pF 1.8        | [g/100 g soil]       | 43.0  | 45.7             | 27.7   |
| Water content at pF 2.5        | [g/100 g soil]       | 29.9  | 31.2             | 13.8   |
| pH (CaCl <sub>2</sub> )        |                      | 5.8   | 7.5              | 6.5  |
| Organic carbon                 | [g/100 g soil]       | 2.0   | 3.0              | 1.3  |
| CEC                            | [meq/100 g soil]     | 8.5   | 36.2             | 10.0   |
| Biomass – start of incubation  | [mg/100 g soil]      | 23.5  | 34.1             | 21.3   |
| Biomass – end of incubation    | [mg/100 g soil]      | 18.7  | 36.5             | 13.9   |

## Results

Mass balance and characterisation of thiophanate-methyl and its metabolites are listed in the tables below. The total recovery in individual replicates ranged from 93.3 to 102.9% AR for all soils and for all sampling intervals. The extractable radioactivity decreased from >98.6% AR at day 0 to 15.3–31.2% AR at the end of the study (day 120). Soxhlet extraction released a maximum of 22.3% AR. The additional harsh extraction performed with sample A at day 120 resulted in high amounts of additionally extracted material; up to 30.3% AR in soil III (5.6% and 15.3% AR in the other two soils). The non-extractable fractions increased continuously from 1.4–3.2% AR at day 0 to 39.7–73.2% AR at day 120. The organic matter fractionation resulted in a relatively equal distribution among fulvic acids, humic acids and humins in the silt loam (Bretagne, soil I) and the sandy loam (Speyer 2.3, soil III). The major part (85.5%) of the non-extractable radioactivity in the clay loam (Mussig, soil II) was bound to humins, 12% to fulvic acids and less than 2% to humic acids. <sup>14</sup>CO<sub>2</sub> reached 7.3–7.6% AR in soils II and III and was 25.7% AR after 120 days in soil I. Other volatile compounds were below 0.1% AR at all sampling intervals and all soils. The microbial biomass measurements showed that the samples were viable throughout the study, although the microbial biomass decreased in two of the soils (Bretagne and Speyer 2.3).

Thiophanate-methyl was almost entirely degraded within the first 7 days. In all soils, the concentration of thiophanate-methyl was <0.5% AR after day 7 with no detection at the last two sampling intervals (day 56 and 120).

The route of degradation as proposed by Völkl (2002) was the same as in Adam (2014); see Figure 8.1.1.1-6.

The metabolite carbendazim was formed in all soils in large amounts already after 1 day (42.2–58.7% AR) and reached maxima of 62.8-75.8% in the different soils after 3 or 7 days, respectively. Two additional metabolites were detected in concentrations ≥5% at a minimum of two consecutive sampling intervals in at least one soil: CM-0237 and 2-AB. The

metabolite CM-0237 was characterised in Völkl (2002) by LC-MS analysis, but referred to as “M2” in the study, while the applicant referred to the metabolite as CM-0237 in documents M-CA, M-CP and N3.

Metabolite CM-0237 (M2) reached a maximum of 9.8% AR (after 3 days). This metabolite together with M1 (named CM-0238 by the applicant) were present as impurities in the application solution. The author, therefore, subtracted the mean amounts before and after application from the amounts of CM-0237 (M2) and M1 detected in soil extracts. These mean amounts were 1.3% in the case of CM-0237 (M2) and 2.7% in the case of CM-0238 (M1). This resulted in originally measured maximum concentrations of 11.1% and 7.7%. M1 was only detected once in a concentration  $\geq 5\%$ . Both metabolites were characterised in Völkl (2002) by LC-MS with molecular masses of 306 and 325 g/mol, respectively. The author furthermore showed that neither of the two metabolites was formed during extraction steps.

The metabolite 2-AB reached a maximum of 6.1% with standard extraction at day 14 in soil Mussig (soil II) and was 5.0% on average at the following sampling interval (day 28). A maximum of 18% AR was extracted with the additional harsh extraction at day 120 from soil Speyer 2.3 (soil III).

Additionally, the metabolite DX-105 and up to 9 unknown other metabolites were detected. None of them exceeded 4.7% AR.

**Table 94: Distribution and characterisation of radioactivity in the Bretagne soil (soil I) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20±2°C and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.\* All values as % of applied radioactivity (AR).**

|                               | days after application |      |      |      |      |      |      |      |
|-------------------------------|------------------------|------|------|------|------|------|------|------|
|                               | 0                      | 1    | 3    | 7    | 14   | 28   | 56   | 120  |
| Parent                        | 85.7                   | 15.9 | 1.4  | 0.9  | -    | 0.6  | -    | -    |
|                               | 81.1                   | 23.5 | 1.7  | 4.6  | -    | -    | -    | -    |
| Mean                          | 83.4                   | 19.7 | 1.6  | 2.7  | -    | 0.3  | -    | -    |
| Carbendazim                   | 8.3                    | 63.0 | 72.6 | 76.2 | 68.0 | 53.5 | 38.9 | 23.0 |
|                               | 10.2                   | 54.5 | 74.2 | 75.4 | 69.1 | 50.8 | 36.7 | 25.2 |
| Mean                          | 9.3                    | 58.7 | 73.4 | 75.8 | 68.5 | 52.1 | 37.8 | 24.1 |
| DX-105                        | 1.7                    | 4.0  | 1.3  | -    | 2.5  | -    | -    | -    |
|                               | 1.6                    | 4.6  | 1.5  | -    | -    | -    | -    | -    |
| Mean                          | 1.6                    | 4.3  | 1.4  | -    | 1.2  | -    | -    | -    |
| 2-AB                          | -                      | -    | 0.6  | 1.3  | 3.4  | 3.2  | 3.3  | 2.6  |
|                               | -                      | -    | 0.4  | -    | 3.7  | 2.9  | 3.2  | 2.8  |
| Mean                          | -                      | -    | 0.5  | 0.6  | 3.6  | 3.0  | 3.3  | 2.7  |
| CM-0237 (M2) <sup>a</sup>     | 1.3                    | 3.6  | 2.3  | 1.3  | 1.0  | 0.3  | -    | -    |
|                               | 1.3                    | 4.7  | 2.0  | 1.6  | 1.0  | 0.2  | 0.1  | -    |
| Mean                          | 1.3                    | 4.1  | 2.1  | 1.4  | 1.0  | 0.3  | 0.1  | -    |
| CM-0238 (M1) <sup>a</sup>     | -                      | -    | -    | -    | -    | -    | -    | -    |
| M3                            | -                      | -    | 1.7  | 1.9  | 1.2  | 0.7  | 0.5  | 0.3  |
| M6                            | -                      | 0.6  | 1.2  | 1.7  | 1.3  | 2.4  | 1.0  | 1.6  |
| M7                            | 0.7                    | 0.8  | 0.6  | -    | 0.5  | 0.3  | 0.5  | 0.3  |
| M8                            | -                      | 1.5  | 0.3  | -    | -    | 0.2  | 0.4  | 0.1  |
|                               |                        |      |      |      |      |      |      |      |
| Extractable                   | 98.6                   | 86.0 | 77.1 | 69.3 | 57.5 | 47.7 | 31.6 | 10.5 |
| Soxhlet                       | n.p.                   | 6.6  | 8.6  | 16.2 | 21.9 | 12.9 | 14.4 | 20.7 |
| Total extractable             | 98.6                   | 92.6 | 85.7 | 85.5 | 79.4 | 60.6 | 46.0 | 31.2 |
| Non-extractable               | 3.2                    | 4.9  | 11.2 | 12.2 | 17.5 | 30.1 | 39.3 | 39.7 |
| <sup>14</sup> CO <sub>2</sub> | n.p.                   | <0.1 | <0.1 | 0.2  | 0.8  | 3.5  | 10.3 | 25.7 |
| Other Volatiles               | n.p.                   | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Total recovery                | 101.8                  | 97.5 | 97.0 | 97.9 | 97.7 | 94.2 | 95.6 | 96.6 |
|                               | <b>97.3 ± 2.1</b>      |      |      |      |      |      |      |      |

\* Results for additional radioactive fractions (M5, M12, M13, M14), individually not exceeding 1.3%, were not presented in detail by the author.

- Not detected

<sup>a</sup> Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%) detected in the application solution before and after application.

**Table 95: Distribution and characterisation of radioactivity in the Mussig soil (soil II) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20±2°C and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.\* All values as % of applied radioactivity (AR).**

|                               | days after application |      |      |      |      |      |      |      |
|-------------------------------|------------------------|------|------|------|------|------|------|------|
|                               | 0                      | 1    | 3    | 7    | 14   | 28   | 56   | 120  |
| Parent                        | 89.9                   | 41.5 | 0.8  | 2.5  | 0.8  | 0.4  | -    | -    |
|                               | 90.4                   | 32.0 | 3.3  | 2.1  | -    | 0.4  | -    | -    |
| Mean                          | 90.1                   | 36.7 | 2.1  | 2.3  | 0.4  | 0.4  | -    | -    |
| Carbendazim                   | 5.2                    | 39.9 | 62.6 | 55.8 | 45.3 | 30.3 | 13.1 | 3.1  |
|                               | 4.6                    | 44.5 | 63.0 | 59.1 | 44.7 | 28.2 | 13.0 | 3.3  |
| Mean                          | 4.9                    | 42.2 | 62.8 | 57.4 | 45.0 | 29.2 | 13.1 | 3.2  |
| DX-105                        | 0.9                    | 1.1  | 0.5  | -    | -    | -    | -    | -    |
|                               | 0.6                    | 1.2  | 0.7  | -    | -    | -    | -    | -    |
| Mean                          | 0.7                    | 1.2  | 0.6  | -    | -    | -    | -    | -    |
| 2-AB                          | -                      | -    | 1.6  | 3.1  | 5.5  | 5.9  | 3.4  | 1.5  |
|                               | -                      | -    | 1.3  | 2.3  | 6.7  | 4.2  | 3.9  | 1.3  |
| Mean                          | -                      | -    | 1.5  | 2.7  | 6.1  | 5.0  | 3.7  | 1.4  |
| CM-0237 (M2) <sup>a</sup>     | -                      | 2.9  | 5.6  | 8.0  | 4.4  | 5.0  | 3.2  | 2.7  |
|                               | -                      | 4.3  | 6.1  | 5.2  | 5.5  | 4.9  | 3.4  | 3.1  |
| Mean                          | -                      | 3.6  | 5.9  | 6.6  | 4.9  | 4.9  | 3.3  | 2.9  |
| CM-0238 (M1) <sup>a</sup>     | 0.2                    | 2.6  | -    | -    | -    | -    | -    | -    |
| M3                            | -                      | -    | 2.8  | -    | 2.3  | 1.3  | 1.3  | 0.6  |
| M6                            | -                      | -    | -    | -    | 0.6  | 0.7  | -    | 1.2  |
| M7                            | -                      | 0.7  | 3.9  | 4.6  | 4.0  | 2.7  | 2.8  | 2.5  |
| M8                            | -                      | 1.7  | -    | -    | 0.3  | 0.2  | 0.4  | 0.4  |
| Extractable                   | 98.7                   | 86   | 69.9 | 57.7 | 48.1 | 32.3 | 11.8 | 3.4  |
| Soxhlet                       | n.p.                   | 6.7  | 11.7 | 17.2 | 17.3 | 13.9 | 16   | 11.9 |
| Total extractable             | 98.7                   | 92.7 | 81.6 | 74.9 | 65.4 | 46.2 | 27.8 | 15.3 |
| Non-extractable               | 2.1                    | 5.1  | 16.3 | 22.5 | 31.0 | 48.1 | 63.3 | 73.2 |
| <sup>14</sup> CO <sub>2</sub> | n.p.                   | <0.1 | 0.2  | 0.5  | 0.9  | 1.5  | 3.7  | 7.6  |
| Other Volatiles               | n.p.                   | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Total recovery                | 100.7                  | 97.8 | 98.1 | 97.9 | 97.4 | 95.8 | 94.8 | 96.1 |
|                               | <b>97.3 ± 1.8</b>      |      |      |      |      |      |      |      |

\* Results for additional radioactive fractions (M5, M11, M12, M13), individually not exceeding 0.8%, were not presented in detail by the author.

- Not detected

<sup>a</sup> Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%) detected in the application solution before and after application.

**Table 96: Distribution and characterisation of radioactivity in the Speyer 2.3 soil (soil III) treated with phenyl-14C-labelled thiophanate-methyl and incubated at  $20 \pm 2^\circ\text{C}$  and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.\* All values as % of applied radioactivity (AR).**

|                               | days after application |      |       |      |      |      |      |      |
|-------------------------------|------------------------|------|-------|------|------|------|------|------|
|                               | 0                      | 1    | 3     | 7    | 14   | 28   | 56   | 120  |
| Parent                        | 89.2                   | 31.1 | 1.5   | 1.0  | -    | -    | -    | -    |
|                               | 90.3                   | 29.0 | 1.6   | -    | -    | -    | -    | -    |
| Mean                          | 89.7                   | 30.0 | 1.6   | 0.5  | -    | -    | -    | -    |
| Carbendazim                   | 5.4                    | 44.6 | 68.0  | 58.4 | 51.1 | 37.6 | 23.1 | 13.0 |
|                               | 5.9                    | 45.4 | 64.3  | 58.3 | 51.6 | 39.1 | 23.2 | 11.7 |
| Mean                          | 5.7                    | 45.0 | 66.1  | 58.4 | 51.4 | 38.3 | 23.1 | 12.3 |
| DX-105                        | 0.7                    | 1.0  | -     | -    | -    | -    | -    | -    |
|                               | 0.6                    | 0.9  | -     | -    | -    | -    | -    | -    |
| Mean                          | 0.6                    | 1.0  | -     | -    | -    | -    | -    | -    |
| 2-AB                          | -                      | -    | 0.7   | -    | 2.3  | 1.6  | 1.9  | 2.6  |
|                               | -                      | -    | 0.7   | -    | 2.2  | 1.8  | 1.8  | -    |
| Mean                          | -                      | -    | 0.7   | -    | 2.3  | 1.7  | 1.8  | 1.3  |
| CM-0237 (M2) <sup>a</sup>     | -                      | 5.4  | 11.2  | 7.2  | 7.9  | 6.6  | 3.3  | 2.3  |
|                               | -                      | 6.0  | 8.5   | 9.6  | 8.3  | 6.2  | 3.7  | 2.3  |
| Mean                          | -                      | 5.7  | 9.8   | 8.4  | 8.1  | 6.4  | 3.5  | 2.3  |
| CM-0238 (M1) <sup>a</sup>     | 0.4                    | 5.0  | -     | 0.5  | -    | -    | -    | -    |
| M3                            | -                      | -    | 2.1   | 0.9  | 1.4  | 1.0  | 0.8  | 0.4  |
| M6                            | -                      | -    | 0.3   | -    | 1.1  | 1.5  | 0.1  | 1.6  |
| M7                            | 0.2                    | 1.1  | 2.0   | 2.2  | 2.1  | 1.5  | 1.4  | 0.8  |
| M8                            | -                      | 0.2  | 0.2   | -    | 0.2  | 0.4  | 0.2  | 0.2  |
| Extractable                   | 99.4                   | 86.3 | 73.3  | 58.5 | 50.8 | 36.8 | 20.7 | 5.1  |
| Soxhlet                       | n.p.                   | 6.5  | 11.8  | 16.4 | 17.7 | 15.9 | 15.6 | 18.2 |
| Total extractable             | 99.4                   | 92.8 | 85.1  | 74.9 | 68.5 | 52.7 | 36.3 | 23.3 |
| Non-extractable               | 1.4                    | 5.7  | 14.8  | 20.1 | 27.2 | 39.8 | 56.7 | 65.9 |
| <sup>14</sup> CO <sub>2</sub> | n.p.                   | <0.1 | 0.1   | 0.4  | 0.7  | 1.4  | 3.4  | 7.3  |
| Other Volatiles               | n.p.                   | <0.1 | <0.1  | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Total recovery                | 100.8                  | 98.5 | 100.1 | 95.4 | 96.4 | 93.9 | 96.4 | 96.5 |
|                               | <b>97.3 ± 2.6</b>      |      |       |      |      |      |      |      |

\* Results for additional radioactive fractions (M5, M11, M12, M13, M15), individually not exceeding 1.9%, were not presented in detail by the author.

- Not detected

a Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%), which were detected in the application solution before and after application.

**Table 97: Recovery of radioactivity as % AR in the different extraction steps, characterisation of the extractable radioactivity after harsh extraction and radioactivity in organic matter fractions, analysed in one sample taken at day 120.**

|   | Bretagne Soil I | Mussig Soil II | Speyer 2.3 Soil III |
|---|-----------------|----------------|---------------------|
| Non-extractable after extraction at room temperature and Soxhlet extraction | 40.3            | 72.5           | 63.8                |
| Extractable radioactivity by "harsh extraction"                             | 14.7            | 5.6            | 30.3                |
| Carbendazim   | 7.2             | 1.1            | 9.0                 |
| 2-AB  | 6.3             | 1.2            | 18.1                |
| CM-0237 (M2)  | 0.8             | 1.9            | 0.6                 |
| CM-0238 (M1)  | 0.2             | 0.1            | 0.3                 |
| Others  | 0.2             | 1.3            | 2.3                 |
| Non-extractable after "harsh extraction"                                    | 25.6            | 66.9           | 33.5                |
| Soluble fraction at low pH (fulvic acids)                                   | 10.1            | 8.3            | 7.7                 |
| Soluble fraction at high pH (humic acids)                                   | 7.5             | 1.1            | 11.7                |
| Insoluble fraction (humins)   | 8.1             | 57.5           | 14.1                |

The author noted that carbendazim was often not retained on the HPLC column and eluting partially after about 5 minutes and after about 15 minutes (see one example in the figure below). The author explained the reduced retention by the low pH value in the samples (pH 5.5) that increased the polarity of carbendazim (pKa = 4.2 according to EFSA conclusion, 2010). The author added that an interaction with the organic solvent present in the sample caused lack of retention. This could be confirmed by partial evaporation of the organic solvent, which the author tested with one sample. The author further mentioned that the use of organic solvent was necessary to obtain complete recoveries. The author added up the two peaks and considered the sum as carbendazim.

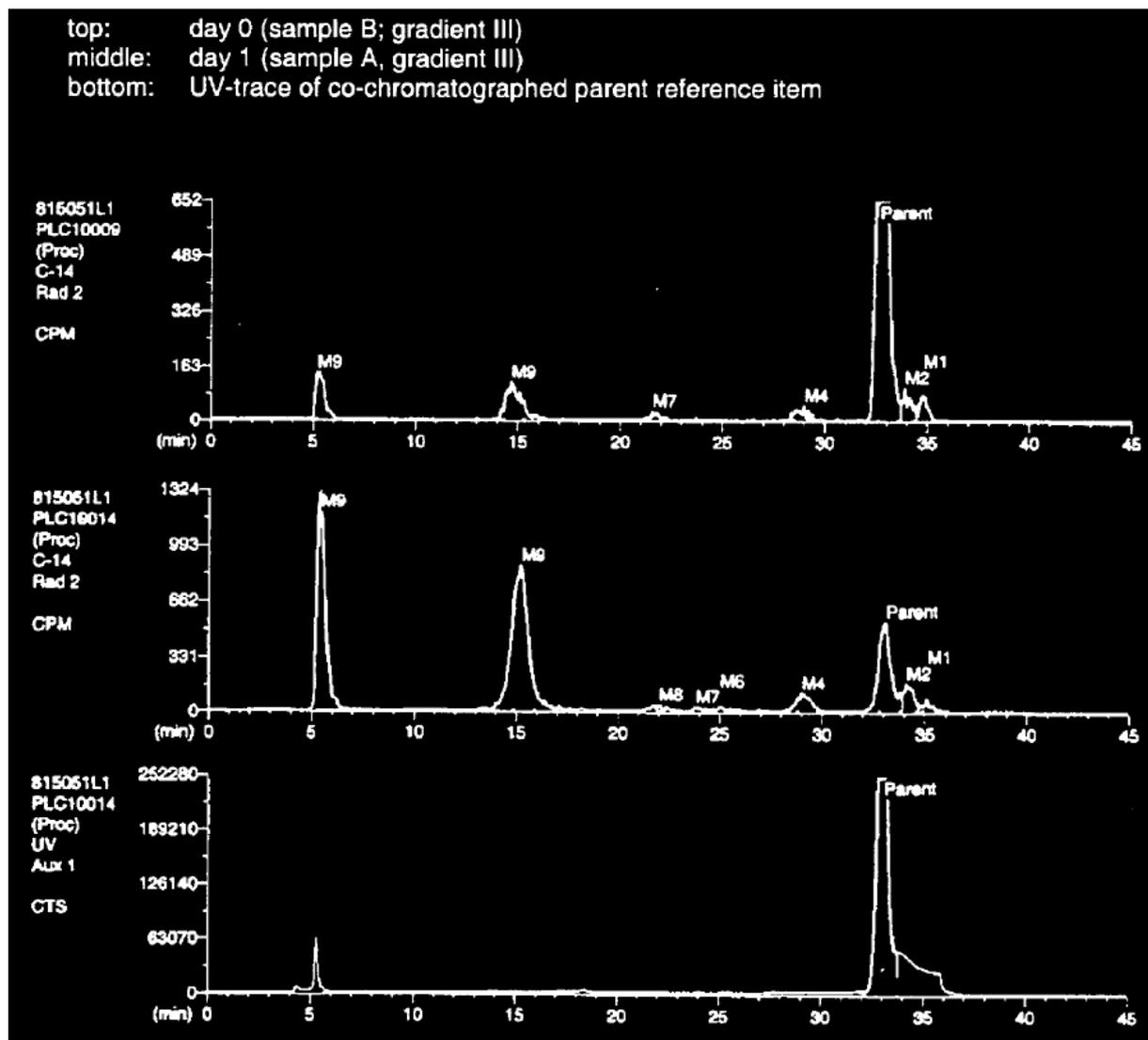
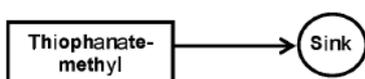


Figure 8.1.1.1-1. HPLC chromatograms of the soil extracts of soil I (Bretagne) on incubation day 0 (top) and day 1 (middle). M9 corresponds to carbendazim. Reference standard of parent (bottom). Figure is taken from Völkl (2002).

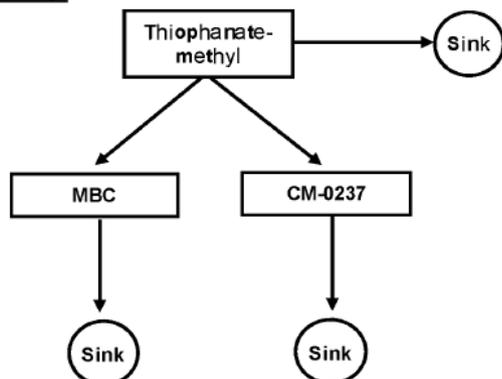
The degradation rates of thiophanate-methyl as well as the metabolites carbendazim and M2 were calculated by Völkl (2002). For the metabolites, decline fits were performed.  $DT_{50}$ s of thiophanate-methyl varied from 0.48 to 0.74 days.  $DT_{50}$ s of carbendazim ranged from 23.1 to 57.8 days and  $DT_{50}$ s of CM-0237 (M2) from 4.5 to 85.6 days.

The kinetic evaluation was superseded by the re-evaluation of Kiesel and Geibel (2016a,b), performed according to FOCUS guidance (2006, 2014). The statistical summaries of the kinetic analysis for thiophanate-methyl as well as the metabolites carbendazim (MBC), CM-0237 and 2-AB are presented in the table below. The degradation of thiophanate-methyl was best described by SFO in all three soils with  $DT_{50}$ s < 1 day (0.48–0.70 d). Additionally, pathway fits were performed that included parent and metabolites carbendazim, CM-0237 and 2-AB according to the below scheme (Evaluation Table Open point 4.3). During peer review, the applicant submitted an additional kinetic evaluation (Kiesel, Drechsler & Geibel, 2017a,b), to provide estimated parameters for the metabolite CM-0237 also for the Bretagne soil (Evaluation table, Data requirement 4.2). As a result of this revised pathway fit for the Bretagne soil, some minor changes were noted for the other metabolites. The below presentation of results have been amended accordingly.

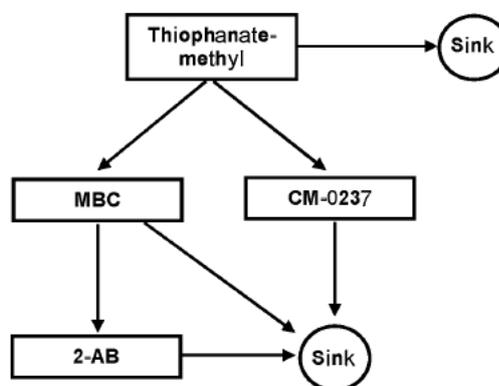
**1. Step:**



**2. Step:**



**3. Step:**



The SFO-SFO results for the metabolites were considered as acceptable by the authors. CM-0237 was originally not included in the kinetic evaluation of the results for the Bretagne soil (I), presumably because the levels were <5% AR. For the metabolite 2-AB the  $\chi^2$ -errors were relatively high, in particular in the soil Speyer 2.3 (III). The visual fits were also not ideal. The RMS nevertheless accepted these results in the first draft RAR but during peer review (experts' consultation September 2017) it was decided to remove the results for 2-AB for the soil Speyer 2.3 from the data set. The estimated formation fractions for the metabolites are presented in table below. The visual assessments of the fits for parent and the metabolites carbendazim, CM-0237 and 2-AB are presented in the figures below.

**Table 98: Summary of kinetic re-analysis by Kiesel and Geibel (2016a,b) of the data from Völkl (2002); for the Bretagne soil the final pathway fit was done by Kiesel, Drechsler & Geibel (2017a,b). For thiophanate-methyl, only results for parent-only fit are shown. For the metabolites, the results from pathway-fit with thiophanate-methyl and the major metabolites (carbendazim, CM-0237 and 2-AB) are given.**

| Substance          | Soil                  | Kinetic model        | Mo    | Parameter                           | $\chi^2$ , %-error | Prob>t  | Lower CI         | Upper CI         | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|--------------------|-----------------------|----------------------|-------|-------------------------------------|--------------------|---------|------------------|------------------|-------------------------|-------------------------|
| Thiophanate-methyl | Bretagne (soil I)     | SFO                  | 96.34 | k=1.5826                            | 5.0                | 1.4E-08 | 1.4              | 1.8              | 0.44                    | 1.5                     |
|                    |                       | FOMC                 | 96.34 | $\alpha=5.584$<br>$\beta=3.034$     | 5.2                | n.r.    | -9.4<br>-6.4     | 20.6<br>12.5     | - <sup>a</sup>          | - <sup>a</sup>          |
|                    | Mussig (soil II)      | SFO                  | 96.22 | k=0.9936                            | 5.8                | 7.0E-11 | 0.9              | 1.1              | 0.70                    | 2.3                     |
|                    |                       | FOMC                 | 96.22 | $\alpha=1.25E+5$<br>$\beta=1.26E+5$ | 6.3                | n.r.    | 1.2E+5<br>1.2E+5 | 1.3E+5<br>1.3E+5 | 0.70                    | 2.3                     |
|                    | Speyer 2.3 (soil III) | SFO                  | 96.77 | k=1.1812                            | 1.9                | 1.9E-10 | 1.1              | 1.2              | 0.59                    | 1.9                     |
|                    |                       | FOMC                 | 96.77 | $\alpha=2.55E+5$<br>$\beta=2.16E+5$ | 2.2                | n.r.    | 2.5E+5<br>2.1E+5 | 2.6E+5<br>2.2E+5 | 0.59                    | 1.9                     |
| Carbendazim        | Bretagne (soil I)     | SFO-SFO              | 0     | k=0.01096                           | 4.7                | 2.0E-16 | 9.3E-3           | 0.013            | 63.2                    | 210                     |
|                    | Mussig (soil II)      | SFO-SFO              | 0     | k=0.03144                           | 2.1                | <2E-16  | 0.029            | 0.034            | 22.0                    | 73.2                    |
|                    | Speyer 2.3 (soil III) | SFO-SFO              | 0     | k=0.01843                           | 5.0                | <2E-16  | 0.016            | 0.021            | 37.6                    | 125                     |
| CM-0237            | Bretagne (soil I)     | SFO-SFO              | 0     | k=0.2438                            | 30.2               | 2.7E-05 | 0.14             | 0.35             | 2.8                     | 9.4                     |
|                    | Mussig (soil II)      | SFO-SFO              | 0     | k=0.00801                           | 8.5                | 2.8E-05 | 0.004            | 0.01             | 86.5                    | 287                     |
|                    | Speyer 2.3 (soil III) | SFO-SFO              | 0     | k=0.01491                           | 7.7                | 1.2E-08 | 0.010            | 0.019            | 46.5                    | 154                     |
| 2-AB               | Bretagne (soil I)     | SFO-SFO <sup>b</sup> | 0     | k=0.05135                           | 20.0               | 1.6E-05 | 0.03             | 0.07             | 13.5                    | 44.8                    |
|                    | Mussig (soil II)      | SFO-SFO <sup>b</sup> | 0     | k=0.06027                           | 16.1               | 1.3E-07 | 0.04             | 0.08             | 11.5                    | 38.2                    |
|                    | Speyer 2.3 (soil III) | SFO-SFO <sup>b</sup> | 0     | k=0.02961                           | 33.6               | 9.7E-3  | 0.0056           | 0.054            | - <sup>c</sup>          | - <sup>c</sup>          |

n.r Not relevant.

n.p Not provided.

a Author considered the results statistically not reliable.

b Carbendazim as pre-cursor.

c Result considered as not reliable at experts' consultation (September, 2017).

**Table 99: Formation fractions of carbendazim, CM-0237 and 2-AB, according to kinetic re-analysis by Kiesel and Geibel (2016a,b) of the data from Völkl (2002); for the Bretagne soil the final pathway fit was done by Kiesel, Drechsler & Geibel (2017a,b).**

| Formation                        | Soil                  | Formation fraction | St. dev.          |
|----------------------------------|-----------------------|--------------------|-------------------|
| Thiophanate-methyl → Carbendazim | Bretagne (soil I)     | 0.79               | 0.02              |
|                                  | Mussig (soil II)      | 0.72               | 0.02              |
|                                  | Speyer 2.3 (soil III) | 0.69               | 0.015             |
| Thiophanate-methyl → CM-0237     | Bretagne (soil I)     | 0.055              | 0.01              |
|                                  | Mussig (soil II)      | 0.064              | 0.007             |
|                                  | Speyer 2.3 (soil III) | 0.099              | 0.008             |
| Carbendazim → 2-AB               | Bretagne (soil I)     | 0.36               | 0.07              |
|                                  | Mussig (soil II)      | 0.33               | 0.04              |
|                                  | Speyer 2.3 (soil III) | 0.12 <sup>a</sup>  | 0.03 <sup>a</sup> |

<sup>a</sup> Result considered as not reliable at experts' consultation (September, 2017).

**Table 100: Summary of modelling endpoints for thiophanate-methyl and its metabolites carbendazim, CM-0237 and 2-AB, as accepted by the RMS.**

| Substance          | Soil                    | Modelled value |                         | Normalised (pF 2, 20°C)<br>SFO DT <sub>50</sub> [days] for modelling |                        |
|--------------------|-------------------------|----------------|-------------------------|--|------------------------|
|                    |                         | Kinetic model  | DT <sub>50</sub> [days] | Correction factor <sup>a</sup>                                       | Norm. DT <sub>50</sub> |
| Thiophanate-methyl | Bretagne (silt loam)    | SFO            | 0.44                    | 1.00   | 0.44                   |
|                    | Mussig (clay loam)      | SFO            | 0.70                    | 1.00   | 0.70                   |
|                    | Speyer 2.3 (sandy loam) | SFO            | 0.59                    | 0.944  | 0.56                   |
| Carbendazim        | Bretagne (silt loam)    | SFO-SFO        | 63.2                    | 1.00   | 63.2                   |
|                    | Mussig (clay loam)      | SFO-SFO        | 22.0                    | 1.00   | 22.0                   |
|                    | Speyer 2.3 (sandy loam) | SFO-SFO        | 37.6                    | 0.944  | 35.5                   |
| CM-0237            | Bretagne (silt loam)    | SFO-SFO        | 2.8                     | 1.00   | 2.8                    |
|                    | Mussig (clay loam)      | SFO-SFO        | 86.5                    | 1.00   | 86.5                   |
|                    | Speyer 2.3 (sandy loam) | SFO-SFO        | 46.5                    | 0.944  | 43.9                   |
| 2-AB               | Bretagne (silt loam)    | SFO-SFO        | 13.5                    | 1.00   | 13.5                   |
|                    | Mussig (clay loam)      | SFO-SFO        | 11.5                    | 1.00   | 11.5                   |
|                    | Speyer 2.3 (sandy loam) | SFO-SFO        | 23.4                    | 0.944  | 22.1                   |

n.a. Not available.

<sup>a</sup> Moisture correction factor calculated according to FOCUS (2000):  $f_{moisture} = (\theta/\theta_{ref})^{0.7}$ . Moisture at 46% MWHC as provided in the study was used for the calculation together with the default moisture content at pF 2 (field capacity). No normalisation for temperature necessary as study was performed at a temperature of 20°C.

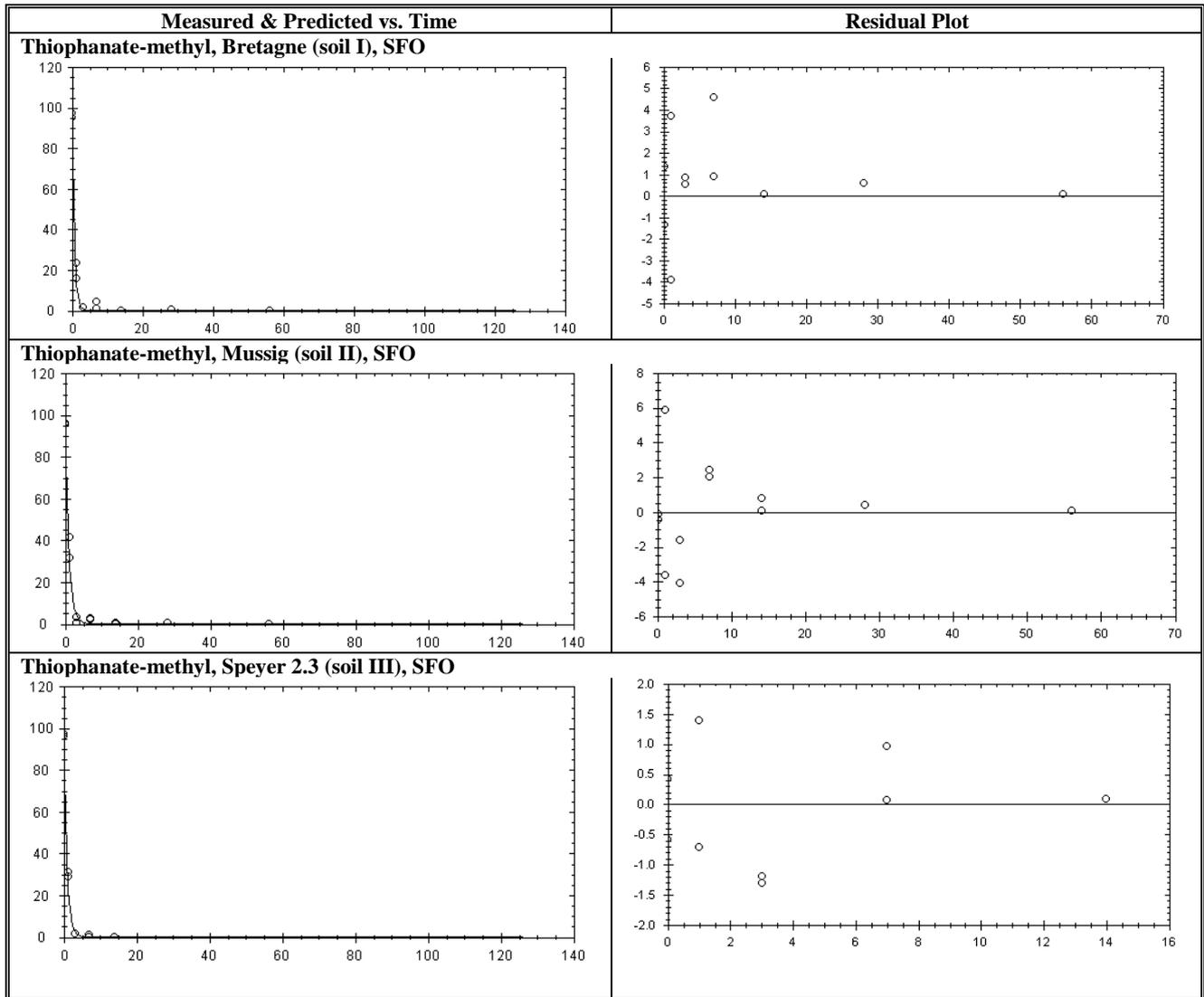


Figure 8.1.1.1-2. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent-only), from Kiesel and Geibel (2016b). Only the best-fit model for each soil is shown.

