Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

thiencarbazone-methyl (ISO); methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonylsulfamoyl]-5-methylthiophene-3-carboxylate

EC Number: -
CAS Number: 317815-83-1

CLH-O-0000001412-86-244/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Thiencarbazone-methyl (ISO)

EC Number: **Not assigned**

CAS Number: **317815-83-1**

Index Number: **Not assigned**

Contact details for dossier submitter: **UK Competent Authority**
Chemicals Regulation Directorate
Health and Safety Executive
United Kingdom

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Thiencarbazone-methyl (ISO)***
EC number:	***None assigned***
CAS number:	***317815-83-1***
Annex VI Index number:	***None assigned***
Degree of purity:	***≥ 95 %***
Impurities:	***The active substance contains a number of impurities. These have been taken into consideration in the CLH proposal and are not considered to be relevant for the classification and labelling. Further information is provided in the technical dossier.***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not currently listed
Current proposal for consideration by RAC	Aquatic Acute 1; H400 – Very toxic to aquatic life Acute M factor = 1000 Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects Chronic M factor = 1000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1; H400 – Very toxic to aquatic life Acute M factor = 1000 Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects Chronic M factor = 1000

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification according to the CLP Regulation

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

3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable		conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Not applicable
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 - Very toxic to aquatic life** **Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects**	**M = 1000** **M = 1000**	Not classified	-conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	No data

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

Labelling:

Pictogram(s): GHS09

Signal word: Warning

Hazard statements: H410 – Very toxic to aquatic life with long lasting effects

Precautionary statements: Not included in Annex VI

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Thiencarbazone-methyl is a pesticidal active substance considered under Directive 91/414/EEC. EFSA first considered the Draft Assessment Report in 2008, but Annex I listing wasn't agreed until 2013. Thiencarbazone-methyl does not have an existing entry on Annex VI of CLP and has not previously been considered in the harmonised classification and labelling process.

At the time of submission the substance has not been registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Thiencarbazone-methyl does not meet the criteria for classification for physical hazards.

It does not meet the criteria for classification for acute toxicity via the oral, dermal or inhalation routes. It does not meet the criteria for classification as a skin irritant/corrosive, eye irritant, skin sensitiser or respiratory sensitiser.

The short-term oral toxicity of thiencarbazone-methyl was investigated in the rat, mouse and dog and was found to be of relatively low toxicity in all three species. The urothelium was identified as the primary target of thiencarbazone-methyl toxicity in all three species investigated; treatment-related findings were apparent in the urinary bladder in all species. In the rat, associated renal findings were also present. The mechanism of toxicity appears to be the deposition of thiencarbazone-methyl crystals in the urine at high dietary concentrations, resulting in urolithiasis.

Urolithiasis in the urinary bladder causes local irritation, inflammation and hyperplasia of the transitional epithelium; similar effects are also seen in the kidney. No classification with STOT-RE is proposed.

Similar effects on the kidney and urinary bladder were observed in rodents after long-term toxicity testing (irritation, inflammation and hyperplasia). In addition, metaplasia and urothelial tumours in mice were also seen. The EFSA expert group (EFSA Journal 2013;11(7):3270) could not reach a consensus on the relevance of the transitional cell tumours observed in mice (urinary bladder and prostatic urethra) or the possibility of classification of thiencarbazone-methyl for carcinogenicity. It is concluded here that the findings in mice are not considered to be relevant to humans and therefore **no classification for carcinogenicity** is proposed.

Thiencarbazone-methyl produced no evidence of reproductive toxicity when tested in a two-generation rat study. Slight reductions in fetal weight and increased incidences of skeletal variations, indicative of delayed ossification, were noted in the rat developmental toxicity study. However, only at the limit dose level of 1000 mg/kg bw/day and in the presence of maternal toxicity. In the rabbit, reduced pup weights and an increased incidence of runts were observed at the top dose level of 500 mg/kg bw/day; again in the presence of maternal toxicity. Overall, the criteria for classification for reproductive toxicity are not met.

Acute toxicity data are available on thiencarbazone-methyl for fish, invertebrates, algae and aquatic plants. Fish and invertebrates showed low sensitivity and, as expected for this herbicide, algae and aquatic plants are the most acutely sensitive groups. The most sensitive algal/diatom species tested was *Pseudokirchneriella subcapitata* with a 72-hour mean measured E_rC_{50} of 1.017 mg/L and a study on the aquatic macrophyte *Lemna gibba* gave a lower 7-day mean measured E_rC_{50} of 0.00131

mg/L. Reliable data are also available from a number of non-standard studies on other aquatic macrophytes. The lowest of these is a 14-day mean measured E_rC_{50} of 0.00094 mg/L for *Myriophyllum spicatum*, which is lower than the endpoint for *Lemna* and is in the range >0.0001 to ≤0.001 mg/L and therefore thiencarbazone-methyl should be classified as: **Aquatic Acute 1: H400 with an Acute M-factor of 1000.**

Chronic toxicity data are available on thiencarbazone-methyl for fish, invertebrates, algae and aquatic plants. Thiencarbazone-methyl again showed low toxicity to fish and invertebrates, with algae and aquatic plants the most chronically sensitive groups. The most sensitive algal/diatom species tested was *Pseudokirchneriella subcapitata* with a 72-hour mean measured NOE_rC of 0.0307 mg/L and the aquatic macrophyte *Lemna gibba* gave a lower 7-day mean measured NOE_rC of 0.00021 mg/L. Reliable data are also available from a number of non-standard studies on other aquatic macrophytes. The lowest chronic endpoint is a 14-day mean measured NOE_rC for *Potamogeton pectinatus*, which is lower than the endpoint for *Lemna* and is within the range >0.0001 to ≤0.001 mg/L. Therefore, since thiencarbazone-methyl is also considered 'not rapidly degradable', it should be classified as: **Aquatic Chronic category 1: H410 with a Chronic M-factor of 1000.**

2.3 Current harmonised classification and labelling

Not applicable, not currently listed on Annex VI of CLP.

2.4 Current self-classification and labelling

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

At the time of submission the following entries were included in the C&L Inventory.

Classification		Labelling			Number
Hazard Class/Cat Code	H-Statement Code	H-Statement Code	Supplementary H-Statement/Code	Pictograms/Signal Word	
Aquatic Chronic 1	H410	H410		GHS09 Wng	30
Aquatic Acute 1	H400	H400		GHS09 Wng	23
Aquatic Acute 1 Aquatic Chronic 1	H400 H410	H410		GHS09 Wng	1

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The purified substance is a white powder at room temperature. It does not have a characteristic odour. The melting point is at 205 °C followed by thermal decomposition in a temperature range of 210 °C – 335 °C with 491 J/g without any evidence of thermal or mechanical (friction, shock) sensitivity. There is no boiling point at atmospheric pressure.

Thiencarbazone-methyl is neither flammable nor pyrophoric, nor does it show any

exothermic reaction up to 401°C. There are no flammable gases in contact with water.

The vapour pressure of purified thiencarbazone-methyl is 8.8×10^{-14} Pa at 20 °C. The solubility of the substance is low and dependent on pH (172 mg/L at pH 4, 436 mg/L at pH 7).

With regard to oxidising properties, a test battery according to EU A.17 method was conducted because the first study was not conclusive. The test item had almost equal maximum burning rates as compared to the reference material and the use of an inert material (Kieselguhr) ignited and propagated combustion although the test item as well as the reference material alone failed to ignite. In a second study, the maximum burning rates of the test item/cellulose mixture were beneath those of the reference material. In an additional study it was shown that the test substance/cellulose mixture was not igniting in an inert atmosphere.

Summing up, the Dossier Submitter (DS) was of the opinion that thiencarbazone-methyl does not warrant classification for physical hazard endpoints.

Comments received during public consultation

No comments were received on physical hazards.

Assessment and comparison with the classification criteria

Explosive properties

Thiencarbazone-methyl showed thermal decomposition in a temperature range of 210 °C – 335 °C with energy 491 J/g in a preliminary DSC (differential scanning calorimetry) screen, but there was no evidence of shock, friction or thermal sensitivity according to EU A.14 test method. The EU A.14 test battery does not entirely cover the requirements of the CLP Regulation. However, the results proved negative in the three relevant key areas: behaviour to heat, shock and friction.

Thiencarbazone-methyl has two contiguous nitrogen atoms in the triazole ring associated with explosive properties, but its exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 °C. Therefore, the screening procedure does not identify thiencarbazone-methyl as a potential explosive and the classification (acceptance) procedure for the class of explosives (see Figure 2.1.2 of the CLP Regulation) does not need to be applied.

Flammability

Thiencarbazone-methyl tested with EEC A.10 method melted but did not ignite on exposure to a flame and therefore, the criteria for classification as a flammable solid are not met.

Oxidising properties

The outcome of two studies according to the EEC A.17 method are considered to be relevant for the classification for the endpoint of oxidising properties of solids. In the first study (Smeykal, 2005) oxidising properties of the substance could not clearly be excluded as the burning rate with 25 % thiencarbazone-methyl/cellulose mixture was quite equal to the burning rate of 55 % barium nitrate/cellulose reference material, and

75 % thiencarbazone-methyl/Kieselguhr mixture was found to ignite and propagate combustion. The second study (Smeykal, 2008) showed that the maximum burning rates of thiencarbazone-methyl/cellulose were beneath those of the reference material in all cases.

The unclear results in the first study conducted in 2005 are considered to be due to the sustained combustion of the test material rather than to an oxidising effect as thiencarbazone-methyl melted but failed to ignite with a flame.

Based on the second study conducted with the EEC A.17 method under an inert atmosphere, thiencarbazone-methyl does not meet the criteria for classification as an oxidising solid.

Self-reactive properties

No exothermic reaction was observed up to a maximum of 401 °C in the test conducted in accordance with the EEC A.16 method and a negative result was obtained in the test (UN Test N.4) using a 10 cm cube sample at 140 °C. Thus thiencarbazone-methyl does not meet the criteria for classification as a self-reactive substance.

Further, experience with handling and use indicates that the material is not pyrophoric and does not ignite in contact with water.

Summing up, **RAC concludes in agreement with the DS that thiencarbazone-methyl does not warrant classification for physical hazards**.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Thiencarbazone-methyl is a new pesticidal active substance considered under Directive 91/414/EEC. EFSA first considered the Draft Assessment Report in 2008, but Annex I listing wasn't agreed until 2013. The EFSA expert group (EFSA Journal 2013;11(7):3270) could not reach a consensus on the relevance of the transitional cell tumours observed in mice (urinary bladder and prostatic urethra) or the possibility of classification of thiencarbazone-methyl for carcinogenicity.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

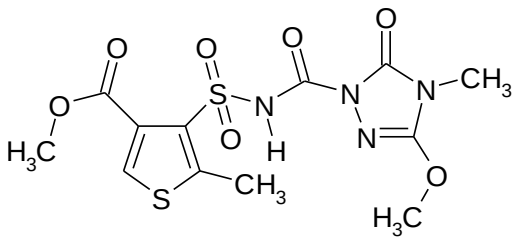
1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	None assigned
EC name:	None assigned
CAS number (EC inventory):	Not listed
CAS number:	317815-83-1
CAS name:	Methyl 4-[[[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate*
IUPAC name:	Methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1*H*- 1,2,4-triazol-1-yl)carbonylsulfamoyl]-5-methylthiophene-3-carboxylate*
CLP Annex VI Index number:	Not listed
Molecular formula:	C_{12} H_{14} N_4 O_7 S_2
Molecular weight range:	390.4

**As provided in the EFSA conclusion*

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Thiencarbazone-methyl	97.6 %	≥ 95 % - < 100 %	

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential refer to IUCLID			

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

Current Annex VI entry: Two of the impurities are listed in Annex VI of CLP. These have been taken into consideration and, given the concentration at which they are present and the available data on thiencarbazone-methyl, these are not considered to impact on the classification proposed in this dossier. Full information is provided in the confidential Annex and the IUCLID.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: None

1.2.1 Composition of test material

The composition of the material in the tested batches is considered to be equivalent to that outlined above.

1.3 Physico-chemical properties

All references are taken from the Draft Assessment Report (DAR) – Thiencarbazone-methyl – volume 3, Annex B.2: Physical and chemical properties. All studies were conducted to appropriate quality standards and are considered valid for classification.

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White crystalline powder	Wiche, A.; 2005 M-255522-01-1	Observation Purity 99.2 %
Melting/freezing point	205 °C	Olenik, B.; 2005 M-259111-01-1	EC A.1 (DSC) OECD 102 purity 99.2 %
Boiling point	Thermal decomposition observed from 231 – 310 °C	Olenik, B.; 2005 M-259111-01-1	EC A.2 (DSC) OECD 103 purity 99.2 %
Relative density	1.52	Bogdoll, B., Lemke,G.; 2005 M-258376-01-1	EC A.3 (pycnometer) OECD 109, OPPTS 830.7300 purity 96.3 %
Vapour pressure	Extrapolated: 8.8 x 10^{-14} Pa for 20 °C 3.7 x 10^{-13} Pa for 25 °C 2.3 x 10^{-10} Pa for 50 °C	Smeykal, H., 2005 M-258349-01-1	EC A.4 (effusion method) OECD 104 OPPTS 830.7950 purity 99.2 %
Surface tension	71.8 mN/m at 20 °C (90% saturated solution)	Bogdoll, B., Lemke, G.; 2005 M-248598-01-1	EC A.5 (OECD harmonised method) OECD 115 Purity 96.3 %
Water solubility	At 20 °C 172 mg/L at pH 4 436 mg/L at pH 7 417 mg/L at pH 9 --- In distilled water at 20 °C 72 mg/L at pH 3.9	Mühlberger, B., Eyrich, U.; 2004 M-127953-01-1 --- Mühlberger, B., Eyrich, U.; 2004 M-127953-01-1	EC A.6 (flask method) OECD 105 purity 99.3 % --- EC A.6 (flask method) OECD 105 purity 99.3 %

Partition coefficient n-octanol/water	-0.13 at pH 4 -1.98 at pH 7 -2.14 at pH 9	Mühlberger, B., Eyrich, U.; 2005 M-248402-01-1	EC A.8 (shake flask) OECD 107 Purity 99.3 %
Flash point	Not applicable substance is a solid with a melting point of 205 °C		
Flammability	The test substance melted but did not ignite on exposure to a flame. Experience with handling and use indicates that the material is not pyrophoric and does not ignite in contact with water.	Smeykal, H.; 2005 M-268423-01-1	EC A.10, OPPTS 830.6315 Purity 96.5 %
Explosive properties	Preliminary DSC screen gave exothermal decomposition in temperature range 210-335 °C with energy of 491 J/g. No evidence of thermal or mechanical (friction or shock) sensitivity in A14 study.	Smeykal, H.; 2005 M-268240-01-1	EC A.14, OPPTS 830.6316, OECD 113 Purity 96.5 %
Self-ignition temperature/Autoflammibility	No exothermic reaction observed up to 401 °C	Smeykal, H.; 2005 M-268841-01-1	EC A.16 Purity 96.5 %

Oxidising properties	The maximum burring rate of the test item/cellulose mixture was 1.08 mm/s, obtained with a 25 % test item/cellulose mixture. This was almost equal to the maximum burning rate (1.10 mm/s) for the reference material. Test item/Kieselguhr mixtures were found to ignite from 40% test item and propagate combustion from 60-80% test item/Kieselguhr ---------- Maximum burning rates of the test item/cellulose mixture were beneath those of the reference material. The test substance could not be ignited under an inert atmosphere.	Smeykal, H.; 2005 M-268594-01-1 ---------- Smeykal, H.; 2008 M-302554-01-1	EC A.17, OPPTS 830.6314 Purity 96.5% ---------- EC, A17 Purity 95.7 %
Granulometry	No information	-	-
Dissociation constant	pKa = 3.0	Wiche, A., Bogdoll,B.; 2005 M-256840-01-1	OECD 112 (Spectrophotometric method) purity 99.2 %
Viscosity	Not relevant, substance is a solid.		

2 MANUFACTURE AND USES

2.1 Manufacture

The substance is manufactured outside of the EU.

2.2 Identified uses

The substance is used as a pesticidal active substance (herbicide) within the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 Physical Hazards

Whilst a preliminary DSC screen gave exothermal decomposition in temperature range 210-335 °C with energy of 491 J/g, there was no evidence of thermal or mechanical (friction or shock) sensitivity in a full A14 study.

The test substance melted but did not ignite on exposure to a flame. Further, experience with handling and use indicates that the material is not pyrophoric and does not ignite in contact with water.

No exothermic reaction observed up to 401 ^{o}C in an autoflammability study conducted in accordance with A.16.