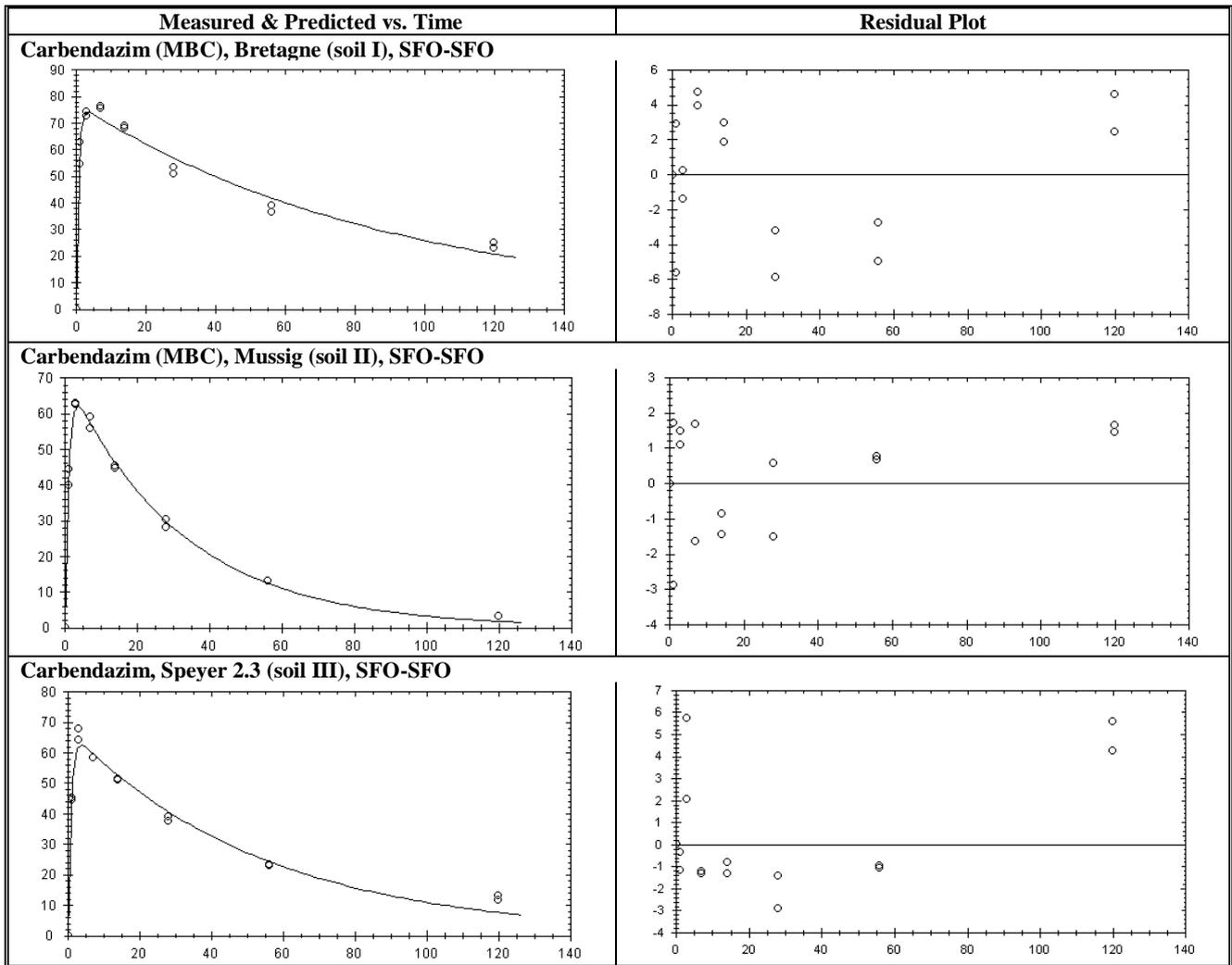


Figure 8.1.1.1-3. Visual fit and plot of residuals for kinetic fitting of the primary metabolite carbendazim (MBC; pathway-fit), from Kiesel and Geibel (2016b).

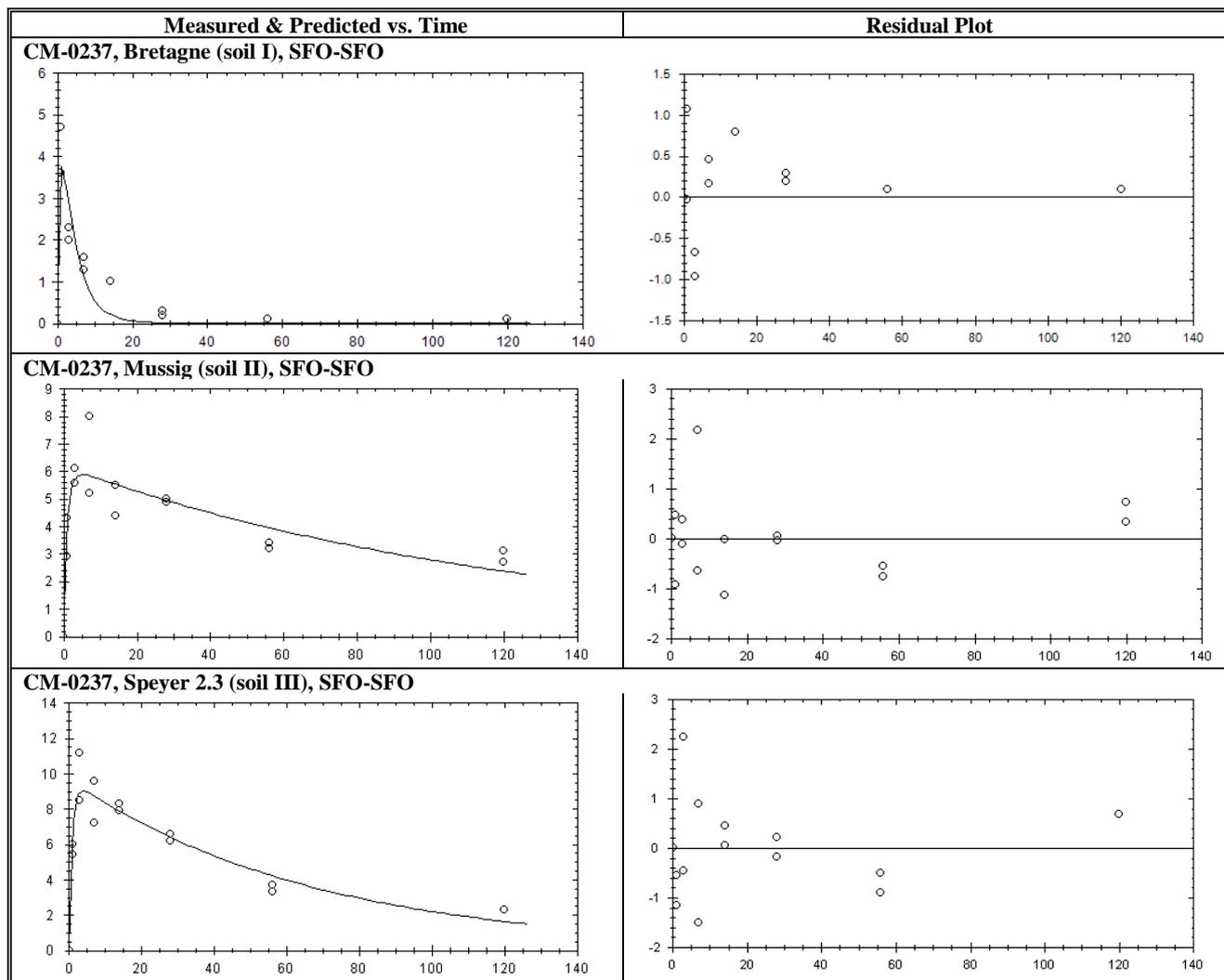


Figure 8.1.1.1-4. Visual fit and plot of residuals for kinetic fitting of the primary metabolite CM-0237 (pathway-fit), from Kiesel and Geibel (2016b). Graphs for the Bretagne soil are from Kiesel, Drechsler & Geibel (2017b).

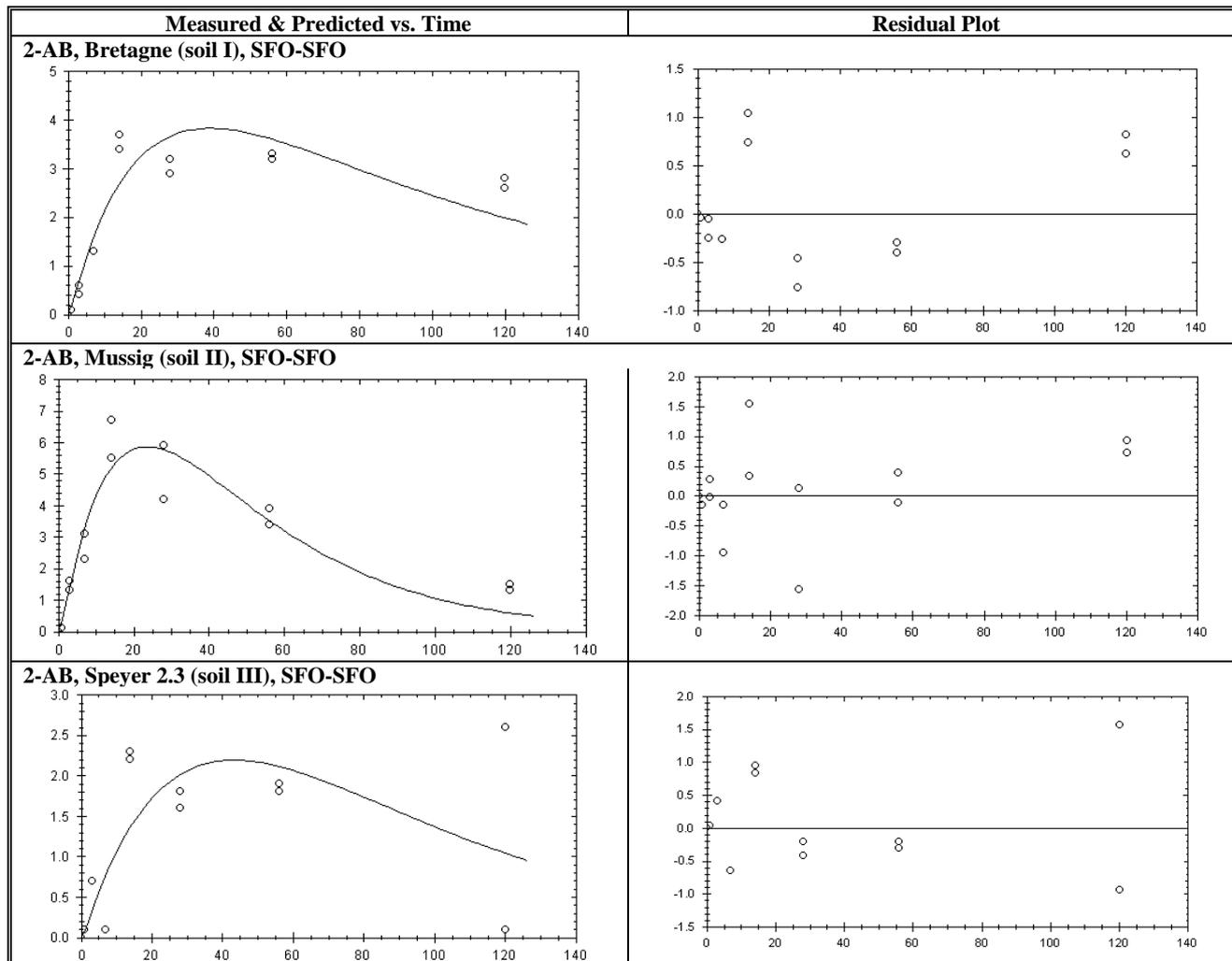


Figure 8.1.1.1-5. Visual fit and plot of residuals for kinetic fitting of the secondary metabolite 2-AB. Results from the pathway-fit with carbendazim as precursor, taken from Kiesel and Geibel (2016b).

#### RMS comments and conclusions

The study by Völkl (2002) was evaluated and accepted in the Addendum (2003) to the DAR (1997), and is also accepted for the purpose of renewal. The study was well performed and reported. The RMS agrees with the proposed degradation pathway and concludes that thiophanate-methyl was rapidly degraded into carbendazim (MBC) and CM-0237 (M2). Carbendazim was likely to be formed via the (minor) metabolite DX-105 and degraded into metabolite 2-AB. The RMS suggests to consider 2-AB further for exposure and risk assessment in accordance with current data requirements (Regulation (EU) No 283/2013). The applicant did not agree to this since one of the measurements was not above 5.0% AR. During peer review it was decided to include 2-AB in the residue definition for soil, groundwater and surface water (Evaluation table Open point 4.1).

The RMS considered it difficult to judge whether the radioactivity eluting after approximately 5 minutes (see text and figure above) corresponded (exclusively) to carbendazim or to another (unknown) metabolite. In the latter case, a potential additional major metabolite might have been missed. However, most of the provided HPLC chromatograms indicated that the first peak was minor compared to the second peak of carbendazim or that there was no peak after 5 minutes. But as there were no HPLC chromatograms for all samples and as at least one of the figures presented

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indicated that the first peak was larger than the second peak (for day 1, sample B), some uncertainty remains. The RMS assumes that it will not be possible to characterise the radioactivity in those first eluting peaks any longer, as the study is almost 15 years old. Therefore, the RMS suggests to accept the approach the author had chosen by adding up the two peaks to carbendazim and use the data for the risk assessment. At least for carbendazim, this approach can be considered conservative.

The re-evaluation of the rate of degradation of the metabolites by Kiesel and Geibel (2016a,b), and for the Bretagne soil by Kiesel, Drechsler & Geibel (2017a,b), was performed according to the FOCUS guidance and is considered acceptable. The RMS agrees with the authors' choice of SFO as best-fit model for parent, and SFO-SFO for the metabolites. As a general principle, the RMS also accepts  $\chi^2$ -%-error above 15% since this level should not be considered as a definitive criterion.

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|                   |   |
|-------------------|---|
| <b>Reference:</b> | <b>1. Adam, D. (2014a)</b> <sup>14</sup> C-Thiophanate-methyl: Route and Rate of Degradation in One Soil under Aerobic Conditions.<br><b>2. Adam, D. (2016)</b> <sup>14</sup> C-Thiophanate-methyl - Route and Rate of Degradation in One Soil under Aerobic Conditions |
| Report No.:       | 1. RD-02985<br>2. RD-03848  |
| Document No.:     | 1. 722-001<br>2. 781-005  |
| Guideline:        | OECD TG No. 307 (2002)  |
| GLP:              | Yes   |

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|                             |                                       |
|-----------------------------|---------------------------------------|
| <b>Previous evaluation:</b> | Submitted for the purpose of renewal. |
|-----------------------------|---------------------------------------|

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|                              |  |
|------------------------------|--|
| <b>Material and methods:</b> |  |
| Test material:               | Phenyl-UL- <sup>14</sup> C-labelled thiophanate-methyl   |
| Lot/Batch No:                | XIX/17/1   |
| Purity:                      | 97.7% (determined by HPLC before treatment; 97.2% after treatment)   |
| Test concentration:          | 1.42 mg/kg, corresponding to 1.1 kg a.s./ha (assuming a distribution of the test item in the top 5 cm soil layer and a bulk density of 1.5 g/cm <sup>3</sup> ). <sup>14</sup> C-thiophanate-methyl was dissolved in acetonitrile ( $\leq 1\%$ ), but the solvent was not evaporated due to the fast degradation of the test item.  |
| Test system:                 | A new study was carried out to fulfil the requirements of determining a fourth degradation rate for thiophanate-methyl.<br><br>100 g dry weight soil were filled into all-glass metabolism flasks in a moist air flow-through systems with absorption traps with ethylene glycol and 2N NaOH for organic volatiles and <sup>14</sup> CO <sub>2</sub> , respectively. Presence of <sup>14</sup> CO <sub>2</sub> in the trapping solution was confirmed by BaCO <sub>3</sub> -precipitation in selected samples. (Figure 1 of the report indicated that an additional trap containing 1 M H <sub>2</sub> SO <sub>4</sub> was used. During the evaluation the applicant submitted a statement (Adam, 2016) in which the study author clarified that Figure 1 was erroneous; no such trap was used.) |
| Test conditions:             | Soils were sieved (2 mm), acclimated at 20°C for 5 days and incubated in the dark at 20.9±0.1°C and at a moisture content of pF 2 under aerobic conditions.  |
| Sampling time points:        | Duplicate samples were taken for extraction and analysis after 0, 1, 3, 7, 14, 28, 55, 90, and 120 days. No sampling of volatiles on day 0.  |
| Method of analysis:          | All samples were extracted with acetonitrile:water (4:1; v/v) up to four times and additionally with a Soxhlet extraction using acetonitrile:water (4:1; v/v) for four hours. Samples from day 120 were subject to an additional harsh extraction with acetonitrile:0.1M hydrochloric acid (4:1; v/v) for four hours under reflux conditions   |

followed by an organic matter fractionation.

Radioactivity of individual extracts as well as radioactivity in the traps was analysed by LSC. Non-extractable radioactivity was analysed by LSC after combustion. Parent and metabolites were characterised with HPLC and/or TLC by co-chromatographic profiling of the pooled extracts and comparison of retention times. The following reference substances were used for metabolite identification: MBC (carbendazim), DX-105, FH-432, CM-0237, AV-1951, 2-AB, CM-0238.

The LOQ of the HPLC was 0.004% AR.

Kinetic analysis:

A pathway fit was performed for thiophanate-methyl and the primary metabolite carbendazim (MBC) with the software tool CAKE v. 1.4 following FOCUS GD (2006, 2014). Only SFO-kinetics were presented and used to determine DT<sub>50</sub> and DT<sub>90</sub> for the two substances and the formation fraction of the metabolite.

**Table 101: Soil characteristics.**

|   |                   | <b>Speyer 5M</b>  |
|---|-------------------|---|
| Origin  |                   | Germany   |
| Soil plot history                               |                   | No pesticides used for at least four years prior to the sampling.   |
| Collection and storage                          |                   | Collected in February 2013. Sieved (2 mm). Stored for about 1.5 months at 4°C. Acclimation at 20°C for about 5 days before study. |
| Soil type (USDA)                                |                   | Sandy loam  |
| Clay <2 µm                                      | [%]               | 11.1  |
| Silt 2–50 µm                                    | [%]               | 29.7  |
| Sand >50 µm                                     | [%]               | 59.2  |
| Water content at pF 2.0                         | [g/100 g soil]    | 23.0  |
| pH (CaCl <sub>2</sub> ) / pH (H <sub>2</sub> O) |                   | 7.9 / 7.3   |
| Organic carbon                                  | [g/100 g soil]    | 1.0   |
| CEC   | [mmol/100 g soil] | 16.6  |
| Biomass – start of incubation                   | [mg/100 g soil]   | 33.7  |
| Biomass – end of incubation                     | [mg/100 g soil]   | 38.6  |

**Results**

The total recovery of individual samples ranged between 92.8 and 107.8% AR. The fraction of extractable radioactivity decreased from 91.4% AR at day 0 to 15.9% AR after 120 days, while the fraction of non-extractables increased from 7.8% AR at day 0 to 72.1% AR by day 120. The samples taken at day 1 deviated from the trend; the fraction of total extractable from samples taken at day 1 was lower than any sample taken up to day 14 and similarly the fraction of non-extractable was higher at day 1 than at day 14. Mineralisation to <sup>14</sup>CO<sub>2</sub> reached a maximum of 7.6% AR at study termination. Other volatiles were below 0.1% AR throughout the study period.

Thiophanate-methyl decreased from 82.9% AR at day 0 to 0.3% AR after 55 days and was not detected thereafter. The concentration of the major metabolite carbendazim peaked at 48.3% AR after 3 days and decreased afterwards to 7.1% AR at the end of the study period. None of the other 19 observed metabolites exceeded 10% once or 5% at two consecutive sampling intervals. An unknown metabolite (M8) was once detected at a concentration of 6.8% AR (day 3) but was below 3% AR at the closest sampling intervals. Neither CM-0237 nor 2-AB exceeded 4.5% AR on average at individual sampling occasions.

Samples taken after 14 and 28 days showed quite a spread between the replicates both for the metabolites carbendazim, CM-0237, 2-AB and the parent. The RMS considers that this suggests some problems with the analysis for those sampling intervals.

The samples taken after 120 days were subject to an additional harsh extraction and the remaining non-extractable radioactivity subject to organic matter fractionation. Of the 64.4% AR that remained non-extractable after the harsh extraction, 56% were bound to the humin fraction (i.e. 36.1% AR), and roughly 20% to fulvic and humic acids, respectively, corresponding to 14% AR.

Adam (2014a) proposed the same route of degradation as Völkl (2002), see Figure 8.1.1.1-6.

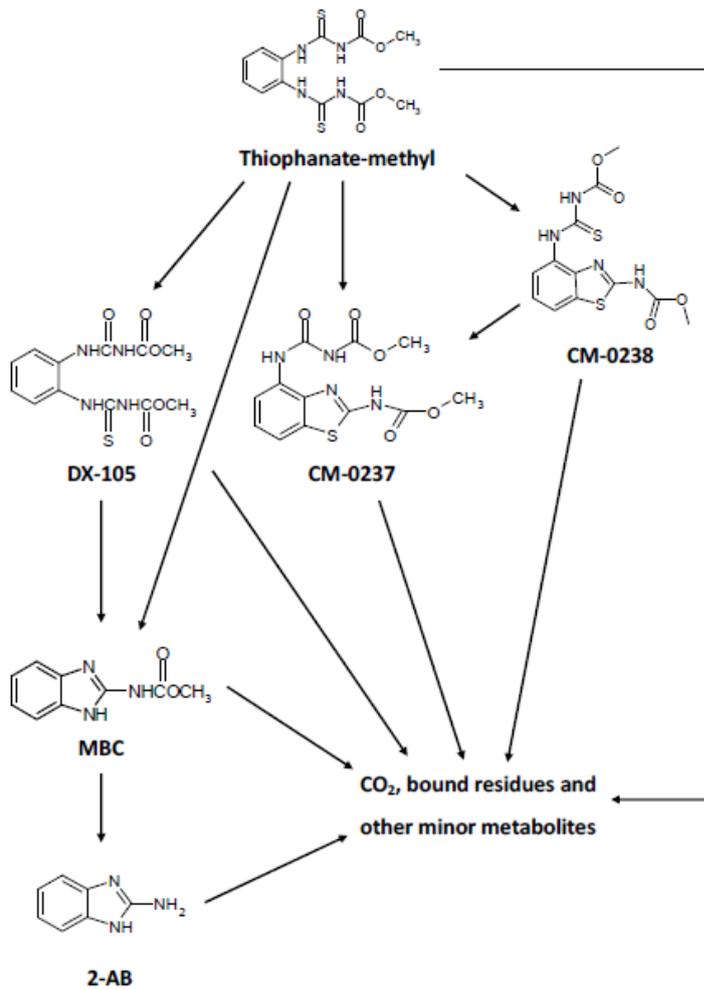


Figure 8.1.1.1-6. Degradation pathway in aerobic soils, as proposed by Adam (2014).

**Table 102: Distribution and characterisation of radioactivity in the Speyer 5M soil (sandy loam) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20.9±0.1°C and at pF 2. Mean of duplicates is given. For the parent and the metabolites carbendazim, 2-AB and CM-0237, results from the two individual replicates are also presented. All values are given as % of applied radioactivity (AR).**

|                                | days after application |      |      |      |      |      |       |       |      |
|--------------------------------|------------------------|------|------|------|------|------|-------|-------|------|
|                                | 0                      | 1    | 3    | 7    | 14   | 28   | 55    | 90    | 120  |
| Parent                         | 81.7                   | 6.9  | 2.1  | 2.3  | 0.7  | 2.6  | 0.7   | -     | -    |
|                                | 84.0                   | 7.6  | 1.7  | 3.1  | -    | 0.4  | -     | -     | -    |
| Mean                           | 82.9                   | 7.3  | 1.9  | 2.7  | 0.4  | 1.5  | 0.3   | -     | -    |
| Carbendazim                    | 3.2                    | 41.1 | 48.9 | 43.1 | 34.8 | 42.7 | 16.2  | 9.7   | 5.8  |
|                                | 3.1                    | 39.2 | 47.7 | 43.7 | 43.9 | 29.4 | 13.3  | 8.5   | 8.3  |
| Mean                           | 3.2                    | 40.1 | 48.3 | 43.4 | 39.3 | 36.0 | 14.8  | 9.1   | 7.1  |
| CM-0237                        | -                      | 2.9  | -    | 3.6  | 3.1  | 3.9  | 4.2   | 2.7   | 2.0  |
|                                | -                      | 3.0  | 0.4  | 3.3  | 5.4  | 5.0  | 4.3   | 2.8   | 2.1  |
| Mean                           | -                      | 3.0  | 0.2  | 3.4  | 4.2  | 4.4  | 4.2   | 2.8   | 2.0  |
| 2-AB                           | -                      | -    | -    | 2.8  | 4.6  | 4.6  | 2.4   | 0.7   | 0.6  |
|                                | -                      | -    | -    | 2.8  | 3.1  | 1.5  | 2.0   | 0.7   | 1.6  |
| Mean                           | -                      | -    | -    | 2.8  | 3.8  | 3.0  | 2.2   | 0.7   | 1.1  |
| DX-105                         | 0.8                    | 1.7  | 1.3  | 1.0  | 0.9  | 0.6  | 0.3   | 0.3   | 0.1  |
| CM-0238                        | -                      | 1.1  | 0.8  | 0.5  | 0.1  | 0.1  | -     | 0.2   | -    |
| AV-1951                        | -                      | -    | -    | -    | 0.5  | 0.9  | 0.6   | 0.3   | 0.3  |
| M8                             | 0.8                    | 1.6  | 6.8  | 2.8  | 2.5  | 2.0  | 2.2   | 1.8   | 1.3  |
| M10                            | -                      | 3.1  | 3.0  | 3.2  | 3.1  | 0.7  | 1.1   | 0.9   | 0.7  |
| M11                            | -                      | 0.7  | 2.2  | -    | -    | -    | -     | -     | -    |
| M15                            | 3.7                    | 0.6  | 1.1  | 1.6  | 2.0  | 2.2  | 0.4   | 3.0   | 0.2  |
| Other metabolites <sup>a</sup> | -                      | 0.7  | 5.0  | 4.9  | 5.2  | 5.2  | 3.5   | 3.8   | 3.0  |
| Extractable                    | 85.3                   | 47.5 | 45.7 | 42.9 | 44.3 | 41.9 | 17.4  | 10.2  | 9.8  |
| Soxhlet                        | 6.1                    | 12.6 | 24.9 | 23.4 | 17.9 | 14.8 | 12.3  | 12.6  | 6.1  |
| Total extractable              | 91.4                   | 60.0 | 70.6 | 66.3 | 62.2 | 56.7 | 29.7  | 22.8  | 15.9 |
| Non-extractable                | 7.8                    | 39.4 | 28.8 | 32.9 | 34.7 | 41.3 | 64.7  | 70.9  | 72.1 |
| <sup>14</sup> CO <sub>2</sub>  | n.p.                   | <0.1 | 0.2  | 0.4  | 0.9  | 1.8  | 5.7   | 7.1   | 7.6  |
| Other Volatiles                | n.p.                   | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1  | <0.1  | <0.1 |
| Total recovery                 | 99.1                   | 99.4 | 99.6 | 99.6 | 97.7 | 99.7 | 100.0 | 100.8 | 95.6 |
| <b>MEAN ± SD</b>               | <b>99.1 ± 3.1</b>      |      |      |      |      |      |       |       |      |

- Not detected.

n.p. Not performed.

SD Standard deviation.

<sup>a</sup> Sum of other minor metabolites identified. None exceeded individually 2% AR.

**Table 103: Radioactivity recovery as % AR in the different extraction steps and in organic matter fractions, analysed at day 120.**

|   | Speyer 5M soil (sandy loam) |
|---|-----------------------------|
| Non-extractable after extraction at room temperature and Soxhlet extraction | 72.1                        |
| Extractable radioactivity by "harsh extraction"                             | 7.7                         |
| Non-extractable after "harsh extraction"                                    | 64.4                        |
| Soluble fraction at low pH (fulvic acids)                                   | 13.9                        |
| Soluble fraction at high pH (humic acids)                                   | 14.4                        |
| Insoluble fraction (humins)   | 36.1                        |

The kinetic evaluation was performed with the software package CAKE with a pathway-fit including thiophanate-methyl and the primary metabolite carbendazim (MBC). The results are presented in the tables and the figure below. No results from parent-only simulations were provided and only SFO-results for parent and the metabolite presented.

Additionally, day zero residues of metabolites were not added to the parent compartment and samples with concentrations <LOD were treated as missing values.

Therefore, the RMS performed new optimisations with the software KinGUI v.2.1, which are presented in tables and figure further below. No reliable results were obtained for thiophanate-methyl with the FOMC model. A pathway fit including CM-0237 could not be derived either. But a pathway fit with parent degrading to the primary metabolite carbendazim and the secondary metabolite 2-AB could be fitted. For 2-AB the  $\chi^2$ -error was relatively high and the fit did not capture the maximum observed. Nevertheless, the RMS suggests that the fit was good enough and the RMS suggests the estimates from the RMS' optimisation should be used for exposure assessment. The results for thiophanate-methyl and carbendazim were similar to those obtained by Adam (2014a).

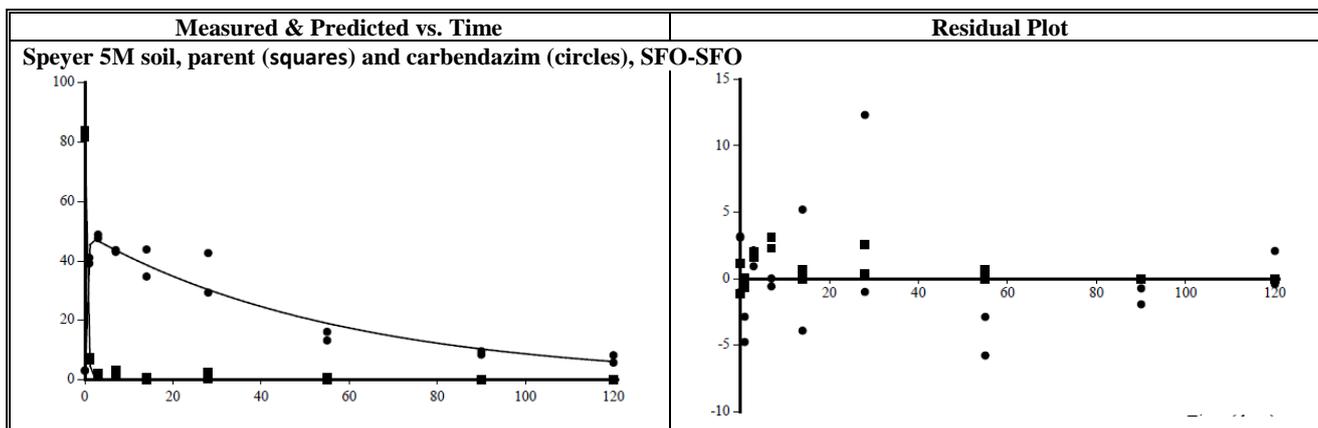
**Table 104: Summary of kinetic analysis by Adam (2014a). Only results from the pathway-fit with all SFO are shown.**

| Substance          | Soil      | Kinetic model | Mo <sup>a</sup> | Parameter | $\chi^2$ , %-error | Prob>t  | Lower CI | Upper CI | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|--------------------|-----------|---------------|-----------------|-----------|--------------------|---------|----------|----------|-------------------------|-------------------------|
| Thiophanate-methyl | Speyer 5M | SFO           | 82.8            | k=2.3968  | 9.0                | 1.2E-18 | 2.1      | 2.7      | 0.29                    | 0.96                    |
| Carbendazim        | Speyer 5M | SFO-SFO       | 3.2             | k=0.01727 | 8.5                | 2.1E-10 | 0.01     | 0.02     | 40.1                    | 133                     |

a The author did neither add the initial concentration of carbendazim (and other metabolites) to the concentration of the parent nor did they fix M0 for the metabolite to 0.

**Table 105: Formation fraction for carbendazim, according to kinetic analysis by Adam (2014a).**

| Formation                        | Soil      | Formation fraction | St. dev. |
|----------------------------------|-----------|--------------------|----------|
| Thiophanate-methyl → Carbendazim | Speyer 5M | 0.59               | 0.024    |



**Figure 8.1.1.1-7. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent) and carbendazim. Results of the pathway-fit, from Adam (2014a).**

**Table 106: Summary of kinetic re-analysis by RMS based on data presented in Adam (2014a).**

| Substance          | Soil      | Kinetic model      | Mo <sup>a</sup> | Parameter                           | $\chi^2$ , %-error | Prob>t  | Lower CI    | Upper CI    | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|--------------------|-----------|--------------------|-----------------|-------------------------------------|--------------------|---------|-------------|-------------|-------------------------|-------------------------|
| Thiophanate-methyl | Speyer 5M | SFO (parent only)  | 91.4            | k=2.5294                            | 7.8                | 1.9E-10 | n.a.        | n.a.        | 0.27                    | 0.91                    |
|                    |           | FOMC (parent only) | 91.4            | $\alpha=0.77566$<br>$\beta=0.03779$ | 4.5                | n.r.    | 0.5<br>0.02 | 1.1<br>0.09 | - <sup>a</sup>          | - <sup>a</sup>          |
| Carbendazim        | Speyer 5M | SFO-SFO            | 0               | k=0.01728                           | 9.1                | 1.3E-10 | n.a.        | n.a.        | 40.1                    | 133                     |
| 2-AB               | Speyer 5M | SFO-SFO            | 0               | k=0.13184                           | 24.2               | 0.013   | n.a.        | n.a.        | 5.3                     | 17.5                    |

n.a. Not available.

n.r. Not relevant.

<sup>a</sup> Statistically not reliable.

**Table 107: Formation fractions of the metabolites carbendazim and 2-AB, according to the kinetic analysis by the RMS, based on data from Adam (2014a).**

| Formation                        | Soil      | Formation fraction | St. dev. |
|----------------------------------|-----------|--------------------|----------|
| Thiophanate-methyl → Carbendazim | Speyer 5M | 0.53               | 0.023    |
| Carbendazim → 2-AB               | Speyer 5M | 0.75               | 0.30     |

**Table 108: Summary of modelling endpoints for thiophanate-methyl and its metabolites carbendazim and 2-AB, as proposed by the RMS.**

| Substance          | Soil      | Modelled value |                         | Normalised (pF 2, 20°C)<br>SFO DT <sub>50</sub> [days] for modelling |                        |
|--------------------|-----------|----------------|-------------------------|--|------------------------|
|                    |           | Kinetic model  | DT <sub>50</sub> [days] | Correction factor <sup>a</sup>                                       | Norm. DT <sub>50</sub> |
| Thiophanate-methyl | Speyer 5M | SFO            | 0.27                    | 1.089  | 0.29                   |
| Carbendazim        | Speyer 5M | SFO            | 40.1                    | 1.089  | 43.6                   |
| 2-AB               | Speyer 5M | SFO            | 5.3                     | 1.089  | 5.8                    |

<sup>a</sup> Moisture correction factor calculated according to FOCUS (2000):  $f_{temp} = Q10^{[(T_{study} - T_{ref})/10]}$  with  $Q10 = 2.58$ ,  $T_{study} = 20.9^\circ\text{C}$  and  $T_{ref} = 20^\circ\text{C}$ . No normalisation for moisture necessary as study was performed at pF 2.