In the first study (conducted in accordance with A.17), the maximum burning rate of the test item/cellulose mixture was 1.08 mm/s, obtained with a 25% test item/cellulose mixture. This was almost equal to the maximum burning rate (1.10 mm/s) for the reference material. As the result was unclear, a further test with an inert material (Kieselguhr) was conducted. In this part of the study, test item/Kieselguhr mixtures were found to ignite and propagate combustion with a maximum burning rate of 1.29 mm/s with a 70% test substance/Kieselguhr mixture. The test item alone melted, but failed to ignite in contact with a flame. The reference material (55% barium nitrate)/Kiselguhr mixture failed to ignite.

In a second study (conducted in accordance with A.17), the maximum burning rates of the test item/cellulose mixture were beneath those of the reference material. In an additional study conducted with an inert atmosphere the test substance/cellulose mixture could not be ignited.

3.1.1 Summary and discussion of physico-chemical properties

See section 3.1

3.1.2 Comparison with criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. Whilst a preliminary DSC screen gave exothermal decomposition in temperature range 210-335 °C with energy of 491 J/g, there was no evidence of shock, friction or thermal sensitivity when thiencarbazone-methyl was tested in a standard explosivity study. Therefore, given that all results were negative, the criteria for classification are not met.

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. Thiencarbazone-methyl melted but did not ignite on exposure to a flame and therefore, the criteria for classification as a flammable solid are not met.

Experience in handling and use indicates that thiencarbazone-methyl is not pyrophoric and does not emit flammable gases on contact with water. Therefore, the criteria for classification in these hazard classes are not met.

A substance is classified as an oxidising solid when the burning time of a sample-to-cellulose mixture is less than or equal to the burning time of the appropriate reference sample. In an initial study, the maximum burning rate of the test item/cellulose mixture was 1.08 mm/s, obtained with a 25% test item/cellulose mixture. This was almost equal to the maximum burning rate (1.10 mm/s) obtained with 55% barium nitrate/cellulose reference material. As the result was unclear, a further test using an inert material (Kieselguhr) instead of cellulose was conducted. In this part of the study, test item/Kieselguhr mixtures were found to ignite and propagate combustion with a maximum burning rate of 1.29 mm/s with a 75% test item/Kieselguhr mixture. The test item alone melted, but failed to ignite with a flame. As Kieselguhr is an inert material, this indicates that the propagation was due to the sustained combustion of the test material (most likely resulting from the melted material soaking into the Kieselguhr or cellulose and creating a greater surface area) rather than an oxidising effect.

In a second study (conducted in accordance with A.17), the maximum burning rates of the test item/cellulose mixture were beneath those of the reference material in all cases. In an additional study, conducted under an inert atmosphere, the test substance/cellulose mixture could not be ignited. Considering the information from all studies, the criteria for classification are not met and the substance is not classified as an oxidising solid.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

4 HUMAN HEALTH HAZARD ASSESSMENT

A detailed summary of the available studies has been provided in the Draft Assessment Report (DAR) – Thiencarbazone-methyl – 2012 and relevant addenda 2013. The key information relevant to determining a classification position is presented below.

Three batches of thiencarbazone-methyl have been used for the human health assessment, with a purity ranging from 94.6-98.0%. These batches are considered to be representative of the technical material. The purity of each batch, as recorded in the study reports, is provided in the summary table for each study.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetics of thiencarbazone-methyl have been well investigated in rats following oral dosing aqueous tragacanth solution. An *in vitro* study in rats is also available investigating the dermal absorption of thiencarbazone-methyl, formulated as a suspension concentrate.

4.1.2 Human information

There is no human information available

4.1.3 Summary and discussion on toxicokinetics

Thiencarbazone-methyl was found to be rapidly absorbed (predominantly within 24 hours) following oral administration. In all dose groups, plasma C_{max} was attained within 1 hour of dosing. Absorption was found to be moderate and ranged between 42-55%. The distribution of radioactivity following dosing was rapid and relatively even, however slightly higher levels of radioactivity were found in the lungs and fat (with the thiophene label) or in the adrenals and thyroids (with the dihydrotriazole label). Quantitative autoradiography also identified persistent but low levels of radioactivity in the nasal mucosa for both radiolabel sites. Total tissue residues at 14 hours following dosing were <1% of the administered dose and do not indicate that thiencarbazone-methyl has the potential to bioaccumulate.

The metabolism of thiencarbazone-methyl was found to be limited, with 91-92% of the administered dose excreted as unchanged parent compound. Three minor metabolites radiolabelled on the thiophene ring were identified at levels of ≤2%. Five minor metabolites radiolabelled on the dihydrotriazole ring were also detected at levels of <1%; three of these metabolites were structurally characterised. The proposed metabolic pathway for thiencarbazone-methyl was initial hydrolysis of the urea group, releasing the thiophene-sulphonamide moiety. Hydrolysis of the methyl ester releases the sulphonamide-carboxylic acid that is subsequently cyclised to the thienosaccharine, following the formation of an intramolecular sulphonamide bond. A second metabolic path starts with the hydrolysis of thiencarbazone-methyl to form the MMT derivative. Demethylation of the MMT derivative forms the MMT derivative with subsequent cleavage of the triazolinone moiety to form methyl carbamate.

There were no marked gender-related differences in absorption, distribution, metabolism or excretion.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**

OECD TG 423 (2001) Rat, female Wistar, 3/group dosed in a stepwise manner (6 total) 2000 mg/kg Observation period: 14 days Purity 96.2% Vehicle: 2% Cremophor EL GLP	> 2000 mg/kg bw	No mortalities, clinical signs, effects on weight gain or gross pathological findings were observed. Anon.; 2004 Report No AT01452
OECD TG 423 (2001) Rat, female Wistar, 3/group dosed in a stepwise manner (6 total) 0, 2000 mg/kg Observation period: 14 days Purity 94.6% Vehicle: 2% Cremophor EL GLP	> 2000 mg/kg bw	No mortalities, clinical signs, effects on weight gain or gross pathological findings were observed. Anon.; *2*006 Report No AT03457
OECD TG 424 (1997) (acute neurotoxicity) Rat, Wistar, 12/sex/ group 0, 125, 500 and 2000 mg/kg (nominal) or 0, 131, 512 or 2180 mg/kg bw (actual) Observation period: 14 days Purity 96.1% Vehicle: 0.5% methylcellulose / 0.4% Tween 80 in deionized water GLP	> 2000 mg/kg	No evidence of specific neurotoxicity or neuropathology. 2000 mg/kg Red nasal staining (2 males and 1 female). Urine staining (1 male and 2 females) on days 1-4. White perigenital area (10 males and 5 females), white substance on bedding (7 males), white substance in urine collection tray (5 males and 3 females) and red substance in urine collection tray (1 female) Present on day 0. Decreased motor (43%) and locomotor (53%) activity in females on day 0 (resolved by the next observation on day 7) 500 mg/kg White perigenital area (6 males and 2 females) and white substance on bedding (8 males and 1 female), Present on day 0. 125 mg/kg No treatment related findings. Anon., 2006 Report No 201512

<table>
<tr><th colspan="3">Acute Inhalation</th></tr>
<tr><th>Method</th><th>LC50</th><th>Observations and remarks</th></tr>
<tr><td>OECD TG 403 (1981) (nose only)

Rat, Sprague-Dawley, 5/sex/group

1060, 2018 and 5158 mg/m^3, 4 hours (solid aerosol) nose only

<table><tr><th>Gravimetric concentration (mg/m^3)</th><th>1060</th><th>2017.5</th><th>5157.5</th></tr><tr><td>MMAD (µm)</td><td>6.28</td><td>2.35</td><td>17.56</td></tr><tr><td>GSD</td><td>3.04</td><td>1.88</td><td>2.73</td></tr><tr><td>Mass < 3 µm (%)</td><td>25.5</td><td>65.2</td><td>4.1</td></tr></table>
Observation period: 14 days

Purity 96.2%

GLP</td><td>2017.5 mg/m^3</td><td>No mortalities, clinical signs, effects on weight gain or gross pathological findings were observed at any of the test concentrations.

Anon.; 2004
Report No AT01473</td></tr>
<tr><th colspan="3">Acute Dermal</th></tr>
<tr><th>Method</th><th>LD50</th><th>Observations and remarks</th></tr>
<tr><td>OECD TG 402 (1987)

Rat, Wistar, 5/sex/group

2000 mg/kg

Observation period: 14 days

Purity 96.2%

Vehicle: test material dosed as received moistened with water

GLP</td><td>2000 mg/kg</td><td>No mortalities occurred; the only clinical sign observed was a partial reddening of the skin in one female from day 5 to day 7.

Anon., 2004
Report No AT01445</td></tr>
</table>

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Two standard acute toxicity studies (TG 423) were provided using thiencarbazone-methyl in a 2% Cremophor EL vehicle. No mortalities, clinical signs or gross abnormalities were observed in either study at a limit dose of 2000 mg/kg bw/day.

In an acute oral neurotoxicity study, treatment related findings were observed although these were limited to non-specific behavioural effects (reduced motor (43%) and locomotor (53%) activity on day 0) secondary to general toxicity at the time of peak effect in females at the highest dose level of 2180 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

In a 4-hour acute inhalation study the LC_{50} value was > 2017.5 mg/m^3. Although the highest concentration tested was 5158 mg/m^3 the majority of particles at this concentration were not of respirable size therefore could not be used to derive an LC_{50} value.

4.2.1.3 Acute toxicity: dermal

There was no evidence of systemic toxicity or mortalities at doses of up to 2000 mg/kg.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

There is no relevant information available.

4.2.3 Summary and discussion of acute toxicity

Thiencarbazone-methyl was found to be of low acute toxicity to the rat by the oral, dermal and inhalation routes. No treatment-related findings were observed in the acute oral toxicity study at the limit dose level of 2000 mg/kg bw. In an acute oral neurotoxicity study, treatment related findings were observed although these were limited to non-specific behavioural effects (reduced motor and locomotor activity) secondary to general toxicity at the time of peak effect in females at the highest dose level of 2180 mg/kg bw.

Treatment-related findings in the acute dermal toxicity were limited to minor bodyweights effects at the limit dose level of 2000 mg/kg bw. No treatment-related findings were observed at the highest concentration of 5158 mg/m^3 (5.158 mg/l) in the acute inhalation toxicity study, however the large particle size (MMAD 17.56 ±2.73 µm) at this concentration means that the majority of particles at this concentration are not of respirable size. The acute inhalation LC50 of thiencarbazone-methyl in the rat was found to be >2018 mg/m^3 (2.018 mg/l) under the conditions of this study. The absence of treatment-related findings at any concentration indicates that thiencarbazone-methyl should not be classified for acute inhalation toxicity according to current EC criteria.

4.2.4 Comparison with criteria

Via the oral route, the LD_{50} was > 2000 mg/kg bw. This is above the value for classification (i.e., 2000 mg/kg bw), therefore no classification is proposed.

Via the inhalation route the LC_{50} was > 2.02 mg/L (no deaths or clinical signs were reported in the study). As there are no data to indicate that the LC_{50} is ≤ 5.0 mg/L (the value for classification of dusts and mists) the criteria for classification are not met.

Via the dermal route, the LD_{50} was > 2000 mg/kg bw. This is above the value for classification (i.e., 2000 mg/kg bw), therefore no classification is proposed.

4.2.5 Conclusions on classification and labelling

Not classified. Conclusive but not sufficient for classification.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented five studies performed with thiencarbazone-methyl in accordance with OECD Test Guidelines (TG) and GLP (good laboratory practice) for acute toxicity: two OECD TG 423 studies via the oral route and one acute oral neurotoxicity study, one OECD TG 402 via the dermal route of exposure, and one OECD TG 403 study via the inhalation route. Based on the outcome of these studies, the DS concluded that thiencarbazone-methyl does not warrant classification for acute toxicity.

Comments received during public consultation

One Member State Competent Authority (MSCA) agreed with the DS to not classify thiencarbazone-methyl for acute toxicity.

Assessment and comparison with the classification criteria

Acute toxicity: oral

In each of the two acute oral toxicity studies on thiencarbazone-methyl (purity 94.6 to 96.2 %), similar to OECD TG 423 (Reports No AT01452 and No AT03457), two groups of three fasted female Wistar rats were given successively a single oral dose of 2 000 mg/kg bw of thiencarbazone-methyl. There were no mortalities, clinical signs, effects on weight gain or gross pathological findings. The oral LD_{50} was > 2 000 mg/kg bw.

In the short-term oral neurotoxicity study in rats (12/sex/dose), similar to OECD TG 424 (Report No 201512), a single dose of thiencarbazone-methyl was administered at doses of 0, 125, 500 and 2 000 mg/kg bw/d by gavage. There were no compound-related deaths at any dose level in either sex. No animals were found dead or sacrificed in extremis during the course of the study

Via the oral route, classification is required where the $LD_{50} \leq 2\,000$ mg/kg bw. Based on the rat acute oral toxicity studies and on the rat oral short-term neurotoxicity study, **RAC agrees with the DS that no classification for acute oral toxicity is warranted**.

Acute toxicity: dermal

In the acute dermal toxicity study in rats (Report No AT01445), carried out according to OECD TG 402, a dose of 2 000 mg/kg bw (purity 96.2 %) moistened test material (thiencarbazone-methyl) was administered as a single occluded dermal application to 10 % of each animal's body surface for 24 hours. The only clinical sign observed was a partial reddening of the skin in one female from day 5 to day 7.

Via the dermal route, classification is required where the LD_{50} is ≤ 2 000 mg/kg bw. The

LD_{50} was > 2 000 mg/kg bw. **RAC agrees with the DS that no classification is warranted for acute dermal toxicity**.

Acute toxicity: inhalation

In an acute inhalation study (Report No AT01473), Wistar rats were exposed by the inhalation route to thiencarbazone-methyl (96.2 % purity) in air for 4 hours (nose only) at concentrations of 1 060, 2 018 and 5 158 mg/m^3. The limit concentration of 5 000 mg/m^3 was attained, however, at the expense of larger particles (no cyclone was used). At 5 158 mg/m^3, the Mass Median Aerodynamic Diameter (MMAD) was 17.56 µm and only 4.1 % of particles had an aerosol mass < 3 µm. As the large majority of particles at this concentration were not of respirable size, this concentration (stated to be the maximum achievable) is not considered to be suitable for the derivation of the LC_{50} value. In order to achieve a particle size < 4 µm, the test was repeated at 2 000 mg/m^3 using the micronized test article and a cyclone. At 2 017.5 mg/m^3, the MMAD was 2.35 µm, and 65.2 % of particles had an aerosol mass < 3 µm. Animals were observed for the following 14 days. No mortality or treatment-related clinical signs occurred up to the maximum technically attainable concentration. No changes in the reflex behaviour were observed. The rectal temperature was not affected by the treatment. No treatment-related significant effects were noted on body weight evaluation. At necropsy no treatment-related findings were reported.

The acute inhalation LC_{50} of thiencarbazone-methyl in the rat was found to be > 2 018 mg/m^3 (2.018 mg/L; MMAD 2.35 ±1.88 µm) under the conditions of this study. However, the absence of treatment-related findings at any technically achievable concentration indicates that thiencarbazone-methyl does not warrant classification for acute inhalation toxicity according to the CLP criteria.

RAC concludes that thiencarbazone-methyl does not warrant classification for acute toxicity via the inhalation route.

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

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

The relevant animal data are summarised in Table 10. No human data are available.

4.3.2 Comparison with criteria

STOT-SE is considered when there is clear evidence of toxicity to a specific organ, especially when observed in the absence of lethality. Substances that produce significant non-lethal toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following single exposure are classified as STOT-SE 1 or 2 under the CLP Regulation.

Classification in STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

There was no clear evidence of any specific toxic effects on a target organ or tissue noted in any of the acute toxicity studies. Clinical signs of toxicity (reduced motor and locomotor activity) were observed after a single oral dose, but these were transient in nature and are considered to be unspecific signs of general acute toxicity. Urine and nasal stains, observed on day 0 resolved within five days after treatment and other findings in high- and mid-dose animals were attributed to the excretion of the parent compound, which did not represent a systemic effect. No classification for STOT-SE 1 or 2 under CLP is proposed.

No definitive signs of respiratory tract irritation or narcotic effects were observed, therefore no classification for STOT-SE 3 is proposed.

4.3.3 Conclusions on classification and labelling

Not classified. Conclusive but not sufficient for classification.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier Submitter's proposal The DS did not propose any classification for STOT SE 1 or 2 as no toxicity to a specific target organ was observed, neither in the acute toxicity studies in rats nor in the short-term oral neurotoxicity rat study. In each of these GLP-compliant studies in accordance with OECD TG, thiencarbazone-methyl (purity 94.6-96.2 %) was administered. In addition, the DS did not propose to classify thiencarbazone-methyl as STOT SE 3 for narcotic effects or respiratory tract irritation considering that no such effects were observed.