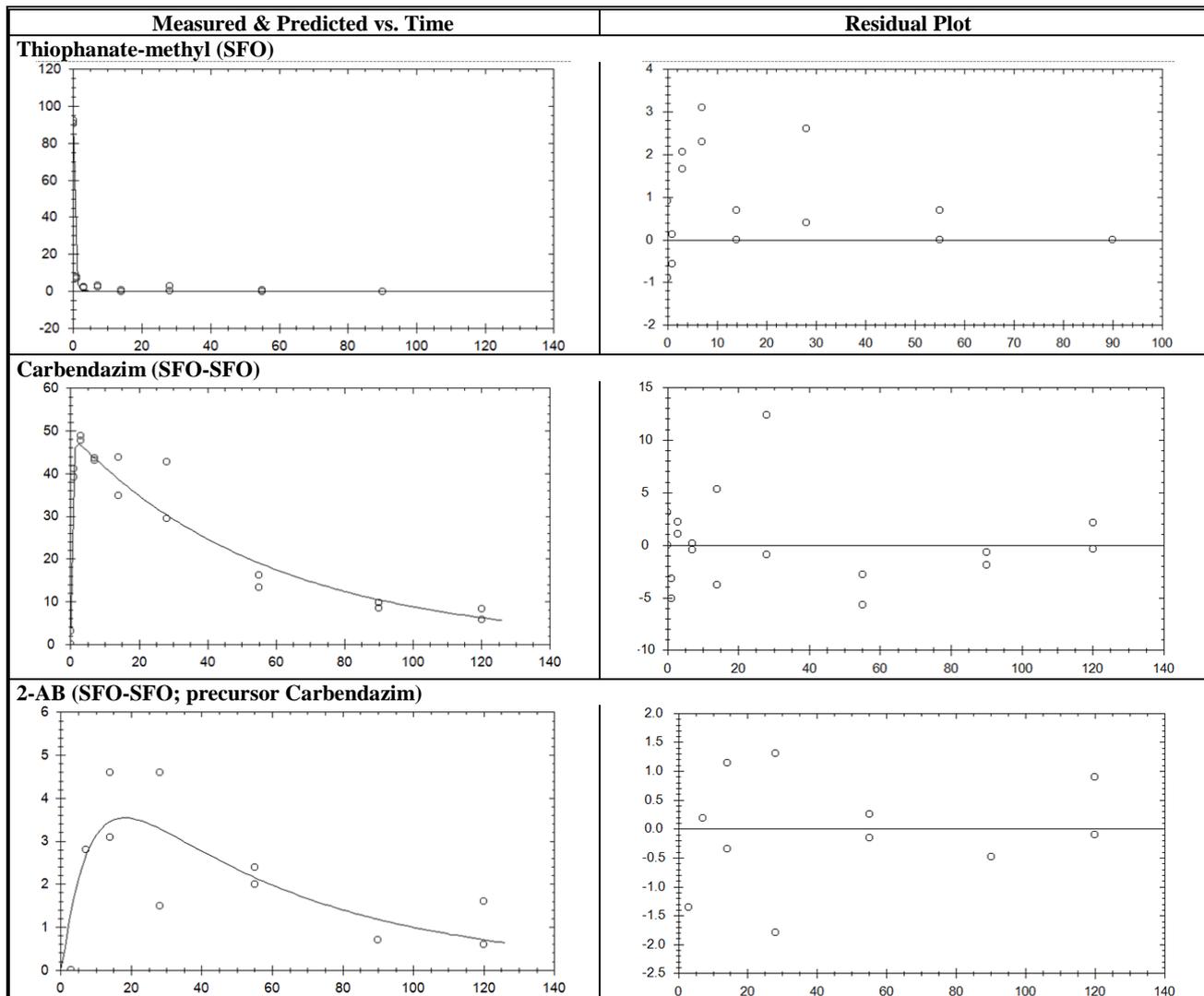
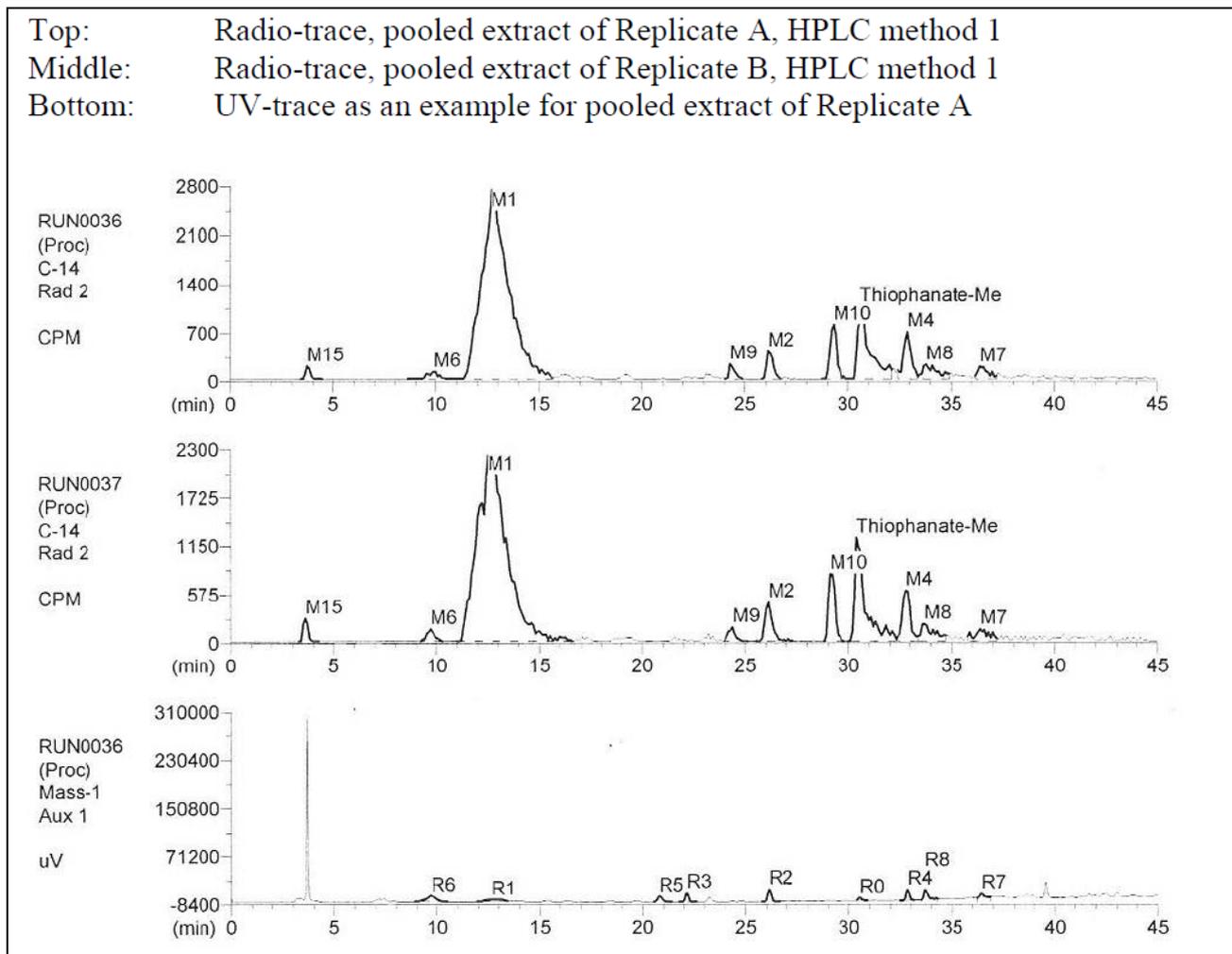


Figure 8.1.1.1-7. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent), carbendazim and 2-AB. Results of the pathway-fit as re-calculated by the RMS, based on data from Adam (2014a).

#### RMS comments and conclusions

The laboratory study was performed according to the OECD TG No. 307 and the results well reported. The study is considered acceptable for the purpose of renewal of the approval of thiophanate-methyl. The kinetic evaluation of the data is not considered fully acceptable due to handling of day 0 values and results <LOD. Nevertheless, there were a few issues that render some uncertainty to the study results. The amount of total extractable and non-extractable radioactivity in samples taken at day 1 suggested some degree of uncertainty as the amount of extractable is higher thereafter and the amount of non-extractable lower (until day 14), respectively. Furthermore, especially the measurements of carbendazim at day 14 and 28 indicated uncertainty in the analyses.

The author did not discuss whether carbendazim was less retained on the HPLC column or might have potentially eluted in several peaks, as it was observed by Völkl (2002); see figure below. The author treated the first peak eluting after roughly four-five minutes as a separate, minor metabolite. The RMS agrees with this.



**Figure 8.1.1.1-8. HPLC chromatograms of day 1 soil samples. Figure taken from Adam (2014). The reference items are denoted as follows: R1 = Carbendazim, R2 = DX-105, R3 = FH-432, R4 = CM-0237, R5 = AV-1951, R6 = 2-AB, R7 = CM-0238.**

The author noted that “Thiophanate-methyl is known to hydrolyse extremely fast. Therefore, extracts were subjected to HPLC with a high concentration of organic solvent. This procedure resulted in an asymmetric peak-shape but had the advantage that only a small amount of Thiophanate-methyl hydrolysed. Hydrolysis during work-up for HPLC could be confirmed by TLC results. However, organic solvent in the sample did not interfere with spot-shape in TLC and therefore, a symmetric test item spot was observed”. A very fast hydrolysis rate is in contrast to the results obtained in the hydrolysis study (with pure water), but hydrolysis rate may be faster in dissolved organic fractions. As it further seems that transformation due to the organic solvent can be excluded, the RMS accepted the procedure chosen by the author to analyse the results.

As the kinetic evaluation by the author did not entirely follow FOCUS degradation kinetics guidance (2006, 2014), the results from the re-evaluations performed by the RMS are considered more appropriate. The results obtained by the RMS for thiophanate-methyl and carbendazim were very similar to the results obtained by the author and provided reliable endpoints. The RMS additionally obtained  $DT_{50}$  and formation fraction (with carbendazim as precursor) for the secondary metabolite 2-AB. Despite a  $\chi^2$ -error  $>15$ , the RMS considered the fit acceptable based on the visual and statistical (p-value) inspection. This is in line with FOCUS degradation kinetics report (2006, 2014) that indicates that a  $\chi^2$ -error of 15% should not be considered a cut-off but rather a guidance value.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

**Reference:** 1. **Otto, S. (1975)** Verhalten des Pflanzenschutzmittel-Wirkstoffes im Boden  
 2. **Zillgens, B. (2007a)** Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model Maker™ 4.0

Report No.: 1 A22991, BOD95-00442  
 2. DuPont-21656

Guideline: BBA leaflet no. 36 (February 1973)

GLP: 1. No

Validity 1. Valid

**Material and methods:**

An aerobic soil degradation study was conducted according to BBA leaflet no. 36. Benzimidazol-2-carbamid-säure-methylester (BCM = Carbendazim) was added to two standard soils characterized in the table below. Each soils was treated with 3 ppm BCM. The amount of applied active substance is about 1.5-fold higher than the maximum application rate of 2 x 250 g as/ha All soil samples were adjusted to 40 % maximum water holding capacity (MWHC) and were incubated in darkness at 22°C up to 380 – 480 days. Samples were taken at time intervals 0, 20, 40, 55, 85, 240 and 380 days (sand) and 0, 10, 25, 75, 120, 240, 380 and 480 days (loamy sand) for subsequent analysis. The samples were extracted with 10 % ethyl acetate in methanol. For the quantification of BCM in the residues of sand soil a detection of <sup>14</sup>C-radioactivity was used followed by radio thin layer chromatography. The detection limit was 0.01 ppm. For the quantification of BCM in the residues of loamy sand soil a UV-spectroscopy was carried out. Detection limit was 0.05 ppm.

Kinetic analyses: DT<sub>50</sub>/DT<sub>90</sub> were determined graphically in the original report (Otto, 1975). DT<sub>50</sub>/DT<sub>90</sub> were re-calculated in accordance with FOCUS guidance (in Zillgens, 2007a) and those results were the final ones.

**Table 109: Soil properties.**

| Soil property                          | Standard soil 1 | Standard soil 2 |
|--|-----------------|-----------------|
| Particle size distribution (DIN) (%)   |                 |                 |
| < 2 µm                                 | 10.1            | 19.5            |
| Classification (BBA)                   | sand            | loamy sand      |
| Organic carbon (%)                     | 2.58            | 1.0             |
| pH (CaCl <sub>2</sub> )                | 6.8             | 5.2             |
| Maximum water holding capacity (w/w %) | n.a.            | n.a.            |

**Results**

The decline of active substance in both soils during the incubation period is summarised in the below table. A mass balance was not completed for the various incubation periods used in this study. Volatilisation was not measured. At the end of the study a recovery rate of 88% and 81% for standard soil 1 and standard soil 2 was determined. In the soil extracts primarily the active substance is detected by radio thin layer chromatography. Extractable degradation products were only formed in traces. The main part of degradation products were probably bound in form of non-extractable residues, mainly in humic material.

**Table 110: Concentration of carbendazim during aerobic incubation of treated test soils.**

| Days after application       | mg carbendazim/kg dry soil | Carbendazim (% of concentration at day 0) |
|------------------------------|----------------------------|---|
| Standard soil 1 (sand)       |                            |   |
| 0                            | 3.00                       | 100                                       |
| 20                           | 2.15                       | 71.7                                      |
| 40                           | 1.53                       | 51.0                                      |
| 55                           | 1.09                       | 36.3                                      |
| 85                           | 0.45                       | 15.0                                      |
| 240                          | 0.19                       | 6.3                                       |
| 380                          | 0.105                      | 3.5                                       |
| Standard soil 2 (loamy sand) |                            |   |
| 0                            | 3.00                       | 100                                       |
| 10                           | 2.57                       | 85.7                                      |
| 25                           | 1.87                       | 60.3                                      |
| 75                           | 0.83                       | 27.7                                      |
| 120                          | 0.57                       | 19.0                                      |
| 240                          | 0.28                       | 9.3                                       |
| 380                          | 0.16                       | 5.3                                       |
| 480                          | 0.11                       | 3.7                                       |

The DT<sub>50</sub> values was determined graphically to be approximately 40 days in both soils. Optimized degradation rates were derived by means of Model Maker™ vers. 4.0 fitting runs and were tested with regard to the acceptability of SFO kinetics in accordance with FOCUS kinetics guidance (2006) (Zillgens, 2007a), see table below.

**Table 111: Re-calculated laboratory DT50 and DT90 for the aerobic degradation of carbendazim in two soils.**

| Soil       | Number of data points | Temp (°C) | Moisture | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) | χ <sup>2</sup> , %-error | Model |
|------------|-----------------------|-----------|----------|-------------------------|-------------------------|--------------------------|-------|
| Sand       | 7                     | 22        | 40% MWHC | 37                      | 123                     | 7                        | SFO   |
| Loamy sand | 8                     | 22        | 40% MWHC | 44                      | 146                     | 9                        | SFO   |

**RMS conclusion**

The soil properties and study conditions are poorly described, but it can be consistently shown that 2-AB or other extractable residues account each for less than 5% of the applied radioactivity throughout the duration of the experiment. The mineralization rate amounts to maximal 12 - 19% (based only on recovery of carbendazim, not specific measurements of <sup>14</sup>CO<sub>2</sub>). Non-extractable residues are not detected quantitatively, but it is suspected that their proportion is relatively high.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

|                   |  |
|-------------------|--|
| <b>Reference:</b> | <b>1. Gildemeister, H. Jordan, H.J. and Remmert, U (1981)</b> Behaviour of the plant protection product HOE 17411 OF AT 102 (Carbendazim) in soil SS 2.2 at 15 °C, 20 °C and 25 °C |
|                   | <b>2. Zillgens, B. (2007a)</b> Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model Maker™ 4.0    |
| Report No.:       | 1 (B) 125/81; A16743, BOD95- 00446   |
|                   | 2. DuPont-21656  |
| Guideline:        | BBA leaflet no. 36   |
| GLP:              | 1. No  |
| Validity          | 1. Valid   |

**Material and methods:**

An aerobic soil degradation study was conducted according to BBA leaflet no. 36. (2-<sup>14</sup>C) 2-(methoxycarbonylamino)-benzimidazole in the formulation Hoe 17411 OF AS 101 was added to a sandy soil at a concentration of 2.674 mg carbendazim/kg corresponding to 711.3 g/ha. The amount of applied active substance is approximately 1.5-fold higher than the maximum application rate of 2 x 250 g as/ha. The specific activity was 16.45 MBq/g. The study was conducted with one sandy soil at 3 different incubation temperatures. A summary of the physical and chemical properties of the soil is provided in the below table.

100 g soil was treated with 2.674 mg/kg Carbendazim in separate containers. All soil samples were adjusted to 40 % maximum water holding capacity (MWHC) and were incubated in darkness at 15, 20 and 25°C for 28 days. Samples were taken at time intervals 0, 7, 14, 21 and 28 days for subsequent analysis.

The samples were extracted with methanol/glacial acetic acid/hydrochloric acid (0.1M) (90+10+5 (v/v/v)). Subsequently the neutralisation with saturated NaHCO<sub>3</sub> solution and a re-extraction with ethyl acetate was carried out. For the quantification of Carbendazim in the residues of soil the <sup>14</sup>C-radioactivity was detected by liquid scintillation counting following TLC separation with silica gel (solvent: methylene chloride/methanol 9+1 (v+v)). The lower limit of determination was 0.01 mg/kg.

Kinetic analyses: DT<sub>50</sub>/DT<sub>90</sub> were determined by linear regression in the original report (Gildemeister et al, 1981). DT<sub>50</sub>/DT<sub>90</sub> were re-calculated in accordance with FOCUS guidance (in Zillgens, 2007a) and those results were the final ones.

**Table 112: Soil properties.**

| Soil property                          | SS 2.2 (Schwanheimer Sand) |
|--|----------------------------|
| Particle size distribution (DIN) (%)   |                            |
| < 2 µm                                 | 37.0                       |
| Classification (BBA)                   | sand                       |
| Organic carbon (%)                     | 2.7                        |
| pH (CaCl <sub>2</sub> )                | 4.7                        |
| Maximum water holding capacity (w/w %) | n.a.                       |

**Results**

The decrease of carbendazim during incubation period was summarized in the table below. Recovery (total radioactivity in extracts and bound residues) over the whole period of the study was between 76 and 90%. Because mineralisation was not detected, a mass balance was not completed for this study. Already on day 0 about 9.5% of the initial applied radioactivity was no longer extractable. By the end of the study (day 28) this value had risen to 48- 56% depending on

the test temperature. Radio-TLC investigation of the extracts at the different sampling points showed that over 92% of these consisted invariably of carbendazim. There were also marginal amounts of unidentified metabolites (three at most). These results indicate either that the active ingredient itself was adsorbed fairly rapidly or that other degradation products, if any, were present almost quantitatively as bound residues.

**Table 113: Decrease of carbendazim during incubation in one soil at three different temperatures over a period of 28 days.**

| Days after application | Carbendazim (mg/kg) |       |       | Carbendazim (% of initial) |      |      |
|------------------------|---------------------|-------|-------|----------------------------|------|------|
|                        | 15°C                | 20°C  | 25°C  | 15°C                       | 20°C | 25°C |
| 0                      | 2.422               | 2.422 | 2.422 | 100                        | 100  | 100  |
| 7                      | 1.882               | 1.776 | 1.728 | 77.7                       | 73.3 | 71.4 |
| 14                     | 1.736               | 1.606 | 1.55  | 71.7                       | 66.3 | 64.0 |
| 21                     | 1.497               | 1.464 | 1.343 | 61.8                       | 60.4 | 55.4 |
| 28                     | 1.38                | 1.291 | 1.16  | 57.0                       | 53.3 | 47.9 |

The DT<sub>50</sub> values in the original study were determined by linear regression to be 28, 25 and 21 days at 15, 20 and 25°C with r<sup>2</sup> range of 0.96 – 0.98. Recalculated values (Zillgens, 2007a) are given in the table below. Optimized degradation rates were derived by means of Model Maker™ vers. 4.0 fitting runs and were tested with regard to the acceptability of SFO kinetics in accordance with FOCUS kinetics guidance (2006). A statistical analysis showed that the degradation of the parent could be generally well described by a SFO kinetic model.

**Table 114: Re-calculated laboratory DT<sub>50</sub> and DT<sub>90</sub> for the aerobic degradation of carbendazim in sand soil at three different temperatures.**

| Soil | Number of data points | Temp (°C) | Moisture | DT <sub>50</sub> (days) | DT <sub>90</sub> (days) | χ <sup>2</sup> , %-error | Model |
|------|-----------------------|-----------|----------|-------------------------|-------------------------|--------------------------|-------|
| Sand | 5                     | 15        | 40% MWHC | 34                      | 112                     | 4                        | SFO   |
|      | 5                     | 20        | 40% MWHC | 31                      | 102                     | 5                        | SFO   |
|      | 5                     | 25        | 40% MWHC | 26                      | 86                      | 5                        | SFO   |

**RMS conclusion**

The soil properties and study conditions are poorly described, but it can be consistently shown that there were only marginal amounts of unidentified metabolites (three at most). So that it can be ascertained, that 2-AB or other extractable residues account each for less than 5% of the applied radioactivity throughout the duration of the experiment. The mineralization rate amounts to maximal 10 - 24% (based only on recovery of applied radioactivity in extracts and bound residues, not specific measurements of <sup>14</sup>CO<sub>2</sub>). Non-extractable residues account 48 - 56% depending on the test temperature.

The study results are plausible.

#### 11.1.4.4 Photochemical degradation

The available data (summarised in RAR Volume 3, B.8.2.1.2) showed that thiophanate-methyl is photolytically degradable under outdoor conditions (Japan, mid-December), with a half-life of 2.3 days under the conditions of the test. The quantum yield ( $\phi$ ) was determined to be  $3.84 \times 10^{-3}$ . From this results  $DT_{50s}$  for water bodies and clear sky conditions at 40°N latitude were calculated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter). The RMS concluded that direct photochemical transformation may contribute to the degradation of thiophanate-methyl under environmental conditions, and that the major product formed is carbendazim, with DX-105 formed in less amounts. Photochemical transformation is not expected to be a significant route of degradation of carbendazim.

|                              |  |
|------------------------------|--|
| <b>Reference:</b>            | <ol style="list-style-type: none"> <li>1. <b>Shiotani, H. (2003c)</b> Photodegradation of Thiophanate-methyl. (A previous study by <b>Soeda &amp; Shiotani (1987b)</b>, Report No. EC-74, RD-8701 (712-001)) was evaluated and considered acceptable in the DAR (1997). The report from the same experiment was re-written by Shiotani (2003c) to fulfil US EPA requirements.)</li> <li>2. <b>Kiesel, A. and Geibel, E. (2015g)</b> Assessment of degradation kinetics of Thiophanate-methyl in a study on aqueous photochemical degradation according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014).</li> <li>3. <b>Kiesel, A. and Geibel, E. (2015h)</b> Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of an aqueous photolysis study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7.</li> </ol> |
| Report No.:                  | <ol style="list-style-type: none"> <li>1. RD-03185</li> <li>2. RD-03367</li> <li>3. RD-03368</li> </ol>  |
| Document No.:                | <ol style="list-style-type: none"> <li>1. 712-002</li> <li>2. 782-039</li> <li>3. 782-085</li> </ol>   |
| Guideline:                   | <ol style="list-style-type: none"> <li>1. US EPA FIFRA guideline 161-2, and US EPA CG-6000</li> <li>2. and 3. FOCUS degradation kinetics (2006, 2014)</li> </ol>   |
| GLP:                         | <ol style="list-style-type: none"> <li>1. No</li> <li>2. and 3. Not applicable</li> </ol>  |
| <b>Previous evaluation:</b>  | All three studies submitted for the purpose of renewal.  |
| <b>Material and methods:</b> |  |
| Test material:               | [Benzene-U- <sup>14</sup> C] thiophanate-methyl  |
| Lot/Batch No:                | CFQ 4519   |
| Radiochemical purity:        | > 99% (TLC)  |
| Test concentration:          | 10 mg/l (thiophanate-methyl was dissolved in chloroform and the solvent was evaporated before the material was dissolved in buffer)  |
| Test system:                 | Quartz tubes >16 mL, sterile.<br>Buffer (pH 5): acetate-acetic acid, passed through a 0.2 µm filter.   |
| Test conditions:             | Outdoors in Japan in mid-December (latitude 35°, longitude 139°E), (temperature and light conditions were provided in tables), 10 dark controls.   |
| Sampling time points:        | Single samples after 0, 0.5, 1, 2, 4, and 7 days. The dark control (two) samples analysed at 0.5 and 1, 2, 4 and 7 days.   |
| Method of analysis:          | Radioactivity in aliquots of the samples were determined by LSC.<br>Characterisation was done by HPLC and TLC. Test solution was extracted twice with 10 dichloromethane. The extract was dried and dissolved in dichloromethane and spotted on thin layer plate and developed two-dimensionally. Major photoproducts were extracted from TLC-plates and purified by HPLC. Mass spectra of these extracts and corresponding standards were determined.   |

Analysis for concentration of thiophanate-methyl was done as area under curve with HPLC with UV-detector. LOD was 5 ng.

Used standards: Thiophanate-methyl, carbendazim (MBC), DX-105 and FH-432

Actinometer: A standard p-nitroacetophenone-pyridine (PNAP/PYR) actinometer was used. Concentration of PYR was adjusted so that experimental half-lives of PNAP and thiophanate-methyl were similar. The solution was irradiated in parallel with the test solutions. After 0, 0.5, 1, 2, 4 and 7 days the concentration of PNAP in duplicate samples was determined by HPLC. Concentrations of thiophanate-methyl and PNAP were used to determine the quantum yield and estimate the rate constant for summer, spring and winter seasons.

Kinetic evaluation: In addition to estimation of half-lives from the quantum yield the study report also estimated the half-life from the measured concentrations using first order kinetic. Degradation rates were re-assessed by Kiesel and Geibel (2015g,h), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.

**Results**

Ambient temperatures varied between 2.9°C and 16.3°C during exposure (as determined 3 times per day).

The recovery ranged from 98.8% to 101.9% of applied radioactivity in both irradiated and dark control samples during the course of the study indicating that no volatile compounds evolved. No significant loss of thiophanate-methyl or PNAP was observed in controls, so the loss in irradiated samples were attributed solely to photolysis. The major degradation products carbendazim (MBC) and DX-105 reached 49.7 and 14.3% AR, respectively.

**Table 115: Irradiated samples: Distribution and characterisation of radioactivity in aqueous samples with 14C-thiophanate-methyl irradiated outdoors in Japan. As % of applied radioactivity.**

| Fraction - compound         | 0 day | 0.5 day | 1.0 day | 2.0 days | 4.0 days | 5.5 days |
|-----------------------------|-------|---------|---------|----------|----------|----------|
| Extractable <sup>14</sup> C | 98.8  | 98.9    | 100.0   | 97.0     | 93.9     | 91.8     |
| TM                          | 92.5  | 85.9    | 81.4    | 63.4     | 28.1     | 12.5     |
| DX-105                      | 2.0   | 3.2     | 6.3     | 8.5      | 11.5     | 14.3     |
| FH-432                      | 0     | 0       | 0       | 0        | 2.8      | 4.4      |
| MBC                         | 1.8   | 4.3     | 5.4     | 13.1     | 38.0     | 49.7     |
| Unknown 1                   | 0.6   | 0.7     | 1.0     | 2.0      | 3.5      | 2.5      |
| Unknown 2                   | 0     | 0       | 2.6     | 5.5      | 5.6      | 4.3      |
| Others *                    | 1.9   | 4.8     | 3.3     | 4.5      | 4.4      | 4.1      |
| Aqueous <sup>14</sup> C     | 1.2   | 1.5     | 1.5     | 3.0      | 4.9      | 7.3      |
| Total <sup>14</sup> C       | 100   | 100.4   | 101.5   | 100      | 98.8     | 99.1     |

\* “Others” consisted of at least six minor compounds including those remaining at the TLC origin.

**Table 116: Dark controls: Distribution and characterisation of radioactivity in aqueous samples with <sup>14</sup>C-thiophanate-methyl kept outdoors in Japan. As % of applied radioactivity.**

| Fraction - compound         | 0 day | 0.5 day | 1.0 day | 2.0 days | 4.0 days | 5.5 days |
|-----------------------------|-------|---------|---------|----------|----------|----------|
| Extractable <sup>14</sup> C | -     | 99.5    | 100.8   | 99.1     | 98.8     | 98.6     |
| TM                          | -     | -       | -       | 91.9     | -        | 90.8     |
| DX-105                      | -     | -       | -       | 2.0      | -        | 2.0      |
| FH-432                      | -     | -       | -       | 0        | -        | 0        |
| MBC                         | -     | -       | -       | 1.8      | -        | 1.8      |
| Others *                    | -     | -       | -       | 3.3      | -        | 4.0      |
| Aqueous <sup>14</sup> C     | -     | 1.3     | 1.1     | 1.8      | 1.6      | 1.7      |
| Total <sup>14</sup> C       | -     | 100.8   | 101.9   | 100.9    | 100.4    | 100.3    |

\* Others consisted of at least six minor compounds including those remaining at the TLC origin.

- No data available.

The quantum yield ( $\phi$ ) was determined to be  $3.84 \times 10^{-3}$ . From this results DT<sub>50</sub>s for water bodies and clear sky conditions at 40°N latitude were calculated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter).

From the regression analysis of the measured concentrations DT<sub>50</sub> was estimated to 2.2 days under the conditions of the test.

The results of the kinetic re-analyses of the data by Kiesel and Geibel (2015g,h) are summarised in the table below.

After optimisation of parameters for the parent only, a pathway fit with flow from the parent to carbendazim and to DX-105 was done. Time zero amounts of transformation products were added to the parent compartment. SFO kinetics represented the best fit for thiophanate-methyl (Chi2 error-% was marginally higher in FOMC). Visual assessments were considered acceptable. Kiesel and Geibel (2015g,h) accepted the results for carbendazim and DX-105 and therefore selected the results from the pathway fit also for the parent. For thiophanate-methyl the RMS has instead selected the results from the parent only fit since the results for the transformation products were not acceptable statistically. For the same reason, no formation fractions are shown.

**Table 117: Summary of kinetic re-analysis by Kiesel and Geibel (2015g,h) of the aquatic photolysis data from Shiotani (2003c). Irradiated buffered solution at pH 5 and ambient temperatures in December outdoors in Japan.**

| Substance          | Kinetic model | Mo    | Parameter                | $\chi^2$ , %-error | Prob>t               | Lower CI | Upper CI | DT <sub>50</sub> [days] | DT <sub>90</sub> [days] |
|--------------------|---------------|-------|--------------------------|--------------------|----------------------|----------|----------|-------------------------|-------------------------|
| <b>Parent only</b> |               |       |                          |                    |                      |          |          |                         |                         |
| Thiophanate-methyl | SFO           | 102.0 | k= 0.2979                | 6.8                | $6.4 \times 10^{-4}$ | 0.2      | 0.4      | 2.3                     | 7.7                     |
| <b>Pathway fit</b> |               |       |                          |                    |                      |          |          |                         |                         |
| Carbendazim        | SFO-SFO       | n.r   | k= $2.3 \times 10^{-14}$ | 24.8               | 0.5                  | -0.2     | 0.2      | >1000                   | >1000                   |
| DX-105             | SFO-SFO       | n.r   | k=0.06637                | 7.1                | 0.09                 | -0.03    | 0.2      | 10.4                    | 34.7                    |

n.r. Not relevant.

### RMS comments and conclusion

The experimental study (Soeda & Shiotani, 1987b) was considered acceptable in the DAR (1997). The report was re-written by Shiotani (2003c) to fulfil US EPA requirements but the description of the experiment and the results were the same. The study is still considered acceptable. The experiment was conducted in compliance with a relevant guideline. This was a non-GLP study but the experiments appeared scientifically sound. It is not considered likely that repetition

of the study would alter the conclusion substantially. It was unclear whether duplicate samples were taken for analyses, but in any case results from individual replicates were not presented.

During peer review the variation in temperature came into question, since modern guidelines (OECD TG No 316) recommend a more stable temperature. However the RMS believes that within an environmentally realistic temperature range such variation is not expected to have any dramatic influence on the rate of photochemical reactions. In addition to estimation of half-life from the measured amounts of thiophanate-methyl, half-lives were also estimated from the quantum yield, and the results were fairly similar. So regarding rate of photolysis the RMS suggest that it can be concluded from the study that the photochemical DT<sub>50</sub> may be in the order of 1-5 days (40°N) depending on season. (Evaluation Table, open point 4.11)

Concerning the relevance of the unknown product 2 the RMS notes that degradation rates were also relatively fast in microbially active systems (DegT<sub>50s</sub> 0.6-3.5 days), and so was the dissipation rate from water (DisT<sub>50s</sub> 1.6-2.8 days). The unknown photo-product 2 was measured as 5.5 and 5.6% AR at sampling points 2 and 4 days, and at the next (and last) sampling point the amount was 4.3% AR. After 2-4 days in a biologically active natural system less than half of the initial amount of thiophanate-methyl would still be present. Therefore it is not expected that the unknown photo-product 2 would be formed as >5% under natural conditions. Since the other major photo-products were identified and given the relatively fast biodegradation the RMS conclude that the available data on photochemical transformation is sufficient. (Evaluation Table, open point 4.11)

### 11.1.5 Summary and discussion on environmental degradation

In a ready biodegradability test in accordance with OECD TG 301C, biodegradation reached only 4% in 28 days. In degradation studies in water only, in water/sediment systems and in soil, the parent compound is transformed with half-lives from less than one day up to a few days to the major environmental metabolite carbendazim. Carbendazim on the other hand, is more persistent with half-lives of 64.8 days, of 15.1-91.6 days and of 22.0-63.2 days in water only, in water/sediment and in soil, respectively. Carbendazim was found as >80% in water and water/sediment studies and as >10% after only one day. In soil studies, carbendazim was found as max 75.8%. Additional metabolites were formed but to a lesser extent compared to carbendazim. Based on the available information, thiophanate-methyl is considered as not rapidly biodegradable.

### 11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant

### 11.3 Environmental fate and other relevant information

Not relevant.

### 11.4 Bioaccumulation

**Table 118: Summary of relevant information on bioaccumulation.**

| Method | Results              | Remarks | Reference |
|--------|----------------------|---------|-----------|
|        | No data, not needed. |         |           |

#### 11.4.1 Estimated bioaccumulation

When experimental data on the bioaccumulation are absent, K<sub>ow</sub> can be used for non-ionised substances to estimate the potential for bioaccumulation according to recommendations in the Guidance on the Application of the CLP Criteria (ECHA-09-G-02-EN. 2009). Since the Log K<sub>ow</sub> values for thiophanate-methyl and the major metabolite carbendazim are ≤ 4, the EG Reg 1272/2008 cut-off value, the potential for bioaccumulation is considered to be low (actual value 1.45 and 1.5 for thiophanate-methyl and carbendazim, respectively, see section 7).

#### 11.4.2 Measured bioaccumulation

No measured bioaccumulation data were available, and not needed.

### 11.5 Acute aquatic hazard

The available and reliable data on acute toxicity of thiophanate-methyl, its major environmental metabolite carbendazim and additional metabolites to aquatic organisms is summarised in the table below.

**Table 119: Summary of relevant information on acute aquatic toxicity. Key data are marked in bold text**

| Group | Test substance | Time-scale<br>(Test type) | End point | Toxicity<br>(mg a.s./L) | Reference |
|-------|----------------|---------------------------|-----------|-------------------------|-----------|
|-------|----------------|---------------------------|-----------|-------------------------|-----------|

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| Group                      | Test substance     | Time-scale<br>(Test type)     | End point                      | Toxicity<br>(mg a.s./L)                | Reference   |
|----------------------------|--------------------|-------------------------------|--------------------------------|--|---|
| Fish                       |                    |                               |                                |  |   |
| <i>Oncorhynchus mykiss</i> | Thiophanate-methyl | Acute 96 hr<br>(flow-through) | Mortality,<br>LC <sub>50</sub> | 11.0 (7.6 – 13) (mm)                   | Report number 821-001                                       |
| <i>Ictalurus punctatus</i> | Carbendazim*       | 96 hr                         | Mortality,<br>LC <sub>50</sub> | <b>0.019</b> (nom)                     | Report number A30119;<br>cited in EFSA (2010) *             |
| <i>Oncorhynchus mykiss</i> | Carbendazim*       | 96 hr                         | Mortality,<br>LC <sub>50</sub> | 0.19 (nom)                             | Report number A52914;<br>cited in EFSA (2010) *             |
| <i>Oncorhynchus mykiss</i> | 4-OH-TM            | 96 h, static                  | Mortality,<br>LC <sub>50</sub> | >10<br>(mm, filtrated)                 | Report number 821-008                                       |
| <i>Oncorhynchus mykiss</i> | CM-0237            | 96 h, static                  | Mortality,<br>LC <sub>50</sub> | >0.14 (mm, filtrated)                  | Report number 821-009                                       |
| Aquatic invertebrates      |                    |                               |                                |  |   |
| <i>Daphnia magna</i>       | Thiophanate-methyl | 48 h, flow-through            | EC <sub>50</sub><br>NOEC       | <b>5.4 (4.4-6.3)</b> (mm)<br><4.2 (mm) | Putt, A.E. (1992)   |
| <i>Daphnia magna</i>       | BAS 325 10 F       | 48 h, static                  | EC <sub>50</sub><br>NOEC       | 4.4 (mm)<br>3.1 (nom)                  | Elendt-Schneider (1991)                                     |
| <i>Daphnia magna</i>       | Carbendazim*       | 48 h                          | EC <sub>50</sub>               | 0.15 (nom)                             | Fisher (1988), cited in<br>EFSA (2010) *                    |
| <i>Daphnia magna</i>       | 4-OH-TM            | 48 h, static                  | EC <sub>50</sub>               | >17.6 (mm, filtrated)                  | Fujikake, N. (2012)   |
| <i>Daphnia magna</i>       | CM-0237            | 48 h, static                  | EC <sub>50</sub>               | >0.27 (mm, filtrated)                  | Fujikake, N. (2012)   |
| <i>Chironomus riparius</i> | 4-OH-TM            | 48 h, static                  | EC <sub>50</sub>               | >14.6 (mm, filtrated)                  | Kley, A., Wydra, V.<br>(2012)                               |
| Algae                      |                    |                               |                                |  |   |
| <i>P. subcapitata</i>      | Thiophanate-methyl | 72 h, static                  | E <sub>r</sub> C <sub>50</sub> | 37.2 (25.6 – 169) (mm)                 | Saito, S. (2002), and<br>Wirzinger, G. (2015)               |
| <i>S. subspicatus</i>      | Topsin 500 SC      | 72 h, static                  | E <sub>r</sub> C <sub>50</sub> | 27.3 (22.4 – 33.2)<br>(mm)             | Kley, A., Wydra, V.<br>(2012); Wirzinger &<br>Ruhnke (2016) |
| <i>P. subcapitata</i>      | Carbendazim*       | 72 h, static                  | E <sub>r</sub> C <sub>50</sub> | > 11 mg/L (mm)                         | Bell (1996), cited in<br>EFSA (2010) *                      |
| <i>P. subcapitata</i>      | 4-OH-TM            | 72 h, static                  | E <sub>r</sub> C <sub>50</sub> | > 15 (mm, dissolved)                   | Baba, K. (2012)   |
| <i>P. subcapitata</i>      | CM-0237            | 72 h, static                  | E <sub>r</sub> C <sub>50</sub> | > 0.182 (mm)                           | Baba, K. (2012)   |
| Higher plant               |                    |                               |                                |  |   |
| No data, not needed        |                    |                               |                                |  |   |

\* Refer to the EFSA conclusion on the peer review of the active substance Carbendazim, EFSA (2010). The study summaries provided below for Carbendazim are derived from the Draft Re-Assessment Report produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not evaluated by Sweden at this stage.