Comments received during public consultation

No comments were received for STOT SE.

Assessment and comparison with the classification criteria

In the short-term oral neurotoxicity study in rats (12/sex/dose), similar to OECD TG 424 (Report No 201512), a single dose of thiencarbazone-methyl was administered at doses of 0, 125, 500 and 2 000 mg/kg bw/d by gavage. Automated measurements of activity (figure-eight maze) and a functional observational battery (FOB) were conducted during the week prior to treatment and on days 0 (the day of treatment at the time of peak effect; approximately 1 hour after administration of the dose) and again 7 and 14 days following the single dose administration. In the high dose female rats, but not in male rats, a transient, decreased motor and locomotor activity (by 43 and 53 %, respectively) were observed on the day of dosing, with a recovery by the next measurement occasion, on days 7 and 14 after dosing (see tables below). There were no compound-related deaths at any dose level in either sex. No animals were found dead or sacrificed in extremis during the course of the study.

***Table**: Motor activity (total activity counts for session)*

Test Day	Dose Level (mg/kg bw/d)			
	Control	125	500	2 000
Males				
Pre-test	452 ± 142	506 ± 182	580 ± 141	547 ± 190
Day 0	459 ± 131	495 ± 179	563 ± 148	455 ± 151
Day 7	486 ± 94	531 ± 101	596 ± 116	605 ± 100
Day 14	476 ± 131	583 ± 153	558 ± 74	591 ± 102
Females				
Pre-test	590 ± 280	621 ± 147	666 ± 165	604 ± 144
Day 0	611 ± 166	525 ± 204	649 ± 169	**347* ± 211**
Day 7	544 ± 143	565 ± 84	599 ± 173	530 ± 234
Day 14	621 ± 177	567 ± 158	654 ± 193	519 ± 138

Values represent mean ± s.d. for 1 h Test Session (hh:mm:ss), n=12, *=p≤0.5 compared with controls

Table: Locomotor activity (total activity counts for session)

Test Day	Dose Level (mg/kg bw/d)			
	Control	125	500	2 000
Males				
Pre-test	259 ± 85	285 ± 101	333 ± 83	315 ± 120
Day 0	254 ± 72	278 ± 108	304 ± 77	231 ± 90
Day 7	255 ± 38	273 ± 64	295 ± 62	302 ± 73

Day 14	239 ± 69	301 ± 100	271 ± 63	298 ± 36
Females				
Pre-test	286 ± 122	304 ± 89	334 ± 88	287 ± 106
Day 0	338 ± 103	272 ± 102	339 ± 103	**160*** ± 119
Day 7	281 ± 61	273 ± 45	298 ± 80	249 ± 126
Day 14	316 ± 78	279 ± 81	328 ± 98	239 ± 67

*Values represent mean ± s.d. for 1 h Test Session (hh:mm:ss), n=12, *=p≤0.5 compared with controls*

Since these abnormalities of motor activity were resolved at the next observation on day 7 and neither signs of neurotoxicity nor compound-related gross or microscopic lesions at the high dose of were found, RAC is of the opinion that these effects detected only by motor and locomotor activity should be seen as slight, transient depression of central nervous system induced by thiencarbazone-methyl at the highest oral dose of 2 000 mg/kg bw/d, but not at single oral dose of 125 and 500 mg/kg bw/d.

In the two oral acute toxicity studies and one dermal acute toxicity study in rats given single dose of 2 000 mg/kg bw/d of thiencarbazone-methyl there were no clinical signs. In the acute inhalation study in rats at concentrations of 1 060, 2 018 and 5 158 mg/m^3 no treatment-related clinical signs occurred up to the maximum technically attainable concentration. Thus, no narcotic effects were detected by eye observation in any acute toxicity studies by oral, inhalation or dermal route. No evidence of neurotoxicity was observed in a 90-day neurotoxicity study in which rats were given thiencarbazone-methyl in diet at doses of 33.1, 137 and 411 mg/kg bw/d (males) or 42.4, 171 and 527 mg/kg bw/d (Report No 201518).

Overall, no specific target organ toxicity after a single exposure was identified at doses within the guidance value range for STOT SE 1-2 listed in the CLP Regulation (Annex I: 3.8.2.1.9.3, Table 3.8.2) for the oral, dermal or inhalation route. Also the criteria for STOT SE 3 for narcotic effects in animals such as lethargy, lack of coordination, loss of righting reflex, and ataxia were not observed in animals receiving single doses of thiencarbazone-methyl by oral, inhalation or dermal route.

Taking into account the above considerations **RAC supports the DS's proposal that no classification for STOT SE is warranted**.

4.4 Irritation

4.4.1 Skin irritation

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks
OECD TG 404 Rabbit (New Zealand White) 3 Females Vehicle: test material dosed as received moistened with water Purity 96.3% GLP Report No. AT01648 Anon.; 2004	No erythema, or oedema were observed at any time point	None

4.4.1.1 Non-human information

The skin irritation potential of thiencarbazone-methyl has been well investigated in a standard study in rabbits. The findings are reported in table 11 above.

4.4.1.2 Human information

There is no information available

4.4.1.3 Summary and discussion of skin irritation

Please see above.

4.4.1.4 Comparison with criteria

No signs of erythema or oedema were observed, therefore the criteria for classification (i.e., average scores of ≥ 2.3 for erythema/oedema in at least 2 out of 3 tested animals) are not met.

4.4.1.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation.

The skin irritation potential of thiencarbazone-methyl was assessed in a standard skin irritation GLP study (OECD TG 404) in three female New Zealand White rabbits (Report No AT01648, 2004).

No skin corrosion/irritation was observed in any rabbit during the study period.

Comments received during public consultation

One MSCA provided comments on the hazard class during the public consultation and agreed with the DS to not classify thiencarbazone-methyl for skin corrosion/irritation.

Assessment and comparison with the classification criteria

In the available study, the CLP criteria for skin corrosion/irritation are not met, and RAC concludes that **thiencarbazone-methyl does not warrant classification for skin corrosion/irritation**.

4.4.2 Eye irritation

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks
Rabbit (New Zealand White) 3 Females OECD TG 405 Purity 96.0% GLP Report No. AT02437 Anon.; 2005	Mean individual scores 24, 48 and 72 hours; Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctival redness: 0.3, 0.3, 0.3 Conjunctival chemosis: 0, 0, 0 1hr observation, redness of the conjunctivae; score of 2 for 2/3 animals and score of 3 for 1/3 animals.	None

4.4.2.1 Non-human information

The eye irritation potential of thiencarbazone-methyl has been investigated in a standard study in rabbits; the findings are reported in table 12 above. Conjunctival redness was observed in all animals at the 24 hour observation only.

4.4.2.2 Human information

There is no information available

4.4.2.3 Summary and discussion of eye irritation

Please see above.

4.4.2.4 Comparison with criteria

No effects were observed in the cornea or iris. Slight conjunctival redness (average score of 0.3 in all animals from observations at 24-72 hours) was observed by scores for chemosis were 0. Therefore the criteria for classification (i.e., average scores in the cornea or iris of ≥ 1 and for corneal redness/chemosis of ≥ 2) are not met.

4.4.2.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS did not propose classification for serious eye damage/irritation based on the results of a reliable GLP study on thiencarbazone-methyl in accordance with OECD TG 405 in 3 female New Zealand White rabbits (Report No AT02437, 2005).

Individual scores for each animal, calculated as mean scores at 24, 48 and 72 hours were:

- Corneal opacity: 0, 0, 0
- iritis: 0, 0, 0
- conjunctival redness: 0.3, 0.3, 0.3
- conjunctival chemosis: 0, 0, 0.

No signs of corneal opacity or iritis were observed. Redness of the conjunctivae was observed after 1 and 24 hours in all females (grade 2 for 2/3 females and grade 3 for 1/3 females after 1 hour, grade 1 for 3/3 females after 24 hours). Reactions declined in severity and were fully reversible within 48 hours.

Comments received during public consultation

One MSCA provided comments on the hazard class during the public consultation and agreed with the DS not to classify thiencarbazone-methyl for serious eye damage/irritation.

Assessment and comparison with the classification criteria

Thiencarbazone-methyl caused reversible eye irritation in an *in vivo* study in the rabbit. However, the mean scores for specific ocular effects do not meet the CLP criteria for classification. Only slight conjunctival redness was observed, but the average scores at 24, 48 and 72 were < 2, thus below the mean score for conjunctival redness that would warrant classification in category 2.

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

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In a single exposure inhalation study in rats, no clinical signs of toxicity or histopathological changes consistent with respiratory tract irritation were observed.

4.4.3.2 Human information

No symptoms of respiratory tract irritation have been reported following routine health surveillance of workers involved in thiencarbazone-methyl manufacture, although exposures may be limited due to control measures.

4.4.3.3 Summary and discussion of respiratory tract irritation

Please see sections 4.4.3.1 and 4.3.3.2

4.4.3.4 Comparison with criteria

No symptoms of respiratory tract irritation were observed in potentially exposed humans. No evidence of respiratory tract irritation was observed in a relevant study in experimental animals. Therefore, it can be concluded that thiencarbazone-methyl does not meet the criteria for classification.

4.4.3.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification

4.5 Corrosivity

As the substance was found to be a non- irritant in a standard animal study, discussion of skin corrosivity is not required.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 13: Summary table of relevant skin sensitisation studies

Method	Doses	Results	Reference
Guinea Pig (Crl: HA) OECD TG 406 Guinea Pig Maximisation test Purity 96.3% 20 test and 10 controls	**Induction**: Intradermal: 5% in PEG 400 Topical: 50% in PEG **Challenge:** 50% in PEG Doses selected from a preliminary study.	Test: 0/20 Negative Control (PEG 400): 0/10 Appropriate historical control data using alpha hexyl cinnamic aldehyde formulated in PEG 400 demonstrated a positive response.	Report No. AT01388 Anon.; 2004

4.6.1.1 Non-human information

Thiencarbazone-methyl tested negative in a standard maximisation test.

4.6.1.2 Human information

No incidences of skin sensitisation have been reported following routine health surveillance, although exposures may be limited due to control measures.

4.6.1.3 Summary and discussion of skin sensitisation

Please see table 13, and sections 4.6.1.1 and 4.6.1.2.

4.6.1.4 Comparison with criteria

The results of a Guinea Pig Maximisation study are considered to be positive if ≥ 30% of the animals respond. None of the animals challenged with thiencarbazone-methyl exhibited a response and as such, the criteria for classification are not met.

4.6.1.5 Conclusions on classification and labelling

Not classified – Conclusive but not sufficient for classification.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The potential of thiencarbazone-methyl to cause skin sensitisation was investigated in a GLP Magnusson and Kligman Guinea Pig Maximisation test (Report No AT01388, 2004), according to the EEC B.6 method (OECD TG 406).

Concentrations used for induction and challenge exposures were based on the results of a preliminary study.

Intradermal induction was performed at a concentration of 5 % of thiencarbazone-methyl in polyethylene glycol 400. Topical induction and challenge were performed at concentrations of 50 % of thiencarbazone methyl in polyethylene glycol 400. Dermal reactions were graded at 24 and 48 hours following the challenge exposure.

There were no skin effects in the animals of the thiencarbazone-methyl treated group and in the vehicle treated control group. Body weight changes were normal in test and control animals.

Appropriate positive control data using alpha hexyl cinnamic aldehyde formulated in polyethylene glycol 400 demonstrated a positive response.

The DS concluded that thiencarbazone-methyl is not a skin sensitiser and does not meet the CLP criteria for skin sensitisation.

Comments received during public consultation

One MSCA provided comments on the hazard class during the public consultation and agreed with the DS not to classify thiencarbazone-methyl for skin sensitisation.

Assessment and comparison with the classification criteria

Skin sensitising properties of thiencarbazone-methyl were studied in Magnusson and Kligman Guinea Pig Maximisation test (Report No AT01388, 2004), claimed to be done according to EC B.6 method (OECD TG 406). According to the CLH report the doses used in the main study were selected based on the results of a preliminary study as required by OECD TG 406 (B.6 method). However, the design and the results of this range finding study were neither reported in the CLH report nor in the DAR. In the description of results of the main study, it was reported that after the intradermal induction with 5 % of thiencarbazone-methyl in polyethylene glycol 400, the animals in the treated group showed strong effects up to encrustation at the injection sites of the first induction. No information was provided in the CLH report or in the DAR whether the concentration of 50 % thiencarbazone-methyl in polyethylene glycol 400 used for topical induction (noted by IND during the RAC plenary meeting to be the maximal achievable concentration in this suspension medium) caused at least mild irritation to skin or whether the pre-treatment with sodium lauryl sulphate had been done. According to OECD TG 406, the concentration of the test substance used for each induction exposure should be well-

tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. Furthermore, in accordance with the OECD TG 406, if the substance is not a skin irritant, the close-clipped and/or shaved test area should be painted with 0.5 ml of 10 % sodium lauryl sulphate in vaseline approximately 24 hours before the topical induction application, in order to create a local irritation. The pre-treatment of the test area with sodium lauryl sulphate was apparently not done based on the test description in the CLH report and in the DAR, although the existing data from skin or eye irritation tests indicate that thiencarbazone-methyl did not induce any irritative effects in skin of rabbits and a very slight irritation of eyelids of rabbits. Since 50 % thiencarbazone-methyl in polyethylene glycol 400 used for topical induction and for challenge did not cause skin irritation, lack of pre-treatment of skin before topical induction with 10 % sodium lauryl sulphate is considered by RAC as a deviation from the test guideline methodology, which could influence the results of the test.

Taking into account the above arguments, RAC notes a considerable uncertainty whether the Magnusson and Kligman Guinea Pig Maximisation test was performed in line with OECD TG 406. However, as the Guinea Pig Maximisation test results were clearly negative up to the tested doses, **RAC is of the opinion that thiencarbazone-methyl does not warrant classification for skin sensitisation**, but notes that the data reported in the CLH report and in the DAR is deficient and therefore not sufficiently conclusive.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

This potential of thiencarbazone-methyl to cause respiratory sensitisation was not investigated directly. However, given that thiencarbazone-methyl does meet the criteria for classification for skin sensitisation it is considered unlikely to be a respiratory sensitiser. Therefore no classification is proposed.

4.6.2.2 Human information

No incidences of respiratory effects have been reported following routine health surveillance, although exposures may be limited due to control measures.

4.6.2.3 Summary and discussion of respiratory sensitisation

See section 4.6.2.1.

4.6.2.4 Comparison with criteria

See section 4.6.2.1.

4.6.2.5 Conclusions on classification and labelling

Not classified – Data lacking.

4.7 Specific Target Organ Toxicity - Repeated Exposure

The repeated dose toxicity of thiencarbazone-methyl has been investigated by the oral route in rats (dietary studies of 90 days and 104 week duration), mice (dietary studies of 90 days and 78 week duration) and dogs (dietary studies of 90 days and 1 year duration). No 28 day studies were available. No repeat dose dermal toxicity or repeated inhalation toxicity studies were conducted.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

Table 14: Summary table of relevant repeated dose toxicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

Method	Results	Reference
Rat, Wistar, 10/sex/ group, **90-days , diet** OECD TG 408 0, 400, 2000 and 7000 ppm ad lib An additional group 10/sex/ group) were given 7000ppm for 90 days then a control diet for a further 30 days to examine the reversibility of any effects seen. Purity 98% GLP **Guideline value for classification: ≤ 100 mg/kg bw/day**	**7000 ppm:** **(Males: 439 mg/kg bw/day** **Females: 543 mg/kg bw/day)** Higher alkaline phosphatase activity in males (+25%, $p<0.05$), not observed at the end the recovery period. Similar findings were not apparent in the chronic rat study (Anon *et al*, 2007), at a slightly lower top dose level. 'Sulfonamide-like' crystals were seen in the urine (9/10 males and 10/10 females), not observed at the end the recovery period. Single premature decedent - urinary tract obstruction was the probable cause of death. Intrapelvic eosinophilic urolithiasis within the kidneys in 3/10 males and 1/10 females. A similar eosinophilic urolithiasis was observed within the lumen of the urinary bladder in 2/10 males, this was correlated with gritty content (stones) observed macroscopically in 3/10 males. Within the urinary bladder, urothelial hyperplasia was found in 3/10 males and 1/10 females. Slight to mild collecting duct hyperplasia was found in 4/10 males and 2/10 females. **2000 ppm:** **(Males: 123 mg/kg bw/day** **Females: 154 mg/kg bw/day)** 'Sulfonamide-like' crystals were seen in the urine (3/10 males and 4/10 females). At the end of the recovery period, no such effects were observed.	Report No. SA 02446 Anon. 2003

	400 ppm: **(Males: 24.7 mg/kg bw/day** **Females: 30.8 mg/kg bw/day)** No treatment-related changes reported. *The NOAEL was 2000 ppm (equivalent to 123 mg/kg/day for males and 154 mg/kg/day for females).*	
Rat, Wistar 12/sex/ group, OECD TG 424 (neurotoxicity) **90-days , diet** 0, 500, 2000 and 6000 ppm Purity 96.4% GLP **Guideline value for classification: ≤100 mg/kg bw/d**	No treatment-related changes reported at any dose *NOAEL: 6000 ppm (equivalent to mean achieved dietary intakes of 411 and 527 mg/kg bw/day in males and females respectively).*	Report No. 201518 Anon., 2006
Rat, Wistar, 60/sex/ dose, plus satellite groups of 10/sex/dose for a planned interim sacrifice OECD TG 453 **2-years , diet** 0, 500, 2500 or 5000 ppm Purity 96% GLP **Guideline value of ≤ 12 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.**	**5000 ppm:** **(Year 1: Males: 268.60 mg/kg bw/day** **Females: 366.6 mg/kg bw/day** **Year 2: Males: 234.0 mg/kg bw/day** **Females: 313.4 mg/kg bw/day)** Lower plasma triglyceride concentrations were observed in females at a number of time points. Crystals present in the urine of the majority of animals, assumed to be thiencarbazone-methyl or a metabolite. No macroscopic or histopathological correlates were present. **2500 ppm:** **(Year 1: Males: 136.4 mg/kg bw/day** **Females: 176.7 mg/kg bw/day** **Year 2: Males: 115.2 mg/kg bw/day** **Females: 152.9 mg/kg bw/day)** Lower plasma triglyceride concentrations in females at 18 months only. Crystals present in the urine of two animals/sex; assumed to be thiencarbazone-methyl or a metabolite. No macroscopic or histopathological correlates were present.	Report No. AT03629 Anon., 2007

	500 ppm: **(Year 1: Males: 10.6 mg/kg bw/day** **Females: 13.2 mg/kg bw/day** **Year 2: Males: 22.8 mg/kg bw/day** **Females: 29.9 mg/kg bw/day)** No treatment-related changes reported. *NOAEL: 2500 ppm (equivalent to 115 and 153 mg/kg bw/day in males and females respectively) based on the clinical chemistry findings (reduced plasma triglyceride concentration) at the top dose level of 5000 ppm (equivalent to 234 and 313 mg/kg bw/day respectively). It is acknowledged that this is a conservative interpretation and that findings at 5000 ppm represent a minimal NOAEL.*	

Mouse

Table: 15 Summary table of relevant repeated dose toxicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

Method	Results	Reference
Mouse, 10/sex/ group, OECD TG 408 **90-days , diet** 0, 500, 2000 and 4000 ppm Purity 98% GLP Deviations from OECD TG 408: The epididymides and ovaries were not weighed, as specified in OECD 408, however this is not considered to affect the integrity of the study in the absence of effects on these organs in other studies in the mouse or in other species **Guideline value of ≤ 100 mg/kg/day is considered for classification: based on the value defined for the rat 90 day study**	**4000 ppm:** **(Males: 637 mg/kg bw/day** **Females: 789 mg/kg bw/day)** Urinary bladder calculus in one male, accompanied by marked submucosal inflammatory cell infiltration, minimal diffuse urothelial inflammation and moderate diffuse urothelial hyperplasia of urinary bladder. **2000 ppm and 500 ppm:** **(Males: 315 mg/kg bw/day** **Females: 409 mg/kg bw/day)** No treatment-related changes reported. **500 ppm:** **(Males: 76 mg/kg bw/day** **Females: 103 mg/kg bw/day)** No treatment-related changes reported. *NOAEL: 2000 ppm for males (equivalent to a mean achieved dietary intake of 315 mg/kg bw/day) can be determined for this study, based on the urinary bladder calculus and associated histopathology seen at 4000 ppm (equivalent to 637 mg/kg bw/day).*	Report No. SA 03086 Anon.;2004

OECD TG 451 **18 months , diet** Mouse C57BL/6J, 50/sex/ group, plus 10/sex/group for a planned interim sacrifice (28 weeks) 0, 200, 1000 or 4000 ppm Purity 96% GLP **Guideline value of ≤ 12 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.**	A number of males (17/50 males) in the high dose group were euthanized due to poor condition. However, survival in all groups exceeded 50%. **<u>4000 ppm</u>** **(Males (m): 599 mg/kg bw/day** **Females (f): 758 mg/kg bw/day)** Mortality; 17/50 m and 9/50 females were euthanised due to poor condition. A further 5 m found dead during the study. Clinical signs included; soiled fur in 16m/7f, skin lesions in 13m (mostly anogenital region), wasted appearance 7m and abnormal penis 23m. Abnormal penis was not associated with any intrinsic histopathological findings, but was associated with chronic ulcerative dermatitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination. Significant overall reduction in mean cumulative body weight gain in males (15% by Study Day 540). <u>Urinary Bladder</u>: At 18 months large, round to oval-shaped stones were found in the urinary bladder of both sexes. The males were more affected than females. The presence of various stone-induced findings, secondary to chronic irritation, were observed in both sexes: • hyperplastic changes (simple and/or nodular/glandular urothelial hyperplasia), • inflammatory changes (interstitial oedema, suburothelial and/or serosal mixed • cell infiltrate, intramuscular inflammatory cell infiltrate and induced arteritis), • focal/multifocal adenomyosis in a few treated males. Detailed summary of findings in urinary bladder shown in Table 15a (below). <u>Kidney:</u> Increased incidence and severity of unilateral and/or bilateral pelvic dilatation (both sexes). Detailed summary of findings in kidney shown in Table 15b (below). <u>Prostatic urethra:</u> Minimal to moderate urothelial hyperplasia (males) Detailed summary of findings in the prostatic urethra (males) shown in Table15c (below). <u>Ureter</u>: Slight increase in simple urothelial hyperplasia (males). <u>Bone marrow</u>: a higher incidence and severity (males only) of myeloid hyperplasia was observed in both sexes. Detailed summary of findings in the bone marrow shown in Table 15d (below). <u>Skin</u>: Significantly higher incidence of chronic ulcerative dermatitis (males).	Report no. SA 04062 Anon., 2006

	1000ppm : **(Males: 147 mg/kg bw/day** **Females: 185 mg/kg bw/day)** Marginally higher incidence of abnormal penis (no treatment-related clinical signs or corresponding histopathology findings). **200 ppm:** **(Males: 29.2 mg/kg bw/day** **Females: 36.8 mg/kg bw/day)** Marginally higher incidence of abnormal penis (no treatment-related clinical signs or corresponding histopathology findings). *NOAEL: 1000 ppm (equivalent to mean intakes of 147 and 185 mg/kg bw/day in males and females respectively) can be determined for this study based on the findings at the top dose level.*	

A detailed summary of findings in urinary bladder, kidney, prostatic urethra, skin and the bone marrow are provided in Tables 15a-d below

Table 15a: Mice: incidence and severity of microscopic changes in the urinary bladder, all animals (18 months study)

Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**200**	**1000**	**4000**	**0**	**200**	**1000**	**4000**
mg/kg bw/day	0	29.2	147	599	0	36.8	185	758
Number of animals	49	49	50	50	48	49	47	49
Stone(s): intraluminal								
Minimal	0	0	0	6	0	0	0	2
Slight	0	0	0	1	0	0	0	3
Moderate	0	0	0	13	0	0	0	2
Marked	0	0	0	11	0	0	0	5
Severe	0	0	0	1	0	0	0	3
Total	**0**	**0**	**0**	**32****	**0**	**0**	**0**	**15****
Stone(s): only noted at necropsy	**1**	**0**	**0**	**9***	**0**	**0**	**0**	**5***
Total incidence of animals with stones	**1**	**0**	**0**	**41****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: simple: multifocal/diffuse								
Minimal	0	0	1	14	0	0	0	10
Slight	0	0	0	22	0	0	0	10
Moderate	0	0	0	2	0	0	0	0
Total	**0**	**0**	**1**	**38****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: nodular/glandular: multifocal/diffuse								
Minimal	0	0	0	12	0	0	0	2
Slight	0	0	0	9	0	0	0	4
Moderate	0	0	0	2	0	0	0	6
Marked	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**23****	**0**	**0**	**0**	**13****
Interstitial oedema: diffuse								
Minimal	0	0	0	16	0	0	0	8
Slight	0	0	0	12	0	0	0	5
Moderate	0	0	0	6	0	0	0	0
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**13****
Suburothelial mixed cell infiltrate: focal/multifocal								
Minimal	1	0	0	21	1	0	0	7

Slight	0	0	0	18	0	0	0	9
Moderate	0	0	0	2	0	0	0	3
Total	**1**	**0**	**0**	**41****	**1**	**0**	**0**	**19****
Intramuscular inflammatory cell infiltrate: focal/multifocal								
Minimal	0	0	0	24	0	0	0	12
Slight	0	0	0	10	0	0	0	7
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**19****
Serosal mixed cell infiltrate: focal/multifocal								
Minimal	0	0	0	3	0	0	0	5
Slight	0	0	0	3	0	0	0	1
Total	**0**	**0**	**0**	**6***	**0**	**0**	**0**	**6***
Induced arteritis								
Minimal	0	0	0	4	0	0	0	3
Total	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**3**
Adenomyosis: focal/multifocal								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Total	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**

*: $p \leq 0.05$; **: $p \leq 0.01$

Table 15b: Mouse: incidence and severity of microscopic changes in the kidney, all animals, 18 month study

Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**200**	**1000**	**4000**	**0**	**200**	**1000**	**4000**
mg/kg bw/day	0	29.2	147	599	0	36.8	185	758
Number of animals	50	50	50	50	50	50	49	50
Pelvic dilatation: unilateral								
Minimal	0	0	0	5	0	0	1	3
Slight	1	0	0	3	1	1	0	1
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**0**	**1**	**8***	**1**	**1**	**2**	**6**
Pelvic dilatation: bilateral								
Minimal	0	1	0	3	0	0	0	2
Slight	0	0	0	4	0	0	0	1
Total	**0**	**1**	**0**	**7***	**0**	**0**	**0**	**3**
Pelvic dilatation: unilateral/bilateral								
Minimal	0	1	0	8	0	0	1	5
Slight	1	0	0	7	1	1	0	2
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**1**	**1**	**15****	**1**	**1**	**2**	**9****

*: $p \leq 0.05$; **: $p \leq 0.01$

Table 15c: Mouse: incidence and severity of microscopic changes in the urethra (prostate), all males (18 month study)

Sex	**Males**			
Dose level (ppm)	**0**	**200**	**1000**	**4000**
mg/kg bw/day	0	29.2	147	599
Number of animals	49	50	48	50
Urothelial hyperplasia: urethra				
Minimal	0	0	0	3
Slight	0	0	0	1
Moderate	0	0	0	1
Total	**0**	**0**	**0**	**5***

*: $p \leq 0.05$

Table 15d: Mouse: incidence and severity of microscopic changes in the skin, all animals (carcinogenicity phase)

Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**200**	**1000**	**4000**	**0**	**200**	**1000**	**4000**
Number of animals	49	48	49	48	48	50	48	47
Chronic ulcerative dermatitis (located in the anogenital region or surrounding area)								
Slight	0	0	1	0	1	1	0	0
Moderate	0	2	1	7	0	2	0	0
Marked	1	3	1	3	0	0	0	0
Total	**1**	**5**	**3**	**10***	**1**	**3**	**0**	**0**

*: $p \leq 0.05$

Table 15e: Mouse: incidence and severity of microscopic changes in the bone marrow, sternum, all animals (18 month study)

Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**200**	**1000**	**4000**	**0**	**200**	**1000**	**4000**
Number of animals	50	50	50	50	50	50	49	50
Myeloid hyperplasia: diffuse								
Minimal	2	1	1	9	3	3	4	7
Slight	1	2	0	2	2	0	0	2
Moderate	1	2	0	6	0	0	0	0
Marked	0	1	0	0	0	0	0	0
Total	**4**	**6**	**1**	**17***	**5**	**3**	**4**	**9**

*: $p \leq 0.05$

Dog

Table 16: Summary table of relevant repeated dose toxicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

Method	Results	Reference
EC B27 **90-days , dietary** Beagle dog, 4/sex/dose 0, 1000, 5000 and 10000 ppm Purity 95.5% **Guideline value of ≤ 100 mg/kg/day is considered for classification: taken from the value defined for the rat 90 day study**.	**10000 ppm:** **(Males: 335 mg/kg bw/day** **Females: 351 mg/kg bw/day)** Urinary bladder calculi in 3/4 males and 2/4 females. Accompanied by marked submucosal inflammatory cell infiltration, minimal diffuse urothelial inflammation (2/4 males), haemorrhage (2/4 males and 1/4 females) and moderate diffuse urothelial hyperplasia (3/4 males and 2/4 females) of urinary bladder. **5000 ppm:** **(Males: 149 mg/kg bw/day** **Females: 159 mg/kg bw/day)** No treatment-related changes reported. **1000 ppm:** **(Males: 34 mg/kg bw/day** **Females: 32 mg/kg bw/day)** No treatment-related changes reported. *NOAEL: 5000 ppm (equivalent to mean achieved dietary intakes of 149 and 159 mg/kg bw/day in males and females respectively) , based on the urinary bladder calculi and associated findings seen in both sexes at the top dose level of 10000 ppm (equivalent to 335 and 351 mg/kg bw/day in males and females respectively).*	Report No 201290-1 Anon., 2005

OECD TG 452

1-year, dietary

Beagle dog, 4/sex/dose

0, 1000, and 4000 ppm for at least 370 days, and 8000 ppm for 21 days which was then reduced to 7000 ppm for males and females on study day 21, due to the presence of urinary calculi in males. Since urinary calculi persisted in males treated at 7000 ppm, a washout period of 4 days was conducted for this group between study days 52 and 55, before continuation of the treatment at 6000 ppm starting on study day 56 for the remainder of the 12-month treatment period.

Guideline value of ≤ 24 mg/kg/day is considered for classification: calculated from the value defined for the rat 90 day study.

Study design

Test group	Sex	Concentrations in the diet (ppm)			
		Days 0 to 20	Days 21 to 51	Days 52 to 55	Days 56 to term.
1	M	0			
	F	0			
2	M	1000			
	F	1000			
3	M	4000			
	F	4000			
4	M	8000	7000	washout	6000
	F	8000	7000		

8000/7000/6000 ppm:
(Males: 179 mg/kg bw/day
Females: 200 mg/kg bw/day)

Reduced bodyweight gain males (up to ~25% between days 7-70, becoming comparable after the was out on day 70).

16%↓ absolute and relative kidney (not statistically significantly) in males.

Calculi in the urinary bladder of 2 males associated with histopathological findings of slight to moderate transitional cell hyperplasia, slight congestion, slight haemorrhage, slight inflammation, minimal calculus, and/or moderate ulceration.

4000 ppm:
(Males: 117 mg/kg bw/day
Females: 127 mg/kg bw/day)

No treatment-related changes.

1000 ppm:
(Males: 29 mg/kg bw/day
Females: 17 mg/kg bw/day)

No treatment-related changes.

NOAEL: 4000 ppm (117 mg/kg bw/day), based on the presence of urinary bladder calculi and associated urinalysis observations and histopathological findings in the transitional epithelium at the top dose level of 8000/7000/6000 ppm (equivalent to a mean achieved dietary intake of 179 mg/kg bw/day).

Report No 201497-1

Anon., 2007

Thiencarbazone-methyl was found to be of relatively low toxicity in all three species tested (rat, mouse and dog).

The urothelium was identified as the primary target of thiencarbazone-methyl toxicity in all species investigated; findings were apparent in the urinary bladder in all species, with associated renal findings also observed in the rat.

The mechanism of toxicity appears to be the deposition of thiencarbazone-methyl crystals in the urine as a result of urinary excretion following the dietary administration of high concentrations, resulting in urolithiasis. Urolithiasis in the urinary bladder causes local irritation, inflammation and hyperplasia of the transitional epithelium; similar effects are also seen in the rat kidney.

Rat

In the 90-day rat study (Anon, 2003), one mortality was seen in a male at the top dose level of 7000 ppm. The death of this animal is considered likely to have been a result of urinary tract obstruction following the deposition of thiencarbazone-methyl crystals. Urine from animals of both sexes administered 2000 and 7000 ppm was noted to be cloudy; microscopic urinalysis revealed the presence of 'sulphonamide-like' crystals at these dose levels at the end of the 90-day dosing period, but not following a 28-day recovery period. Histopathological examination of rats at the top dose level revealed renal intrapelvic and urinary bladder eosinophilic urolithiasis, renal collecting duct and bladder urothelial hyperplasia. Treatment-related findings at 2000 ppm were limited to the presence of crystals in the urine of a small number of animals of both sexes: this finding is clearly a consequence of treatment but is not considered to be of toxicological significance in the absence of histopathological correlates. In the two year rat study, in common with the 90-day study, treatment-related effects were observed in the urinary tract. Survival was unaffected by treatment and findings were limited to the deposition of crystals (presumed to be of thiencarbazone-methyl) and are not considered to be of toxicological significance in the absence of macroscopic or histopathological correlates. Treatment-related findings of potential toxicological relevance were limited to a minimal effect on plasma triglyceride concentration at the top dose level of 5000 ppm.