### 11.5.1 Acute (short-term) toxicity to fish

|                              |   |
|------------------------------|---|
| <b>Reference:</b>            | <b>xxxx (1993):</b> Thiophanate-methyl - Acute toxicity to rainbow trout ( <i>Oncorhynchus mykiss</i> ) under flow-through conditions |
| Report number:               | RD-9330, 93-4-4738; 12681.1292.6101.108 (821-001)   |
| Guideline:                   | US EPA 72-1   |
| GLP:                         | Yes   |
| <b>Previous evaluation:</b>  | In DAR 1997.  |
| <b>Material and methods:</b> |   |
| Test material:               | Thiophanate-methyl  |
| Lot/Batch No:                | Lot# TIF-01016 (non-radiolabelled), Lot# 109F9209 (radiolabelled)   |

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|                              |   |
|------------------------------|---|
| Purity:                      | 95.95±0.36% (non-radiolabelled), >98% radiopurity (radiolabelled)   |
| Species:                     | Rainbow trout ( <i>Oncorhynchus mykiss</i> ), length 37 (30-48) mm, body weight 0.47 (0.28-0.75) g w.w.   |
| Test system/test conditions: | <p>Flow-through conditions. Twenty organisms (ten per replicate) were exposed in duplicate test aquaria to each of five concentrations of Thiophanate-methyl, a solvent control (dimethylformamide, DMF), and a dilution water control for 96-hours. The test solutions were prepared with a combination of non-radiolabelled and radiolabelled (<sup>14</sup>C) test material.</p> <p>Nominal concentrations of 2.6, 4.3, 7.2, 12 and 20 mg a.i./L were maintained by introducing approximately 6.4 aquarium volumes per day of newly prepared test solution via an intermittent-flow proportional diluter apparatus. Each replicate solution was sampled and analyzed for Thiophanate-methyl concentration at 0-hour (test initiation) and 96-hours (test termination) of exposure.</p> <p>Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Test temperature was 12±1°C, pH 6.8-7.2, oxygen saturation 80-90%. Hardness and alkalinity ranged 25-26 mg/L and 20-23 mg/L, respectively (as CaCO<sub>3</sub>). Photoperiod was 16 hours light per day.</p> |

### Results:

Based on the results of the chemical analyses, the mean measured exposure concentrations were defined as 2.4, 4.7, 7.6, 13 and 20 mg a.i./L, corresponding to 92, 109, 106, 108 and 100% of the nominal values.

**Table 120: Concentration of <sup>14</sup>C-Thiophanate-methyl measured in replicate test solutions during the 96 hour flow-through exposure of rainbow trout (*Oncorhynchus mykiss*)**

| Nominal Concentration mg a.i./L | Measured Concentration after [mg a.i./ L] |       |         |       |      |
|---------------------------------|---|-------|---------|-------|------|
|                                 | 0-Hour                                    |       | 96-Hour |       | Mean |
|                                 | A   | B     | A       | B     |      |
| Control                         | <0.36                                     | <0.36 | <0.36   | <0.36 |      |
| Solvent control                 | <0.36                                     | <0.36 | <0.36   | <0.36 |      |
| 2.6                             | 2.4                                       | 2.4   | 2.3     | 2.4   | 2.4  |
| 4.3                             | 4.6                                       | 4.7   | 5.1     | 4.3   | 4.7  |
| 7.2                             | 7.1                                       | 7.9   | 7.5     | 7.9   | 7.6  |
| 12                              | 13  | 13    | 13      | 13    | 13   |
| 20                              | 20  | 21    | 20      | 20    | 20   |

After 96 hours, 100 and 75% mortality was observed at 20 and 13 mg a.i./L, respectively. At lower test concentrations, no mortality was observed. Sublethal effects were observed at 4.7 mg a.i./L and higher. The resulting 96 hour LC<sub>50</sub> was determined to be 11 (7.6 – 13) mg a.i./L, and a NOEC could be set to 2.4 mg a.i./L based on mean measured test concentrations.

**Table 121: Mortality during the 96 hour flow-through exposure of *Oncorhynchus mykiss* to Thiophanate-methyl**

| Mean measured Conc. [mg/L] | Cumulative mortality(%) |    |      |         |    |      |         |     |      |         |     |      |
|----------------------------|-------------------------|----|------|---------|----|------|---------|-----|------|---------|-----|------|
|                            | 24-hour                 |    |      | 48-hour |    |      | 72-hour |     |      | 96-hour |     |      |
|                            | A                       | B  | Mean | A       | B  | Mean | A       | B   | Mean | A       | B   | Mean |
| Control                    | 0                       | 0  | 0    | 0       | 0  | 0    | 0       | 0   | 0    | 0       | 0   | 0    |
| Solvent Control            | 0                       | 0  | 0    | 0       | 0  | 0    | 0       | 0   | 0    | 0       | 0   | 0    |
| 2.4                        | 0                       | 0  | 0    | 0       | 0  | 0    | 0       | 0   | 0    | 0       | 0   | 0    |
| 4.7                        | 0                       | 0  | 0    | 0       | 0  | 0    | 0       | 0   | 0    | 0       | 0   | 0    |
| 7.6                        | 0                       | 0  | 0    | 0       | 0  | 0    | 0       | 0   | 0    | 0       | 0   | 0    |
| 13                         | 10                      | 0  | 5    | 20      | 0  | 10   | 40      | 40  | 40   | 70      | 80  | 75   |
| 20                         | 0                       | 10 | 5    | 40      | 50 | 45   | 90      | 100 | 95   | 100     | 100 | 100  |

**RMS comments:**

The study was re-evaluated against the validity criteria of the corresponding OECD TG 203 (1992):

1. mortality in the control(s) should not exceed 10% at the end of the test;
2. constant conditions maintained as far as possible throughout the test (eg. semi-static or flow-through)
3. dissolved oxygen concentration at least 60 per cent of the air saturation value throughout the test;
4. concentrations of test substance were satisfactorily maintained, and preferably at least 80% of nominal.

All these criteria were fulfilled, and the study is considered to be valid and useful for the risk assessment.

**Author:** xxxxx  
**Title:** Toxicological Studies of Benomyl and Carbendazim in Fish  
**Date:** Dec 1984  
**Doc ID:** A30119; BVL No. 1758267  
**Guidelines:** ASTM  
**GLP:** Not applicable  
**Validity:** Results considered to be valid

**Methods**

Conducted according to prevailing standards and subsequently accepted for international registration.

10 animals per tested concentration (0.3 g to 1.2 g)

Rainbow trout (*Oncorhynchus mykiss*): 12° C

Channel catfish (*Ictalurus punctatus*): 22° C

Bluegill (*Lepomis macrochirus*): 22° C

Test concentrations and application of test substance: Not reported; static testing

Additional series of acute tests were performed to determine the effects of temperature (rainbow trout 7° C, 12° C and 17° C; channel catfish: 12° C, 17° C and 22° C), pH (rainbow trout and channel catfish: 6.5, 7.5 and 8.5), hardness (rainbow trout: soft water of pH 8, hardness 40 mg/l CaCO<sub>3</sub> and hard water of pH 8, hardness 320 mg/L CaCO<sub>3</sub>) and exposure of early life stages (rainbow trout and channel catfish) yolk-sac fry, swim-up fry and 0.2 g fry.

**Results**

**Table 122: Acute toxicity of carbendazim for rainbow trout (*Oncorhynchus mykiss*) channel catfish (*Ictalurus punctatus*) and bluegill (*Lepomis macrochirus*):**

| Species                    | Endpoint         | Value (mg/L)        | time | ai | n/r/i |
|----------------------------|------------------|---------------------|------|----|-------|
| <i>Oncorhynchus mykiss</i> | LC <sub>50</sub> | 0.87 (0.63-1.19)    | 96 h |    | n     |
| <i>Lepomis macrochirus</i> | LC <sub>50</sub> | >3.2                | 96 h |    | n     |
| <i>Ictalurus punctatus</i> | LC <sub>50</sub> | 0.019 (0.013-0.027) | 96 h |    | n     |

n = nominal; r = real; i = initial

**Table 123: Acute toxicity of carbendazim for *Ictalurus punctatus* –results of additional test series**

| Early life stages        | LC <sub>50</sub> (mg/L) |
|--------------------------|-------------------------|
| yolk-sac fry:            | 0.007 (0.006 - 0.009)   |
| swim-up fry :            | 0.012 (0.009 - 0.015)   |
| 0.2 g fry:               | 0.01 (0.008 - 0.013)    |
| water hardness           |                         |
| 40 mg CaCO <sub>3</sub>  | 0.018 (0.011 - 0.028)   |
| 320 mg CaCO <sub>3</sub> | 0.024 (0.018 - 0.032)   |
| pH                       |                         |
| 6.5                      | 0.023 (0.018 - 0.031)   |
| 7.5                      | 0.014 (0.011 - 0.018)   |
| 8.5                      | 0.023 (0.018 - 0.029)   |
| temperature              |                         |
| 12° C                    | > 0.56                  |
| 17° C                    | 0.14 (0.091 - 0.216)    |
| 22° C                    | 0.032 (0.023 - 0.044)   |

**Table 124: Acute toxicity of carbendazim for *Oncorhynchus mykiss* –results of additional test series**

| Early life stages        | LC <sub>50</sub> (mg/L) |
|--------------------------|-------------------------|
| yolk-sac fry:            | 0.145 (0.109 - 0.192)   |
| swim-up fry :            | 0.32 (0.24 - 0.43)      |
| 0.2 g fry:               | 0.37 (0.27 - 0.51)      |
| water hardness           |                         |
| 40 mg CaCO <sub>3</sub>  | 0.78 (0.58 - 1.05)      |
| 320 mg CaCO <sub>3</sub> | 0.88 (0.64 - 1.21)      |
| pH                       |                         |
| 6.5                      | 0.64 (0.46 - 0.90)      |
| 7.5                      | 0.41 (0.31 - 0.44)      |
| 8.5                      | 0.34 (0.26 - 0.44)      |
| temperature              |                         |
| 7° C                     | > 1.8                   |
| 12° C                    | 0.87 (0.63 - 1.19)      |
| 17° C                    | 0.10 (0.07 - 0.14)      |

**Conclusion**

Report results listed and evaluated in the carbendazim monograph of 13.11.1997. The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfil the objectives of the study and there is no reason to doubt the validity of the results. Critical point: raw data missing (esp. control treatments). Determined median lethal concentrations after 96 h are for *Oncorhynchus mykiss* LC<sub>50</sub> = 0.87 mg carbendazim/L, for *Lepomis macrochirus* LC<sub>50</sub> > 3.2 mg carbendazim/L and for *Ictalurus punctatus* LC<sub>50</sub> = 0.019 mg carbendazim/L.

**Author:** xxxx  
**Title:** Acute (96-hour) toxicity of H-9910 to rainbow trout  
**Date:** Jun 1976  
**Doc ID:** A52914, Report no: HLO486-76; BVL No. 1840602  
**Guidelines:** Not applicable  
**GLP:** Not applicable  
**Validity:** Results considered to be valid

**Methods**

Conducted according to prevailing standards and subsequently accepted for international registration. The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfil the objectives of the study and there is no reason to doubt the validity of the results.

Test species: Rainbow trout (*Oncorhynchus mykiss*). Number of animals: 10 per tested concentration (static, stock solution in acetone)

control, 0.10, 0.14, 0.24, 0.32, 0.42, 0.56, 0.75, 1 and 2.1 mg/L

Maintenance: 15 L tanks; loading: 3.7 cm fish/L; 12° C ±1.0° C. Duration of exposure: 96 h

Observed parameters: Mortality and symptoms every 24 hours after start of exposure

**Results**

The reported values are based on nominal concentrations.

**Table 125: Observed mortality in *Oncorhynchus mykiss* during exposure to carbendazim**

| Concentrations | Survival (No. animals) |    |    |
|----------------|------------------------|----|----|
|                | Control                | 10 | 10 |
| 0.10           | 10                     | 10 | 10 |
| 0.14           | 10                     | 10 | 9  |
| 0.24           | 10                     | 10 | 2  |
| 0.32           | 10                     | 9  | 0  |
| 0.42           | 10                     | 9  | 0  |
| 0.56           | 10                     | 10 | 0  |
| 0.75           | 10                     | 3  | 0  |
| 1.4            | 10                     | 1  | 0  |
| 2.1            | 10                     | 0  | 0  |

**Table 126: Acute toxicity of carbendazim for *Oncorhynchus mykiss* as given in the report summary and recalculated LC<sub>50</sub>-values**

| Species                    | Endpoint         | Value (mg/L)<br>in the report | Value (mg/L)<br>recalculated | time | ai | n/r/i |
|----------------------------|------------------|-------------------------------|------------------------------|------|----|-------|
| <i>Oncorhynchus mykiss</i> | LC <sub>50</sub> | > 2.1                         | > 2.1                        | 24 h |    | n     |
|                            | LC <sub>50</sub> | 0.84 (0.55 – 1.30)            | 0.75 (0.61-0.93)             | 48 h |    | n     |
|                            | LC <sub>50</sub> | 0.34 (0.26 – 0.45)            | 0.19 (-)                     | 96 h |    | n     |

n = nominal; r = real; i = initial

**Conclusion**

Report results not listed in the carbendazim monograph of 13.11.1997. The LC<sub>50</sub> (96 h) of carbendazim, technical, in an acute static test with rainbow trout was reported to be 0.34 mg/L. Recalculated values according to the data given in the summary of the study report are LC<sub>50</sub> (48 h) = 0.75 mg carbendazim/L and LC<sub>50</sub> (96 h) = 0.19 mg carbendazim/L.

Reference: **Kley, A., Wydra, V. (2012):** Acute Toxicity of 4-OH-TM to Rainbow trout (*Oncorhynchus mykiss*) in a 96-hour Static Test

## CLH REPORT FOR THIOPHANATE-METHYL

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Report number: RD-02401, 67761230 (821-008)  
Guideline: OECD TG 203 (1992)  
GLP: Yes

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**Previous evaluation:** No, submitted for the purpose of renewal.

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**Material and methods:**

Test material: 4-OH-TM (Thiophanate-methyl metabolite)  
Lot/Batch No: 31-10193-N.UMEDA  
Purity: 95.3 %  
Species: Rainbow trout (*Oncorhynchus mykiss*), juveniles; average length  $4.84 \pm 0.2$  cm; average weight 1.09 – 0.16 g  
Treatments: Nominal concentrations: 0, 4.3, 9.4, 21, 45 and 100 mg/L  
Controls: Reconstituted water  
Replication: Seven fish each were prepared for each experimental group.  
Duration: 96 hours  
Test conditions: Static test conditions.  
Temperature 13 – 14°C; pH:7.7 – 8.0  
Hardness: 250 mg/L as CaCO<sub>3</sub>; Conductivity  $\leq 10 \mu\text{S/cm}$   
Photoperiod 16 h light : 8 h dark; Light intensity 490 – 950 lux  
Fish were fed three times per week or daily until 24 h prior to test initiation or during the exposure period. The toxicity test was conducted in 12 L glass aquaria, containing 10 L of test solution.  
Observations: Records of mortality and sublethal effects of exposure were made after 0, 2, 24, 48, 72 and 96 hours.  
Chemical analysis: Test substance concentrations were analysed at the test start and end were analysed via HPLC-UV method. LOD was 0.025 mg/L and LOQ was 1 mg/L.  
Data analysis: The determined endpoints were the NOEC and LOEC after 96 h as well as the LC<sub>50</sub> after 96 h.

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**Results:**

*Analytical results*

At test start, turbidity of the test medium caused by the test item was observed at nominal 21, 45 and 100 mg/L, with the strongest turbidity observed at 100 mg/L. After 2 hours of test duration, some of the test item was lying at the bottom of the aquaria of all test item treatment groups and the test medium of the 21, 45 and 100 mg/L test item treatment groups was still turbid. After 24, 48, 72 and 96 hours of duration, the test item was still observed at the bottom of the aquaria of all test item treatment groups and the test medium of the 45 and 100 mg/L test item treatment groups was still turbid.

At the start of the test, on average 95 % of the nominal concentrations were found in the non-filtrated samples. After 96 hours, on average 51 % of the nominal concentrations were determined in the non-filtrated samples. These results were caused by settlement of the test item during the test (with exception of the lowest test item concentration).

In the filtrated samples, on average 23 % of the nominal concentrations were found at the start of the test and 46 % were found at test termination. Hence, the concentration of dissolved test item increased during the course of the test. This was however not the case at the highest treatment level, where all measured values were 10% of the nominal test concentration.

All reported biological results were related to the mean measured concentrations of the test item in non-filtrated samples.

**Table 127: Arithmetic mean measured 4-OH-TM concentrations in the exposure solutions during the 96 h exposure of rainbow trout**

| Nominal concentration [mg/L] | Mean measured concentration [% of nominal] <sup>1</sup> |                    |                   |                    |
|------------------------------|---|--------------------|-------------------|--------------------|
|                              | Non-filtrated samples                                   | Standard deviation | Filtrated samples | Standard deviation |
| 0                            | n.a.  | n.a.               | n.a.              | n.a.               |
| 4.3                          | 94  | 3                  | 58                | 56                 |
| 9.4                          | 85  | 15                 | 49                | 52                 |
| 21                           | 68  | 38                 | 32                | 32                 |
| 45                           | 62  | 66                 | 20                | 9                  |
| 100                          | 55  | 99                 | 10                | 0                  |

<sup>1</sup> Arithmetic mean value of all measured samples per treatment group.

*n.a.* = not applicable

Measurements of pH, dissolved oxygen and temperature were unaffected by the concentrations of 4-OH-TM tested and remained within acceptable ranges.

#### *Biological results*

In the control and all treatment groups, all fish survived until the end of the experiment. After 2 hours of exposure, one fish of the nominal 100 mg/L test item treatment group was observed to be swimming mainly at the bottom of the aquarium. No other sublethal effects or signs of intoxication were observed. Since no mortality was observed in any of the test item treatment groups, the 96 h LC<sub>50</sub> was empirically estimated to be > 55 mg/L based on mean measured concentrations (non-filtrated samples). The NOEC was determined to be at least 55 mg/L.

#### **RMS comments:**

The study was well performed and reported. The study was evaluated against the validity criteria of OECD TG 203 (1992):

1. mortality in the control(s) should not exceed 10% at the end of the test;
2. constant conditions maintained as far as possible throughout the test (eg. semi-static or flow-through)
3. dissolved oxygen concentration at least 60 per cent of the air saturation value throughout the test;

4. concentrations of the test substance satisfactorily maintained, and preferably at least 80% of nominal.

Except for the fourth criterium regarding the test substance satisfactorily maintained, these criteria were fulfilled. However, this was compensated by using the analytically measured test concentration for the results. Hence, the study is considered to be valid and useful for the risk assessment.

It was noted that the measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 96 hour LC<sub>50</sub> of >10 mg/L.

|                              |   |
|------------------------------|---|
| Reference:                   | <b>Kley, A., Wydra, V. (2012):</b> Acute Toxicity of CM-0237 to Rainbow trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour Static Test   |
| Report number:               | RD-02403, 67771230 (821-009)  |
| Guideline:                   | OECD TG 203 (1992)  |
| GLP:                         | Yes   |
| <b>Previous evaluation:</b>  | No, submitted for the purpose of renewal.   |
| <b>Material and methods:</b> |   |
| Test material:               | CM-0237   |
| Lot/Batch No:                | 31-11145-S.KOIZUMI  |
| Purity:                      | 99.1%   |
| Species:                     | Rainbow trout ( <i>Oncorhynchus mykiss</i> ), Juveniles; average length 4.84 ± 0.2 cm; average weight 1.09 – 0.16 g.  |
| Treatments:                  | Control, 4.3, 9.4, 21, 45 and 100 mg/L (nominal)  |
| Replication:                 | 7 individuals per treatment group   |
| Controls:                    | Reconstituted water   |
| Duration:                    | 96 hours  |
| Test conditions:             | Static test. Temperature: 13 – 14°C, Dissolved oxygen: 95 – 101 % of air saturation.<br>pH: 7.7 – 8.0; Hardness: 250 mg/L as CaCO <sub>3</sub><br>Conductivity: ≤ 10 µS/cm<br>Photoperiod: 16 h light : 8 h dark<br>Light intensity: 490 – 1000 lux |
| Observations:                | Records of mortality and sublethal effects of exposure were made after 0, 2, 24, 48, 72 and 96 hours.   |
| Chemical analysis:           | Test substance concentrations were analysed at the test start and end were analysed via HPLC-UV method. LOQ 1 mg/L.   |
| Data analysis:               | Due to the low mortality, no LC <sub>50</sub> could be calculated.  |

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**Results:***Analytical results*

Turbidity of the test medium caused by the test item was observed in all test item treatment groups during the whole test. Additionally, the test item was swimming at the water surface and lying at the bottom of the aquaria of the 21, 45 and 100 mg/L test item treatment groups during the whole test. In the 4.3 and 9.4 mg/L test item treatment groups, some test item was lying at the bottom of the aquaria after 24 hours until the end of the test.

At the start of the test, 91 % of the nominal concentrations were found in the non-filtrated samples. After 96 hours, on average 26 % of the nominal concentrations were determined in the non-filtrated samples. In the filtrated samples, the concentrations of the test item were above the limit of detection (0.006 mg/L) but below the limit of quantification (1 mg/L), which indicates the low water solubility of the test item. Reported biological results below are related to the mean measured concentrations of the test item (non-filtrated samples).

**Table 128: Mean measured CM-0237 concentrations in the exposure solutions during the 96 h exposure of rainbow trout**

| Nominal concentration [mg/L] | Mean measured concentration [% of nominal] <sup>1</sup> |                    |                    |                   |
|------------------------------|---|--------------------|--------------------|-------------------|
|                              | Non-filtrated samples                                   | Standard deviation | Filtrated samples  | Number of samples |
| 0                            | n.a.  | n.a.               | n.a.               | 2                 |
| 4.3                          | 59  | 60                 | < LOQ (0.067 mg/L) | 4                 |
| 9.4                          | 59  | 68                 | < LOQ (0.087 mg/L) | 4                 |
| 21                           | 61  | 53                 | < LOQ (0.092 mg/L) | 4                 |
| 45                           | 55  | 65                 | < LOQ (0.10 mg/L)  | 4                 |
| 100                          | 58  | 77                 | < LOQ (0.14 mg/L)  | 4                 |

<sup>1</sup> Mean value of all measured samples per treatment group

n.a. = not applicable

Measurements of pH, dissolved oxygen and temperature were unaffected by the concentrations of CM-0237 tested and remained within acceptable ranges for the survival of *O. mykiss*.

*Biological results*

In the control and all test item treatment groups, all fish survived until the end of the experiment. No signs of intoxication were observed.

**RMS comments:**

The study generally fulfilled the validity criteria of OECD TG 203 (1992). However, the results must be regarded as uncertain due to the unknown bioavailability of the test substance since the dissolved portion of the test substance was below the limit of quantification at all treatment levels. The RMS proposes that, although uncertain, the 96 hour LC<sub>50</sub> can be estimated to the mean measured concentrations (higher than

LOD, below LOQ, therefore uncertain) at the highest treatment level, or >0.14 mg/L. Given that no mortality was observed in the test, this approach is considered as sufficiently protective for the risk assessment.

### 11.5.2 Acute (short-term) toxicity to aquatic invertebrates

|                              |   |
|------------------------------|---|
| Reference:                   | <b>Putt, A.E. (1992):</b> Thiophanate-methyl - Acute toxicity to Daphnids ( <i>Daphnia magna</i> ) under flow-through conditions (FIFRA Guideline no.: 72-2)                    |
| Report number:               | 92-4-4217; 12681.1191.6100.115. RD-9229 (822-004)   |
| Guideline:                   | U.S. EPA 72-2   |
| GLP:                         | Yes   |
| <b>Previous evaluation:</b>  | In DAR 1997.  |
| <b>Material and methods:</b> |   |
| Test material:               | Non-radiolabelled and radiolabelled thiophanate-methyl.   |
| Lot/Batch No:                | TIF-01016 and 109F9209  |
| Purity:                      | 97.57% for non-radiolabelled. >98% for radiolabelled.   |
| Species:                     | <i>Daphnia magna</i> , less than 24 hours old.  |
| Treatments:                  | 3.1, 5.2, 8.6, 14 and 24 mg/L (nominal).  |
| Replication:                 | Two replicates per treatment and controls, ten daphnids per test level.   |
| Controls:                    | Untreated and solvent (DMF) control   |
| Duration:                    | 48 hours  |
| Test conditions:             | Flow-through test.<br>Temperature: 18-22°C<br>Dissolved oxygen: >60% of saturation<br>pH: 7.8 - 8.3<br>Photoperiod: 16 h light : 8 h dark<br>Light intensity: 30-50 footcandles |
| Observations:                | Number of immobilised daphnids were monitored after 24 and 48 hours.  |
| Chemical analysis:           | At start and end of exposure, by means of LSC.  |
| Data analysis:               | Moving angle and probit analysis.   |

#### Results:

Mean measured test concentrations were 4.2, 5.3, 9.5, 15 and 24 mg/L, or 100 – 135% of the nominal values. The results were based on mean measured values.

**Table 129: Concentration of <sup>14</sup>C-Thiophanate-methyl measured in replicate test solutions during the 48 hour flow-through exposure of daphnids (*Daphnia magna*)**

| Nominal Concentration<br>mg a.i./L | Measured Concentration after [mg a.i./ L] |       |         |       |         |
|------------------------------------|---|-------|---------|-------|---------|
|                                    | 0 hour                                    |       | 48 hour |       | geomean |
|                                    | A   | B     | A       | B     | (SD)    |
| Control                            | <0.29                                     | <0.29 | <0.29   | <0.29 |         |
| Solvent control                    | <0.29                                     | <0.29 | <0.29   | <0.29 |         |
| 3.1                                | 4.2                                       | 4.3   | 4.1     | 4.1   | 4.2     |
| 5.2                                | 5.4                                       | 5.4   | 5.1     | 5.2   | 5.3     |
| 8.6                                | 9.6                                       | 10    | 9.1     | 9.4   | 9.5     |
| 14                                 | 16  | 15    | 14      | 15    | 15      |
| 24                                 | 23  | 24    | 24      | 23    | 24      |

The cumulative percent immobilisation is summarised in the table below.

**Table 130: Cumulative percent of immobilised *Daphnia magna* exposed to Thiophanate-methyl**

| Mean Measured Cone. of Thiophanate-methyl [mg/1] | Cumulative percent immobilised organisms |    |      |          |     |      |
|--|--|----|------|----------|-----|------|
|  | 24 hours                                 |    |      | 48 hours |     |      |
|  | A  | B  | Mean | A        | B   | Mean |
| 24   | 60                                       | 60 | 60   | 100      | 100 | 100  |
| 15   | 80                                       | 40 | 60   | 100      | 90  | 95   |
| 9.5  | 40                                       | 60 | 50   | 100      | 100 | 100  |
| 5.3  | 20                                       | 0  | 10   | 60       | 40  | 50   |
| 4.2  | 0  | 0  | 0    | 30       | 10  | 20   |
| Solvent Control                                  | 0  | 0  | 0    | 0        | 0   | 0    |
| Control  | 0  | 0  | 0    | 0        | 0   | 0    |

In addition, sublethal effects (e.g. lethargy) were observed among all of the mobile daphnids exposed to the 15, 5.3 and 4.2 mg/L test concentrations. The 24 and 48 hour EC<sub>50</sub> values were 14 (95% CL 11-20) and 5.4 (95% CL 4.4-6.3) mg/L, respectively. NOEC was <4.2 mg/L, due to the observed sublethal effects (lethargy) at all treatment levels.

**RMS comments:**

The study was re-evaluated against the validity criteria of OECD 202:

– less than 10% immobilization in the control

– dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled and the study is considered as still valid for risk assessment.

|                              |   |
|------------------------------|---|
| <b>Reference:</b>            | <b>Elendt-Schneider (1991):</b> Determination of the acute toxicity of BAS 325 10 F on the water flea <i>Daphnia magna</i> STRAUS   |
| Report number:               | 1/90/1119/50/1; 1991/10277; RD-00035 (822-001)  |
| Guideline:                   | OECD TG 202 (1984)  |
| GLP:                         | GLP, unpublished  |
| <b>Previous evaluation:</b>  | In DAR 1997.  |
| <b>Material and methods:</b> |   |
| Test material:               | BAS 325 10 F, 500 g/L Thiophanate-methyl  |
| Lot/Batch:                   | 90-1  |
| Test species:                | Water flea ( <i>Daphnia magna</i> STRAUS), neonates at test initiation 2 to 24 hours old  |
| Test design:                 | Static system (48 hours), 7 treatment groups (6 test item rates, control), 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.  |
| Treatments:                  | Control, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg formulation/L (nominal).   |
| Replication:                 | 5 animals per vessel, 20 per test concentration.  |
| Test conditions:             | Dilution water "M4" Elendt medium; test volume 10 mL, temperature 20-21°C; pH 7.8 - 8.1; oxygen content: 8.4 mg/L - 9.5 mg/L; total hardness: 2.2-3.2 mmol/L at test initiation; photoperiod: 16 h light : 8 h dark, light intensity: 5 - 6 µE/(m <sup>2</sup> *s); no feeding, no ventilation. |
| Chemical analysis:           | The test item concentrations were analyzed using reversed-phase HPLC with UV-detection.   |
| Data analysis:               | Descriptive statistics, moving average method for determination of the EC <sub>50</sub> values.   |

**Results:**

Mean measured concentrations were in the range 42.3 – 97.5% of the nominal values. Mean measured concentrations and the biological results are given in the table below.

**Table 131: Results from acute toxicity study with *Daphnia magna***

| Nominal test concentration (mg/L) | Mean measured test concentration (mg/L) | Number of live daphnids 24 hours (%) | Number of live daphnids 48 hours (%) |
|-----------------------------------|---|--------------------------------------|--------------------------------------|
| Control                           | Not detectable                          | 20                                   | 20                                   |
| 0.39                              | Not analysed                            | 20                                   | 20                                   |
| 0.78                              | 0.48 (61.5%)                            | 20                                   | 20                                   |

| Nominal test concentration (mg/L) | Mean measured test concentration (mg/L) | Number of live daphnids 24 hours (%) | Number of live daphnids 48 hours (%) |
|-----------------------------------|---|--------------------------------------|--------------------------------------|
| 1.56                              | Not analysed                            | 20                                   | 20                                   |
| 3.13                              | Not analysed                            | 20                                   | 20                                   |
| 6.25                              | Not analysed                            | 20                                   | 19                                   |
| 12.5                              | 10.1 (80.8%)                            | 18                                   | 6                                    |
| 25                                | Not analysed                            | 16                                   | 3                                    |
| 50                                | Not analysed                            | 16                                   | 3                                    |
| 100                               | 95.3 (95.3%)                            | 14                                   | 0                                    |

The 48 hour EC<sub>50</sub> value was 11.0 (95% CL 9.3-12.1) mg/L, corresponding to 5.5 (4.7 – 6.1) mg a.s./L. The 48 hour NOEC was set to 3.13 mg/L (corresponding to 1.6 mg a.s./L) due to the observed effects at all higher treatment levels.

#### RMS comments:

The study was re-evaluated against the validity criteria of OECD 202 (2004):

- < 10% immobilization in the control
- dissolved oxygen concentration > 60% of saturation

These validity criteria were fulfilled and the study is considered as still valid for risk assessment.

Measured test concentrations were below 80% of the nominal values within the test period. Therefore the results should be based on mean measured values. Since only three treatment levels were analysed, it is however not possible to re-calculate the EC<sub>50</sub> value. Instead, it is proposed that the mean measured at the level closest to the 50% effect level is used for estimation of a mean measured EC<sub>50</sub>, ie. 80.8% at 12.5 mg formulation/L. Hence, the 48 hour EC<sub>50</sub> is 8.9 mg formulation/L (80.8% of 11.0 mg /L), corresponding to ca 4.4 mg a.s./L.

**Author:** Fischer, R.  
**Title:** The Effect of Carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Daphnia magna* (waterflea) in a static-acute toxicity  
**Date:** 04 Aug 1988  
**Doc ID:** A39285; Report no: OEK88/091E; BVL No. 1758297  
**Guidelines:** OECD 202  
**GLP:** Yes  
**Validity:** Yes

Aim was to assess the effect of carbendazim - substance, technical (identification code: Hoe 017411 OF ZD99 0010) to *Daphnia magna* (water flea) in a static-acute toxicity test (method OECD).

#### Methods

Test material: carbendazim; substance, technical; Code: Hoe 017411 OF ZD99 0010

Specification: Analytical certificate no. AZ 03819, dated 25 March 1988; batch 3/86; purity 99.4 %, solid, white crystalline powder. Stability of the test substance was shown by chemical analysis of test medium. Dosing vehicle was 37 % HCl or acetone.

Guideline: OECD 202 (1984)

Animals: *Daphnia magna* was bred by the testing laboratory. Number of animals in the test: 2 x 10 animals per tested concentration (6 x 10 in the range of 0.001 to 10 mg/L) and control. Age: max. 24 hours; maintenance conditions: 300 ml glass jars filled with 200 ml medium; 20° C ±1° C; photoperiod 16 h light.

Test medium: 70 % filtered (sand and activated charcoal) and 30 % deionised water

Test concentrations:

25 concentrations between 0.0010 mg/L and 1000 mg/L

Stock solution (in HCl or acetone) added to the test medium; at 180 to 1000 mg/L, the test substance was directly added to the medium.

Duration of exposure/recording: 48 hours

Measured/Observed parameters: Immobilization: 24 and 48 hours

Chemical analysis of test substance: 0 and 48 hours, analytical monitoring: HPLC

### Results

Due to varying results when acetone was used as a solvent and a recovery of > 80 % of nominal concentration when HCl was taken, all concentrations refer to nominal values. Data on immobilization and the calculated EC<sub>50</sub> values are given in the tables below.

In the test systems with Acetone as solvent, the NOEC ranged from 0.032 (test system C) to 0.0056 (test system B). However, in these experiments the substance recovery ranged between 16 and 3247 %.(vierstellige %-Zahl ?)

Results of test system A (HCl as solvent) have to be regarded as invalid, since the control vessel showed a contamination with the tested substance when analyzed, even if no immobilization of the daphnids was observed. Report, page 14 '... In spite of all analytical ambiguities, the biological responses were quite comparable'.

In the test systems C with HCl as solvent, the NOEC for *Daphnia magna* exposed for 48 h to carbendazim was 0.010 mg/L.