No evidence of neurotoxicity was seen in the 90-day neurotoxicity study (Anon, 2006). No treatment-related findings were apparent at the highest dose level of 6000 ppm.

Mouse

Findings in the 90-day mouse study (Anon, 2004) indicate that this species is less sensitive than rats to the toxicity of thiencarbazone-methyl. Treatment-related findings were limited to urinary bladder calculi observed in one male at the top dose level of 4000 ppm. This finding was accompanied by marked submucosal inflammatory cell infiltration; diffuse urothelial inflammation and urothelial hyperplasia of the urinary bladder. In the 18 month mouse study urinary tract findings were also limited to the top dose level and consisted of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter. Increased mortality in males at this dose level is also associated with urolithiasis. Analysis of the urinary bladder stones revealed that they consisted of approximately 70-75% thiencarbazone-methyl.

Dog

In the 90-day dog study (Anon, 2005); treatment-related findings were limited to the top dose level of 10000 ppm in both sexes. Urinary bladder calculi in males and females were associated with haemorrhage, inflammation and hyperplasia of the transitional epithelium. In the one year dog study (Anon, 2007); the top dose level of 8000 ppm was reduced to 7000 ppm after three weeks due to the presence of urinary calculi. Findings persisted and the dose level in males was subsequently

further reduced to 6000 ppm after eight weeks and following a four day 'washout' period. Urinary bladder calculi were noted at termination in 6000 ppm males. Findings were associated with macroscopic observations of 'abnormal' bladder consistency; and histopathologically with congestion, haemorrhage, inflammation and ulceration of the transitional epithelium.

4.7.1.2 Repeated dose toxicity: inhalation

No studies provided.

4.7.1.3 Repeated dose toxicity: dermal

No studies provided

4.7.1.4 Repeated dose toxicity: other routes

No other relevant information.

4.7.1.5 Human information

There is no information available.

4.7.1.6 Other relevant information

All relevant information is summarised above.

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

A substance is classified for STOT-RE when specific target organ toxicity arises from repeated exposure to concentrations at or below the specified guidance values.

Oral

The urothelium was identified as the primary target of thiencarbazone-methyl toxicity in all three species investigated; findings were apparent in the urinary bladder in all species, with associated renal findings also observed in the rat. The mechanism of toxicity appears to be the deposition of thiencarbazone-methyl crystals in the urine as a result of urinary excretion following the dietary administration of high concentrations, resulting in urolithiasis. Urolithiasis in the urinary bladder causes local irritation, inflammation and hyperplasia of the transitional epithelium; similar effects are also seen in the rat kidney.

No classification is proposed as there were no signs of significant or severe toxic effects in rats, mice or dogs at doses within the guideline range.

Table 17: Summary of treatment related effects in the repeated dose oral studies

Type of study	Treatment related effects	Dose at which effects were noted	Guideline value for classification
		mg/kg bw/day	mg/kg bw/day
90-day rat study	Sulfonamide-like crystals in the urine	**123/154 M/F**	≤ 100
	urolithiasis and hyperplasia within the kidneys and the urinary bladder	**439/543 M/F**	
2-year rat	Clinical chemistry findings (reduced plasma triglyceride concentration) Crystals (assumed to be thiencarbazone-methyl or a metabolite) in the urine	**115/153 M/F**	≤ 12 (calculated from the value defined for the rat 90 day study)
90-day mouse study	Urinary bladder calculus in one male, accompanied by inflammation and urothelial hyperplasia of the urinary bladder. Nothing in females	**637/789 M/F**	≤ 100
18-month mouse	Slightly increased mortality (associated with urolithiasis) and slight reductions in bodyweight gain in top dose males. Effects on the urinary tract system consisting of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter.	**599/758 M/F**	≤ 12 (calculated from the value defined for the rat 90 day study)
90-day dog study	Calculi in the urinary bladder, inflammatory changes and hyperplasia in the urinary bladder	**335/351 M/F**	≤ 100 (taken from the value defined for the rat 90 day study)
1 year dog study	Urinary bladder calculi in two males, accompanied by inflammation, haemorrhage, ulceration and transitional cell hyperplasia of the urinary bladder. Nothing in females	**179/>200 M/F**	≤ 24 (calculated from the value defined for the rat 90 day study)

Dermal

No information provided. No classification is proposed for repeated dermal toxicity.

Inhalation

No information provided. No classification is proposed for repeated inhalation toxicity.

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

Not classified – conclusive but not sufficient for classification.

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

Summary of the Dossier Submitter's proposal

The CLH dossier contained several repeated dose toxicity studies on thiencarbazone-methyl in rats (90-day and 2-year dietary studies), in mice (90-day and 78-week dietary studies) and in dogs (90-day and 1-year dietary studies). No 28-day studies were available. No repeated-dose toxicity studies via dermal or inhalation route were conducted according to the DS.

Thiencarbazone-methyl was found to be of relatively low toxicity in all three species tested (rat, mouse and dog).

The urothelium was identified as the primary target organ of thiencarbazone-methyl in all species investigated; findings were apparent in the urinary bladder in all species, with also associated renal findings observed in the rat.

The mechanism of toxicity was considered to be the deposition of thiencarbazone-methyl crystals in the urine as a result of urinary excretion following the dietary administration of high concentrations of the substance, resulting in urolithiasis. Urolithiasis in the urinary bladder was concluded to cause local irritation, inflammation and hyperplasia of the transitional epithelium, and similar effects were also seen in the rat kidney.

Rat

In the 90-day rat study (Report No SA 02446, 2003), in accordance with GLP and OECD TG 408, one mortality occurred in a male at the top dose level of 7 000 ppm (males: 439 mg/kg bw/d, females: 543 mg/kg bw/d). The death of this animal was assumed to be a result of urinary tract obstruction following the deposition of thiencarbazone-methyl crystals. Urine from animals of both sexes administered 2 000 (males: 123 mg/kg bw/d, females: 154 mg/kg bw/d) and 7 a000 ppm (males: 439 mg/kg bw/d, females: 543 mg/kg bw/d) was noted to be cloudy, and microscopic urinalysis revealed the presence of 'sulphonamide-like' crystals at these dose levels at the end of the 90-day dosing period, but not following a 28-day recovery period. Histopathological examination of rats at the top dose level revealed renal intrapelvic and urinary bladder eosinophilic urolithiasis, as well as renal collecting duct and bladder urothelial hyperplasia. Treatment-related findings at 2 000 ppm were limited to the presence of crystals in the urine of a small number of animals of both sexes. This finding was clearly a consequence of treatment, but was not considered to be of toxicological significance in the absence of histopathological correlates.

In the 2-year rat study (Report No AT03629; 2007) in accordance with GLP and OECD TG 453, treatment-related effects were observed in the urinary tract. Survival was unaffected by treatment, and findings were limited to the deposition of crystals (presumed to be of thiencarbazone-methyl) that were not considered to be of toxicological significance in the absence of macroscopic or histopathological correlates. Treatment-related findings of potential toxicological relevance were limited to a minimal effect on plasma triglyceride concentration at the top dose level of 5 000 ppm (Year 1: males: 269 mg/kg bw/d, females: 367 mg/kg bw/d; Year 2: males: 234.0 mg/kg bw/d,

females: 313 mg/kg bw/d).

No evidence of neurotoxicity or other treatment-related effects were seen in the 90-day neurotoxicity study (Report No 201518; 2006) performed in accordance with GLP and OECD TG 424 up to the highest dose level of 6 000 ppm (males: 411 mg/kg bw/d, females: 527 mg/kg bw/d).

Mouse

Findings in the 90-day mouse study (Report No SA 03086, 2004), performed in accordance with GLP and OECD TG 408, indicated according to the DS that this species was less sensitive than rats to the toxicity of thiencarbazone-methyl. Treatment-related findings were limited to urinary bladder calculi observed in one male at the top dose level of 4 000 ppm (males: 637 mg/kg bw/d, females: 789 mg/kg bw/d). This finding was accompanied by marked submucosal inflammatory cell infiltration, diffuse urothelial inflammation and urothelial hyperplasia of the urinary bladder.

In the 18-month mouse study (Report No SA 04062; 2006) performed in accordance with GLP and OECD TG 451, urinary tract findings were also limited to the top dose level of 4 000 ppm (males: 599 mg/kg bw/d, females: 758 mg/kg bw/d) and consisted of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter. Increased mortality in males at this dose level was also associated with urolithiasis. Analysis of the urinary bladder stones revealed that they consisted of approximately 70-75 % thiencarbazone-methyl.

Dog

In the 90-day dog study (Report No 201290-1; 2005) performed in accordance with the EEC B.27 method, treatment-related findings were limited to the top dose level of 10 000 ppm (males: 335 mg/kg bw/d, females: 351 mg/kg bw/d) in both sexes. Urinary bladder calculi were associated with haemorrhage, inflammation and hyperplasia of the transitional epithelium.

In the 1-year dog study (Report No 201497-1, 2007) performed in accordance with OECD TG 452, the top dose level of 8 000 ppm was reduced to 7 000 ppm after three weeks due to the presence of urinary calculi. Findings persisted and the dose level in males was subsequently further reduced to 6 000 ppm (males: 179 mg/kg bw/d, females: 200 mg/kg bw/d) after eight weeks and following a four-day 'washout' period. Urinary bladder calculi were noted in males at study termination at 6 000 ppm. The findings were associated with macroscopic observations of 'abnormal' bladder consistency and histopathologically with congestion, haemorrhage, inflammation and ulceration of the transitional epithelium.

The DS concluded that thiencarbazone-methyl did not meet the CLP criteria for specific target organ toxicity – repeated exposure (STOT RE).

Comments received during public consultation

One MSCA provided comments on the hazard class during the public consultation and agreed with the DS not to classify thiencarbazone-methyl for specific target organ toxicity – repeated exposure.

Assessment and comparison with the classification criteria

There is no information on the repeated dose toxicity of thiencarbazone-methyl in humans. However, there is a 90-day study and a 2-year study available in the rat and a 90-day and 18-month study in the mouse and a 90-day study and 1-year study in the dog. All of those studies were diet studies.

The effects observed in the repeated dose toxicity studies did not meet the CLP criteria.

Type of study	Treatment-related effects	Dose at which effects were noted	Guidance value for classification
		mg/kg bw/d males/females	mg/kg bw/d
90-day rat study	Sulfonamide-like crystals in urine.	123/154	≤ 100
	Urolithiasis and hyperplasia in the kidneys and urinary bladder.	439/543	
2-year rat study	Clinical chemistry findings (reduced plasma triglyceride concentration). Crystals (assumed to be thiencarbazone-methyl or a metabolite) in urine.	115/153	≤ 12*
90-day mouse study	Urinary bladder calculus in one male, accompanied by inflammation and urothelial hyperplasia of the urinary bladder. No effects in females.	637/789	≤ 100
18-month mouse study	Slightly increased mortality (associated with urolithiasis) and slight reductions in bodyweight gain in top dose males. Effects on the urinary tract system consisting of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter.	599/758	≤ 12*
90-day dog study	Calculi in the urinary bladder, inflammatory changes and hyperplasia in the urinary bladder.	335/351	≤ 100
1-year dog study	Urinary bladder calculi in two males accompanied by inflammation, haemorrhage, ulceration and transitional cell hyperplasia of the urinary bladder. No effects in females.	179/>200	≤ 24*

* = value extrapolated from a 90-day study using Haber's rule.

The urothelium is identified as the primary target organ of thiencarbazone-methyl toxicity in all three species investigated. The findings were apparent in the urinary bladder in all species, with also associated renal findings observed in the rat. The mechanism of toxicity appears to be the deposition of thiencarbazone-methyl crystals in the urine as a result of urinary excretion following the dietary administration of high concentrations of the substance, resulting in urolithiasis. Urolithiasis in the urinary bladder causes local irritation, inflammation and hyperplasia of the transitional epithelium. Similar effects are

also seen in the rat kidney.

However, there were no signs of significant or severe toxic effects in rats, mice or dogs at doses within the guidance value ranges for classification for STOT RE.

Taking into account the available data, RAC concludes that thiencarbazone-methyl **does not warrant classification for specific target organ toxicity – repeated exposure (STOT RE)**.

4.8 Germ cell mutagenicity

4.8.1 Non-human information

4.8.1.1 In vitro data

Table 18: Summary of relevant *in vitro* data

Test system/ Method/Guideline	Organism/ Strain	Concs. tested	Result		Remarks (e.g. cytotoxicity)	Reference
			+ S9	- S9		
Bacterial Reverse Mutation Test OECD TG 471 GLP	*S.typhimurium*: TA 1535, TA 1537, TA 98, TA 100 and TA 102	Plate incorporation assay: First assay for all strains with or without S9 mix: 16 - 5000 µg/plate Second assay for TA 1535, TA 1537 and TA 102 with or without S9 mix: 20, -100 µg/plate Second assay for TA 100 with or without S9 mix: 400- 1200 µg/plate Second assay for TA 98 with or without S9 mix: 100, - 400 µg/plate, Third assay for TA 98 with S9 mix: 50, - 400 µg/plate. Pre-incubation assay: For TA 1535, TA 1537 and TA 102 with or without S9 mix: 10-100 µg/plate, For TA 100 and TA 98 with or without S9 mix: 30-400 µg/plate.	-ve	-ve	Doses up to 20 µg/plate did not cause any bacteriotoxic effects. At higher doses, there was a strong, strain-specific bacteriotoxic effect, so that the range could only to be used to a limited extent up to 400 µg/plate for assessment purposes. Substance precipitation occurred at the dose of 1581 µg/plate and above. No evidence of genotoxicity was seen under the conditions of this study. However the sensitivity of this study is considered to be limited in the absence of a consistent response to the positive control compounds in strain TA100 and the magnitude of the response to the positive control compounds in strain TA102.	Report No AT02274 Wirnitzer U., 2005

Bacterial Reverse Mutation Test OECD TG 471 GLP	*S.typhimurium:* TA 1535, TA 1537, TA 98, TA 100 and TA 102	Plate incorporation assay: For all strains with or without S9 mix: 15, - 480 µg/plate Pre-incubation assay: For TA 1535, TA 1537 and TA 98 with or without S9 mix: 8-256 µg/plate For TA 100 with or without S9 mix: 16-512 µg/plate For TA 102 with or without S9 mix: 4- 128 µg/plate.	-ve	-ve	The highest concentrations of the test material used were limited by cytotoxicity. In contrast to the previous study (Wirnitzer, 2005); the positive controls demonstrated the sensitivity of the assay adequately.	Report No AT03630 Herbold B., 2007
Bacterial Reverse Mutation Test Exogenous metabolic activation system (Aroclor 1254-induced male NMRI mouse liver S9 fraction used due to the possibility of a mouse-specific mutagenic metabolite being involved in the urothelial carcinogenicity seen in the mouse OECD TG 471 GLP	*S.typhimurium:* TA1535, TA100, TA1537, TA98 and TA102	Plate incorporation assay: For all strains with or without S9 mix: 16- 512 µg/plate Pre-incubation assay: For all strains with or without S9 mix: 3- 384 µg/plate.	-ve	-ve	Cytotoxicity observed at concentrations of $\geq$64 µg/plate (plate incorporation) and $\geq$24 µg/plate (pre-incubation).	Report No AT04414 Herbold B., 2008
Chromosomal aberration test OECD TG 473 GLP	Chinese Hamster V79 cells	100 – 400 µg/mL	-ve	-ve	Only limited cytotoxicity (~25% reduction in the survival index) was seen at the highest test concentration in the initial assay, however the highest concentrations were limited by its solubility in the vehicle (40 mg/ml in DMSO).	Report No AT02499 Thum M., 2005

<table>
<tr>
<td>Chromosomal aberration test
OECD TG 473
GLP</td>
<td>Chinese Hamster V79 cells</td>
<td>100 – 400 µg/mL</td>
<td>-ve</td>
<td>-ve</td>
<td>The study is essentially a repeat of the previous study (Thum, 2005) using material of lower purity (94.6% compared to 96.3-96.4% in the previous study).
Only limited cytotoxicity (10-25% reduction in the survival index) was seen at the highest concentration of the test material use in the initial assay, however the highest concentrations of the test material were limited by its solubility in the vehicle (40 mg/ml in DMSO).</td>
<td>Report No AT03625
Thum M., 2007</td>
</tr>
<tr>
<td>In vitro gene mutation assay (HPRT) in mammalian cells