**Table 132: Immobilization of *Daphnia magna* when exposed to carbendazim in an acute test**

| Concentration<br>(mg/L) | Immobilization (%)     |      |                      |      |                      |      |                        |      |
|-------------------------|------------------------|------|----------------------|------|----------------------|------|------------------------|------|
|                         | Test system A<br>(HCL) |      | Test system B<br>(A) |      | Test system C<br>(A) |      | Test system C<br>(HCL) |      |
|                         | 24 h                   | 48 h | 24 h                 | 48 h | 24 h                 | 48 h | 24 h                   | 48 h |
| control                 | 0                      | 0    | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| HCl control             | 0                      | 0    | -                    | -    | -                    | -    | 0                      | 0    |
| Acetone control         | -                      | -    | 0                    | 0    | 0                    | 0    | -                      | -    |
| 0.0010                  | -                      | -    | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| 0.0018                  | -                      | -    | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| 0.0032                  | -                      | -    | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| 0.0056                  | -                      | -    | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| 0.010                   | -                      | -    | 0                    | 5    | 0                    | 0    | 0                      | 0    |
| 0.018                   | -                      | -    | 0                    | 5    | 0                    | 0    | 0                      | 5    |
| 0.032                   | -                      | -    | 0                    | 10   | 0                    | 0    | 5                      | 5    |
| 0.056                   | -                      | -    | 0                    | 0    | 5                    | 5    | 0                      | 0    |
| 0.10                    | 0                      | 35   | 0                    | 0    | 0                    | 0    | 0                      | 0    |
| 0.18                    | 0                      | 90   | 15                   | 40   | 65                   | 100  | 20                     | 80   |
| 0.32                    | 10                     | 95   | 45                   | 70   | 70                   | 100  | 35                     | 100  |
| 0.56                    | 35                     | 100  | 65                   | 100  | 70                   | 100  | 50                     | 100  |
| 1.0                     | 30                     | 100  | 95                   | 100  | 100                  | 100  | 35                     | 100  |
| 1.8                     | 25                     | 100  | 95                   | 100  | 95                   | 100  | 85                     | 100  |
| 3.2                     | 55                     | 100  | 100                  | 100  | 80                   | 100  | 90                     | 100  |
| 5.6                     | 90                     | 100  | 100                  | 100  | 95                   | 100  | 85                     | 100  |
| 10                      | 85                     | 100  | 100                  | 100  | 100                  | 100  | 90                     | 100  |
| 18                      | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 32                      | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 56                      | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 100                     | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 180                     | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 320                     | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 560                     | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |
| 1000                    | 100                    | 100  | -                    | -    | -                    | -    | -                      | -    |

HCl = Hydrochloric acid min. 37 % as solvent, A = acetone as solvent

**Table 133: EC50 values for *Daphnia magna* following exposure to carbendazim**

| Endpoint         | Value (mg/L) | 95 % confidence interval (mg/L) | time | solvent | ai | n/r/i |
|------------------|--------------|---------------------------------|------|---------|----|-------|
| EC <sub>50</sub> | 0.15         | 0.10 - 0.18                     | 2 d  | HCl     |    | n     |
| EC <sub>50</sub> | 0.80         | 0.59 - 1.12                     | 1 d  | HCl     |    | n     |
| EC <sub>50</sub> | 0.13         | 0.10 - 0.18                     | 2 d  | Acetone |    | n     |
| EC <sub>50</sub> | 0.16         | 0.10 - 1.0                      | 1 d  | Acetone |    | n     |

n = nominal; r = real; i = initial

### Conclusion

Report results listed and evaluated in the carbendazim monograph of 13.11.1997. For *Daphnia magna* exposed to carbendazim in a static test system, the EC<sub>50</sub> (48 h) was 0.15 mg/L.

|                |   |
|----------------|---|
| Reference:     | <b>Fujikake, N. (2012):</b> 4-OH-TM: Acute immobilization Study in <i>Daphnia magna</i> |
| Report number: | RD-02410. NCAS 11-179 (822-007)   |
| Guideline:     | OECD 202 (1984)   |
| GLP:           | Yes   |

**Previous evaluation:** No, submitted for the purpose of renewal.

### Material and methods:

Test material: 4-OH-TM  
 Lot/Batch No: 31-5267-DT  
 Purity: 99.2%  
 Species: *Daphnia magna*, less than 24 hours old.  
 Treatments: Control, 25, 50 and 100 mg/L (nominal)  
 Controls: Elendt M4 medium  
 Duration: 48 hours  
 Test conditions: Static test.  
 Temperature: 20 ± 1°C  
 Dissolved oxygen: 7.5 – 7.9 mg/L (87 - 89%)  
 pH: 7.8 – 8.0  
 Photoperiod: 16 h light : 8 h dark  
 Light intensity: 520 – 880 lux

Observations: The number of immobilised *D. magna* in each replicate test vessel was recorded at 24 and 48 h of exposure. Immobilisation was defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made

and recorded at 0, 24 and 48 h.

Chemical analysis: The dissolved test substance concentrations (dissolved fluid concentrations) and the dissolved and non-dissolved but suspended test substance concentrations (suspended fluid concentrations) were measured with HPLC at test initiation and end.

Data analysis: Since the concentrations of 4-OH-TM tested did not produce  $\geq 50$  % immobilisation, the  $EC_{50}$  was empirically estimated to be greater than the mean measured concentration for the highest treatment level tested.

## Results:

### Analytical results

The test medium was stained slightly milky white or milky white in a concentration-dependent manner at the test initiation in all test item treatment groups. The test medium became clear and colourless in all test item treatment groups at the end of the exposure period. White precipitates were observed in all of the exposure groups after 24 and 48 hours of exposure.

**Table 134: Measured concentrations of 4-OH-TM in the exposure solutions during the 48 h exposure of *Daphnia magna***

| Nominal concentration [mg/L] | Measured concentration [mg/L] |       |      |              |
|------------------------------|-------------------------------|-------|------|--------------|
|                              | 0 h                           | 48 h  | Mean | % of nominal |
| Suspended fluid              |                               |       |      |              |
| 0                            | < LOQ                         | < LOQ | n.a. | n.a.         |
| 25                           | 20.9                          | 12.1  | 16.1 | 64.4         |
| 50                           | 38.6                          | 15.8  | 25.5 | 51.1         |
| 100                          | 50.4                          | 17.5  | 31.1 | 31.1         |
| Dissolved fluid              |                               |       |      |              |
| 0                            | < LOQ                         | < LOQ | n.a. | n.a.         |
| 25                           | 13.6                          | 11.7  | 12.6 | 50.5         |
| 50                           | 16.3                          | 14.6  | 15.4 | 30.9         |
| 100                          | 18.7                          | 16.6  | 17.6 | 17.6         |

LOQ = limit of quantification. i.e. 0.1 mg/L

n.a. = not applicable

The suspended fluid concentrations of the test substance were 50.4 – 83.6 % of the nominal concentrations at test initiation and 17.5 – 48.4 % at the end of the test. The mean concentrations during the exposure period were 16.1, 25.5 and 31.1 mg/L in the 25, 50 and 100 mg/L test item treatment groups, respectively. The dissolved fluid concentrations of the test substance were 13.6, 16.3 and 18.7 mg/L at test initiation and 11.7, 14.6 and 16.6 mg/L at the end of the test in the 25, 50 and 100 mg/L test item treatment groups, respectively.

All chemical and physical parameters (dissolved oxygen concentration, pH. and temperature) in the definitive test were within expected ranges.

*Biological results*

In the latest test with the reference substance, the EC<sub>50</sub> value determined in three tests was 0.66 – 1.3 mg/L.

The rate of immobilisation was 45 % in the 100 mg/L test item treatment group after 48 hours of exposure. Thus, the EC<sub>50</sub> was estimated to be > 31.1 mg/L based on suspended fluid (mean measured concentration) and > 17.6 mg/L based on dissolved fluid (mean measured concentration).

**Table 135: Immobilisation of *Daphnia magna* exposed to 4-OH-TM for 48 h in a static test**

| Nominal concentration [mg/L] | Cumulative number of immobilised daphnids |                |
|------------------------------|---|----------------|
|                              | After 24 hours                            | After 48 hours |
| 0                            | 0   | 0              |
| 25                           | 0   | 0              |
| 50                           | 0   | 4              |
| 100                          | 0   | 9              |

Since the concentrations tested did not result in  $\geq 50$  % immobilization, the 48 h EC<sub>50</sub> value for *D. magna* exposed to 4-OH-TM was empirically estimated to be greater than the highest test concentration (> 31.1 mg/L. mean measured concentration when based on suspended fluid and > 17.6 mg/L, mean measured concentration when based on dissolved fluid).

**RMS comments:**

The study was well performed and reported. The study was evaluated against the validity criteria of OECD 202:

- less than 10% immobilization in the control
- dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled.

The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. It can be assumed that undissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the geomean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC<sub>50</sub> of >17.6 mg/L.

|                             |   |
|-----------------------------|---|
| Reference:                  | <b>Fujikake. N. (2012):</b> CM-0237: Acute immobilization Study in <i>Daphnia magna</i> |
| Report number:              | RD-02409. NCAS 11-178 (822-006)   |
| Guideline:                  | OECD TG 202 (1984)  |
| GLP:                        | Yes   |
| <b>Previous evaluation:</b> | No, submitted for the purpose of renewal.   |

**Material and methods:**

Test material: CM-0237 (Thiophanate-methyl metabolite)

Lot/Batch No: 31-02122-I.TAKEUCHI

Purity: 98.1 %

Species: Water flea (*Daphnia magna*), < 24 hours old

Treatments: 0 (control), 25, 50 and 100 mg/L (nominal)

Controls: Elendt M4 medium

Replication: Four replicate test vessels with five individuals each

Duration: 48 hours

Test conditions: Static test design  
 Temperature: 20 ± 1°C  
 Dissolved oxygen: 7.3 – 7.9 mg/L (83-90% of saturation)  
 pH: 7.8 – 8.0  
 Photoperiod: 16 h light : 8 h dark  
 Light intensity 500 – 900 lux

Observations: The number of immobilised *D. magna* in each replicate test vessel was recorded at 24 and 48 h of exposure. Immobilisation was defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 h.

Chemical analysis: The dissolved test substance concentrations (dissolved fluid concentrations) and the dissolved and non-dissolved but suspended test substance concentrations (suspended fluid concentrations) were measured with HPLC at test initiation and end.

Data analysis: Since the concentrations of CM-0237 tested did not produce ≥ 50 % immobilisation, the EC<sub>50</sub> was empirically estimated to be greater than the mean measured concentration for the highest treatment level tested.

**Results:**

*Analytical results*

The test medium was stained milky white in a concentration-dependent manner at the test initiation in all test item treatment groups. The test medium became clear and colourless in the 50 and 100 mg/L test item treatment groups at the end of the exposure period but a slightly milky white colour was still observed in the 25 mg/L test item treatment group. Precipitates were observed in all of the exposure groups after 24 and 48 hours of exposure in a concentration-dependent manner.

**Table 136: Measured concentrations of CM-0237 in the exposure solutions during the 48 h exposure of *Daphnia magna***

| Nominal concentration [mg/L] | Measured concentration [mg/L] |      |      |              |
|------------------------------|-------------------------------|------|------|--------------|
|                              | 0 h                           | 48 h | Mean | % of nominal |
| Suspended fluid              |                               |      |      |              |

| Nominal concentration [mg/L] | Measured concentration [mg/L] |       |       |              |
|------------------------------|-------------------------------|-------|-------|--------------|
|                              | 0 h                           | 48 h  | Mean  | % of nominal |
| 0                            | < LOQ                         | < LOQ | n.a.  | n.a.         |
| 25                           | 20.8                          | 5.18  | 11.2  | 44.9         |
| 50                           | 45.6                          | 1.83  | 13.6  | 27.2         |
| 100                          | 85.0                          | 1.68  | 21.2  | 21.2         |
| Dissolved fluid              |                               |       |       |              |
| 0                            | < LOQ                         | < LOQ | n.a.  | n.a.         |
| 25                           | 0.304                         | 0.242 | 0.272 | 1.09         |
| 50                           | 0.289                         | 0.222 | 0.254 | 0.508        |
| 100                          | 0.296                         | 0.219 | 0.256 | 0.256        |

LOQ = limit of quantification. i.e. 0.1 mg/L

n.a. = not applicable

#### Biological results

In the latest test with the reference substance, the EC<sub>50</sub> value determined in three tests was 0.66 – 1.3 mg/L.

**Table 137: Immobilisation of *Daphnia magna* exposed to CM-0237 for 48 h in a static test**

| Nominal concentration [mg/L] | Cumulative number of immobilised daphnids |                |
|------------------------------|---|----------------|
|                              | After 24 hours                            | After 48 hours |
| 0                            | 0   | 0              |
| 25                           | 0   | 5              |
| 50                           | 0   | 5              |
| 100                          | 0   | 9              |

The rate of immobilisation was 45 % in the 100 mg/L test item treatment group after 48 hours of exposure. Thus, the EC<sub>50</sub> was estimated to be > 21.2 mg/L based on suspended fluid (mean measured concentration) and >0.256 mg/L based on dissolved fluid (mean measured concentration).

#### RMS comments:

The study was well performed and reported. The study was evaluated against the validity criteria of OECD 202:

- less than 10% immobilization in the control
- dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled.

The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. The dissolved concentrations

were practically the same at all treatment levels. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore the RMS propose that the geometric mean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC<sub>50</sub> of >0.256 mg/L.

|                              |  |
|------------------------------|--|
| Reference:                   | <b>Kley, A., Wydra. V. (2012):</b> Acute Toxicity of 4-OH-TM to Larvae of <i>Chironomus riparius</i> in a Static 48-hour Immobilisation Test                               |
| Report number:               | RD-02402. 67762251 (824-001)   |
| Guideline:                   | OECD TG 235 (2011)   |
| GLP:                         | Yes  |
| <b>Previous evaluation:</b>  | No, submitted for the purpose of renewal.  |
| <b>Material and methods:</b> |  |
| Test material:               | 4-OH-TM  |
| Lot/Batch No:                | 31-10193-N.UMEDA   |
| Purity:                      | 95.3%  |
| Species:                     | Larvae of <i>Chironomus riparius</i> , 2 days old.   |
| Treatments:                  | 0.51, 1.9, 7.1, 27 and 100 mg/L (nominal).   |
| Controls:                    | Reconstituted water (Elendt M4)  |
| Replication:                 | 20 larvae per treatment level and control, divided into 4 groups of 5 animals.   |
| Duration:                    | 48 hours   |
| Test conditions:             | Static test design.<br>Temperature: 19 – 20°C<br>Dissolved oxygen: 8.3 – 8.9 mg/L<br>pH: 7.7 – 7.9<br>Photoperiod: 16 h light : 8 h dark<br>Light intensity: 580 – 850 lux |
| Observations:                | Number of immobile organisms were observed after 24 and 48 hours.  |
| Chemical analysis:           | At start and end of exposure period by HPLC-UV method. LOQ was 0.5 mg/L. Filtrated and non-filtrated samples were analysed.  |
| Data analysis:               | Due to the low effect. EC <sub>50</sub> , EC <sub>20</sub> and EC <sub>10</sub> could not be calculated. NOEC and LOEC determined directly from raw data.                  |

**Results:**

*Analytical results*

At the start of the test, a mean of 93 % of the nominal test concentrations was found in the non-filtrated samples. After 48 hours a mean of 64 % of the nominal value was determined. These results were caused by the settlement of the test item during the test in the test media of 27 and 100 mg/L.

In the filtrated samples, a mean of 40 % of the nominal test concentrations was found in the filtrated samples. After 48 hours a mean of 55 % of the nominal value was determined.

**Table 138: Measured concentrations of 4-OH-TM in the exposure solutions during the 48 h exposure of *Chironomus riparius***

| Nominal concentration [mg/L] | Measured concentration [mg/L] |       |                   |              |
|------------------------------|-------------------------------|-------|-------------------|--------------|
|                              | 0 h                           | 48 h  | Mean <sup>1</sup> | % of nominal |
| Non-filtrated samples        |                               |       |                   |              |
| 0                            | < LOQ                         | < LOQ | n.a.              | n.a.         |
| 0.51                         | 0.483                         | 0.389 | 0.436             | 86           |
| 1.9                          | 1.70                          | 1.51  | 1.61              | 85           |
| 7.1                          | 6.59                          | 5.70  | 6.14              | 87           |
| 27                           | 25.5                          | 12.9  | 19.2              | 71           |
| 100                          | 92.8                          | 48.6  | 70.7              | 71           |
| Filtrated samples            |                               |       |                   |              |
| 0                            | < LOQ                         | < LOQ | n.a.              | n.a.         |
| 0.51                         | 0.308                         | 0.387 | 0.347             | 68           |
| 1.9                          | 1.04                          | 1.54  | 1.29              | 68           |
| 7.1                          | 3.66                          | 5.62  | 4.64              | 65           |
| 27                           | 10.4                          | 12.0  | 11.2              | 41           |
| 100                          | 15.3                          | 14.0  | 14.6              | 15           |

LOQ = limit of quantification. i.e. 0.009 mg/L

n.a. = not applicable

<sup>1</sup> mean value of all replicate measurements after 0 and 48 h

All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within expected ranges.

*Biological results*

After 48 hours of exposure, no immobilisation was observed in the control and in the test item treatment groups up to and including 7.1 mg/L (nominal concentration). In the test item treatment groups of nominal 27 and 100 mg/L, an immobilisation of 5 and 10 %, respectively, was observed after 48 hours of exposure.

**Table 139: Immobilisation of *Chironomus riparius* exposed to 4-OH-TM for 48 h in a static test**

| Mean measured concentration [mg/L] | Cumulative number (%) of immobilised larvae |                |
|------------------------------------|---|----------------|
|                                    | After 24 hours                              | After 48 hours |
|                                    |   |                |

| Mean measured concentration [mg/L] | Cumulative number (%) of immobilised larvae |                |
|------------------------------------|---|----------------|
|                                    | After 24 hours                              | After 48 hours |
| 0                                  | 0 (0)                                       | 0 (0)          |
| 0.439                              | 0 (0)                                       | 0 (0)          |
| 1.62                               | 0 (0)                                       | 0 (0)          |
| 6.18                               | 0 (0)                                       | 0 (0)          |
| 19.2                               | 0 (0)                                       | 1 (5)          |
| 71                                 | 0 (0)                                       | 2 (10)         |

Since the concentrations tested did not result in  $\geq 50\%$  immobilization, the 48 h EC<sub>50</sub> value for *Chironomus riparius* exposed to 4-OH-TM was empirically estimated to be greater than the highest test concentration ( $> 71$  mg/L, mean measured concentration when based on non-filtrated samples).

#### RMS comments:

The study was evaluated against the validity criteria of OECD 235:

-Less than 15% immobilisation or other signs of toxicity in the control at the end of the test.

-Dissolved oxygen concentration at the end of the test should be  $\geq 3$  mg/L in control and test vessels.

Both these criteria were met and the study is considered as valid. The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the mean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC<sub>50</sub> of  $>14.6$  mg/L.

#### 11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

|                             |   |
|-----------------------------|---|
| Reference:                  | <b>Saito, S. (2002):</b> Thiophanate-methyl - Inhibition of growth to the alga <i>Pseudokirchneriella subcapitata</i> |
| Report number:              | NCAS 02-160. RD-II 02438 (823-003)  |
| Guideline:                  | OECD TG 201 (1984)  |
| GLP:                        | Yes   |
| <b>Previous evaluation:</b> | In Addendum 7 to the DAR, 2003  |

#### Material and methods:

|                |   |
|----------------|---|
| Test material: | Thiophanate-methyl technical  |
| Lot/Batch No:  | TBC- 035G   |
| Purity:        | 98.2%   |
| Species:       | The green alga <i>Pseudokirchneriella subcapitata</i> , initial cell volume of 10000 cells/mL |
| Test media:    | OECD medium, pH 7.8-9.8   |

## CLH REPORT FOR THIOPHANATE-METHYL

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|                    |   |
|--------------------|---|
| Treatments:        | 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg/L (nominal)   |
| Controls:          | Untreated and solvent control (acetone)   |
| Replication:       | Three test vessels containing a volume of 100 ml of water were used per concentration, control and solvent control. |
| Duration:          | 96 hours  |
| Test conditions:   | Temperature ca 24°C, fluorescent light of 4000-4500 lux continuous illumination                                     |
| Observations:      | Cells concentrations in each solution were determined at 0, 24, 48, 72 and 96 hours of exposure.                    |
| Chemical analyses: | The concentrations of Thiophanate-methyl in the test solutions was determined at test start and end.                |
| Data analysis:     | Probit analysis for EC <sub>50</sub> and Dunnet's test for NOEC.  |

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### Results:

The mean measured concentrations of dissolved Thiophanate-methyl ranged from 25 to 73 % of the nominal values.

**Table 140: Summary of measured dissolved concentrations of Thiophanate-methyl in the test solutions.**

| Nominal test conc. (mg/L) | 0 hours | 96 hours | Mean (geomean) | % of nominal |
|---------------------------|---------|----------|----------------|--------------|
| 1.56                      | 1.38    | 0.386    | 0.780 (0.730)  | 50 (47)      |
| 3.13                      | 2.78    | 1.33     | 1.97 (1.92)    | 63 (61)      |
| 6.25                      | 4.93    | 3.87     | 4.38 (4.37)    | 70 (70)      |
| 12.5                      | 10.4    | 7.95     | 9.12 (9.09)    | 73 (73)      |
| 25                        | 14.6    | 13.7     | 14.1 (14.1)    | 56 (56)      |
| 50                        | 21.5    | 19.9     | 20.7 (20.7)    | 41 (41)      |
| 125                       | 24.8    | 26.0     | 25.4 (25.4)    | 25 (25)      |

Based on the arithmetic mean measured test concentrations, it was concluded that the E<sub>b</sub>C<sub>50</sub> values at 72 and 96 hours were 12 (95% CL 10-13) and 13 (95% CL 12-15) mg/L, respectively, and E<sub>r</sub>C<sub>50</sub> values at 24-48, 24-72 and 24-96 hours were >25.4 mg/L. NOEC was determined to be 4.38 for biomass and 9.12 mg/L after 72 hours.

### RMS comments:

The study was conducted according to the OECD TG 201 from 1984. In 2006 and 2011, the OECD TG 201 was revised, including additional validity criteria and the additional endpoint of yield. Therefore, the study was re-evaluated by Wirzinger (2015, see below) in order to see whether the study is still valid and complies with current guideline requirements with regard to other parameters (e.g. test conditions, cell number and pH values).

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Reference: **Wirzinger, G. (2015):** Effects of Thiophanate-methyl on the growth of green algae (*Pseudokirchneriella subcapitata*) - Re-calculation of toxicity

## CLH REPORT FOR THIOPHANATE-METHYL

|                             |   |
|-----------------------------|---|
|                             | endpoints of the algal growth study by Saito (2002. Doc. No. 823-003) |
| Report number:              | RD-03363. PP146-00061 (882-007)                                       |
| Guideline:                  | OECD TG 201 (2011)  |
| GLP:                        | No  |
| <b>Previous evaluation:</b> | No, submitted for the purpose of renewal.                             |

### Summary:

-Specific growth rate  $>0.92 \text{ day}^{-1}$ , or actual increase  $>16$  times in 72 hours. Actual increase was 92.3 times.

-Mean coefficient of variation for section-by-section specific growth rates in the control cultures  $< 35\%$ . Actual value was reported to be 33.9%.

-Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures  $< 7\%$  (for *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*). Actual value was reported to be 1.8%.

According to the current guideline, the pH of the control should not increase by more than 1.5 units during the test. In the study, the pH increased by more than 1.5 units at the end of the test in both controls and in the test item treatment groups up to and including 9.12 mg a.s./L (actual increase 1.6 to 1.9 units). According to the study director, this was due to the photosynthesis by the algae and had no impact on the assessment of the test substance.

Algal growth in terms of yield showed a decrease of more than 50 % compared to the solvent control at the three highest test item treatment groups after 72 hours (see table below).

**Table 141: Yield of *Pseudokirchneriella subcapitata* after exposure to Thiophanate-methyl**

| Mean measured concentration [mg a.s./L] | Yield [ $\times 10^4$ cells/mL] (percent inhibition) after |               |                |
|---|--|---------------|----------------|
|   | 24 h   | 48 h          | 72 h           |
| Solvent control <sup>1</sup>            | 2.78   | 24.8          | 90.9           |
| 0.780                                   | 2.75 (1.1) *   | 25.6 (-3.4) * | 96.5 (-6.2)    |
| 1.97                                    | 2.16 (22.2) *  | 21.3 (13.8) * | 91.3 (-0.5)    |
| 4.38                                    | 1.68 (39.6) *  | 17.7 (28.4) * | 83.6 (8.0)     |
| 9.12                                    | 1.07 (61.6) *  | 11.3 (54.4) * | 49.9 (45.1) ** |
| 14.1                                    | 1.64 (41.1) *  | 11.1 (55.2) * | 35.4 (61.0) ** |
| 20.7                                    | -2.46 (189) *  | 3.57 (85.6) * | 27.5 (69.8) ** |
| 25.4                                    | 0.823 (70.4) *   | 5.79 (76.6) * | 25.1 (72.4) ** |

Values in parentheses represent percent inhibition compared to solvent control; negative values indicate a higher growth rate in treatment group compared to solvent control

<sup>1</sup> Inhibition of yield was compared to the solvent control as there were not statistically significant differences between the water control and the solvent control

\* statistically significantly different compared to solvent control ( $p \leq 0.05$ , Bonferroni-Holm U-test)

\*\* statistically significantly different compared to solvent control ( $p \leq 0.05$ , Williams t-test)

Algal growth results are summarised in the table below. After 72 hours, the maximum growth inhibition relative to the solvent control at the second highest test concentration was 42.6 %.

**Table 142: Growth rate of *Pseudokirchneriella subcapitata* after exposure to Thiophanate-methyl**

| Mean measured concentration [mg a.s./L] | Growth rate [ $\times 10^4$ cells/mL/d] (percent inhibition) after |                |                |
|---|--|----------------|----------------|
|   | 24 h   | 48 h           | 72 h           |
| Solvent control <sup>1</sup>            | 1.32   | 1.62           | 1.50           |
| 0.780                                   | 1.30 (1.6) *   | 1.63 (-0.5) *  | 1.52 (-0.9) *  |
| 1.97                                    | 1.11 (15.6) *  | 1.53 (5.6) *   | 1.49 (1.0) *   |
| 4.38                                    | 0.962 (27.1) *   | 1.45 (10.6) *  | 1.47 (2.4) *   |
| 9.12                                    | 0.684 (48.2) *   | 1.22 (24.7) *  | 1.28 (14.7) *  |
| 14.1                                    | 0.930 (29.5) *   | 1.21 (25.3) *  | 1.17 (21.9) *  |
| 20.7                                    | -0.151 (112) *   | 0.604 (62.7) * | 0.862 (42.6) * |
| 25.4                                    | 0.526 (60.1) *   | 0.888 (45.1) * | 1.03 (31.4) *  |

Values in parentheses represent percent inhibition compared to control; negative values indicate a higher growth rate in treatment group compared to control

<sup>1</sup> Inhibition of growth rate was compared to the solvent control as there were not statistically significant differences between the water control and the solvent control

\* statistically significantly different compared to control ( $p \leq 0.05$ , Bonferroni-Holm U-test)

The calculated toxicity endpoints for yield and growth rate are summarised in the table below. The  $E_yC_{10}$ ,  $E_yC_{20}$  and  $E_yC_{50}$  after 72 hours based on mean measured concentrations were 3.13, 4.93 and 11.8 mg a.s./L, respectively. The  $E_rC_{10}$ ,  $E_rC_{20}$  and  $E_rC_{50}$  after 72 hours based on mean measured concentrations were 6.74, 12.1 and 37.2 mg a.s./L, respectively. The NOEC and LOEC for growth rate could not be determined due to mathematical reasons. Based on yield, the NOEC and the LOEC were 4.38 and 9.12 mg a.s./L, respectively.

**Table 143: ECx, LOEC and NOEC values for growth rate of *Pseudokirchneriella subcapitata* after 72 hours of exposure to Thiophanate-methyl in a static test system**

| Growth Function                          | EC <sub>10</sub> [mg a.s./L] | EC <sub>20</sub> [mg a.s./L] | EC <sub>50</sub> [mg a.s./L] | LOEC [mg a.s./L] | NOEC [mg a.s./L] |
|--|------------------------------|------------------------------|------------------------------|------------------|------------------|
| <b>Toxicity endpoints after 72 hours</b> |                              |                              |                              |                  |                  |
| Yield                                    | 3.13 (1.89 – 4.28)           | 4.93 (3.43 – 6.22)           | 11.8 (10.1 – 13.6)           | 9.12             | 4.38             |
| Growth rate                              | 6.74 (1.00 – 10.5)           | 12.1 (5.14 – 16.1)           | 37.2 (25.6 – 169)            | n.d.             | n.d.             |

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

**RMS comments:**

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Based on the growth curves from the study, exponential growth in the control and solvent control was limited to the first 72 hours of the test. Therefore, the 72 hour values are considered most relevant for the further assessment. It was noted that the arithmetic mean values were used by the applicant in the re-analysis. In principle, the geometric mean are regarded as more appropriate, however in this case the values were similar. The RMS generally agree with the proposed re-evaluation but has made a check against the validity criteria beside that presented by the applicant.

**Table 144: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures. Control and solvent control were pooled in the calculations below.**

| Replicates | 0-72 h                                   |        |        |        | Section by section (day 0-1. 1-2. 2-3)   |        |      |         |             |
|------------|--|--------|--------|--------|--|--------|------|---------|-------------|
|            | Average growth rate (day <sup>-1</sup> ) | St Dev | CV     | CV (%) | Average growth rate (day <sup>-1</sup> ) | St Dev | CV   | Mean CV | Mean CV (%) |
| A          | 1.50                                     | 0.0256 | 0.0170 | 2      | 1.48                                     | 0.56   | 0.38 | 0.29    | 29          |
| B          |  |        |        |        | 1.54                                     | 0.45   | 0.29 |         |             |
| C          |  |        |        |        | 1.50                                     | 0.51   | 0.34 |         |             |
| D          |  |        |        |        | 1.53                                     | 0.36   | 0.24 |         |             |
| E          |  |        |        |        | 1.49                                     | 0.35   | 0.23 |         |             |
| F          |  |        |        |        | 1.48                                     | 0.38   | 0.25 |         |             |

In conclusion, the RMS could confirm that the study fulfilled the validity criteria and can be considered as useful for the risk assessment.

|                             |   |
|-----------------------------|---|
| <b>Reference:</b>           | <b>Kley, A., Wydra, V. (2012):</b> Toxicity of Thiophanate-methyl 500 SC to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test |
| Report number:              | RD-02400, 67723210 (823-007)  |
| Guideline:                  | OECD TG 201 (2006)  |
| GLP:                        | Yes   |
| <b>Previous evaluation:</b> | Submitted for the purpose of renewal.   |

### Material and methods:

|                  |   |
|------------------|---|
| Test material :  | Thiophanate-methyl 500 SC   |
| Lot/Batch #:     | 0151107   |
| Content of a.s.: | 500 g Thiophanate-methyl/L (nominal), 41.0 % w/w (analysed)               |
| Treatments:      | Nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg formulation/L. |
| Control:         | Reconstituted water (OECD medium)   |
| Species:         | <i>Pseudokirchneriella subcapitata</i> Strain 61.81 SAG                   |
| Source:          | In-house culture  |
| Test unit:       | 50 mL Erlenmeyer flasks with 50 mL test medium, 5000 algal cells/mL at    |

test start.

- Replication:** Three replicate flasks of the test concentrations and six replicates for the control were included in the test. Additionally, one replicate of each test concentration and of the control was prepared without algae to provide a blank for the spectrophotometrical measurements.
- Duration:** 72 hours
- Test conditions:** Static conditions  
 Temperature: 21 – 23°C  
 pH: 7.8 – 8.0 (test start); 8.1 – 8.6 (test end)  
 Photoperiod: continuous uniform illumination  
 Light intensity: 7820 lux (7370 – 8240 lux)
- Observations:** At each subsequent 24-hour interval, the cell densities were determined by spectrophotometrical measurements. One sample was removed from each flask for counting. Observations of the health of the algal cells and appearance of the test item in the test media were also made and recorded at each 24-hour interval.
- Chemical analysis:** The samples of the test media (containing algae) sampled at the start and at the end of the test after 72 hours of exposure were analysed with high performance liquid chromatography with ultraviolet detection (HPLC/UV).
- Data analysis:** The inhibition of algal growth was determined from yield and average specific growth rate. Based on the calculated cell densities, the 72-hour  $E_rC_{50}$  and  $E_yC_{50}$ , the corresponding  $EC_{10}$  values and where possible their 95 % confidence limits were calculated by Probit analysis. For the determination of the 72-hour LOEC and NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by the Williams Test, respectively.

**Results:**

*Analytical results*

At the start of the test, 96 % of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 74 % of the nominal value was determined (average of all test concentrations). Mean measured concentrations were in the range of 81.3 to 90.0 % of the nominal test concentrations, therefore all endpoints refer to nominal concentrations.

A slight turbidity caused by the test item was observed at the test start in the 32 mg/L test item treatment group. In the 100 mg/L test item treatment group, turbidity of the test medium was observed during the whole test.

**Table 145: Measured Thiophanate-methyl concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata* to Topsin M 500 SC**

| Nominal concentration [mg/L] | Thiophanate-methyl | Measured concentration <sup>1</sup> [mg a.s./L] |              |          |              | Geomean measured [mg a.s./L] | % of nominal |
|------------------------------|--------------------|---|--------------|----------|--------------|------------------------------|--------------|
|                              |                    | 0 hours   | % of nominal | 72 hours | % of nominal |                              |              |
| Thiophanate-methyl 500 SC    |                    |   |              |          |              |                              |              |

| Nominal concentration [mg/L] |                    | Measured concentration <sup>1</sup> [mg a.s./L] |              |                    |              |                              |              |
|------------------------------|--------------------|---|--------------|--------------------|--------------|------------------------------|--------------|
| Thiophanate-methyl 500 SC    | Thiophanate-methyl | 0 hours   | % of nominal | 72 hours           | % of nominal | Geomean measured [mg a.s./L] | % of nominal |
| 0 (control)                  | 0 (control)        | < LOD <sup>2</sup>                              | n.a.         | < LOD <sub>2</sub> | n.a.         | <LOD                         | n.a.         |
| 1.0                          | 0.410              | 0.394   | 96           | 0.273              | 66.5         | 0.328                        | 80.0         |
| 3.2                          | 1.31               | 1.24  | 95           | 0.900              | 68.5         | 1.06                         | 80.9         |
| 10                           | 4.10               | 3.84  | 93.5         | 2.84               | 69.5         | 3.30                         | 80.4         |
| 32                           | 13.1               | 13.7  | 104          | 9.96               | 76.0         | 11.7                         | 89.3         |
| 100                          | 41.0               | 36.5  | 89.0         | 35.8               | 87.5         | 36.1                         | 88.0         |

<sup>1</sup> Results represent rounded results calculated on the exact analytical data

<sup>2</sup> LOD = limit of detection (7 µg a.s./L) n.a. = not applicable

All chemical and physical parameters (dissolved oxygen concentration, pH, temperature) in the definitive test were within expected ranges.

*Biological results*

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 177); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.1 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.7 %).

Cell densities determined at each observation interval are presented in the table below. Cells appeared to be normal throughout the exposure period for the control and test item concentrations up to and including 32 mg/L. At a nominal concentration of 100 mg/L, the cells were smaller and showed a round shape compared to the cells of the control.

**Table 146: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC**

| Nominal concentration of Topsin M 500 SC [mg/L] | Mean Cell Concentration (x 10 <sup>4</sup> cells/mL) after |              |              |             |
|---|--|--------------|--------------|-------------|
|   | 0 h  | 24 h         | 48 h         | 72 h        |
| 0 (control)                                     | 0.5  | 2.94 ± 0.411 | 13.8 ± 1.11  | 88.5 ± 7.66 |
| 1.0   | 0.5  | 2.98 ± 0.424 | 13.1 ± 0.883 | 77.8 ± 6.18 |
| 3.2   | 0.5  | 2.41 ± 0.245 | 13.2 ± 2.36  | 77.2 ± 5.65 |
| 10  | 0.5  | 1.84 ± 0.122 | 14.8 ± 2.36  | 73.6 ± 7.37 |
| 32  | 0.5  | 1.14 ± 0.122 | 11.2 ± 0.490 | 50.5 ± 1.29 |

|     |     |              |             |             |
|-----|-----|--------------|-------------|-------------|
| 100 | 0.5 | 0.500 ± 0.00 | 8.98 ± 1.05 | 6.37 ± 4.29 |
|-----|-----|--------------|-------------|-------------|

Cell biomass, expressed as yield, is presented in the table below. Based on the results, Williams Test detected a significant reduction in yield in treatment levels  $\geq 1.0$  mg/L compared to the control data. Therefore, the 72-hour NOEC for yield was determined to be  $\leq 1.0$  mg a.s./L. The 72-hour  $E_yC_{50}$  value was determined to be 31.8 mg/L.