OECD TG 476
EC B17
GLP</td>
<td>Chinese Hamster V79/ HPRT locus</td>
<td>Thiencarbazone-methyl was tested at 25-600 µg/ml -/+ S9 in the clonal cytotoxicity assay and at 60-600 µg/ml -/+ S9 in the mutagenic assays (2 Trials –S9; 3 Trials +S9)</td>
<td>-ve</td>
<td>-ve</td>
<td>No cytotoxicity was seen in this study: the highest tested concentration was limited by solubility in the vehicle.
<table>
<tr><th rowspan="2">[µg/ml]</th><th colspan="5">Mutation frequency (x10^{-6} cells)</th></tr>
<tr><th colspan="2">Experiment 1</th><th colspan="2">Experiment 2</th><th>Experiment 3</th></tr>
<tr><th>-S9</th><th>+S9</th><th>-S9</th><th>+S9</th><th>+S9</th></tr>
<tr><td rowspan="2">0</td><td>0.5</td><td>7.6</td><td>3.7</td><td>5.8</td><td>3.4</td></tr>
<tr><td>2.1</td><td>7.4</td><td>1.4</td><td>8.4</td><td>2.2</td></tr>
<tr><td rowspan="2">DMSO</td><td>0.5</td><td>8.0</td><td>1.5</td><td>3.9</td><td>2.4</td></tr>
<tr><td>1.0</td><td>7.8</td><td>4.5</td><td>6.7</td><td>2.4</td></tr>
<tr><td rowspan="2">60</td><td>0.6</td><td>8.9</td><td>6.1</td><td>5.6</td><td>1.3</td></tr>
<tr><td>0.5</td><td>10.6</td><td>5.5</td><td>7.6</td><td>2.9</td></tr>
<tr><td rowspan="2">120</td><td>0.5</td><td>19.3</td><td>2.0</td><td>8.8</td><td>1.1</td></tr>
<tr><td>0.5</td><td>9.5</td><td>3.0</td><td>8.4</td><td>0.6</td></tr>
<tr><td rowspan="2">240</td><td>0.5</td><td>11.2</td><td>4.9</td><td>2.6</td><td>4.5</td></tr>
<tr><td>0.5</td><td>24.3</td><td>5.2</td><td>6.7</td><td>1.3</td></tr>
<tr><td rowspan="2">360</td><td>0.6</td><td>16.1</td><td>4.5</td><td>10.4</td><td>2.8</td></tr>
<tr><td>1.1</td><td>17.1</td><td>3.8</td><td>4.5</td><td>1.7</td></tr>
<tr><td rowspan="2">480</td><td>0.5</td><td>7.1</td><td>8.8</td><td>7.5</td><td>0.5</td></tr>
<tr><td>1.0</td><td>9.5</td><td>3.8</td><td>4.1</td><td>1.5</td></tr>
<tr><td rowspan="2">600</td><td>0.5</td><td>21.7</td><td>2.4</td><td>12.7</td><td>1.0</td></tr>
<tr><td>1.6</td><td>14.3</td><td>-</td><td>4.1</td><td>0.7</td></tr>
<tr><td rowspan="2">EMS 900</td><td>256.6</td><td>-</td><td>617.1</td><td>-</td><td>-</td></tr>
<tr><td>216.8</td><td>-</td><td>643.1</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">DMBA 20</td><td>-</td><td>36.0</td><td>-</td><td>27.2</td><td>62.4</td></tr>
<tr><td>-</td><td>30.7</td><td>-</td><td>35.3</td><td>57.6</td></tr>
</table>
</td>
<td>Report No AT02752
Herbold B., 2005</td>
</tr>
</table>

<table>
<tr>
<td>*In vitro* gene mutation assay (HPRT) in mammalian cells

OECD TG 476
EC B17
GLP</td>
<td>Chinese Hamster V79/ HPRT locus</td>
<td>Thiencarbazone-methyl at 30- 600 µg/ml -/+ S9 in the mutagenic assays (2 Trials).</td>
<td>-ve</td>
<td>-ve</td>
<td>
<table>
<tr><th rowspan="3">[µg/ml]</th><th colspan="4">Mutation frequency (x10^{-6} cells)</th></tr>
<tr><th colspan="2">Experiment 1</th><th colspan="2">Experiment 2</th></tr>
<tr><th>-S9</th><th>+S9</th><th>-S9</th><th>+S9</th></tr>
<tr><td>0</td><td>3.2
1.1</td><td>1.9
0.7</td><td>11.2
5.0</td><td>5.6
4.7</td></tr>
<tr><td>DMSO</td><td>5.3
2.1</td><td>1.6
0.0</td><td>6.5
5.0</td><td>2.2
4.1</td></tr>
<tr><td>30</td><td>1.9
4.4</td><td>0.6
1.9</td><td>10.7
3.5</td><td>4.9
2.6</td></tr>
<tr><td>60</td><td>2.2
3.3</td><td>1.3
0.6</td><td>10.9
14.6</td><td>2.3
5.4</td></tr>
<tr><td>120</td><td>0.6
0.5</td><td>2.0
1.3</td><td>7.6
4.8</td><td>1.9
5.3</td></tr>
<tr><td>240</td><td>1.3
1.1</td><td>0.0
1.0</td><td>11.7
6.2</td><td>1.3
6.3</td></tr>
<tr><td>360</td><td>1.2
0.0</td><td>3.8
0.0</td><td>9.5
4.0</td><td>3.9
1.2</td></tr>
<tr><td>480</td><td>0.8
2.6</td><td>2.0
0.7</td><td>10.0
8.1</td><td>2.5
5.3</td></tr>
<tr><td>600</td><td>5.2
3.9</td><td>0.7
0.0</td><td>15.7
2.6</td><td>3.0
3.0</td></tr>
<tr><td>EMS 900</td><td>563.5
579.3</td><td></td><td>923.7
835.7</td><td></td></tr>
<tr><td>DMBA 20</td><td></td><td>101.4
110.4</td><td></td><td>51.1
61.9</td></tr>
</table>
No evidence of cytotoxicity was seen in this study: the highest tested concentration of thiencarbazone-methyl was limited by solubility in the vehicle.
</td>
<td>Report No AT03686
Herbold B., 2007</td>
</tr>
</table>

4.8.1.2 *In vivo* data

Table 19: Summary of relevant *in vivo* data

<table>
<tr><th>Test method/
Guideline</th><th>Sampling times</th><th>Dose levels</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>Micronucleus Test
Mouse, Hsd/Win: NMRI,
5 males/ group

OECD TG 474
EC B12
GLP</td>
<td>24 hours after last IP injection</td>
<td>125, 250 and 500 mg/kg bw</td>
<td>Negative.

At 500 mg/kg, 3/10 males died. Clinical signs included: apathy, roughened fur, loss of weight, spasm, periodically stretching of body, difficulty in breathing and slitted eyes.

There was no evidence of target cell cytotoxicity (a significant decrease in the PCE/total erythrocyte ratio) at any dose level.

<table>
<tr><th colspan="5">Micronucleated PCEs per 2000 [range]</th></tr>
<tr><th>-control</th><th>125 mg/kg bw</th><th>250 mg/kg bw</th><th>500 mg/kg bw</th><th>+control</th></tr>
<tr><td>1.6 ± 1.1
[0-3]</td><td>2.4 ± 1.5
[1-4]</td><td>2.8 ± 2.0
[1-6]</td><td>4.2* ± 1.5
[2-6]</td><td>31.0** ± 5.8
[21-36]</td></tr>
</table>
* p < 0.05; ** p < 0.01 in non-parametric Wilcoxon ranking test

Laboratory’s background range of 2.0-5.6 (figures from 27 studies performed in 2002-2003)</td>
<td>Dose levels were based on a pilot study, where groups of 3 males and 3 females received two IP injections (1000 mg/kg of thiencarbazone-methyl) separated by 24 hours. A dose was used. The test animals displayed various clinical signs and 1 of 3 animals of each sex died. Based on these results, as no substantial differences between sexes in toxicity were observed, males only were used in this study and 500 mg/kg of BYH 18636 was chosen as MTD</td>
<td>Report No AT01568
Anon.; 2004</td>
</tr>
</table>

4.8.2 Human information

There is no human information available.

4.8.3 Other relevant information

4.8.4 Summary and discussion of Mutagenicity

The genotoxicity of thiencarbazone-methyl was investigated in an appropriate battery of studies *in vitro* and *in vivo* (Tables 18-19). All studies were compliant with the relevant OECD guidelines; however there were some reservations about the sensitivity of some of the *in vitro* studies.

Studies *in vitro* were performed in duplicate due to the relatively high purity of the thiencarbazone-methyl used in initial testing.

No evidence of mutagenicity was seen in two bacterial mutation tests. A third Ames Assay was performed to verify the negative results of the first two studies. The activation system used in the third study was an Aroclor 1254-induced male NMRI mouse liver S9 fraction due to the possibility of a mouse-specific mutagenic metabolite being involved in the urothelial carcinogenicity seen in the mouse. This study was negative and the positive controls gave appropriate responses.

No evidence of mutagenicity was seen in mammalian cells *in vitro* (HPRT assay); the performance of the positive control compound in one of these assays (using material of higher purity) was considered to have limited its sensitivity, however performance in the second assay (using lower purity material) was acceptable. No evidence of clastogenicity was seen *in vitro* in two chromosomal aberration tests (Chinese Hamster V79 cells).

No evidence of genotoxicity was seen *in vivo* in a mouse bone marrow micronucleus assay, using the higher purity material. A slight (but statistically significant) increase in the proportion of micronucleated polychromatic erythrocytes in the top dose group (4.2±1.5) was observed. However, this was within the laboratory's background range of 2.0-5.6 (figures from 27 studies performed in 2002-2003) and was associated with an unusually low concurrent control value of 1.6 (control range 0-3). This finding is therefore not considered to be of toxicological significance.

It is therefore concluded, based on the results of these studies, that thiencarbazone-methyl is not genotoxic.

4.8.5 Comparison with criteria

As thiencarbazone-methyl tested negative *in vitro* and *in vivo*, and there are no human data available, classification for genotoxicity is not justified.

4.8.6 Conclusions on classification and labelling

Not Classified – Conclusive but not sufficient for classification.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS provided results of a battery of seven *in vitro* studies and one *in vivo* study to assess the mutagenic potential of thiencarbazone-methyl. All studies were compliant with the relevant OECD test guidelines, but there were some reservations about the sensitivity of some of the *in vitro* studies.

No evidence of mutagenicity was seen in two bacterial mutation tests (Report No AT02274, 2005; Report No AT03630, 2007). The third Ames Assay (Report No AT04414, 2008) was performed to verify the negative results of the first two studies. The activation system used in the third study was an Aroclor 1254-induced male NMRI mouse liver S9 fraction due to the possibility of a mouse-specific mutagenic metabolite being involved in the mouse urothelial carcinogenicity. This study was negative and the positive controls gave appropriate responses.

No evidence of mutagenicity was seen in mammalian cells in an *in vitro* HPRT assay (Report No AT02752, 2005; Report No AT03686, 2007). No evidence of clastogenicity was seen *in vitro* in two chromosomal aberration tests in Chinese Hamster V79 cells (Report No AT02499, 2005; Report No AT03625, 2007).

No evidence of genotoxicity was seen *in vivo* in a mouse bone marrow micronucleus assay using a test substance of higher purity (Report No AT01568, 2004).

The DS concluded, based on the results of these studies, that classification of thiencarbazone-methyl for mutagenicity was not required.

Comments received during public consultation

One MSCA provided comments on the hazard class during the public consultation and agreed with the DS not to classify thiencarbazone-methyl for germ cell mutagenicity.

Assessment and comparison with the classification criteria

The *in vitro* gene mutation assays in bacteria (Report No AT02274, 2005; Report No AT03630, 2007; Report No AT04414, 2008), *in vitro* chromosomal aberration tests (Report No AT02499, 2005; Report No AT03625, 2007) and *in vitro* gene mutation assay (HPRT) in mammalian cells (Report No AT02752, 2005; Report No AT03686, 2007) on thiencarbazone-methyl were negative. In the micronucleus test on male mice thiencarbazone-methyl did not induce micronucleated polychromatic erythrocytes (Report No AT01568, 2004) at doses up to 500 mg/kg bw. The top dose level used in this study is considered to be sufficiently high. In the range-finding study at 1 000 mg/kg bw, 1 of 3 tested animals of each sex died and signs of toxicity were seen.

RAC concludes that thiencarbazone-methyl does not induce chromosomal aberrations in somatic cells under *in vivo* conditions and **does not warrant classification as a germ cell mutagen**.

4.9 Carcinogenicity

The carcinogenic potential of thiencarbazone-methyl has been investigated by the oral route, in dietary studies in rats (2 year duration) and mice (18 month duration).

Table 20: Summary table of relevant carcinogenicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

Method	Results/Remarks	Reference
OECD TG 453 2-years , diet Rat, Wistar, 50/sex/ dose, plus satellite groups of 10/sex/dose for a planned interim sacrifice 0, 500, 2500 or 5000 ppm One year doses estimated to be approximately: 0, 10.6, 27.2, 136.4 and 268.60 mg/kg bw/day in males and 0, 13.2, 35.8, 176.7 and 366.6 mg/kg bw/day in females Two year doses estimated to be approximately: 0, 22.8, 115.2 and 234.0 mg/kg bw/day in males and 0, 29.9, 152.9 and 313.4 mg/kg bw/day in females	*Non-neoplastic findings are summarised in table 14* ***Neoplastic Findings:*** Thyroid: Increase in C-cell adenoma (6.7, 6.9, 3.3 and 10% at 0, 500, 2500 and 5000 ppm) and C-cell carcinoma (0, 0, 0 and 3.3% at 0, 500, 2500 and 5000 ppm) in males only. *Laboratory Historical Control Data (6 studies conducted between 2003-2007)* *Thyroid:* *C-cell adenoma: 4.0 – 6.7%; mean 4.8%* *C-cell carcinoma: 0 – 2.0%; mean 0.3%* *Broader Laboratory Historical Control Data (16 studies conducted between 1986-1999)* *Thyroid:* *C-cell carcinoma: 0.0 - 6.0%; mean 0.9%* *C-cell adenoma: 2.1 - 24.0%; mean: 11.1%* Uterus: Increase in uterine adenocarcinomas (3.4, 1.7, 6.7 and 8.3% at 0, 500, 2500 and 5000 ppm) *Laboratory Historical Control Data (6 studies conducted between 2003-2007)* *Uterine adenocarcinoma: 3.4 – 10%; mean 5.6%*	Report No. AT03629 Anon., 2007

<table>
<tr>
<td>OECD TG 451
18 months , diet

Mouse C57BL/6J, 50/sex/ group, plus 10/sex/group for a planned interim sacrifice (28 weeks)
0, 200, 1000 or 4000 ppm

Estimated to be approximately:

0, 29.2, 147 and 599 mg/kg/day in males and

0, 0, 36.8, 185 and 758 mg/kg/day in females

GLP</td>
<td>Non-neoplastic findings are summarised in table 15

<u>Neoplastic findings</u>:

Tumours of the transitional cell epithelium (papilloma and/or carcinoma) were observed in the urinary bladder of both sexes and the prostatic urethra in males at 4000 ppm.

<u>Incidence of neoplastic microscopic changes in the urinary bladder and urethra (prostate), all animals, carcinogenicity phase</u>
<table>
<tr><th colspan="2">Sex</th><th>Male</th><th>Female</th></tr>
<tr><td colspan="2">Number of animals</td><td>50</td><td>49</td></tr>
<tr><td rowspan="2">urinary bladder</td><td>M-Transitional cell carcinoma</td><td>0</td><td>1</td></tr>
<tr><td>B-Transitional cell papilloma</td><td>1</td><td>2</td></tr>
<tr><td>urethra (prostate)</td><td>M-Urethral transitional cell carcinoma</td><td>1</td><td>-</td></tr>
</table>
NOAEL: 1000 ppm (equivalent to mean intakes of 147 and 185 mg/kg bw/day in males and females respectively)</td>
<td>Report no. SA 04062
Anon., 2006</td>
</tr>
</table>

4.9.1 Non-human information

There is no information on the carcinogenic potential of thiencarbazone-methyl in humans.