**Table 147: Mean cell biomass (expressed as yield) of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC**

| Nominal concentration of Topsin M 500 SC [mg/L] | Mean yield (x 10 <sup>4</sup> cells/mL) after |                |               |                             |
|---|---|----------------|---------------|-----------------------------|
|   | 24 h  | 48 h           | 72 h          | Inhibition [%] <sup>1</sup> |
| 0 (control)                                     | 2.44 ± 0.411                                  | 13.3 ± 1.11    | 88.0 ± 7.66   | -                           |
| 1.0   | 2.48 ± 0.424                                  | 12.6 ± 0.883   | 77.3 ± 6.18 * | 12.2                        |
| 3.2   | 1.91 ± 0.245 *                                | 12.7 ± 2.36    | 76.7 ± 5.65 * | 12.8                        |
| 10  | 1.34 ± 0.122 *                                | 14.3 ± 2.36    | 73.1 ± 7.37 * | 16.9                        |
| 32  | 0.638 ± 0.122 *                               | 10.7 ± 0.490 * | 50.0 ± 1.29 * | 43.3                        |
| 100   | 0.00 ± 0.00 *                                 | 8.48 ± 1.05 *  | 5.87 ± 4.29 * | 93.3                        |

<sup>1</sup> Percent inhibition after 72 hours compared to control.

\* Significantly reduced compared to the control, based on Williams Test,  $\alpha = 0.05$ , one-sided

Growth rates are presented in the table below. Based on the results of the Williams Test a significant reduction in average growth rate in treatment levels 32 mg/L compared to the control data was detected. Therefore, the 72-hour NOEC for average growth rate was determined to be 10 mg/L. The 72-hour  $E_rC_{50}$  value was determined to be 88.3 mg/L.

**Table 148: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC**

| Nominal concentration of Topsin M 500 SC [mg/L] | Growth rate (days <sup>-1</sup> ) after |                |                 |                             |
|---|---|----------------|-----------------|-----------------------------|
|   | 0 – 24 h                                | 0 – 48 h       | 0 – 72 h        | Inhibition [%] <sup>1</sup> |
| 0 (control)                                     | 1.76 ± 0.136                            | 1.66 ± 0.040   | 1.72 ± 0.029    | -                           |
| 1.0   | 1.78 ± 0.144                            | 1.63 ± 0.034   | 1.68 ± 0.027    | 2.5                         |
| 3.2   | 1.57 ± 0.105 *                          | 1.64 ± 0.052   | 1.68 ± 0.024    | 2.6                         |
| 10  | 1.30 ± 0.068 *                          | 1.69 ± 0.082   | 1.66 ± 0.034    | 3.6                         |
| 32  | 0.818 ± 0.105 *                         | 1.55 ± 0.022 * | 1.54 ± 0.009 *  | 10.8                        |
| 100   | 0.00 ± 0.00 *                           | 1.44 ± 0.057 * | 0.758 ± 0.342 * | 56.0                        |

<sup>1</sup> Percent inhibition after 72 hours compared to control.

\* Significantly reduced compared to the control, based on Williams Test,  $\alpha = 0.05$ , one-sided

The 72-hour  $E_yC_{10}$ ,  $E_yC_{20}$  and  $E_yC_{50}$  and NOEC values determined for this test as well as the 72-hour  $E_rC_{10}$ ,  $E_rC_{20}$  and  $E_rC_{50}$  and NOEC values are presented in the table below.

**Table 149: Toxicity endpoints for growth rate and yield of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC based on nominal test concentrations.**

| Parameter   | EC <sub>10</sub><br>[mg/L] | EC <sub>20</sub><br>[mg/L] | EC <sub>50</sub><br>[mg/L] | NOEC<br>[mg/L] |
|---|----------------------------|----------------------------|----------------------------|----------------|
| 0 – 72 h yield<br>(95 % confidence intervals)       | 8.12<br>(3.20 – 12.7)      | 13.0<br>(6.66 – 18.4)      | 31.8<br>(23.4 – 43.3)      | ≤ 1.0          |
| 0 – 72 h growth rate<br>(95 % confidence intervals) | 30.2<br>(13.8 – 42.4)      | 43.6<br>(25.7 – 56.0)      | 88.3<br>(72.2 – 112.6)     | 10             |

**RMS comments:**

The study was well performed and reported and the validity criteria of OECD TG 201 (2011) were met, except that since the measured test concentrations declined below 80%, the results should have been based on the measured values and not the nominal. The applicant (Wirzinger and Ruhnke, 2016; 882-008) has re-evaluated the endpoints based on the geometric mean measured concentrations of the test item, i.e. 0.328, 1.06, 3.30, 11.7 and 36.1 mg a.s./L. The results of the re-evaluation of the study using ToxRat (version 3.2.1) are presented in the table below.

**Table 150: Toxicity endpoints for effects on yield and growth rate of the *Pseudokirchneriella subcapitata* study (Kley & Wydra, 2012) after exposure to Thiophanate-methyl 500 SC**

| Parameter <sup>1</sup>                              | EC <sub>10</sub><br>[mg a.s./L] | EC <sub>20</sub><br>[mg a.s./L] | EC <sub>50</sub><br>[mg a.s./L] | NOEC<br>[mg a.s./L] |
|---|---------------------------------|---------------------------------|---------------------------------|---------------------|
| 0 – 72 h yield<br>(95 % confidence intervals)       | 2.7<br>(2.0 – 3.4)              | 4.4<br>(3.5 – 5.2)              | 11.2<br>(9.9 – 12.7)            | < 0.33              |
| 0 – 72 h growth rate<br>(95 % confidence intervals) | 10.5<br>(6.8 – 16.3)            | 14.6<br>(10.5 – 20.2)           | 27.3<br>(22.4 – 33.2)           | 3.3                 |

<sup>1</sup> EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> calculated with linear regression using probit analysis

The results indicate that the formulation (expressed per content of active substance) is of similar toxicity to algae as Thiophanate-methyl alone.

**Author:** Bell, G.  
**Title:** Caarbendazim techn. – Algal growth inhibition  
**Date:** 31 July 96  
**Doc ID:** SNG 44(a)/960463; BVL No. 1843621

**Guidelines:** OECD 201  
**GLP:** Yes  
**Validity:** Yes

Aim of the study was to assess the toxicity of carbendazim to the algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a static test.

**Methods**

Test substance: Carbendazim tech, 99.5% purity

Test concentrations:

0.46, 1.0, 2.2, 4.6 und 10 mg carbendazim/L

The solubility limit of carbendazim in the test medium was 10 mg/L. Carbendazim was applied directly to the medium then sonicated and stirred with magnetic bars for 24 h.

**Results**

The measured test concentrations were:

0.51, 1.1, 2.5, 4.9 und 11 mg carbendazim/L, respectively (recovery 106 – 113 %)

**Table 151: Toxicity of carbendazim to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)**

| Endpoint    | Value (mg/L) | 95 % confidence interval (mg/L) | Time (d) | ai | n/r/i |
|-------------|--------------|---------------------------------|----------|----|-------|
| NOECbiomass | 2.5          |                                 | 3        |    | r     |
| EbC50       | 7.7          | 6.6 – 8.9                       | 3        |    | r     |
| ErC50       | >11          |                                 | 5        |    | r     |

n = nominal; r = real; i = initial

**Conclusion**

Report results listed and evaluated in the Addendum 2 to the monograph (26.01.2000).

For *Pseudokirchneriella subcapitata* exposed to carbendazim in a static test, the NOEC (72 h) was 2.5 mg/L, the EbC<sub>50</sub> (72 h) = 7.7 mg/L and the ErC<sub>50</sub> >11 mg/L.

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|                |  |
|----------------|--|
| Reference:     | <b>Baba, K. (2012): 4-OH-TM:</b> Growth Inhibition Study in <i>Pseudokirchneriella subcapitata</i> |
| Report number: | RD-02399. NCAS 11-177 (823-006)  |
| Guideline:     | OECD TG 201 (2011)   |
| GLP:           | Yes.   |

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**Previous evaluation:** No. submitted for the purpose of renewal.

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**Material and methods:**

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|                    |  |
|--------------------|--|
| Test material:     | 4-OH-TM (Thiophanate-methyl metabolite)  |
| Lot/Batch No:      | 31-5267-DT   |
| Purity:            | 99.2%  |
| Species:           | The freshwater green alga <i>Pseudokirchneriella subcapitata</i> (Strain ATCC 22662), cell density 5000 cells per mL.  |
| Test media:        | OECD medium  |
| Treatments:        | 0.294, 0.960, 3.06, 9.80, 31.4 and 100 mg/L (nominal)  |
| Controls:          | Untreated  |
| Replication:       | Three replicate flasks of the test concentrations and six replicates for the control were included in the test.  |
| Duration:          | 72 hours   |
| Test conditions:   | Temperature: $23 \pm 2$ °C, pH 8.0 – 8.1, continuous uniform illumination.<br>Light intensity was 63 - 82 $\mu\text{E}/\text{m}^2/\text{s}$  |
| Observations:      | At each subsequent 24-hour interval, cell counts were conducted on the replicate vessels using an electronic particle counter or a microscope with a counting chamber. Observations of the health of the algal cells and on the appearance of the test media were also made and recorded at each 24-hour interval.   |
| Chemical analyses: | At test initiation (0 hour) and test termination (72 hours), a single sample was removed from each test concentration and the control and analysed for 4-OH-TM with HPLC. For the determination of 4-OH-TM in dissolved fluids, an aliquot of the test medium was collected and centrifuged. The supernatant fluid was filtrated, mixed with methanol in the same volume and analysed with HPLC.   |
| Data analysis:     | <p>The growth curves were prepared by plotting the time of measurement against the mean measured cell density. The average specific growth rate for a specific period is calculated as the logarithmic increase in biomass. Since the per cent inhibition of the growth rate was less than 50 % even at the highest concentration tested, the 72 h <math>E_rC_{50}</math> was not calculated statistically.</p> <p>The No-Observed-Effect Concentration (NOEC) is the highest test concentration which demonstrated no statistically adverse effect on growth. The data were first checked for homogeneity of variance using Bartlett's Test. If the data sets passed the test, then 1-way ANOVA and Dunnett's test were used to determine the NOEC.</p> |

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### Results:

#### *Analytical results*

At the start of the exposure period, the control and the 0.294, 0.960, 3.06 and 9.80 mg/L treatment groups were clear and colourless with no precipitate or undissolved particles present, whereas the test medium of the 31.4 and 100 mg/L test item treatment groups was a white, cloudy dispersion with precipitates and floating particles attached to the upper part of the test vessels. After 48 and 72 hours a small amount of floating particles attached to the upper part of the test vessels was observed in the 9.80 mg/L test item treatment group. After 72 hours, the colour of the test medium in the 31.4 and 100 mg/L test item treatment groups became clearer and no precipitate was present, but the amount of floating particles increased.

The geomean measured concentrations of the suspended fluids and of the dissolved fluids are given in the table below. The endpoints were based on the mean measured concentrations of dissolved fluids (from centrifuged samples).

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

**Table 152: Measured 4-OH-TM concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata***

| Nominal concentration [mg/L] | Measured concentration [mg/L] |       |         |              |
|------------------------------|-------------------------------|-------|---------|--------------|
|                              | 0 h                           | 72 h  | Geomean | % of nominal |
| Suspended fluid              |                               |       |         |              |
| 0                            | < LOD                         | < LOD | n.a.    | n.a.         |
| 0.294                        | 0.311                         | 0.190 | 0.246   | 83.7         |
| 0.960                        | 0.875                         | 0.688 | 0.778   | 81.0         |
| 3.06                         | 2.82                          | 2.48  | 2.65    | 86.6         |
| 9.80                         | 9.56                          | 8.32  | 8.93    | 91.1         |
| 31.4                         | 30.4                          | 22.3  | 26.1    | 83.1         |
| 100                          | 104                           | 51.5  | 74.7    | 74.7         |
| Dissolved fluid              |                               |       |         |              |
| 0                            | < LOD                         | < LOD | n.a.    | n.a.         |
| 0.294                        | 0.296                         | 0.186 | 0.237   | 80.6         |
| 0.960                        | 0.856                         | 0.681 | 0.765   | 79.7         |
| 3.06                         | 2.75                          | 2.43  | 2.59    | 84.6         |
| 9.80                         | 8.32                          | 8.10  | 8.21    | 83.8         |
| 31.4                         | 13.0                          | 13.7  | 13.3    | 42.4         |
| 100                          | 14.6                          | 15.5  | 15.0    | 15.0         |

LOD = limit of detection, i.e. 0.050 mg/L

n.a. = not applicable

*Biological results*

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 200); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.5 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.44 %).

Cell densities and growth rates determined at each observation interval are presented in the tables below. There were no morphological abnormalities in any of the treatment groups after 72 hours of exposure.

**Table 153: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to 4-OH-TM**

| Mean measured concentration <sup>1</sup> [mg/L] | Mean Cell Concentration [x 10 <sup>4</sup> cells/mL] after |              |              |             |
|---|--|--------------|--------------|-------------|
|   | 0 h  | 24 h         | 48 h         | 72 h        |
| 0 (control)                                     | 0.5  | 2.45 ± 0.251 | 16.3 ± 1.45  | 99.8 ± 7.37 |
| 0.237   | 0.5  | 2.53 ± 0.176 | 18.3 ± 0.208 | 121 ± 3.79  |
| 0.765   | 0.5  | 2.86 ± 0.283 | 17.4 ± 0.611 | 112 ± 2.08  |
| 2.59  | 0.5  | 2.89 ± 0.191 | 19.0 ± 0.889 | 109 ± 4.16  |
| 8.21  | 0.5  | 2.74 ± 0.325 | 15.5 ± 0.116 | 92.2 ± 3.19 |
| 13.3  | 0.5  | 2.50 ± 0.346 | 18.4 ± 0.346 | 94.7 ± 2.89 |
| 15.0  | 0.5  | 2.77 ± 0.404 | 18.3 ± 1.70  | 96.7 ± 13.3 |

<sup>1</sup> Based on dissolved fluids

**Table 154: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to 4-OH-TM**

| Mean measured concentration <sup>1</sup> [mg/L] | Growth rate (days <sup>-1</sup> ) after |               |               |                  |                             |
|---|---|---------------|---------------|------------------|-----------------------------|
|   | 0 – 24 h                                | 24 – 48 h     | 48 – 72 h     | 0 – 72 h         | Inhibition [%] <sup>2</sup> |
| Control   | 0.066 ± 0.004                           | 0.079 ± 0.006 | 0.076 ± 0.004 | 0.074 ± 0.001    | -                           |
| 0.237   | 0.067 ± 0.003                           | 0.083 ± 0.003 | 0.079 ± 0.001 | 0.076 ± 0.0004 * | -3.67                       |
| 0.765   | 0.073 ± 0.004                           | 0.075 ± 0.004 | 0.078 ± 0.001 | 0.075 ± 0.0003   | -2.31                       |
| 2.59  | 0.073 ± 0.003                           | 0.079 ± 0.001 | 0.073 ± 0.001 | 0.075 ± 0.001    | -1.81                       |
| 8.21  | 0.071 ± 0.005                           | 0.072 ± 0.005 | 0.074 ± 0.002 | 0.073 ± 0.001    | 1.41                        |
| 13.3  | 0.067 ± 0.006                           | 0.083 ± 0.007 | 0.068 ± 0.002 | 0.073 ± 0.0004   | 0.904                       |
| 15.0  | 0.071 ± 0.006                           | 0.079 ± 0.002 | 0.069 ± 0.009 | 0.073 ± 0.002    | 0.590                       |

<sup>1</sup> Based on dissolved fluids

<sup>2</sup> Per cent inhibition compared to control.

\* Significantly different compared to the control, based on Dunnett's Test.

Since the per cent inhibition based on mean measured concentrations of the dissolved fluids was less than 50 % even at the highest test item concentration, the E<sub>r</sub>C<sub>50</sub> was determined to be > 15.0 mg/L. Based on the results of Dunnett's test, there was a significant difference in growth compared to the control in the test item treatment group of 0.237 mg/L. As the growth rate in this test item treatment group was higher than in the control, this was not considered to be treatment-related. Therefore, the 72-hour NOEC for growth rate was determined to be 15.0 mg/L.

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In conclusion, based on mean measured concentrations of dissolved fluids the 72-hour EC<sub>50</sub> value for growth rate (E<sub>r</sub>C<sub>50</sub>) of *Pseudokirchneriella subcapitata* exposed to 4-OH-TM was determined to be >15.0 mg/L. The 72 h NOEC value for growth rate (NOEC<sub>r</sub>) was calculated to be 15.0 mg/L based on mean measured values of dissolved fluids.

The EC<sub>10</sub> and EC<sub>20</sub> values were not reported. However, the E<sub>r</sub>C<sub>50</sub> is the relevant endpoint for the risk assessment for algae.

### RMS comments:

The study was well performed and reported. The RMS agree with the validity assessment provided by the applicant, and that the results should be based on the mean measured dissolved test concentrations. An overview of the validity check is given in the table below.

**Table 155: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures.**

| Replicates | 0-72 h                                   |        |       |        | Section by section (day 0-1. 1-2. 2-3)   |        |      |         |             |
|------------|--|--------|-------|--------|--|--------|------|---------|-------------|
|            | Average growth rate (day <sup>-1</sup> ) | St Dev | CV    | CV (%) | Average growth rate (day <sup>-1</sup> ) | St Dev | CV   | Mean CV | Mean CV (%) |
| A          | 1.76                                     | 0.025  | 0.014 | 1.40   | 1.79                                     | 0.09   | 0.05 | 0.105   | 11          |
| B          |  |        |       |        | 1.75                                     | 0.19   | 0.11 |         |             |
| C          |  |        |       |        | 1.75                                     | 0.35   | 0.20 |         |             |
| D          |  |        |       |        | 1.73                                     | 0.18   | 0.10 |         |             |
| E          |  |        |       |        | 1.79                                     | 0.11   | 0.06 |         |             |
| F          |  |        |       |        | 1.78                                     | 0.20   | 0.11 |         |             |

**Reference:** Baba, K. (2012): CM-0237: Growth Inhibition Study in *Pseudokirchneriella subcapitata*

Company Report No.: RD-02398, NCAS 11-176 (823-005)

Guideline: OECD TG 201 (2011)

GLP: Yes

**Previous evaluation:** No, submitted for the purpose of renewal

### Material and methods:

Test material: CM-0237 (Thiophanate-methyl metabolite)

Lot/Batch No: 31-02122-I.TAKEUCHI

Purity: 98.1%

Species: *Pseudokirchneriella subcapitata* (Strain ATCC 22662), initial cell density

|                    |   |
|--------------------|---|
|                    | 5000 cells/mL   |
| Test media:        | OECD medium   |
| Treatments:        | 0.150, 0.300, 0.600, 1.20 and 2.4 mg/L (nominal)  |
| Controls:          | Untreated   |
| Replication:       | Three replicate flasks of the test concentrations and six replicates for the control were included in the test.   |
| Duration:          | 72 hours  |
| Test conditions:   | Temperature: 23.0-23.3°C, pH 7.9 – 8.0, continuous uniform illumination.<br>Light intensity was 70 - 82 $\mu\text{E}/\text{m}^2/\text{s}$   |
| Observations:      | At each subsequent 24-hour interval, cell counts were conducted on the replicate vessels using an electronic particle counter or a microscope with a counting chamber. Observations of the health of the algal cells and on the appearance of the test media were also made and recorded at each 24-hour interval.  |
| Chemical analyses: | At test initiation (0 hour) and test termination (72 hours), a single sample was removed from each test concentration and the control and analysed for CM-0237. For the determination of CM-0237 in suspended fluids, an aliquot of the test medium was collected and mixed with methanol. The mixture was centrifuged and the supernatant fluid was analysed with HPLC. For the determination of CM-0237 in dissolved fluids, an aliquot of the test medium was collected and centrifuged. The supernatant fluid was filtrated, mixed with methanol in the same volume and analysed with HPLC.   |
| Data analysis:     | The growth curves were prepared by plotting the time of measurement against the mean measured cell density. The average specific growth rate for a specific period is calculated as the logarithmic increase in biomass. Since the per cent inhibition of the growth rate was less than 50% even at the highest concentration tested, the 72 h $\text{ErC}_{50}$ was not calculated statistically.<br><br>The No-Observed-Effect Concentration (NOEC) is the highest test concentration which demonstrated no statistically adverse effect on growth. The data were first checked for homogeneity of variance using Bartlett's Test. If the data sets passed the test, then 1-way ANOVA and Dunnett test were used to determine the NOEC. All statistical determinations were performed by using the Yukms software Statlight 2000. |

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### Results:

#### *Analytical Results*

During the exposure period, the control and the 0.150, 0.300, 0.600 and 1.20 mg/L test item treatment groups were clear and colourless. The test medium of the 2.40 mg/L test item treatment group was white with cloudy dispersions attributed to the test substance at test initiation and after 24 hours. After 24 hours, a small amount of floating particles attached to the upper part of the test vessels were observed in all exposure groups.

The mean measured concentrations of the suspended fluids and of the dissolved fluids are presented in the table below.

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

**Table 156: Measured CM-0237 concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata***

| Nominal concentration [mg/L] | Measured concentration [mg/L] |       |       |              |
|------------------------------|-------------------------------|-------|-------|--------------|
|                              | 0 h                           | 72 h  | Mean  | % of nominal |
| Suspended fluid              |                               |       |       |              |
| 0                            | < LOD                         | < LOD | n.a.  | n.a.         |
| 0.150                        | 0.110                         | 0.091 | 0.100 | 66.7         |
| 0.300                        | 0.212                         | 0.143 | 0.175 | 58.3         |
| 0.600                        | 0.427                         | 0.211 | 0.306 | 51.0         |
| 1.20                         | 0.893                         | 0.325 | 0.562 | 46.8         |
| 2.40                         | 1.71                          | 0.411 | 0.911 | 38.0         |
| Dissolved fluid              |                               |       |       |              |
| 0                            | < LOD                         | < LOD | n.a.  | n.a.         |
| 0.150                        | 0.0596                        | 0.107 | 0.081 | 54.0         |
| 0.300                        | 0.104                         | 0.128 | 0.116 | 38.7         |
| 0.600                        | 0.141                         | 0.153 | 0.147 | 24.5         |
| 1.20                         | 0.154                         | 0.171 | 0.162 | 13.5         |
| 2.40                         | 0.185                         | 0.179 | 0.182 | 7.58         |

LOD = limit of detection, i.e. 0.050 mg/L

n.a. = not applicable

*Biological results*

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 201); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.7 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.82 %).

Cell densities determined at each observation interval, and corresponding growth rates are presented in the tables below.

**Table 157: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to CM-0237**

| Mean measured concentration <sup>1</sup> | Mean Cell Concentration [x 10 <sup>4</sup> cells/mL] after |
|--|--|
|--|--|

| [mg/L]  | 0 h | 24 h         | 48 h        | 72 h       |
|---------|-----|--------------|-------------|------------|
| Control | 0.5 | 2.44 ± 0.367 | 15.9 ± 1.59 | 101 ± 9.46 |
| 0.081   | 0.5 | 2.7 ± 0.380  | 18.3 ± 1.36 | 126 ± 15.4 |
| 0.116   | 0.5 | 2.48 ± 0.032 | 16.5 ± 1.21 | 123 ± 14.0 |
| 0.147   | 0.5 | 2.64 ± 0.334 | 20.0 ± 1.97 | 131 ± 13.6 |
| 0.162   | 0.5 | 2.70 ± 0.557 | 18.1 ± 1.62 | 101 ± 9.54 |
| 0.182   | 0.5 | 2.67 ± 0.551 | 20.5 ± 3.93 | 108 ± 15.0 |

<sup>1</sup> Based on dissolved fluids

**Table 158: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to CM-0237**

| Mean measured concentration <sup>1</sup><br>[mg/L] | Growth rate (days <sup>-1</sup> )<br>after |               |               |                 | Inhibition [%] <sup>2</sup> |
|--|--|---------------|---------------|-----------------|-----------------------------|
|  | 0 – 24 h                                   | 24 – 48 h     | 48 – 72 h     | 0 – 72 h        |                             |
| Control  | 0.066 ± 0.006                              | 0.078 ± 0.007 | 0.077 ± 0.004 | 0.074 ± 0.001   | -                           |
| 0.081  | 0.070 ± 0.006                              | 0.080 ± 0.006 | 0.080 ± 0.002 | 0.077 ± 0.002 * | -4.30                       |
| 0.116  | 0.067 ± 0.001                              | 0.079 ± 0.003 | 0.084 ± 0.004 | 0.077 ± 0.002   | -3.90                       |
| 0.147  | 0.069 ± 0.005                              | 0.085 ± 0.001 | 0.078 ± 0.003 | 0.077 ± 0.001 * | -4.94                       |
| 0.162  | 0.070 ± 0.009                              | 0.080 ± 0.012 | 0.072 ± 0.006 | 0.074 ± 0.001   | -0.135                      |
| 0.182  | 0.069 ± 0.009                              | 0.085 ± 0.016 | 0.070 ± 0.007 | 0.075 ± 0.002   | -1.36                       |

<sup>1</sup> Based on dissolved fluids

<sup>2</sup> Per cent inhibition compared to control.

\* Significantly different compared to the control, based on Dunnett's Test.

Cells in the control were observed to be normal throughout the exposure period. Swelled cells were observed in the 0.150, 0.300 and 0.600 mg/L treatment groups, whereas atrophied cells were observed in the 1.2 and 2.4 mg/L treatment groups. Since the per cent inhibition based on mean measured concentrations of the dissolved fluids was less than 50 % even at the highest test item concentration, the E<sub>r</sub>C<sub>50</sub> was determined to be > 0.182 mg/L.

Based on the results of Dunnett's test, there was a significant difference in growth compared to the control in the test item treatment groups of 0.150 and 1.2 mg/L. As the growth rates in these test item treatment groups were higher than in the control, this was not considered to be treatment-related. Therefore, the 72-hour NOEC for growth rate (NOEC<sub>r</sub>) was determined to be 0.182 mg/L.

In conclusion, based on mean measured concentrations of dissolved fluids the 72-hour EC<sub>50</sub> value for growth rate (E<sub>r</sub>C<sub>50</sub>) of *Pseudokirchneriella subcapitata* exposed to CM-0237 was determined to be > 0.182 mg/L. The 72 h NOEC value for growth rate (NOEC<sub>r</sub>) was calculated to be 0.182 mg/L based on mean measured values of dissolved fluids.

The EC<sub>10</sub> and EC<sub>20</sub> values were not reported. However, the E<sub>r</sub>C<sub>50</sub> is the relevant endpoint for the risk assessment for algae.

**RMS comments:**

The study was well performed and reported. The RMS agree with the validity assessment provided by the applicant, and that the results should be based on the mean measured dissolved test concentrations. It is proposed, though, that the NOEC is set to <0.081 mg/L, due to the observed effects (swelled/atrophied cells) at all treatment levels. However, this will have no impact on the risk assessment. An overview of the validity check is given in the table below.

**Table 159: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures.**

| Replicates | 0-72 h                                   |        |       |        | Section by section (day 0-1. 1-2. 2-3)   |        |      |         |             |
|------------|--|--------|-------|--------|--|--------|------|---------|-------------|
|            | Average growth rate (day <sup>-1</sup> ) | St Dev | CV    | CV (%) | Average growth rate (day <sup>-1</sup> ) | St Dev | CV   | Mean CV | Mean CV (%) |
| A          | 1.77                                     | 0.032  | 0.018 | 1.83   | 1.76                                     | 0.28   | 0.16 | 0.107   | 11          |
| B          |  |        |       |        | 1.77                                     | 0.31   | 0.17 |         |             |
| C          |  |        |       |        | 1.76                                     | 0.31   | 0.18 |         |             |
| D          |  |        |       |        | 1.71                                     | 0.05   | 0.03 |         |             |
| E          |  |        |       |        | 1.79                                     | 0.13   | 0.08 |         |             |
| F          |  |        |       |        | 1.81                                     | 0.05   | 0.03 |         |             |

**11.5.4 Acute (short-term) toxicity to other aquatic organisms**

Not relevant, no data available.

**11.6 Long-term aquatic hazard**

The available and reliable data on chronic toxicity of thiophanate-methyl, its major environmental metabolite carbendazim and additional metabolites to aquatic organisms is summarised in the table below.

**Table 160: Summary of relevant information on chronic aquatic toxicity. Key data are marked in bold text.**

| Group                      | Test substance     | Time-scale (Test type)     | End point                | Toxicity (mg a.s./L)             | Reference                                    |
|----------------------------|--------------------|----------------------------|--------------------------|----------------------------------|--|
| Laboratory tests           |                    |                            |                          |                                  |  |
| Fish                       |                    |                            |                          |                                  |  |
| <i>Danio rerio</i>         | Thiophanate-methyl | 35 days, ELS, flow-through | NOEC<br>EC <sub>10</sub> | not reported<br><b>0.39</b> (mm) | Report number 826-002 (2014)                 |
| <i>Danio rerio</i>         | Carbendazim        | 35 days, ELS, flow-through | NOEC<br>EC <sub>10</sub> | Not reported                     | Report number 826-002 (2014)                 |
| <i>Oncorhynchus mykiss</i> | Carbendazim*       | 21 days, flow-through      | NOEC                     | <b>0.0032</b> (nom)              | Report number A40788, cited in EFSA (2010) * |

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| Group                       | Test substance                    | Time-scale<br>(Test type) | End point                                    | Toxicity<br>(mg a.s./L)                             | Reference   |
|-----------------------------|-----------------------------------|---------------------------|--|---|---|
| Aquatic invertebrates       |                                   |                           |  |   |   |
| <i>Daphnia magna</i>        | Thiophanate-methyl                | 21 d, semi-static         | NOEC<br>EC <sub>10</sub><br>EC <sub>20</sub> | <b>0.16</b> (mm)<br>Not reported<br>Not reported    | Handley, J.W. et al. (1990) ; Wirzinger and Ruhnke (2016) |
| <i>Daphnia magna</i>        | TOPSIN M WDG (Thiophanate-methyl) | 21 d, aged test item*     | NOEC<br>EC <sub>10</sub>                     | 0.0373 (mm)<br>0.0285 (mm)                          | Schäfers, C. (2007); Schäfers, C. (2016)                  |
| <i>Daphnia magna</i>        | TOPSIN M WDG (Carbendazim)        | 21 d, aged test item*     | NOEC<br>EC <sub>10</sub>                     | 0.0177 (mm)<br>0.0149 (mm)                          | Schäfers, C. (2007); Schäfers, C. (2016)                  |
| <i>Daphnia magna</i>        | Carbendazim*                      | 21 d, semi-static         | NOEC<br>EC <sub>10</sub><br>EC <sub>20</sub> | <b>0.0015</b> (nom)<br>Not reported<br>Not reported | Kelly et al. (1997), cited in EFSA (2010) *               |
| Sediment-dwelling organisms |                                   |                           |  |   |   |
| <i>Chironomus riparius</i>  | Thiophanate-methyl                | 28 d, water spiked        | NOEC<br>EC <sub>10</sub><br>EC <sub>20</sub> | 0.44 (init. meas.)<br>Not reported<br>Not reported  | Memmert. U. (2002)  |
| <i>Chironomus riparius</i>  | Carbendazim*                      | 28 d, water spiked        | NOEC<br>EC <sub>10</sub><br>EC <sub>20</sub> | 0.0133 (nom)<br>Not reported<br>Not reported        | Sowig & Gosch (2002), cited in EFSA (2010) *              |
| Algae                       |                                   |                           |  |   |   |
| <i>P. subcapitata</i>       | Thiophanate-methyl                | 72 h, static              | NOEC   | n.d. (mm)   | Saito, S. (2002), and Wirzinger, G. (2015)                |
| <i>S. subspicatus</i>       | Topsin 500 SC                     | 72 h, static              | NOEC   | 3.28 (mm)   | Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)     |
| <i>P. subcapitata</i>       | Carbendazim*                      | 72 h, static              | NOEC   | Not reported  | Bell (1996), cited in EFSA (2010) *                       |
| <i>P. subcapitata</i>       | 4-OH-TM                           | 72 h, static              | NOEC   | 15 (mm, dissolved)                                  | Baba, K. (2012)   |
| <i>P. subcapitata</i>       | CM-0237                           | 72 h, static              | NOEC   | 0.182 (mm, dissolved)                               | Baba, K. (2012)   |

\* Refer to the EFSA conclusion on the peer review of the active substance Carbendazim, EFSA (2010). The study summaries provided below for Carbendazim are derived from the Draft Re-Assessment Report produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not evaluated by Sweden at this stage.

### 11.6.1 Chronic toxicity to fish

Reference: **xxxx (2014):** Zebrafish (*Danio rerio*), Early Life Stage Toxicity Test, Flow through conditions - Test item: Thiophanate-methyl

Report number: RD-02818, NIS-002/4-43/A (826-002)

Guideline: OECD TG 210 (1992)

GLP: Yes

**Previous evaluation:** No, submitted for the purpose of renewal.

#### Material and methods:

Test material: Thiophanate-methyl

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|                    |  |
|--------------------|--|
| Lot/Batch No:      | TBC-035G   |
| Purity:            | 97.9 %   |
| Species:           | Zebrafish ( <i>Danio rerio</i> ). Freshly fertilised eggs, less than 4 hours old.  |
| Treatments:        | The nominal test concentrations of Thiophanate-methyl were 125, 250, 500, 1000 and 2000 µg a.s./L.   |
| Controls:          | Purified drinking water  |
| Replication:       | Four replicates per test level, 60 eggs per test level, 15 eggs per test vessel.   |
| Duration:          | 35 days  |
| Test conditions:   | <p>Flow-through. Each group of 15 eggs was placed into a fry chamber suspended in each test vessel. After hatching, the larvae were fed daily ad libitum with newly hatched brine shrimp nauplii (<i>Artemia salina</i>). Temperature: 24.3 – 25.7°C</p> <p>Dissolved oxygen: 93 – 103 % of saturation</p> <p>pH: 7.9 – 8.4</p> <p>Hardness: 1.1 – 1.2 mg/L</p> <p>Photoperiod: Light/dark cycle of 12 hours/12 hours</p>  |
| Observations:      | <p>Qualitative observations on hatching and survival were made daily. Dead embryos, larvae and juvenile fish were removed as soon as observed. Observations on abnormal appearance of behaviour were made daily, too. After 14, 21, 28 and 35 days post fertilisation (pf), the fish larvae were digitally photographed. Post hatch survival rates were estimated by evaluating photographs using electronically supported counting and analysis. At test end, fish lengths and group dry weights of replicate fish groups were measured.</p> <p>Oxygen concentration and pH were measured in each test vessel directly before adding the eggs and at least twice a week thereafter. The water temperature was measured each working day in all test vessels. Additionally, the water temperature was continuously measured in two control test vessels.</p> |
| Chemical analysis: | LC-MS/MS. LOQ was 50 µg/L for thiophanate-methyl and 1.0 µg/L for MBC (carbendazim).   |
| Data analysis:     | The evaluation of the effect concentrations was based on measured test item concentrations of Thiophanate-methyl. For each endpoint the arithmetic mean of all replicates (n=4) was calculated per dose level and control. To confirm normal distribution, Shapiro Wilk's test was performed with each data set. To detect homogeneity of variances, Levene's test was performed. The data were analysed for statistical differences as compared with the untreated control by performing ANOVA, followed by Williams' test to calculate NOECs. William's  |

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test, as a trend based test, was chosen, since monotonous dose responses were observed or biologically expected for all endpoints. All data sets were tested against the control. Beside the NOEC determination, a probit analysis was performed to determine EC<sub>10</sub> and EC<sub>20</sub>.

**Results:**

All validity criteria for controls mentioned by the OECD guideline were met: The dissolved oxygen concentration was between 60 and 100 % throughout the test; the water temperature did not differ by more than ± 1.5 °C between successive days at any time during the test and was kept within 25 °C ± 2 °C the survival rate (post hatch) in the controls was greater than 70 %.

*Analytical results*

The chemical analyses of the test media revealed mean concentrations for the single test vessels between 81 and 123 % of the nominal concentrations. The effect data evaluation was based on the mean measured concentrations. The mean measured test concentrations were between 93.5 % and 103 % of the nominal values. Thus, the treatment levels were defined as 123, 256, 509, 939 and 1870 µg a.s./L.

In addition to the chemical analysis of Thiophanate-methyl the concentrations of Carbendazim in the different treatments were also measured. For Carbendazim the mean concentrations per treatment were found to be between 6.3 and 21.0 % of the Carbendazim equivalents of the nominal concentrations of Thiophanate-methyl (the nominal concentrations of Carbendazim were calculated using the molar weight quotient of Carbendazim : Thiophanate methyl of 0.55838) and were calculated to be 14.6, 20.9, 28.1, 44.2 and 69.9 µg Carbendazim/L.