4.9.1.1 Carcinogenicity: oral

<u>Rats</u>

Thyroid gland nodules were observed in males. The correlating histopathological changes were diverse and included C-cell tumours, follicular cell tumours and hyperplasias of the adjacent parathyroid gland. C-cell adenoma and C-cell carcinoma was observed in males of the high dose group only (C-cell adenoma; 6.7, 6.9, 3.3 and 10% and C-cell carcinoma; 0, 0, 0 and 3.3% at 0, 500, 2500 and 5000ppm respectively). Such effects were not seen in females. The laboratory historical control data from 6 studies conducted within 5 years of the current study gives a range of 0-2% (mean; 0.3%) for C-cell carcinoma and a range of 4.0 – 6.7% (mean 4.8%) for C-cell adenoma. However, wider laboratory historical control data (taken from 16 studies with the same rat strain from 1986-1999) gives a range for C-cell carcinoma of 0.0 - 6.0% (mean 0.9%) and for C-cell adenoma of 2.1 - 24.0% (mean: 11.1%). It is noted that the incidence of adenomas did not show a dose-related response and that the incidence in the concurrent control and low dose groups was already relatively high compared to the historical control range. Further, the focal C-cell hyperplasia, as a precursor lesion, was not elevated accordingly (incidence 13.3, 6.9, 15.0 and 6.7% at 0, 500, 2500 and 5000ppm). There were no other findings in the thyroid. Consequently, the thyroid tumours observed in top-dose males are not considered to represent a treatment-related effect.

Nodules in the uterus were also noted, the vast majority of which correlated with stromal polyps which are a frequent finding in aged Wistar rats. Whilst uterine adenocarcinomas were noted (incidences; 3.4, 1.7, 6.7 and 8.3% at 0, 500, 2500 and 5000ppm respectively), these findings were

not statistically significant and were all within the incidence of the laboratory historical data (range; 3.4 – 10.0%, mean: 5.6%, from 6 studies). It is therefore considered that these findings are not related to treatment.

Overall, it is concluded that there is no evidence of carcinogenicity in the rat.

Mice

Clear evidence of carcinogenicity was seen in the mouse carcinogenicity study. Low incidences of benign and malignant tumours of the transitional epithelium were observed in both sexes at the top dose level of 4000 ppm (equivalent to mean achieved dietary intakes of 599 and 758 mg/kg bw/d in males and females respectively). This carcinogenic response is considered to have been secondary to the hyperplastic changes associated with the urolithiasis in the mice. Additional urinary tract findings were also limited to the top dose level and consisted of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter. Increased mortality in males at this dose level was also associated with urolithiasis. Analysis of the urinary bladder stones revealed that they consisted of approximately 70-75% thiencarbazone-methyl.

Table 21: Incidence of urinary tract tumours and urinary bladder stones

Animal	Macroscopic examination	Microscopic findings in the urinary tract considered treatment-related
Male1636	Multiple stones (N=17, up to 0.3 cm in diameter) in the urinary bladder. Thickened mucosa, slight, diffuse	Urinary bladder: simple and nodular/glandular urothelial hyperplasia, minimal to slight Suburothelial mixed cell infiltrate, focal/multifocal, slight Intramuscular inflammatory cell infiltrate, focal/multifocal, slight Interstitial oedema: diffuse, moderate Prostate: M-urethral transitional cell carcinoma
Male1669	Multiple stones (N=11, up to 0.3 cm in diameter) in the urinary bladder. Thickened mucosa, slight, diffuse	Urinary bladder: B-transitional cell papilloma simple urothelial hyperplasia, slight Suburothelial mixed cell infiltrate, focal/multifocal, minimal Intramuscular inflammatory cell infiltrate, focal/multifocal, minimal Interstitial oedema: diffuse, slight
Female1698	Multiple stones (N=2, up to 0.7 cm in diameter) in the urinary bladder	Kidney: pelvic dilatation, unilateral, minimum Urinary bladder: M-transitional cell carcinoma Stones, intraluminal, severe Suburothelial mixed cell infiltrate, focal/multifocal, minimal Intramuscular inflammatory cell infiltrate, focal/multifocal, minimal
Female1706	Single stone in the urinary bladder, 0.8 x 0.6 x 0.6 cm	Urinary bladder: B-transitional cell papilloma Stones, intraluminal, severe Simple urothelial hyperplasia, slight Suburothelial mixed cell infiltrate, focal/multifocal, moderate Intramuscular inflammatory cell infiltrate, focal/multifocal, minimal
Female1718	Single stone of 1 cm in diameter	Kidney: bilateral pelvic dilatation, minimal B-transitional cell papilloma Stones, intraluminal, marked Simple and nodular/glandular urothelial hyperplasia, minimal to slight Suburothelial mixed cell infiltrate, focal/multifocal, slight Intramuscular inflammatory cell infiltrate, focal/multifocal, minimal Interstitial oedema: diffuse, minimal

4.9.1.2 Carcinogenicity: inhalation

There are no data available.

4.9.1.3 Carcinogenicity: dermal

There are no data available.

4.9.2 Human Information

None available.

4.9.3 Other relevant information

No other relevant information.

4.9.4 Summary and discussion

(i) Incidence of tumours

In the mouse carcinogenicity study, a very small number of treatment-related tumours were evident at the high dose level only (4000 ppm; males 599 mg/kg/day, females 758 mg/kg/day). They were only seen in the transitional epithelium and there was a clear association with hyperplastic changes in the urinary tract. Three mice had benign papilloma of the urinary bladder transitional epithelium and a further two high dose animals had malignant carcinoma of the transitional epithelium (one of the bladder, and the other of the prostatic urethra).

Uroliths (stones) were identified at macroscopic and/or microscopic examinations for all the animals presenting urinary tract tumours. The company concluded that based on the mouse carcinogenicity study the crystals are not in themselves sufficient to induce tumours. The applicant suggests that over the lifespan of mice, the uroliths caused mechanical abrasion of the urothelium and associated tissues. The resulting regenerative hyperplasia eventually led to tumour formation in a small number of cases.

(ii) The high dose level from the mouse carcinogenicity study (4000 ppm) exceeded the Maximum Tolerated Dose (MTD)

The study authors described how the findings at 4000 ppm of increased mortality and decreased bodyweight in males, and the attainment of a threshold urinary concentration of thiencarbazone-methyl which led to formation of uroliths in both sexes, indicated that the MTD was exceeded. The toxicity was seen in mice and not rats, and tumours were only seen in mice. Presumably, the same events did not occur in the rat carcinogenicity study because the threshold concentration of thiencarbazone- methyl necessary to produce uroliths was not reached.

(iii) Thiencarbazone methyl was non-genotoxic; therefore the induction of tumours was via a non-genotoxic mechanism

The absence of genotoxicity of thiencarbazone-methyl was demonstrated in a range of standard studies, notably including a bacterial mutation assay using mouse S9 metabolic activation. Given the absence of genotoxicity for thiencarbazone-methyl, it can further be concluded that the induction of urinary tract pathology, which ultimately led to a very low incidence of benign and malignant tumours in mice at a very high dose, occurred via a non-genotoxic and non-physiologically relevant mechanism.

(iv) Lack of relevance to humans of the mouse urinary tract tumours

The induction of rodent tumours caused by crystal formation in the bladder is cited specifically in the ECHA Guidance on the Application of the CLP Criteria as an example of a mechanism not relevant for humans.

The key events in the mode of action for induction of urinary tract tumours in mice were;

- the exceeding of the urinary concentration necessary for formation of thiencarbazone-methyl crystals,
- the formation of uroliths,
- uroliths causing the chronic mechanical irritation of the urinary tract urothelium leading to regenerative hyperplasia,
- ultimately the induction of a low incidence of tumours.

The applicant has made a number of points based on published data:

The direct mechanical mode of action for the induction of urinary tract pathology crystals and uroliths in rodents is well-established in the scientific literature, and follows this pathway: the presence of uroliths of sufficient size produce abrasion of the mucosal surface of the bladder, resulting in erosion and ulceration. This is frequently accompanied by an acute inflammatory reaction, and is always accompanied by marked regenerative hyperplasia. Each of these effects was observed in rodent and dog studies conducted with thiencarbazone-methyl.

The potential for chronic irritation of the rat urothelium due to increased urinary solids is exacerbated because rats are quadrupeds and this orientation favours settling of solids to the anteroventral regions of the urinary bladder due to gravity. Excessive crystals or uroliths are present at the urothelial surface; the urothelium in the ventral aspects of the urinary bladder is readily irritated with bladder contraction during urination. Since the internal urethral orifice is along the same plane as the anteroventral wall of the rat bladder, urinary precipitates, crystals, aggregates, and uroliths can remain in the bladder and irritate the urothelium for prolonged periods without interfering with the outflow of urine.

Although urinary crystals and uroliths predispose to urinary bladder tumorigenesis in rodents, there are no strong epidemiologic data implicating persistent crystalluria (i.e. as seen in individuals with inborn errors of metabolism such as cystinuria, xanthinuria, and hyperoxaluria) as a risk factor for bladder cancer in humans. The apparent disparity in susceptibility between laboratory animals and humans to irritation-induced bladder tumours is considered, in part, due to postural and anatomic differences in the orientation of the urinary bladder in biped humans compared to quadruped rodents. Unlike the rat and mouse, it appears that the anatomic orientation of the urinary bladder in humans favours clearance of potentially irritating urinary solids.

Weight of evidence summary

The key events in mode of action for the induction of urinary tract tumours in mice were the exceeding of the urinary concentration necessary for formation of thiencarbazone-methyl crystals, the formation of uroliths, the chronic mechanical irritation of the urinary tract urothelium leading to regenerative hyperplasia, and ultimately the induction of a low incidence of tumours. The key events in mice are not plausible in humans. Repeated exposure of humans to toxic levels of thiencarbazone-methyl are unlikely to occur without medical or other intervention; levels of human exposure will not lead to the formation of uroliths, and the induction of chronic mechanical damage to the urothelium will not occur.

It is possible that the erect posture of humans and general anatomic differences to laboratory animals will additionally make it unlikely humans will share the same propensity as mice for the induction of urinary tract pathology by uroliths and crystals.

Table 22: Key events in the mode of action

Key Event	Concordance			Confidence/Uncertainty
	Mice	**Rats**	**Humans**	
Formation of crystals or uroliths	Yes	Yes	Unlikely, unless exceptionally high urinary concentrations are achieved	Uroliths are composed of 70 – 75 % thiencarbazone-methyl
Mechanical abrasion of the urothelium and associated tissues	Yes	Yes	Unlikely, due to high dose/high urinary concentration phenomenon, and postural and anatomic differences in the orientation of the urinary bladder in biped humans compared to quadruped rodents	Confirmed in rodents by submucosal inflammatory cell infiltration, minimal diffuse urothelial inflammation
Regenerative hyperplasia	Yes	Yes	Unlikely	
Urinary bladder tumours	Yes	Not observed but plausible at high doses	Highly unlikely	Due to quantitative and qualitative differences between rodent and humans

The proposed mechanism is considered to be plausible and fulfils the critical criteria of the IPCS conceptual framework for analysis of the relevance of a cancer mode of action for humans (IPCS, 2001). While there is little in the way of mechanistic data, key aspects of the IPCS criteria including a dose-response relationship with a clear threshold, biological plausibility and coherence and temporal association are satisfied.

The IPCS consideration of species differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis (IARC Scientific Publications No. 147) makes a number of pertinent observations:

- That urinary bladder calculi, irrespective of composition, cause irritation and cell proliferation in humans.
- That there is some epidemiological evidence that urinary tract cancer in humans is associated with a history of calculi in the bladder.
- That the risk in humans may not be as great as that in rodents because the calculi are usually voided spontaneously or removed by surgical procedures.
- Therefore although there are quantitative differences in the carcinogenic response to calculi between species, the effect is not species-specific.
- That calculus formation is dependent on attainment in the urine of critically high concentrations of the constituent chemicals which form the calculus. The carcinogenic effects are therefore dependent on reaching a threshold concentration for calculus formation.

4.9.5 Comparison with criteria

As a treatment-related increase in tumours was seen in mice, it is appropriate to consider classification of thiencarbazone for carcinogenicity.

Classification in Category 1A is not appropriate, as there is no evidence of carcinogenicity in humans. Similarly, Category 1B is not justified as the animal data are limited rather than sufficient due to tumour incidence being restricted to one species (mouse), in a single tissue, with no evidence of a genotoxic mode of action.

The observed tumour profile would be sufficient to justify classification in Category 2. However, as indicated in the Guidance on the Application of the CLP Criteria (Version 4.1 June 2015), urinary bladder tumours due to crystals in the bladder (IARC, 1999) may be considered not relevant for humans. This has been discussed (above) for thiencarbazone-methyl and a conclusion of non-relevance to humans reached. Accordingly, no classification for carcinogenicity seems appropriate.

4.9.6 Conclusions on classification and labelling

Not Classified – Conclusive but not sufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There were no data on the carcinogenic potential of thiencarbazone-methyl in humans.

The carcinogenicity of thiencarbazone-methyl was investigated in two acceptable animal studies, in which the substance was administered in diet.

In the 2-year study in rats according to OECD TG 453 (Report No AT03629, 2007), the substance was administered in diet at concentrations of 0, 500, 2 500 and 5 000 ppm corresponding to doses of 0, 23, 115 and 234 mg/kg bw/d in males and 0, 30, 153 and 313 mg/kg bw/d in females. The choice of the top dose had been considered justified based on the results of the 90-day rat study, in which mortality (secondary to urinary tract toxicity) was observed at 7 000 ppm. However, at the top dose of 5 000 ppm in the present rat carcinogenicity study, no clear adverse effects were noted in either sex, indicating that systemic toxicity at that dose was limited. After analysing the results, the DS concluded that there was no evidence of carcinogenicity in this study.

In the 18-month study in mice according to OECD TG 451 (Report No SA04062, 2006) and GLP, thiencarbazone-methyl was given in diet at concentrations of 0, 200, 1 000 and 4 000 ppm corresponding to doses of 0, 29, 147 and 599 mg/kg bw/d in males and 0, 37, 185 and 758 mg/kg bw/d in females. No evidence of treatment-related tumours was found in animals exposed at 200 ppm and 1 000 ppm. The only observed neoplastic effects in mice were noted in the 4 000 ppm groups in both sexes. M-transitional carcinoma occurred in 1 out of 49 females, and B-transitional cell papilloma was observed in 2 out of 49 females and in 1 out of 50 males. In addition, M-urethral transitional cell carcinoma was found in urethra (prostate) in 1 out of 50 males. Uroliths

(stones) were identified at macroscopic and/or microscopic examinations in all the animals having urinary tract tumours. Additional urinary tract findings were also limited to the top dose level and consisted of hyperplastic, inflammatory and irritant effects on the kidneys, bladder, urethra and ureter. Increased number of unscheduled deaths in males at this dose level was also associated with urolithiasis. Analysis of the urinary bladder stones revealed that they consisted of approximately 70-75 % thiencarbazone-methyl.

The low incidence of tumours in the transitional epithelium of the urinary bladder and urethra of mice exposed to 4 000 ppm was considered by DS to be secondary to the hyperplastic changes associated with the urolithiasis. In the opinion of DS, data on carcinogenicity of thiencarbazone-methyl was conclusive, but not sufficient for classification; thus, no classification for carcinogenicity was proposed.

Comments received during public consultation

Three MSCAs supported the DS's proposal to not classify thiencarbazone-methyl for carcinogenicity. They were of the view that the induction of tumours in transitional epithelium of bladder and urethra was caused by crystals (stones) consisting of precipitated thiencarbazone-methyl in urine, and that this mechanism of carcinogenicity was not relevant to humans.

Assessment and comparison with the classification criteria

In the 2-year study in rats according to OECD TG 453 (Report No AT03629, 2007) no treatment-related tumours were found after 12 months.

After a 2-year dietary exposure, the incidence of fibroadenomas in the mammary gland of females at 5 000 ppm (234 mg/kg bw/d) was statistically significantly lower compared to the control incidence and a few tumours (brain granular cell tumours and malignant pheochromocytomas of the adrenal medulla in males and mammary gland fibroadenoma in females) showed a negative trend in the trend test statistics according to Peto.

In male rats the focal C-cell hyperplasia and adenoma as precursor lesions of the C-cell carcinoma were not elevated accordingly after the 2-year exposure. The incidence of C-cell adenoma in the concurrent control group was at the upper limit of the historical control range in years 2003-2007 suggesting that the animals selected for this study had an elevated background incidence of this tumour. The C-cell carcinomas were observed only in males of the high dose group (incidence in males including decedents: 0/60 – 0/60 – 0/60 – 2/60 or in %: 0 – 0 – 0 – 3.3). The increase in the top dose group was not statistically significant by pair comparison with the concurrent control group by Fisher exact probability test ($P < 0.01$), and the incidence in the top dose (3.3 %) was only slightly above the historical control data (HCD) range (up to 2 %) for years 2003-2007. Comparison of all these findings with the broader HCD from 16 studies from the same laboratory in 1986-1999 with the same rat strain demonstrates a high variation in their incidences (the table below). It is concluded that the small increase of C-cell carcinoma at the top dose was a chance finding, because the effect did not show a dose-response relationship at lower doses, it was not statistically significant in comparison with the concurrent control and the incidence was in a similar range with the HCD of the laboratory in years 2003-2007 and within the broader laboratory HCD.