**Table 161: Measured Thiophanate-methyl and Carbendazim concentrations in the exposure solutions during the exposure of zebrafish**

| Nominal concentration<br>[µg/L] |              | Mean measured concentration |              |             |              |
|---------------------------------|--------------|-----------------------------|--------------|-------------|--------------|
|                                 |              | Thiophanate-methyl          |              | Carbendazim |              |
| Thiophanate-methyl              | Carbendazim* | µg/L                        | % of nominal | µg/L        | % of nominal |
| 0 (control)                     | 0 (control)  | > LOQ                       | -            | > LOQ       | -            |
| 125                             | 70           | 123 ± 9.1                   | 98.1 ± 7.2   | 14.6 ± 0.3  | 21.0 ± 0.5   |
| 250                             | 140          | 256 ± 6.4                   | 103 ± 2.6    | 20.9 ± 0.6  | 15.0 ± 0.4   |
| 500                             | 279          | 509 ± 38.9                  | 102 ± 7.8    | 28.1 ± 3.3  | 10.0 ± 1.2   |
| 1000                            | 558          | 939 ± 44.5                  | 93.9 ± 4.4   | 44.2 ± 2.5  | 7.9 ± 0.4    |
| 2000                            | 1117         | 1870 ±                      | 93.5 ± 1.8   | 69.9 ± 4.2  | 6.3 ± 0.4    |

| Nominal concentration |              | Mean measured concentration |              |             |              |
|-----------------------|--------------|-----------------------------|--------------|-------------|--------------|
| [µg/L]                |              | Thiophanate-methyl          |              | Carbendazim |              |
| Thiophanate-methyl    | Carbendazim* | µg/L                        | % of nominal | µg/L        | % of nominal |
|                       |              | 36.0                        |              |             |              |

*LOQ of Thiophanate-methyl: 50 µg/L, LOQ for Carbendazim: 1 µg/L*

*\* Carbendazim equivalents of the nominal concentrations of Thiophanate-methyl (the nominal concentrations of Carbendazim were calculated using the molar weight quotient of Carbendazim : Thiophanate methyl of 0.55838)*

All chemical and physical parameters in the test were within expected ranges. The oxygen saturation was between 93 and 103 %. The water temperature was measured to be in a range of 24.3 to 25.7 °C throughout the study. The pH in the test vessels was between 7.9 and 8.4. The test media was of clear appearance throughout the whole study.

#### *Biological results*

Hatch of the fish larvae was not affected by the test item. The hatching success was 100 % at all test concentrations of Thiophanate-methyl (NOEC  $\geq$  1870 µg a.s./L). The evaluation of post hatch survival revealed a significant decrease at 939 and 1870 µg a.s./L after 28 and 35 days (test end). Statistically significant reduced post hatch survival at the test item treatment groups of 256 and 509 µg a.s./L was observed after 14 and 21 days pf. The respective rates of post hatch survival thereafter were almost comparable to those of after 21 days pf, but no statistically significant differences to the control were found at all later observation dates. Based on this observation, the decreased survival at 256 and 509 µg a.s./L is not considered as biologically relevant. The EC<sub>10</sub> for this endpoint was calculated to be 390 µg a.s./L (95 % confidence intervals 147 – 645 µg a.s./L) and the EC<sub>20</sub> was determined to be 1279 µg a.s./L (95 % confidence intervals 769 – 3877 µg a.s./L).

Fish lengths were recorded on day 35 (test end). The single dry weights were calculated by dividing the group dry weights by the number of remaining fish on day 35 pf. Fish lengths and the single dry weights were found to be not significantly inhibited by Thiophanate-methyl (NOEC  $\geq$  1870 µg a.s./L). An increase of both lengths and weights was observed at all treatments of Thiophanate-methyl, which was not concentration dependent. The single dry weight was between 1.7 to 2.0 times higher than the single dry weight of the control. The observed increase in growth was not considered by the applicant as negative effect on the fish. No abnormality in the fish behaviour was observed.

A summary of egg hatch, post hatch survival, length and wet weight is presented in the table below.

**Table 162: Summary of hatching, post hatch survival, length and wet weight of early life stages of zebrafish following exposure to Thiophanate-methyl under flow through conditions**

| Mean measured concentration<br>[µg a.s./L]      | 0<br>(control) | 123         | 256          | 509          | 939           | 1870         |
|---|----------------|-------------|--------------|--------------|---------------|--------------|
| Number of introduced eggs (n)                   | 60             | 60          | 60           | 60           | 60            | 60           |
| Embryo hatching success [%]                     | 100            | 100         | 100          | 100          | 100           | 100          |
| Hatching day 3 pf (%)                           | 26.7 ± 9.4     | 28.3 ± 6.4  | 28.3 ± 14.8  | 33.3 ± 14.4  | 26.7 ± 21.1   | 16.7 ± 3.8   |
| Hatching day 4 pf (%)                           | 90.0 ± 8.6     | 83.3 ± 15.9 | 86.7 ± 5.4   | 76.7 ± 8.6   | 70.0 ± 12.8   | 78.3 ± 17.5  |
| Hatching day 5 pf (%)                           | 98.3 ± 3.3     | 93.3 ± 5.4  | 98.3 ± 3.3   | 95.0 ± 6.4   | 91.7 ± 12.6   | 96.7 ± 6.7   |
| Hatching day 6 pf (%)                           | 100 ± 0.0      | 100 ± 0.0   | 95.0 ± 10.0  | 100 ± 0.0    | 98.3 ± 3.3    | 100 ± 0.0    |
| Hatching day 7 pf (%)                           | 100 ± 0.0      | 100 ± 0.0   | 100 ± 0.0    | 100 ± 0.0    | 100 ± 0.0     | 100 ± 0.0    |
| Normal fry at hatch [%]                         | 100            | 100         | 100          | 100          | 100           | 100          |
| Larval survival on day 14 pf [%]                | 95.0 ± 3.3     | 90.0 ± 6.7  | 88.3 ± 6.4   | 85.0 ± 6.4 * | 75.0 ± 11.3 * | 70.0 ± 3.8 * |
| Larval survival on day 21 pf [%]                | 91.7 ± 6.4     | 85.0 ± 6.4  | 83.3 ± 8.6 * | 83.3 ± 6.7 * | 75.0 ± 11.3 * | 68.3 ± 6.4 * |
| Larval survival on day 28 pf [%]                | 90.0 ± 8.6     | 85.0 ± 6.4  | 83.3 ± 8.6   | 81.7 ± 8.4   | 75.0 ± 11.3 * | 68.3 ± 6.4 * |
| Larval survival on day 35 pf [%]                | 90.0 ± 8.6     | 85.0 ± 6.4  | 83.3 ± 8.6   | 81.7 ± 8.4   | 73.3 ± 12.4 * | 66.7 ± 5.4 * |
| Larval length (day 35 pf) [cm]                  | 1.40 ± 0.04    | 1.54 ± 0.05 | 1.58 ± 0.06  | 1.57 ± 0.09  | 1.55 ± 0.09   | 1.52 ± 0.09  |
| Larval dry weight (day 35 pf) [mg] <sup>1</sup> | 5.0 ± 0.3      | 8.5 ± 1.4   | 10.1 ± 0.7   | 9.8 ± 2.5    | 9.6 ± 2.4     | 9.2 ± 1.6    |

<sup>1</sup> mean single dry weights

\* Statistically reduced compared to the pooled control based on Williams' Test

No statistically significant treatment related effect could be observed on the fish larvae concerning the endpoint hatching success. For post hatch survival a statistically significant decrease was observed at mean measured concentrations of 939 and 1870 µg a.s./L at test end. Lengths and single dry weights were found to be elevated at all investigated test concentrations. However, the authors proposed that this should not be considered as negative effect on the test organisms. Thus, the lowest no effect concentration, based on the mean measured concentrations, was proposed to be 509 µg a.s./L for the endpoint post hatch survival after 35 days. The EC<sub>10</sub> and EC<sub>20</sub> for post hatch survival were calculated to be 390 (95%CL 147 – 645) and 1279 (95%CL 769 – 3877) µg a.s./L, respectively.

**RMS comments:**

The study fulfils the validity criteria of OECD TG 210 (2013):

- dissolved oxygen concentration should be >60% of the air saturation value throughout the test;
- water temperature should not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (Annex 2);
- analytical measurements of the test concentrations.
- overall survival of fertilised eggs and post-hatch success in the controls >70%

In the table below, the RMS has summarised the measured concentrations of Thiophanate-methyl and Carbendazim in the test solutions at different time points during the study. It was noted that in most cases the concentrations of Carbendazim increased during the test, indicating that using the mean measured may be an over-estimation of the initial concentration potentially resulting in effects from short term exposure.

**Table 163: Measured concentrations of Thiophanate-methyl and Carbendazim (MBC) in the ELS study under flow-through conditions. Mean of four replicates.**

| Treatment group | Thiophanate-methyl Measured (µg/L) |       |       |       |       |        | MBC Measured (µg/L) |      |      |      |      |      |
|-----------------|------------------------------------|-------|-------|-------|-------|--------|---------------------|------|------|------|------|------|
|                 | Control                            | 1     | 2     | 3     | 4     | 5      | Control             | 1    | 2    | 3    | 4    | 5    |
| Start           | 0                                  | 113.3 | 232.5 | 474.5 | 926.0 | 1892.0 | 0                   | 14.0 | 19.0 | 25.3 | 38.5 | 59.7 |
| Day 7           | 0                                  | 128.8 | 264.0 | 550.0 | 996.3 | 1960.3 | 0                   | 13.4 | 19.0 | 25.9 | 50.0 | 86.1 |
| Day 13          | 0                                  | 128.0 | 276.8 | 537.3 | 974.8 | 1960.3 | 0                   | 13.2 | 19.4 | 26.5 | 47.0 | 80.0 |
| Day 21          | 0                                  | 121.5 | 256.3 | 495.3 | 906.3 | 1835.0 | 0                   | 16.1 | 23.0 | 29.1 | 48.2 | 78.5 |
| Day 27          | 0                                  | 119.8 | 240.8 | 478.8 | 880.3 | 1706.8 | 0                   | 14.9 | 22.9 | 31.0 | 40.6 | 56.2 |
| Day 35          | 0                                  | 124.0 | 267.5 | 519.5 | 953.8 | 1863.5 | 0                   | 16.1 | 22.2 | 30.6 | 40.7 | 58.9 |
| Mean measured   | 0                                  | 122.5 | 256.3 | 509.2 | 939.5 | 1869.6 | 0.0                 | 14.6 | 20.9 | 28.0 | 44.2 | 69.9 |
| Std dev         | 0                                  | 5.8   | 16.8  | 31.3  | 43.5  | 94.5   | 0.00                | 1.3  | 2.0  | 2.5  | 4.8  | 13.0 |

From the results, although not statistically significant at all concentrations, there seems to be treatment related effect on larval survival throughout the test at all treatment levels. Therefore, the RMS would support the EC<sub>10</sub> (390 µg a.s./L for Thiophanate-methyl, not reported for Carbendazim but may be estimated to be ca 20 µg/L) as endpoint for this effect. The steepness of the curve cannot be calculated in accordance with EFSA 2015 as no EC<sub>50</sub> could be calculated. However, as the EC<sub>10</sub> is lower than the lower limit of the EC<sub>20</sub> the applicant proposed that there is a high certainty of the protection level. The RMS agree with this conclusion regarding the effects on larval survival. Regarding the observed effects on larval length (>10% increase at all treatment levels) and dry weight (>70% increase at all treatment levels), the RMS proposed that this may be a relevant effect. However, this was questioned by EFSA during the commenting period (“*relevance of selecting positive effects on fish for setting a NOEC seems not fully justified*”) and it was concluded that the risk assessment should be based on the EC<sub>10</sub> of 390 µg a.s./L for Thiophanate-methyl. No further discussion was foreseen.

Due to concerns during the review process a statement was provided by the applicant on the impact of the effects seen on fish length and weight in the test item treatment groups of the fish early life stage test with Thiophanate-methyl (Report number 826-002). The conclusion of this statement (Report number 881-021) is submitted below.

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|                             |   |
|-----------------------------|---|
| <b>Reference:</b>           | <b>xxxx (2017):</b> Scientific statement on the correlation of fish size and reproduction, for interpretation of observed effects of the following study: Zebrafish ( <i>Danio rerio</i> ), Early Life Stage Toxicity Test, Flow through conditions |
| <b>Report number:</b>       | 881-021   |
| <b>Guideline:</b>           | Not relevant  |
| <b>GLP:</b>                 | Not relevant  |
| <b>Previous evaluation:</b> | No, submitted for the purpose of renewal.   |

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**Summary:**

In the fish early life stage study (Report number 826-002), increased growth in terms of length and single fish dry weight at test end was observed. The mean length of fish was 14 mm in the control and up to 15.8 mm at a treatment level of 250 µg a.s./L. At the highest treatment level of 2000 µg a.s./L, a mean size of 15.2 mm was observed.

The mean single dry weight in controls was determined to be 5.0 mg. The maximum mean single dry weight of 10.1 mg was observed at a treatment level of 250 µg a.s./L. At the highest treatment level, a mean single dry weight of 9.2 mg was observed.

It was observed that the increased growth in terms of length and weight was not concentration-dependent, as it was observed across treatment levels in a similar range. If the test item would have been responsible for increased size, a dose dependent increase of growth could be assumed in correspondence to increasing Thiophanate-methyl concentrations. Thus, it is assumed that this effect was not treatment related.

An increased size is not considered by the author as a negative effect. A review article by Lawrence (2007) indicates that for example good water quality promotes zebrafish growth and reproduction. Sub-optimal conditions result in a decrease in growth rates and the number and quality of offspring. No correlation of large size and reduced reproductive performance was described. Furthermore, the Lawrence (2007) repeatedly correlated increased growth with an optimal health status of fish. Thus, for unknown reasons, the health status under treatment conditions in Report number 826-002 was rather advantageous compared to control conditions. No hint to any adverse effect related to increased growth rate is given in this review.

Control fish as well as fish under Thiophanate-methyl treatment conditions reached an overall mean length of 14 mm in the GLP-study of Report number 826-002, which exceeded the minimum mean size of 11 mm as suggested by the OECD TG 210. It can thus be postulated that the growth rate observed in this study indicates that test conditions were optimal for fish growth.

The author referred to several studies examining the correlation of reproductive success and size of male and female fish. In many fish species, a relationship exists between female body size and fecundity, following a linear regression (Wootton, 1998). For example, Uusi-Heikkilä *et al.* (2010) reported that large female zebrafish spawned more frequently and had significantly greater clutch sizes than small females. Furthermore, eggs from small fish suffered from higher egg mortality than eggs from large individuals. In another study, it was reported that females showed a clear preference for high quality, i.e. large males (Uusi-Heikkilä *et al.*, 2012). In consequence, a higher spawning probability and a larger clutch size were observed. These findings indicate that increased size of fish did not only enhance own survival but also reproductive performance and survival of the offspring. A negative impact of increased size on reproduction could not be concluded from this study.

A detailed comparison of the lengths and weights observed in the fish early life stage study to historical control data was also performed. Only data with comparable number of introduced eggs per vessel (15 to 20 eggs per vessel) were included in order to rule out any potential effect due to different fish density.

**Table 164: Historical control data \* on fish size**

| Year of conduct  |      | 2013                                   | 2012                  | 2014                  | 2016                  |
|--|------|--|-----------------------|-----------------------|-----------------------|
| No of introduced eggs at test start  |      | 15                                     | 15                    | 20                    | 20                    |
|  |      | Control fish Study #1 (NIS-002-4-43-A) | Control fish study #2 | Control fish study #3 | Control fish study #4 |
| Single dry weight in controls during fish early life stage studies [mg/fish] | A    | 5.0                                    | 4.8                   | 7.9                   | 6.3                   |
|  | B    | 5.1                                    | 4.6                   | 7.8                   | 5.8                   |
|  | C    | 4.6                                    | 7.4                   | 8.3                   | 8.3                   |
|  | D    | 5.4                                    | 7.4                   | 8.6                   | 6.3                   |
|  | Mean | 5.0                                    | 6.1                   | 8.2                   | 6.7                   |
|  | SD   | 0.3                                    | 1.6                   | 0.4                   | 1.1                   |
| Single wet weight in controls during fish early life stage studies [mg/fish] | A    | 24.71                                  | 25.00                 | 33.26                 | 27.05                 |
|  | B    | 23.66                                  | 26.50                 | 33.17                 | 25.18                 |
|  | C    | 23.18                                  | 33.20                 | 34.23                 | 34.76                 |
|  | D    | 23.76                                  | 31.90                 | 33.05                 | 26.57                 |
|  | Mean | 23.8                                   | 29.2                  | 33.4                  | 28.4                  |
|  | SD   | 0.6                                    | 4.0                   | 0.5                   | 4.3                   |
| Mean length in controls during fish early life stage studies [mm]            | A    | 13.5                                   | 13.3                  | 15.5                  | 14.1                  |
|  | B    | 14.3                                   | 13.2                  | 15.4                  | 13.4                  |
|  | C    | 14.3                                   | 15.4                  | 15.4                  | 15.4                  |
|  | D    | 13.7                                   | 14.7                  | 15.0                  | 13.9                  |
|  | Mean | 14.0                                   | 14.2                  | 15.3                  | 14.2                  |
|  | SD   | 0.4                                    | 1.1                   | 0.2                   | 0.9                   |

\* Historical control data were derived from FELS studies conducted at the Fraunhofer IME and reared under similar conditions as the study NIS-002/4-43/A

Comparison of the size data of the study of Report number 826-002 to the historical control size data indicate that fish in controls were considerably small compared to controls of similar studies. For example, the single dry weight of controls in the here discussed study are between 4.6 mg and 5.4 mg, while historical control values range between 4.6 mg and 8.6 mg. Fish length of controls of the here discussed study varies between 13.5 mm and 14.3 mm, while historical control values range between 13.2 mm and 15.5 mm. Even though for the test item treatment groups there are still values for length and weight above the values observed in controls of similar studies, the differences are not as prominent as compared to controls of the study of Report number 826-002. The values of the study Report number 826-002, which are above the values of all individual replicates of historical control data, are furthermore distributed across all treatment levels, with highest values at 250 and 2000 µg a.s./L, ruling out any concentration-related effect. Nevertheless, it was proposed that there is no concern about the performance of the study as fish in controls reached the minimum value of 11 mm.

#### RMS comments:

The statement by the applicant supports the conclusion that the observed positive effect on fish size should not be regarded as biologically relevant. Hence, the EC<sub>10</sub> of 390 µg a.s./L based on effects on post hatch survival is regarded as the relevant endpoint from this study.

**Author:** xxxxx  
**Title:** The effect of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Salmo gairdneri* (Rainbow trout) in a 21-day Prolonged Toxicity Test (method OECD)  
**Date:** 17 Apr 1989  
**Doc ID:** A40788, Report no: CE89/021; BVL No. 1758283  
**Guidelines:** OECD 204 (Draft)  
**GLP:** Yes  
**Validity:** Yes

Aim was to assess the effects of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Oncorhynchus mykiss* (Rainbow trout, formerly *Salmo gairdneri*) in a 21-day prolonged toxicity test (method OECD).

### Methods

Carbendazim; substance, technical; Code: Hoe 017411 00 ZD99 0010, purity 99.4 %. Physical form of test substance: Solid, white crystalline. Lacking stability data are not considered to affect the validity of the study as chemical analysis in test medium showed expected values. Dosing vehicle: 1 NHCL

Test method: OECD 204 (1984)

Test system: Rainbow trout (*O. mykiss*, formerly *Salmo gairdneri*)

Number of animals: 10 fish per tested concentration and control

Age/size/weight: Approx. 10 months /6.1 cm (n = 70)/4.2 g (n = 70)

Maintenance conditions: Stainless steel tanks; photoperiod 16 h light; 14° C ±1° C; loading 0.09 g/L - 0.12 cm/L at start and 1.19 g/L - 1.63 cm/L as a mean value.

Test concentrations (flow through):

0 (control and solvent control), 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L

Application of test substance: Automatic dosage pumps during flow through phase

Duration of exposure/recording: 21 days

Measured/observed parameters: Chemical analysis in medium: 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21 days after initiation; mortality and symptoms: every 24 hours

### Results

Analyses of test substance concentration in medium showed mean recoveries of 94.3 % to 102.7 % during the exposure phase. Hence, all calculations are based on nominal values. The findings of the study are summarized in the table below.

**Table 165: Mortality and intoxication symptoms in rainbow trout when exposed to carbendazim for a period of 21 days**

| Test day | Parameter            | Control | Solvent Control | Carbendazim (nom mg/L)    |            |            |            |        |
|----------|----------------------|---------|-----------------|---------------------------|------------|------------|------------|--------|
|          |                      |         |                 | 0.032                     | 0.018      | 0.010      | 0.0056     | 0.0032 |
| 0        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 1        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 2        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 3        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 4        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 5        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 6        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>-                    | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 7        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 8        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 9        | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 10       | %M<br>IS             | 0<br>-  | 0<br>*          | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 11       | %M<br>IS             | 0<br>-  | 10<br>-         | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 12       | %M<br>IS             | 0<br>-  | 10<br>-         | 0<br>S1/10                | 0<br>-     | 0<br>-     | 0<br>-     | 0<br>- |
| 13       | %M<br>IS#<br>IS# IS# | 0       | 10              | 0<br>S1/10<br>S3/10 S4/10 | 0<br>S4/10 | 0<br>S4/8  | 0<br>S4/8  | 0      |
| 14       | %M<br>IS#<br>IS# IS# | 0       | 10              | 0<br>S1/9<br>S3/9 S4/9    | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0      |
| 15       | %M<br>IS#<br>IS# IS# | 0       | 10              | 0<br>S1/9<br>S3/9 S4/9    | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0      |
| 16       | %M<br>IS#<br>IS# IS# | 0       | 10              | 10<br>S1/9                | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/5  | 0      |

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|    |                             |   |    |                                      |            |            |            |   |
|----|-----------------------------|---|----|--------------------------------------|------------|------------|------------|---|
|    |                             |   |    | S3/9 S4/9                            |            |            |            |   |
| 17 | %M<br>IS#<br>IS# IS#        | 0 | 10 | 10<br>S1/9<br>S3/9 S4/9              | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0 |
| 18 | %M<br>IS#<br>IS# IS#        | 0 | 10 | 10<br>S1/9<br>S3/9 S4/9              | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0 |
| 19 | %M<br>IS#<br>IS# IS#        | 0 | 10 | 10<br>S1/9<br>S3/9 S4/9              | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0 |
| 20 | %M<br>IS#<br>IS# IS#<br>IS# | 0 | 10 | 10<br>S1/9 S3/9<br>S5/1 S4/9<br>S6/9 | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0 |
| 21 | %M<br>IS#<br>IS# IS#        | 0 | 10 | 30<br>S1/7<br>S3/7 S4/7              | 0<br>S4/10 | 0<br>S4/10 | 0<br>S4/10 | 0 |

\* One fish appeared to be damaged and died on day 10 in the solvent control group

% M: percent mortality

IS: Intoxication symptoms, type and number of affected fish

S1 delayed uptake of feed and feed residues on the bottom of the test tanks

S2 swimming on back on the surface and slow reaction

S3 indifferent, reduced escape behaviour

S4 fish stay mostly near outflow

S5 narcotic conditions

S6 damaged tail fin and reduced mobility

**Table 166: Toxicity endpoints assessed in a fish juvenile growth test with *O. mykiss* exposed to carbendazim for 21 day**

| Endpoint    | Value (mg/L) | Time (d) | ai | n/r/i |
|-------------|--------------|----------|----|-------|
| NOEC        | 0.0032       | 21       |    | n     |
| LOEC growth | 0.032        | 21       |    | r     |

n = nominal; r = real; i = initial

The NOEC after 21 days was 0.0032 mg carbendazim/L. Mortality was only observed at the highest concentration level. Differences in size and weight of the fish in comparison to control animals were ascertained to be significantly influenced by the test substance at 0.032 mg/L, only.

**Conclusion**

Report results listed and evaluated in the carbendazim monograph of 13.11.1997.

The NOEC in a 21 d prolonged toxicity test with rainbow trout *O. mykiss* was 0.0032 mg carbendazim/L. Mortality of 30 % was only observed at 0.032 mg/L, the highest tested concentration. At this concentration, fish size and weight was also influenced in comparison to control animals.

### 11.6.2 Chronic toxicity to aquatic invertebrates

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|                |  |
|----------------|--|
| Reference:     | <b>Handley, J.W. et al. (1990):</b> An assessment of the effects of Thiophanate-methyl on the reproduction of <i>Daphnia magna</i> |
| Report number: | 235/4. RD-9010 (827-001)   |
| Guideline:     | OECD TG 202, Part 2 (1984)   |
| GLP:           | Yes.   |

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**Previous evaluation:** In DAR 1997.

---

#### **Material and methods:**

|                  |   |
|------------------|---|
| Test material:   | Thiophanate-methyl  |
| Lot/Batch No:    | TP-569 (impurities: 3.0% sodium chloride, 0.69% sulphur   |
| Purity:          | 95.2%   |
| Species:         | <i>Daphnia magna</i> (Straus)   |
| Treatments:      | 0.18, 0.56, 1.8, 5.6 and 18 mg/L (nominal).   |
| Controls:        | Untreated and solvent control (DMF, dimethylformamide)  |
| Replication:     | Four test vessels per treatment level, 10 daphnids per vessel   |
| Duration:        | 21 days   |
| Test conditions: | Semi-static, renewal 3 times per week.<br>Temperature: 20 - 21°C<br>Dissolved oxygen: 7.9 – 8.8 mg/L<br>pH: 7.7 – 7.8<br>Photoperiod: 16 h light : 8 h dark<br>Light intensity: not reported<br>Food was supplied continuously.   |
| Observations:    | Live and dead animals of the parental generation was counted daily together with the general condition and size compared to the control. The number of daphnids with eggs or young in the brood pouch was determined at each test media renewal together with the numbers of live and dead F1 daphnia. The number or discarded unhatched eggs |

was also determined at this time.

Chemical analysis: Water samples of fresh media were taken on Day 0 and expired media were taken on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21 from each test and solvent control vessel. Analysis by HPLC.

Data analysis: One way Analysis of Variance ( $F_{\max}$  test for homogeneity of variance)

**Results:**

Measured test concentrations were in the range 51 – 174% of the nominal values. Arithmetic mean measured concentrations were 0.168, 0.486, 1.65, 5.24 and 16.3 mg/L (86.8 – 93.5% of nominal). The results were based on nominal test concentrations.

The biological results are summarised in the table below.

**Table 167: Summary of effects on survival and reproductive parameters of Thiophanate-methyl to *Daphnia magna*.**

| Nominal test concentration (mg/L) | % survival of P1 | % adults with eggs in the brood pouch (%) | No. of live young |            | No. of dead young |            | No. of unhatched eggs |            |
|-----------------------------------|------------------|---|-------------------|------------|-------------------|------------|-----------------------|------------|
|                                   |                  |   | Total             | Per female | Total             | Per female | Total                 | Per female |
| Control                           | 100              | 100                                       | 3011              | 75         | 3                 | <1         | 1                     | <1         |
| Solvent control                   | 100              | 100                                       | 2998              | 75         | 0                 | 0          | 3                     | <1         |
| 0.18                              | 100              | 100                                       | 2916              | 73         | 1                 | <1         | 14                    | <1         |
| 0.56                              | 100              | 100                                       | 73                | 2          | 0                 | 0          | 1723                  | 43         |
| 1.8                               | 98               | 100                                       | 0                 | 0          | 0                 | 0          | 1673                  | 43         |
| 5.6                               | 0                | 0   | 0                 | 0          | 0                 | 0          | 0                     | 0          |
| 18                                | 0                | 0   | 0                 | 0          | 0                 | 0          | 0                     | 0          |

There were no significant effects on reproduction of *Daphnia magna* at the test concentration of 0.18 mg/L after 21 days exposure as determined by one-way analysis of variance comparing the numbers of young produced per adult, from the solvent control and test groups.

Apart from the initial acute effect on the parental generation (48 hours  $EC_{50}$  for adult survival was reported to be 12.7 mg/L) no other adverse effects of exposure were observed during the study in those groups which survived through to maturation. All control groups and the 0.18 mg/L produced

significant numbers of young by Day 7 and all surviving *Daphnia* appeared similar in terms of size and condition.

A toxicological significant effect on reproduction was observed in the 0.56 and 1.8 mg/L test groups. Despite the adult generation surviving through to maturation and all the surviving adults in each group showing the presence of eggs in the brood pouch these eggs once shed failed to hatch resulting in significant numbers of unhatched eggs being recorded in these test groups. The numbers of adults showing the presence of eggs in the brood pouch in the 0.56 and 1.8 mg/L test groups was equal to that exhibited by the control groups.

Numbers of unhatched eggs and dead young were low in the controls and 0.18 mg/L test groups.

A NOEC was determined to 0.18 mg/L (nominal) based on the effects on reproduction at 0.56 mg/L and higher test concentrations.

Based on a re-evaluation of the endpoints from this study provided by the applicant during the evaluation (Wirzinger and Ruhnke, 2016, Doc. No. 882-009), the EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values for immobility were 1.63 (95%CL 1.36 – 1.89), 1.96 (95%CL 1.67 – 2.24) and 2.77 (95%CL 2.43 – 3.17) mg/L, respectively. For reproduction, the EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values were 0.190 (95%CL 0.181 – 0.199), 0.215 (95%CL 0.205 – 0.225) and 0.273 (95%CL 0.261 – 0.285) mg/L, respectively. All values were based on geometric mean measured test concentrations.

**RMS comments:**

The study was re-evaluated against the validity criteria in OECD TG 211 (2012):

- control mortality of parent animals (female *Daphnia*) does not exceed 20% at the end of the test;
- mean number of living offspring per parent animal surviving in the control at the end of the test is > 60.

These validity criteria were fulfilled, and the study is considered as still valid for risk assessment.

Since individual measured concentrations deviated more than 20% from the control (51 – 174%, see table below), the RMS proposes that the results are based on geometric mean measured concentrations, resulting in a NOEC of 0.16 mg/L.

Further, it was noted that at Day 0 and Day 21 of the test, levels of test compound was observed in the negative control, 0.036 and 0.043 mg/L, respectively. Given the low margin to the highest concentration with no observed effects, 0.16 mg/L, this may affect the reliability of the results. However, since the statistical analysis was made against the solvent control the results are considered as sufficiently robust.

**Table 168: Results of the analytical determination of Thiophanate-methyl concentrations in the control and in the lowest test level.**

| Nominal test concentration of Thiophanate-methyl [mg/L] | Day  | Measured concentration [mg/L] | %of nominal values    |
|---|------|-------------------------------|-----------------------|
| Control   | 0    | 0.036                         | -                     |
|   | 2    | <0.01                         | -                     |
|   | 4    | <0.01                         | -                     |
|   | 7    | <0.01                         | -                     |
|   | 9    | <0.01                         | -                     |
|   | 11   | <0.01                         | -                     |
|   | 14   | <0.01                         | -                     |
|   | 16   | <0.01                         | -                     |
|   | 18   | <0.01                         | -                     |
|   | 21   | 0.043                         | -                     |
|   | 0.18 | 0 (fresh)                     | 0.189 (geomean 0.160) |
| 2 (old)   |      | 0.138                         | 77                    |
| 4 (old)   |      | 0.148                         | 82                    |
| 7 (old)   |      | 0.144                         | 80                    |
| 9 (old)   |      | 0.183                         | 102                   |
| 11 (old)  |      | 0.092                         | 51                    |
| 14 (old)  |      | 0.156                         | 87                    |
| 16 (old)  |      | 0.184                         | 102                   |
| 18 (old)  |      | 0.314                         | 174                   |
| 21 (old)  |      | 0.135                         | 75                    |

**Reference:** Schäfers, C. (2007): *Daphnia magna* - Reproduction test (OECD 211) with aging test item - TOPSIN-M WDG  
Amendment: Schäfers 2016, Doc. No. 827-007

Report number: RD-01133, NIS-003/4-21 (827-006)

Guideline: OECD TG 211 (1998)

GLP: Yes

|                              |  |
|------------------------------|--|
| <b>Previous evaluation:</b>  | Submitted for the purpose of renewal.  |
| <hr/>                        |  |
| <b>Material and methods:</b> |  |
| Test Material:               | TOPSIN M WDG containing 70.5 % a.s.  |
| Lot/Batch #:                 | G000008868   |
| Test species:                | <i>Daphnia magna</i> STRAUS, 4 – 24 hours old.   |
| Treatments:                  | Control, 24, 43, 78, 140 and 252 µg formulation/L  |
| Control:                     | Dilution water   |
| Replication:                 | For each test concentration ten replicates with 1 daphnid each were exposed.   |
| Duration:                    | 21 days exposure; started at the peak of formed Carbendazim in the ageing solution.  |
| Test conditions:             | Semi-static, with a renewal of the test solutions three times weekly. Test vessels were 60 mL glass beakers, covered with a glass pane. The daphnids were fed during the test with suspensions of unicellular green algae.<br><br>Temperature: 20.5°C ± 1°C<br>Dissolved oxygen: 8.1 – 8.3 mg/L<br>pH: 7.71 – 8.65<br>Photoperiod: 16 h light : 8 h dark (600 – 700 lux)   |
| Observations:                | The following endpoints observed in the reproduction test were evaluated quantitatively: <ul style="list-style-type: none"><li>•Mortality (immobility) of parental generation daphnids</li><li>•Time to the first brood</li><li>•Total number of offspring per replicate</li><li>•Cumulative number of live offspring per surviving female at the time of recording</li><li>•Intrinsic rate of increase r</li></ul>  |
| Chemical analysis:           | Once weekly, samples were taken in old and fresh test medium from each vessel and pooled for replicates 1 to 5 and 6 to 10. The water samples were analysed for Thiophanate-methyl and the metabolite Carbendazim.   |
| Data analysis:               | For each endpoint, the NOEC, LOEC were determined. A LOEC was calculated by using ANOVA followed by Williams' test or Mann-Whitney U-test with Bonferroni correction as appropriate non-parametric test as suggested by the ToxRat program. As the test results for reproduction and intrinsic rate showed a concentration-response relationship, the data were analysed by regression to determine the EC <sub>50</sub> including the 95 % confidence interval as well as the EC <sub>10</sub> using Probit-analysis assuming log-normal distribution of the values by using a computer program (ToxRat). |

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**Results:***Analytical results*

The measured concentrations of Thiophanate-methyl and Carbendazim in the test vessels are presented in the table below. Thiophanate-methyl concentrations increased during the pre-treatment phase depending on the nominal concentration in the aging aquaria. During pre-exposure and exposure, the development of the formation rate of Carbendazim was similar in all aging aquaria, reaching equilibrium between 60 and 70 % of initial Thiophanate-methyl loadings around day 8, when the *Daphnia magna* reproduction test was started.

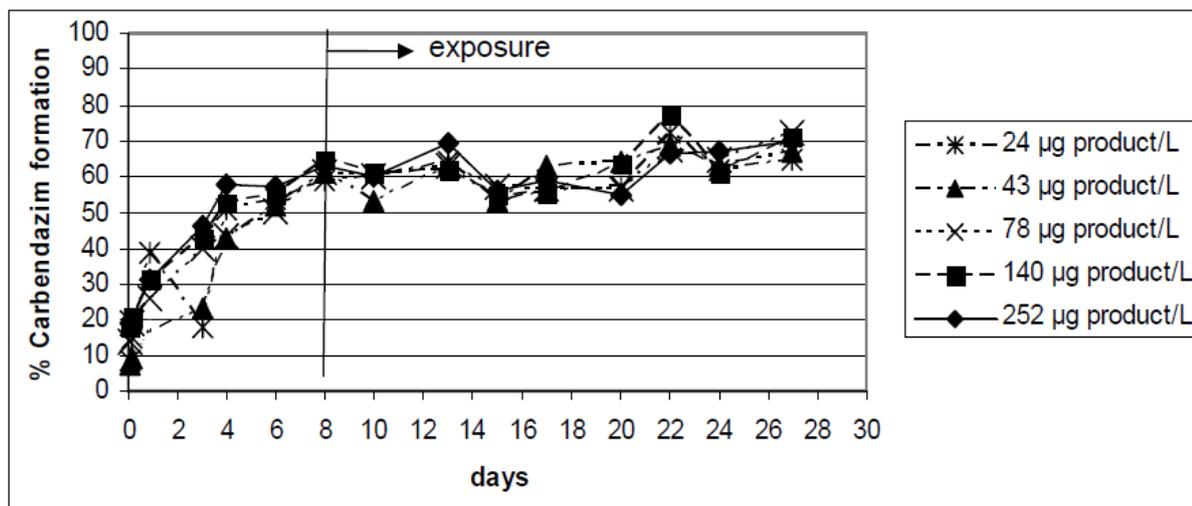


Figure 1: Formation rate of Carbendazim from Thiophanate-methyl (molar equivalents)

**Table 169: Analytical results for the determination of the concentrations of Carbendazim during the 21 d exposure period**

| Nominal Topsin concentration [µg/L] | Nominal Thiophanate-methyl concentration [µg/L] | Measured Thiophanate-methyl concentration at day 0 [µg/L] |       | Measured Carbendazim concentration during exposure [µg/L] |
|-------------------------------------|---|---|-------|---|
|                                     |   | Fresh   | Old   |   |
| 24                                  | 16.9  | 23.7  | 24.7  | 5.3±0.5   |
| 43                                  | 30.3  | 40.4  | 48.0  | 9.7±0.9   |
| 78                                  | 55.0  | 101.4   | 101.3 | 17.7±1.5  |
| 140                                 | 98.7  | 178.4   | 178.4 | 32.4±3.7  |
| 252                                 | 177.7   | 318.3   | 318.0 | 57.7±9.2  |

Fresh: taken from aging tanks before introduction into exposure vessels

Old: taken from exposure vessels immediately before exchange of medium

When re-calculating the applied concentrations of the formulation by adding the active substance equivalents generated by the Thiophanate-methyl and Carbendazim measurements and considering the active substance content of the product, concentrations of the formulation of the freshly prepared and the aged test solutions were 94 – 130 % and 103 – 130 % of nominal concentrations, respectively. The effect concentrations for Thiophanate-methyl were related to nominal test concentrations, which was proposed to be a conservative approach since measured concentrations were higher than the nominal in the day 0 samples. Effect concentrations for Carbendazim were based on mean measured concentrations over the exposure period of 21 days (day 8 – 28). For the latter, arithmetic means were used instead of geometric means, as there was variability due to dynamic equilibrium events or variability of sampling, rather than to dissipation.

All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within the ranges required by the guideline.

*Biological results*

All validity criteria for the performance of the controls were reported as fulfilled: Survival was above 80 %, the mean number of offspring per female over 21 days was above the criterion of 60. The dissolved oxygen concentration was always above 3 mg/L.

No treatment related mortality occurred up to and including the highest test concentration. Adult body length was not affected. Age at the first brood was significantly increased at the highest treatment. No offspring mortality occurred. The mean cumulative number of offspring showed a treatment-related reduction.

Effects on survival and reproduction are integrated by the intrinsic rate of population increase. The intrinsic rate of population increase was significantly reduced at the two highest test concentrations. The inhibition of reproduction measured as the reduction in the cumulative number of offspring per surviving female was the most sensitive endpoint.

A summary of the observed effects is presented in the table below.

**Table 170: Summary of effects on *Daphnia magna* exposed to TOPSIN M 70% WDG for 21 days**

| Nominal Topsisin conc. [ $\mu\text{g/L}$ ] | Mean conc. Thiophanate-methyl conc. [ $\mu\text{g/L}$ ] | Mean conc. Carbendazim [ $\mu\text{g/L}$ ] | Survival (%) | Growth (length day 21, mm) | Mean number of young per female | Age at first brood [days] | Intrinsic rate of population increase [ $\text{day}^{-1}$ ] | % effect mean number of young /female |
|--|---|--|--------------|----------------------------|---------------------------------|---------------------------|---|---------------------------------------|
| Control                                    | -   | -  | 90           | 4.80 $\pm$ 0.25            | 82.4 $\pm$ 19.0                 | 9.6 $\pm$ 0.6             | 0.31 $\pm$ 0.05   | -                                     |
| 24.0                                       | 6.4 $\pm$ 1.3   | 5.3 $\pm$ 0.5                              | 100          | 4.81 $\pm$ 0.44            | 88.7 $\pm$ 10.4                 | 9.5 $\pm$ 0.0             | 0.32 $\pm$ 0.02   | -7.6                                  |
| 43.0                                       | 9.8 $\pm$ 1.5   | 9.7 $\pm$ 0.9                              | 100          | 4.91 $\pm$ 0.40            | 76.4 $\pm$ 15.6                 | 9.7 $\pm$ 0.6             | 0.31 $\pm$ 0.03   | 7.3                                   |
| 78.0                                       | 37.3 $\pm$ 3.5  | 17.7 $\pm$ 1.5                             | 100          | 4.80 $\pm$ 0.43            | 73.6 $\pm$ 14.6                 | 10.4 $\pm$ 1.4            | 0.30 $\pm$ 0.03   | 10.7                                  |
| 140.0                                      | 63.2 $\pm$ 6.0  | 32.4 $\pm$ 3.7                             | 90           | 4.62 $\pm$ 0.30            | 55.8 $\pm$ 14.1*                | 10.8 $\pm$ 1.7            | 0.27 $\pm$ 0.05*  | 32.3*                                 |
| 252  | 113.0 $\pm$ 7.5   | 57.7 $\pm$ 5.3                             | 100          | 4.79 $\pm$ 0.27            | 38.7 $\pm$ 17.8*                | 14.3 $\pm$ 1.1*           | 0.22 $\pm$ 0.04*  | 53.1*                                 |

\* statistical difference to control based on multiple testing

The overall NOEC for the most sensitive endpoint (mean number of young per female) after 21 days was determined to be 78  $\mu\text{g}$  Topsisin/L, corresponding to 54  $\mu\text{g}$  Thiophanate-methyl/L (based on nominal, 37.3  $\mu\text{g/L}$  based on mean measured concentrations). The corresponding NOEC for Carbendazim was 17.7  $\mu\text{g/L}$  based on mean measured concentrations, and  $\text{EC}_{10}$  for the same endpoint was calculated to 14.9 (95% CL 9.9 – 18.9)  $\mu\text{g/L}$ .

#### RMS comments:

The validity criteria of the updated OECD TG 211 (2012) were fulfilled (parent mortality < 20% at the end of the test; mean number of living offspring produced per parent animal > 60. It should be noted that the test compound was a different formulation type than the representative formulation for this evaluation. Information on the composition of both formulations is included in Annex C and a comparison based on the aquatic toxicity of the two formulations was provided by the applicant (Sewell and Mullee, 2002; 822-005, cited by Nieslony and Heidemann, 2007; 881-009). It was concluded that the toxicity of active substance and the formulations is comparable and that the toxicity towards aquatic organisms results only from the active substance itself and not from the formulants within a certain formulation. The formulations revealed comparable acute toxicity, the WDG formulation being slightly higher in toxicity than the SC formulation. Consequently, it was proposed that the higher tier study with *Daphnia magna* which was performed with a Thiophanate-methyl containing WDG-formulation is also representative for the 500 SC formulation. This was tentatively accepted by the RMS, however, it should be noted that the analysis was based solely on comparison of acute toxicity and there may be an uncertainty in extrapolation to chronic toxicity. Member states are asked to give their views on this issue during the peer review.

## CLH REPORT FOR THIOPHANATE-METHYL

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Based on other available data on aquatic invertebrates, the toxicity of Thiophanate-methyl can be concluded to be lower than that of Carbendazim. The RMS would therefore propose that the effects observed in this test are likely due to the presence of Carbendazim.

The proposed NOEC values from this study were based on statistical significance, however, due to the observed non-statistically significant effects (10.7% reduction in number of young per female) at this treatment level it would be more appropriate to use the EC<sub>10</sub> as an alternative endpoint for the risk assessment. The EC<sub>10</sub> for the most sensitive endpoint (mean cumulative number of offspring) was 14.9 (95% CL 9.9 – 18.9) µg/L based on mean measured concentrations of Carbendazim during the exposure phase.

In order to support the use of the chronic *Daphnia magna* study (Schäfers, 2007, see above) conducted with TOPSIN-M WDG (70 % Thiophanate-methyl) for the risk assessment for Topsin M 500 SC (50 % Thiophanate-methyl), the acute *Daphnia magna* study with TOPSIN M 70 % WDG (70 % Thiophanate-methyl), cited by Nieslony & Heidemann (2007), was submitted by the applicant. A summary is given below.

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|                       |   |
|-----------------------|---|
| <b>Reference:</b>     | <b>Sewell, I.G., Mullee, D. M. (2002):</b> TOPSIN M 70% WDG: Acute Toxicity to <i>Daphnia magna</i> |
| <b>Report number:</b> | 235/402 (822-005)   |
| <b>Guideline:</b>     | OECD TG 202 (1984)  |
| <b>GLP:</b>           | Yes   |

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|                             |                                       |
|-----------------------------|---------------------------------------|
| <b>Previous evaluation:</b> | Submitted for the purpose of renewal. |
|-----------------------------|---------------------------------------|

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|                              |  |
|------------------------------|--|
| <b>Material and methods:</b> |  |
| Test Material:               | TOPSIN M WDG containing 71.8 % a.s.  |
| Lot/Batch #:                 | 540/0000004/13   |
| Test species:                | <i>Daphnia magna</i> , in-house culture <24 hours old.   |
| Treatments:                  | Daphnids were exposed to an aqueous dispersion of TOPSIN M 70% WDG at nominal concentrations of 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L |
| Control:                     | Freshwater   |
| Replication:                 | Duplicate vessels, each containing 10 first instars  |
| Duration:                    | 48 hours   |
| Test conditions:             | Semi-static.<br>Temperature: 21°C<br>Dissolved oxygen: 8.3 – 8.6 mg/L<br>pH: 7.5 – 7.8<br>Photoperiod: 16 h light : 8 h dark                           |
| Observations:                | The number of immobilized <i>Daphnia</i> was recorded after 24 and 48 hours.   |
| Chemical analysis:           | Measured concentrations of Thiophanate-methyl were determined at 0, 24 and 48 hours by chemical analysis.  |
| Data analysis:               | Probit analysis  |

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Results:

### Analytical results

The mean measured concentration of Thiophanate-methyl in the test vessels ranged from 83 to 106 % after test start and was between 60 and 92 % of nominal values after 24 hours. Analysis of the 24 h old preparations at 24 and 48 h showed measured concentrations in a range of 61 to 79 % and 53 to 100 % of nominal values, respectively. The variation in measured test concentrations was considered to be due to the problems associated with the sampling and analysis of dispersions rather than true solutions. Therefore, the test results were based on the nominal exposure concentrations.

**Table 171: Measured Thiophanate-methyl concentrations in the exposure solutions during the 48-hour exposure of *Daphnia magna* to Topsin M 70 % WDG**

| Nominal concentration<br>[mg product/L] | Measured concentration <sup>1</sup><br>[mg product/L] |             |                         |             |
|---|---|-------------|-------------------------|-------------|
|   | 0 hours   | 24 h (old)  | 24 h (new)              | 48 h (old)  |
| Thiophanate-methyl 70 % WDG             |   |             |                         |             |
| 0 (control)                             | < LOQ   | < LOQ       | < LOQ                   | < LOQ       |
| 0.10                                    | 0.088 (88)  | 0.0605 (61) | 0.0602 (60)             | 0.0525 (53) |
| 0.18                                    | 0.190 (106)   | 0.126 (70)  | 0.129 (72)              | 0.117 (65)  |
| 0.32                                    | 0.270 (84)  | 0.206 (64)  | 0.235 (73)              | 0.178 (56)  |
| 0.56                                    | 0.482 (86)  | 0.443 (79)  | 0.495 (79)              | 0.386 (69)  |
| 1.0                                     | 0.831 (83)  | 0.652 (65)  | 0.711 (88)              | 0.588 (59)  |
| 1.8                                     | 1.58 (88)   | 1.35 (75)   | 1.34 (75) / 1.66 (92) * | 1.24 (69)   |
| 3.2                                     | 2.97 (93)   | 2.46 (77)   | 2.66 (83)               | 2.16 (67)   |
| 5.6                                     | 4.98 (89)   | 4.27 (76)   | 3.91 (70) / 4.06 (72) * | 3.84 (69)   |
| 10                                      | 10.2 (102)  | 7.90 (79)   | 7.37 (74) / 8.33 (83) * | 10.0 (100)  |

<sup>1</sup> values in parentheses represent % of nominal concentration

\* analysis of frozen sample

Water quality parameters like temperature, dissolved oxygen and pH value were within ranges requested by guideline OECD 202.

Biological results

After 24 hours, there were no immobile daphnids in the control and in the test item treatment groups up to and including 3.2 mg product/L. After 48 hours, there were no immobile daphnids in the control and in the test item treatment groups up to and including 1.8 mg product/L.

**Table 172: Observed mortality of *Daphnia magna* exposed to a WDG formulation containing 70 % Thiophanate-methyl for 48 hours in a static acute test**

| Nominal Concentration<br>[mg product/L] | Number of <i>Daphnia magna</i> | Number immobilised<br>(% immobilised) |          |
|---|--------------------------------|---------------------------------------|----------|
|   |                                | 24 hr                                 | 48 hr    |
| Control                                 | 20                             | 0 (0)                                 | 0 (0)    |
| 0.10                                    | 20                             | 0 (0)                                 | 0 (0)    |
| 0.18                                    | 20                             | 0 (0)                                 | 0 (0)    |
| 0.32                                    | 20                             | 0 (0)                                 | 0 (0)    |
| 0.56                                    | 20                             | 0 (0)                                 | 0 (0)    |
| 1.0                                     | 20                             | 0 (0)                                 | 0 (0)    |
| 1.8                                     | 20                             | 0 (0)                                 | 0 (0)    |
| 3.2                                     | 20                             | 0 (0)                                 | 3 (15)   |
| 5.6                                     | 20                             | 4 (20)                                | 17 (85)  |
| 10                                      | 20                             | 10 (50)                               | 20 (100) |

The 48-hour no observed effect concentration (NOEC) of TOPSIN M 70% WG to first instar *Daphnia magna* was found to be 1.8 mg product/L; the 48-hour EC<sub>50</sub> was 4.2 mg product/L (95 % confidence intervals: 3.7 – 4.9 mg product/L), corresponding to 3.02 mg a.s./L (based on nominal concentrations).

**RMS comments**

The following validity criteria were met: not more than 10 % immobile daphnids in the control (actual 0 %); and the dissolved oxygen concentration at the end of the test should be  $\geq 3$  mg/L in control and test vessels (actual  $\geq 8.3$  mg/L). However, analytical measurements must be considered to be of low reliability due to problems associated with sampling of the dispersed test material. Although not providing any useful endpoint for the risk assessment, the study can be considered as supportive information for the study by Schäfers (2007). The results indicate that the tested WDG-formulation is also representative for the 500 SC formulation, however, it should be noted that the analysis was based solely on comparison of acute toxicity and there may be an uncertainty in extrapolation to chronic toxicity.

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|                   |  |
|-------------------|--|
| <b>Reference:</b> | <b>Memmert. U. (2002):</b> Effects of <sup>14</sup> C-Thiophanate-methyl on the development of sediment-dwelling larvae of <i>Chironomus riparius</i> in a water-sediment system |
| Report number:    | 831148. RD-II 02133 (828-001)  |
| Guideline:        | OECD Draft TG 219 (2001)   |
| GLP:              | Yes  |

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**Previous evaluation:** Addendum 7 to the Monograph (DAR), 2003.

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|                              |  |
|------------------------------|--|
| <b>Material and methods:</b> |  |
| Test material:               | Technical Thiophanate-methyl and phenyl-C <sup>14</sup> -labelled thiophanate-methyl   |
| Lot/Batch No:                | NSTM-98824 and EC-01-01  |
| Purity:                      | 99.93% and 96.9%, specific radioactivity 1.64 MBq/mg   |
| Species:                     | <i>Chironomus riparius</i> , first larval stage, 3 days after hatching.  |
| Treatments:                  | 0.125, 0.25, 0.50, 1.00, 2.00 mg/L (nominal), spiked water.  |
| Controls:                    | Reconstituted water, initial pH ca. 7.9  |
| Replication:                 | 20 larvae per test vessel, 3 replicates.   |
| Duration:                    | 28 days, 7 days after emergence of the last test animal in the control.  |
| Test conditions:             | Artificial sediment; 4% sphagnum peat, 20% kaolin clay, 76% sand, 0.26% CaCO <sub>3</sub> , total organic carbon 2% dry weight.<br><br>Glass beakers, 600 mL were used as test vessels. 130 g wet sediment (corresponding to 89 g dry sediment) and 250 mL test water was used per beaker. |

Water temperature 20.0 – 21.8°C, 16 hours light per day (light intensity 560 – 680 Lux). pH was in the range 6.0 – 8.0, and oxygen concentration 6.4 – 8.3 mg/L (>75% of saturation) throughout the test. Fish food was added three times per week.

Observations: Number of emerged adults and their sex was recorded daily from day 10 after application until test termination day 28. Number of visible pupae that had failed to emerge was counted separately for each beaker. Any other signs of intoxication was recorded.

Chemical analysis: Water, pore water and sediment was sampled in duplicate for analysis on day 0, 7 and 28 in the test. Radioactivity was measured by means of LSC, HPLC and TLC.

Data analysis: The emergence ratio and the development time and rate were calculated for each test vessel as the sum of fully emerged midges divided by the number of inserted larvae.

**Results:**

Measured test concentrations in the water phase were 87 – 89% of the nominal values after 1 hour, 6 – 17% after 7 days, and 0 – 2% after 28 days. The results were based on nominal test concentrations.

**Table 173: Mean measured concentrations at different time points during the test.**

| Nominal (µg a.s./L) | Fraction | Day 0 measured (% of applied)         | Day 7 measured (% of applied) | Day 28 measured (% of applied)       |
|---------------------|----------|---------------------------------------|-------------------------------|--------------------------------------|
| 500                 | Parent   | A: 87.2<br>B: n.a.<br>C: n.a.         | A: 6.2<br>B: n.a.<br>C: 2.3   | A: not detected<br>B: n.a.<br>C: 4.8 |
| 2000                |          | A: 88.7<br>B: n.a.<br>C: n.a.         | A: 17.2<br>B: n.a.<br>C: 4.6  | A: 1.5<br>B: n.a.<br>C: 4.5          |
| 500                 | CF-27    | A: not detected<br>B: n.a.<br>C: n.a. | A: 44.7<br>B: n.a.<br>C: 35.7 | A: 6.0<br>B: n.a.<br>C: 27.6         |
| 2000                |          | A: not detected<br>B: n.a.<br>C: n.a. | A: 24.8<br>B: n.a.<br>C: 20.8 | A: 7.4<br>B: n.a.<br>C: 21.3         |
| 500                 | 2-AB     | A: not detected                       | A: not detected               | A: 7.9                               |

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|      |                  |                                       |   |   |
|------|------------------|---------------------------------------|---|---|
|      |                  | B: n.a.<br>C: n.a.                    | B: n.a.<br>C: not detected                    | B: n.a.<br>C: 1.3                             |
| 2000 |                  | A: not detected<br>B: n.a.<br>C: n.a. | A: 7.5<br>B: n.a.<br>C: 2.8                   | A: 5.4<br>B: n.a.<br>C: 5.7                   |
| 500  | M2 unknown       | A: not detected<br>B: n.a.<br>C: n.a. | A: not detected<br>B: n.a.<br>C: not detected | A: not detected<br>B: n.a.<br>C: not detected |
| 2000 |                  | A: not detected<br>B: n.a.<br>C: n.a. | A: not detected<br>B: n.a.<br>C: 0.6          | A: not detected<br>B: n.a.<br>C: 0.9          |
| 500  | M4 impurity      | A: 1.8<br>B: n.a.<br>C: n.a.          | A: not detected<br>B: n.a.<br>C: not detected | A: not detected<br>B: n.a.<br>C: 1.3          |
| 2000 |                  | A: 1.4<br>B: n.a.<br>C: n.a.          | A: 1.2<br>B: n.a.<br>C: 2.2                   | A: not detected<br>B: n.a.<br>C: 1.6          |
| 500  | Non-extractables | 1.4                                   | 10.7  | 46.9  |
| 2000 |                  | 1.9                                   | 7.4   | 39.5  |

A: water phase

B: sediment pore water

C: sediment extract

**Table 174: Summary of results on emergence and development of *Chironomus riparius*, mean of four replicates.**

| Nominal test concentrations (ug/L)                     | Control | 125 | 250 | 500  | 1000 | 2000 |
|--|---------|-----|-----|------|------|------|
| Initial measured (ug/L)                                |         |     |     | 436  |      | 1774 |
| <b>Number of emerged midges during the test period</b> |         |     |     |      |      |      |
| Sum of males   | 7.5     | 9.8 | 10  | 9.3  | 12.5 | 0    |
| Sum of females   | 11.3    | 9.3 | 9.0 | 10.3 | 3.4  | 0    |

| Nominal test concentrations (ug/L)                     | Control           | 125               | 250               | 500               | 1000              | 2000 |
|--|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| Initial measured (ug/L)                                |                   |                   |                   | 436               |                   | 1774 |
| <b>Number of emerged midges during the test period</b> |                   |                   |                   |                   |                   |      |
| Sum of both sexes                                      | 18.8              | 19.0              | 19.0              | 19.5              | 16.3              | 0    |
| <b>Emergence ratio</b>                                 |                   |                   |                   |                   |                   |      |
| Sum of inserted larvae per treatment                   | 80                | 80                | 80                | 80                | 80                | 80   |
| Sum of emerged midges per treatment                    | 75                | 76                | 76                | 78                | 65                | 0    |
| % of emerged midges per treatment (mean)               | 94                | 95                | 95                | 98                | 81                | 0    |
| % of control   | 100               | 104               | 104               | 107               | 84*               | 0*   |
| <b>Development rate per treatment (d<sup>-1</sup>)</b> |                   |                   |                   |                   |                   |      |
| Males  | 0.066<br>(0.0017) | 0.064<br>(0.0035) | 0.063<br>(0.0019) | 0.065<br>(0.0039) | 0.063<br>(0.0003) | n.a. |
| Females  | 0.062<br>(0.0027) | 0.058<br>(0.0024) | 0.059<br>(0.0017) | 0.061<br>(0.0006) | 0.057<br>(0.0085) | n.a. |

\*statistically significant based on William's test ( $\alpha=0.05$ , one-sided smaller)

Based on the results presented above and the nominal concentrations, a 28 d NOEC of 1.0 mg a.s./L was obtained for effects on development and the NOEC for effects on emergence was 0.5 mg a.s./L. The EC<sub>15</sub> values for emergence ratio and development rate were determined to be 0.952 and > 1.0 mg a.s./L, respectively.

The EC<sub>10</sub> and EC<sub>20</sub> values were not reported, but the EC<sub>15</sub> values were determined. However, the NOEC is the relevant endpoint for the chronic risk assessment for chironomids.

#### RMS comments:

The study fulfilled the validity criteria of OECD TG 219 (2004):

- emergence in the controls > 70% at the end of the test
- emergence to adults from control vessels between 12 and 23 days after their insertion into the vessels
- oxygen concentration > 60% of the air saturation value, and the pH of overlying water in the range 6-9; the water temperature should not differ by more than  $\pm 1.0$  °C.

The results were based on the nominal concentrations in the water phase, although the levels declined to below 20% within a week. Although this is consistent with OECD TG 219, it is acknowledged that the toxicity might be under-estimated, especially since the major exposure route for *Chironomus* is considered to be via the water phase. The RMS proposes that the measured initial concentration in the water phase is used, resulting in a NOEC of 0.44 mg a.s./L.

**Author:** Kelly, C., Thirkettle, K., Smith, B., Graham, F.H.  
**Title:** Carbendazim technical prolonged toxicity to *Daphnia magna*  
**Date:** 15 July 1997  
**Doc ID:** SNG 80/970692; BVL No. 1840691  
**Guidelines:** OECD Guideline 202  
**GLP:** Yes  
**Validity:** Yes

Semi static chronic toxicity test with *Daphnia magna* (Straus)

**Methods**

Carbendazim technical, purity 99,5 %

Exposure mode: semi static, solutions were renewed three times per week

Nominal test concentrations:

0, 0.0018, 0.0056, 0.018, 0.056, 0.18 mg/L

**Results**

Measured test concentrations in the test were:

0, 0.0015, 0.0046, 0.015, 0.045, 0.19 (81 – 106 % recovery)

The carbendazim concentration-response curve regarding the effects on the reproductive performance of *Daphnia magna* shows in the lower concentration range tested (< 0.015 mg/L) a gentle slope (control = 71 juvenile/adult; 0.0015 = -13 %; 0.0046 = -21 %; 0.015 = -23 %). When exposed to the next tested concentration (0.045 mg carbendazim/L), *Daphnia magna* reproduction was reduced by 94 % of control values (5 juveniles/adult). At 0.19 mg/L the mortality was 100% and hence no reproduction could be assessed.

**Table 175: Chronic toxicity endpoints for *Daphnia magna* exposed to carbendazim**

| Endpoint                        | Value (mg/L) | 95 % confidence interval (mg/L) | Time (d) | ai | n/r/i |
|---------------------------------|--------------|---------------------------------|----------|----|-------|
| NOEC reproduction               | 0.0015       |                                 | 21       |    | r     |
| EC <sub>50</sub> reproduction   | 0.025        | 0.019 – 0.032                   | 21       |    | r     |
| EC <sub>50</sub> Immobilisation | 0.074        | 0.048 – 0.111                   | 21       |    | r     |

The acute toxicity of carbendazim was additionally assessed at 48 h after start of the test. The EC<sub>50</sub> (48 h) for *Daphnia magna* was 0.092 mg carbendazim/L (0.045 - 0.19 mg/L), pointing at a very acute toxic effect of

carbendazim: the difference to the 21 d values are minimal and at 0.019 mg/L the observed 100% mortality could be observed already 2 days after beginning of exposure.

### Conclusion

Report results listed and evaluated in the Addendum 8 to the monograph (03 May 2004). The NOEC concerning the reproduction performance of *Daphnia magna* exposed to carbendazim in a chronic test was 0.0015 mg/L. EC<sub>50</sub> (21 d) reproduction = 0.0025 mg carbendazim/L (0.019 – 0.032 mg/L) and EC<sub>50</sub> (21 d) immobilisation = 0.074 mg/L (0.048 – 0.011 mg/L).

**Author:** Sowig, P., Gosch, H.  
**Title:** Chronic toxicity to the sediment dwelling chironomid larvae *Chironomus riparius* - Carbendazim; water miscible suspension  
**Date:** 25 Apr 2002  
**Doc ID:** C018793; BVL No. 1758317  
**Guidelines:** OECD Draft Guideline No 219 (Draft February 2001)  
**GLP:** Yes  
**Validity:** Yes

### Methods

Test substance: Formulation AE F017411 00 SC42 A208, Carbendazim 41.6 %  
 Guideline: OECD Draft Guideline No 219 (Draft February 2001)  
 Test species: *Chironomus riparius*  
 Exposure mode: Spiked water  
 Concentration levels, nominal:  
 0, 0.001, 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032 mg/L

### Results

Measured concentration levels in the water phase: recovery between 69.4 - 74.8 % related to nominal concentrations. Since dissipation of the test item from the water phase to the sediment is to be expected in water-sediment systems, the biological results are based on initial nominal figures.

**Table 176: Chronic toxicity of carbendazim to the sediment dweller *Chironomus riparius***

| Endpoint       | Value (mg/L) | time | ai | n/r/i |
|----------------|--------------|------|----|-------|
| NOEC emergence | 0.032        | 28 d |    | n     |
| LOEC emergence | ≥ 0.032      | 28 d |    | n     |

n = nominal; r = real; i = initial

### Conclusion

The NOEC for *Chironomus riparius* exposed to carbendazim in a 28 d chronic toxicity test was 0.032 mg /L, corresponding to 0.0133 mg Carbendazim/L.

### 11.6.3 Chronic toxicity to algae or other aquatic plants

See section 11.5.3 above.

### 11.6.4 Chronic toxicity to other aquatic organisms

No further data available.

## 11.7 Comparison with the CLP criteria

### 11.7.1 Acute aquatic hazard

According to the CLP criteria a substance should be assigned to hazard class Aquatic Acute 1 (H400) if EC<sub>50</sub> of the most sensitive organism is lower than 1 mg/L. According to the conclusions above (section 11.5) this is considered fulfilled for thiophanate-methyl including its major environmental metabolite carbendazim. This metabolite was formed as 82.8% after 14 days in study on biodegradation in surface water and as maximum 81.6% after 8 days in study on biodegradation in water/sediment systems. In both test systems (water-only and water/sediment) carbendazim could be observed as >10% already after 1 day. It was also found as maximum 75.6% (day 7) in studies on biodegradation in soil. Carbendazim was also a major product formed in abiotic studies (58.7% after 4 days in hydrolysis study at alkaline pH 9, 22°C, and 49.7% after 5.5 days of exposure to natural sunlight in Japan, mid-December).

The lowest acute toxicity value for the parent compound thiophanate-methyl was 4.4 mg/L, determined with *Daphnia magna*. However, the lowest acute toxicity value for the major metabolite carbendazim was 0.019 mg/L, determined with the fish species *Ictalurus punctatus*. As the EC<sub>50</sub> is between 0.01 and 0.1 mg/L, a M-factor of 10 according to Regulation EC 1272/2008 is proposed.

### 11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Based on available data relevant for classification (section 11.1) it can be concluded that thiophanate-methyl and its major metabolite carbendazim are not rapidly degraded in the aquatic environment. In a ready biodegradability test in accordance with OECD 301C, 4% was biodegraded in 28 days. In biodegradation studies in surface water, in water/sediment systems and in soil, thiophanate-methyl was transformed with half-lives from less than one day up to a few days, while carbendazim was more persistent with half-lives of 64.8 days (water only), 15.1-91.6 days (water/sediment) and 22.0-63.2 days (soil). Carbendazim was found as 82.8% after 14 days in study on biodegradation in surface water, as maximum 81.6% after 8 days in study on biodegradation in water/sediment systems, and as maximum 75.6% after 7 days in studies in soil. According to the CLP criteria a substance should be assigned to hazard class Aquatic Chronic 1 (H410) if NOEC or EC<sub>x</sub> of the most sensitive organism is lower than 0.1 mg/L. According to the conclusions above this is considered fulfilled for thiophanate-methyl including its major environmental metabolite carbendazim.

Based on the available data, the substance has a low potential for bioaccumulation.

The lowest chronic toxicity value for the parent compound thiophanate-methyl was the NOEC = 0.16 mg/L, determined with *Daphnia magna*. However, the lowest chronic toxicity value for the major metabolite carbendazim was the NOEC = 0.0015 mg/L, determined with *Daphnia magna*. As

the NOEC is between 0.001 and 0.01 mg/L and thiophanate-methyl does not fulfil the criteria for rapid degradation, a M-factor of 10 according to Regulation EC 1272/2008 is proposed.

## 11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The suggested classification for thiophanate-methyl is Acute Category 1 (H400), M-factor 10 and Chronic Category 1 (H410), M-factor 10.

## 12 EVALUATION OF ADDITIONAL HAZARDS

### 12.1 Hazardous to the ozone layer

No available data.

## 13 ADDITIONAL LABELLING

Not relevant.

## 14 REFERENCES

See non-confidential Annex 2 and confidential Annex 3.

In addition to the references included in the annex, study summaries for the references in the below table were derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany in their re-evaluation of carbendazim (major metabolite to thiophanate-methyl) under Regulation 1107/2009.

| Data point                  | Author(s)  | Year | Title<br>Company Report No<br>Source (where different from<br>company)<br>GLP or GEP status<br>Published or not  | Vertebrate<br>study<br><br>Y/N | Data Protec-<br>tion<br>Claimed<br><br>Y/N | Justification if<br>data protection is<br>claimed | Owner* |
|-----------------------------|--|------|--|--------------------------------|--|---|--------|
| KIIA 7.1.1,<br>7.2.1, 7.2.2 | Otto, S.   | 1975 | Verhalten des<br>Pflanzenschutzmittelwirkstoffes<br>im Boden<br>k.A. ,<br>A22991<br>GLP: N,<br>published: N  | N                              | Y  |   | CTF    |
| KIIA 7.1.1,<br>7.2.1, 7.2.2 | Gildemeister<br>H.; Jordan H.<br>J.; Remmert<br>U. | 1981 | Behaviour of the plant protection<br>product Hoe 17411 OF AT 102<br>(carbendazim) in soil SS 2.2 at<br>15°C, 20°C and 25°C<br>k.A. ,<br>A47457<br>GLP: N,<br>published: N  | N                              | Y  |   | CTF    |
| KIIA 7.8.3                  | Knoch E.   | 2001 | Degradability and fate of (U-14C-<br>phenyl) car-bendazim in the<br>aquatic environment (wa-<br>ter/sediment system)<br>Aventis CropScience GmbH,<br>DEU, Oekochem-ie, Frankfurt ,<br>C017201<br>GLP: Y,<br>published: N | N                              | Y  |   | CTF    |

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| Data point  | Author(s)   | Year  | Title<br>Company Report No<br>Source (where different from<br>company)<br>GLP or GEP status<br>Published or not   | Verte-<br>brate<br>study<br><br>Y/N | Data<br>Protec-<br>tion<br>Claimed<br><br>Y/N | Justification if<br>data protection is<br>claimed | Owner* |
|---|-------------|-------|---|-------------------------------------|---|---|--------|
| KIIIA1 9.4  | Zillgens B. | 2007a | Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model Maker™ 4.0<br>E.I. du Pont de Nemours and Company,<br>DuPont-21656<br>GLP: N,<br>published: N                             | N                                   | Y   |   | CTF    |
| KIIIA1 9.7  | Zillgens B. | 2007c | Predicted environmental concentrations of carbendazim in surface water and sediment associated with uses in arable crops: Modeling for the European Union<br>E.I. du Pont de Nemours and Company ,<br>DuPont-23452<br>GLP: N,<br>published: N                   | N                                   | Y   |   | CTF    |
| IIA<br>8.2.1/02,<br>IIA<br>8.2.4/02,<br>IIA<br>8.2.6/02 | xxxx        | 1984  | Toxicological Studies of Benomyl and Carbendazim in Fish<br>A30119<br>GLP: not applicable<br>Published: yes<br>1758267  | Y                                   | Y   |   | CTF    |
| IIA<br>8.2.1/03   | xxxx        | 1976  | Acute (96-hour) toxicity of H-9910 to rainbow trout<br>A52914, Report no: HLO486-76<br>GLP: not applicable<br>Published: no<br>1840602  | Y                                   | N   |   | AVO    |
| IIA<br>8.2.3/01   | xxxx        | 1989  | The effect of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to <i>Salmo gairdneri</i> (Rainbow trout) in a 21-day Prolonged Toxicity Test (method OECD) A40788, Report no: CE89/021<br>GLP: yes<br>Published: no<br>1758283 | Y                                   | Y   |   | CTF    |

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| Data point      | Author(s)   | Year | Title<br>Company Report No<br>Source (where different from<br>company)<br>GLP or GEP status<br>Published or not   | Verte-<br>brate<br>study<br><br>Y/N | Data<br>Protec-<br>tion<br>Claimed<br><br>Y/N | Justification if<br>data protection is<br>claimed | Owner* |
|-----------------|---|------|---|-------------------------------------|---|---|--------|
| IIA<br>8.3.1/03 | Fischer, R.   | 1988 | The Effect of Carbendazim -<br>substance, technical<br>(Identification code: Hoe<br>017411 OF ZD99 0010) to<br><i>Daphnia magna</i> (Waterflea) in<br>a Static-Acute Toxicity<br>A39285; Report no:<br>OEK88/091E<br>GLP: yes<br>Published: no<br>1758297 | N                                   | Y   |   | AVO    |
| IIA<br>8.3.2/05 | Kelly,C.,<br>Thirkettle,<br>K., Smith,<br>B., Graham,<br>F.H. | 1997 | Carbendazim technical<br>prolonged toxicity to <i>Daphnia<br/>magna</i><br>SNG 80/970692<br>GLP: yes<br>Published: no<br>1840691  | N                                   | Y   |   | SIN    |
| IIA 8.4/04      | Bell, G.  | 1996 | Carbendazim technical: Algal<br>growth inhibiton<br>SNG 44(a)/960463<br>GLP: yes<br>Published: no<br>1843621  | N                                   | Y   |   | SIN    |
| IIA<br>8.5.2/01 | Sowig, P.,<br>Gosch, H.                                       | 2002 | Chronic toxicity to the<br>sediment dwelling chironomid<br>larvae <i>Chironomus riparius</i> -<br>Carbendazim; water miscible<br>suspension<br>C018793<br>GLP: yes<br>Published: no<br>1758317  | N                                   | Y   |   | CTF    |

\* CTF = Carbendazim Task Force (DuPont de Nemours (Deutschland), BASF Aktiengesellschaft, Bayer CropScience AG), according to information in Draft Re-Assessment Report (dated July, 2009) produced by Germany.

AVO = Hoechst Schering AgrEvo GmbH

SIN = Sinon EU Corporation

## 15 ANNEXES

Annex 1 Detailed study summaries on the toxicity data referred to in this CLH report

Annex 2 References: List of studies relied upon

Annex 3 CONFIDENTIAL References: List of vertebrate studies relied upon

Annex 4 CONFIDENTIAL Detailed information on tested formulated products.