***Table**: Neoplastic findings in thyroid of male rats and related laboratory HCD.*

		Laboratory HCD (6 studies in 2003-2007)	**Broader Laboratory HCD (16 studies in 1986-1999)**	**0 ppm 0 mg/kg bw/d**	**500 ppm 23 mg/kg bw/d**	**2 500 ppm 115 mg/kg bw/d**	**5 000 ppm 234 mg/kg bw/d**
Thyroid	C-cell hyperplasia (%)	-	3.4 - 34.0 %, mean 14.7 %;	13.3 %	6.9 %	15.0 %	6.7 %
	C-cell adenoma (%)	4.0-6.7 %; mean 4.8 %	2.1 - 24.0 %, mean 11.1 %;	6.7 %	6.9 %	3.3 %	10 %
	C-cell carcinoma (%)	0-2.0 %; mean 0.3 %	0.0-6.0 %, mean 0.9 %	0/60 0 %	0/60 0 %	0/60 0 %	2/60 3.3 %

Nodules in the uterus were present at necropsy at an incidence of 8, 13, 13 and 15 at 0, 500, 2 500 and 5 000 ppm, respectively. The vast majority of them correlated with stromal polyps which are frequent findings in aged Wistar rats. The incidence of uterine adenocarcinomas was slightly higher at the high dose level as compared to the concurrent control (2, 1, 4 and 5 at 0, 500, 2 500 and 5 000 ppm, respectively); however, it was not statistically significant and it was well within the HCD range (the table below). RAC concludes that the observed tumours are not considered to be treatment-related.

***Table**: The incidence of uterine adenocarcinomas and related laboratory HCD.*

		Laboratory HCD (6 studies in 2003-2007)	**0 ppm 0 mg/kg bw/d**	**500 ppm 23 mg/kg bw/d**	**2 500 ppm 115 mg/kg bw/d**	**5 000 ppm 234 mg/kg bw/d**
Uterus	Adeno-carcinoma (%)	3.4-10 %; mean 5.6 %	3.4	1.7	6.7	8.3

Treatment-related effects on the urinary tract were limited to the presence of crystals (assumed to be thiencarbazone-methyl or its metabolite) in the urine of the majority of animals at the top dose level of 5 000 ppm and in two animals/sex at 2 500 ppm. These findings are not considered to be of toxicological significance in the absence of macroscopic or histopathological correlates.

Summing up, no evidence of carcinogenicity was seen in this study. A NOAEL of 2 500 ppm (equivalent to mean achieved dietary intakes of 115 and 153 mg/kg bw/d in males and females, respectively) can be determined based on the clinical chemistry findings (reduced plasma triglyceride concentration) at the top dose level of 5 000 ppm (equivalent to 234 and 313 mg/kg bw/d, respectively).

In the second study (Report No SA04062, 2006) in mice, 60 male and 60 female C57BL/6J mice per group were fed a diet containing 0, 200, 1 000 or 4 000 ppm of thiencarbazone-methyl (mix-batch 702-73-06-0001) for at least 28 weeks. After 28 weeks, 10 males and 10 females from each group were necropsied at the scheduled interim sacrifice. The remaining 50 animals/sex/group continued to be treated until the scheduled final sacrifice at week 78. The mean intake of thiencarbazone-methyl over 18 months was calculated to be 0, 29, 147 and 599 mg/kg bw/d in males and 0, 37, 185 and 758 mg/kg bw/d in females, at 0, 200, 1 000 and 4 000 ppm, respectively.

The mean body weight of male mice at 4 000 ppm was 5 % lower than that of the control males at the end of the study on day 540. The mean body weight and body weight gain of females, the mean food consumption and haematology parameters of both sexes were unaffected by the treatment.

In animals exposed to 4 000 ppm, the clinical symptoms consisted of an increased incidence of generalized soiled fur in both sexes and of an increased incidence of skin lesions in males only, principally in the anogenital region, abnormal penis and wasted appearance. Abnormal penis was not associated with any intrinsic histopathological findings, but was associated with chronic ulcerative dermatitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination. A statistically significantly higher incidence of unscheduled deaths (17/22) attributable to killing for humane reasons was observed in males at 4 000 ppm (the table below). The majority of these animals had skin lesions in the anogenital region on the day of sacrifice.

***Table**: Mortality incidence of animals of the carcinogenicity phase (unscheduled deaths).*

Sex	**Males**				**Females**			
thiencarbazone-methyl (ppm)	0	200	1 000	4 000	0	200	1 000	4 000
Number of animals	50	50	50	50	50	50	50	50
Killed for humane reasons	5 (10 %)	7 (14 %)	5 (10 %)	17 (34 %)	6 (12 %)	11 (22 %)	3 (6 %)	9 (18 %)
Found dead	6 (12 %)	9 (18 %)	6 (12 %)	5 (10 %)	0 (0 %)	3 (6 %)	1 (2 %)	0 (0 %)
Total number of unscheduled deaths	11 (22 %)	16 (32 %)	11 (22 %)	22* (44 %)	6 (12 %)	14 (28 %)	4 (8 %)	9 (18 %)

At 4 000 ppm treatment-related macroscopic findings at unscheduled sacrifice consisted of stones in the urinary bladder of 19/22 males and 3/9 females. The stones were often multiple (0.1 to 0.6 cm in diameter), yellow (mainly), greenish or white and firm. Thickening of the mucosa in the urinary bladder was observed in 8/22 males and 2/9 females. This finding was often correlated with urothelial hyperplasia and/or interstitial oedema at the microscopic examination. Pelvic dilatation of the kidney was observed in 4/22 males, and was correlated with treatment-related pelvic dilatation at the microscopic examination.

In the skin, a statistically significantly higher incidence of chronic ulcerative dermatitis was observed in males, but not in females, at 4 000 ppm. As this finding was located in the anogenital region or surrounding area, it was considered to be probably related to a

stone-induced dysuria and thus indirectly treatment-related.

At 4 000 ppm, tumours of the transitional cell epithelium (papilloma and/or carcinoma) were observed in the urinary bladder of both sexes and in the prostatic urethra in males, and they were considered to be treatment-related (the tables below). The incidence of these tumours was very low and it was considered to be secondary to the chronic hyperplastic changes.

***Table**: Incidence of neoplastic microscopic changes in the urinary bladder, all animals, carcinogenicity phase.*

Sex	**Males**				**Females**			
thiencarbazone-methyl (ppm)	0	200	1 000	4 000	0	200	1 000	4 000
Number of animals	49	49	50	50	48	49	47	49
M-transitional cell carcinoma	0	0	0	0	0	0	0	1
B-transitional cell papilloma	0	0	0	1	0	0	0	2

***Table**: Incidence of neoplastic microscopic changes in the urethra (prostate), all animals, carcinogenicity phase.*

Sex	**Males**			
thiencarbazone-methyl (ppm)	0	200	1 000	4 000
Number of animals	49	50	48	50
M-urethral transitional cell carcinoma	0	0	0	1

The other tumours in treated animals were those commonly observed in this mouse strain and age of mice, and they were considered to be incidental.

Summing up, dietary administration of thiencarbazone-methyl for an 18-month period to the C57BL/6J mouse strain, at dose levels up to 4 000 ppm (equivalent to 599 mg/kg bw/d in males and 758 mg/kg bw/d in females) produced transitional cell epithelium tumours in the urinary bladder in one male (2 %) and three females (6 %) and in the prostatic urethra in one male (2 %). The incidence of these tumours was very low and it was considered by the authors of the study to be secondary to the chronic hyperplastic changes resulting from chronic irritation due to the presence of stones in the urinary bladder.

According to the CLH report, the authors of the 18-month mouse study (Report No SA04062, 2006) had indicated that the MTD was exceeded in both sexes at 4 000 ppm because of an increased mortality and decreased body weight. RAC concludes that that MTD was not exceeded in either sex, since systemic toxicity was low as judged by low or no effect on body weight and by lack of specific adverse effects in internal organs other than urogenital system. There was no increase in number of animals found dead at the high dose as compared to the concurrent controls. However, the high dose used in the study was considered sufficiently high by RAC as indicated by the number of unscheduled deaths of males due to ulcerative skin lesions in the anogenital region leading to killing for humane reasons.

The toxicity on the urogenital system was observed in mice but not in rats, and the tumours in the urinary bladder and prostatic urethra were only seen in mice. Presumably,

the same events did not occur in the rat carcinogenicity study because the threshold concentration of thiencarbazone-methyl to produce uroliths was not reached.

Noting the absence of genotoxicity for thiencarbazone-methyl, it can be further concluded that the induction of urinary tract pathology, which ultimately led to a very low incidence of benign and malignant tumours in the bladder and urethra of mice, occurred via a non-genotoxic mechanism, only at the high dose producing considerable toxicity in the urinary system and skin of the anogenital region of males, leading to an increased number of unscheduled deaths due to humane killing.

In the scientific analysis made for Thyroid, Kidney and Urinary Bladder Carcinogenesis (IARC Scientific Publications No 147) the following conclusions were reached:

- the urinary bladder calculi, irrespective of composition, cause irritation and cell proliferation in humans.
- there is some epidemiological evidence that urinary tract cancer in humans may be associated with a history of calculi in the bladder.
- the calculus formation is dependent on attainment in the urine of critically high concentrations of the constituent chemicals which form the calculus. The carcinogenic effects are therefore dependent on reaching a threshold concentration for calculus formation.
- that the risk in humans may not be as great as that in rodents because the calculi are usually voided spontaneously or removed by surgical procedures.
- therefore, although there are quantitative differences in the carcinogenic response to calculi between species, the effect is not species-specific.

Summing up, the crystallisation of thiencarbazone-methyl precipitating from urine develops calculi in the urinary tract. The calculi cause chronic mechanical irritation of the epithelium leading to regenerative hyperplasia and ultimately to a low incidence of tumours. However, thiencarbazone-methyl caused these effects only at the highest tested dose (599 mg/kg bw/d in males and 758 mg/kg bw/d in females) as a result of a very high concentration of thiencarbazone-methyl in urine. This led to severe ulcerative skin lesions in the anogenital region and consequently to an increased number of unscheduled deaths of males killed for humane reasons.

In summary, RAC concludes that the very low incidence of urinary bladder tumours in mice found only at the top dose, induced by the mechanism with potential quantitative differences between species, does not provide sufficient evidence for classification of thiencarbazone-methyl as carcinogenic. Furthermore, no evidence of thiencarbazone-methyl-induced carcinogenicity was found in the acceptable 2-year carcinogenicity study in rats.

Taking into account the evidence from these both carcinogenicity studies, RAC concludes in line with the DS, that thiencarbazone-methyl **does not warrant classification for carcinogenicity**.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

The potential for thiencarbazone-methyl to adversely affect fertility has been investigated in rats in a standard dietary multigenerational study and non-standard gavage studies.

4.10.1.1 Non-human information

Table 23: Summary table of relevant reproductive toxicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

Method	Results/Remarks	Reference
2-Generation study OECD TG 416 Rats (Wistar), 25/sex/dose Diet Test diets were fed continuously to male and female rats in the F0 parental generation throughout premating, mating, gestation, and lactation periods. After weaning of the F1 generation at 4 weeks of age, selected weanlings were maintained in their same dietary groups through maturation, mating, gestation, and lactation. The final necropsy took place when the F2 offspring were weaned at 4 weeks of age. 0, 500, 2500, or 10000 ppm **In F0 animals estimated to be:** **<u>Pre-mating</u>** 0, 46.0, 245.0, 945.8 mg/kg bw/day in males 0, 55.6, 263.7 or 968.4 mg/kg bw/day in females **<u>Gestation and lactation (females)</u>** <u>day 14 to 20 p.c</u> 0, 36.1, 181.6 or 696.8 mg/kg bw/day <u>0 to 4 p.p</u> 0, 57.8, 270.4 or 1175.7 mg/kg bw/day **In F1 animals estimated to be:** **<u>Pre-mating</u>** 0, 50.2, 260.5 or 992.1 mg/kg bw/day in males	**10000 ppm** ***<u>Parental animals</u>*** **F0** ↑ abs/rel kidney weight (ca 15 %) females ↑ rel liver weight (10%) females <u>Kidneys</u>: Transitional cell hyperplasia (10 females), pyelitis and/or pelvic degeneration (4 males and 2 females), pyelonephritis (1 male), dilation of cortical and/or medullary tubules (9 females), pelvic dilation (9 females), dilation of papillary tubules (3 males), inflammation (8 females), basophilic tubules (16 females) and urolithiasis (4 males and 6 females) <u>Ureter</u>: transitional cell hyperplasia (6 females), oedema (3 females), inflammatory cell infiltration (5 females), dilation (8 males and 7 females) , urolitiasis (1 female) and hypertrophy (3 males and 6 females) <u>Urinary bladder</u>: transitional cell hyperplasia (5 males and 7 females), submucosal oedema (4 males and 1 female) submucosal infiltration (4 males and 2 females) and urolithiasis (2 males). 3 males with no sperm (cf. 1 male in controls , 0 at 500 ppm and 5 at 2500ppm) **F1** 1 male was found dead at the end of the pre-mating period. The male was emaciated, had reduced water intake and exhibited morphological degenerative findings in the kidneys (papillary necroses, transitional cell hyperplasia and tubulus dilations), ureters (hypertrophy) and urinary bladder (transitional cell hyperplasia). This death was considered to be treatment related. Titled head (2 males), sunken flanks (1 male) and/or emaciation (3 males). ↑ urination during lactation (2 females) ↓ food consumption (8% males and females). ↓ bodyweight at necropsy (7% females), ↓ bodyweight gain during gestation 9%. ↑abs/rel kidney weight (8/17%) females ↓abs/rel liver weight (14/13%) males <u>Kidneys</u>: Transitional cell hyperplasia (13 females), pyelitis and/or pelvic degeneration (1 male and 9 females), pyelonephritis (4 females), dilation of cortical and/or medullary tubules (11 females), pelvic dilation (12 females), dilation of papillary tubules (4 males and 9 females), basophilic tubules (13 females), interstitial fibrosis (7 females) and urolithiasis (3 males and 9 females). <u>Urinary bladder</u>: transitional cell hyperplasia (6 males and 11 females), submucosal oedema (2 males and 4 female) submucosal infiltration (13 males and 12 females) and urolithiasis (2 males) <u>Ureter</u>: transitional cell hyperplasia (8 females), oedema (1 female), inflammatory cell infiltration (5 females), dilation (2 males and 9	Report No. AT 03180 Anon., 2006

0, 68.0, 353.1 or 1284.0 in females **<u>Gestation and lactation (females)</u>** <u>day 14 to 20 p.c</u> 0, 40.4, 226.6 or 742.28 mg/kg bw/day <u>0 to 4 p.p</u> 0, 85.9, 460.3 or 1832.8 mg/kg bw/day GLP	females) and hypertrophy (3 males and 12 females). ***<u>Offspring</u>*** ↓ total number of F1 pups (not statistically significant); 217 compared to 249, 282 and 220 at 0, 500 and 2500 ppm respectively) F2 pups with no milk in stomach (7 compared to 3 or 4 in control and low dose groups) 2F1 and 2F2 weanlings with dilated and/or enlarged kidneys 1F2 weanling with stones in the kidney 2F2 weanlings with stones in the urinary bladder	

	2500 ppm ***Parental animals*** **F0** 5 males with no sperm (c.f., 1 male in controls and 0 at 500 ppm) **F1** ↓abs/rel kidney weights (males). ***Offspring*** ↓ total number of F1 pups (not statistically significant); 220 compared to 249 and 282 at 0 and 500 ppm respectively) **500 ppm** ***Parental animals*** F1 ↓ rel kidney weights (males). ***Offspring*** No treatment related effects *NOAEL (reproductive toxicity): 10000 ppm (equivalent to mean achieved pre-mating dietary intakes of 946 and 968 mg/kg bw/day in males and females respectively).* *NOAEL (parental toxicity, males): 2500 ppm for males (equivalent to 245 mg/kg bw/day) based on the mortality, signs of toxicity and histopathological findings in the kidney, ureter and bladder seen at the top dose level of 10000 ppm.* *NOAEL Parental toxicity, females): 500 ppm (equivalent to 56 mg/kg bw/day), based on the histopathological findings in the kidney and ureter seen at 2500 ppm.*	

Treatment-related findings in parental animals were comparable to those seen in other studies in the rat and are consistent with the mode of action of thiencarbazone-methyl. Effects at the high dose level (10000 ppm) are associated with urolithiasis: mortality in one male at this dose level was secondary to renal necrosis. Findings in other parental animals in this dose group were less severe but were largely limited to characteristic histopathological changes in the urinary system. Slight reductions in weight gain and food consumption and increased kidney weight (in females) may also be related to the mode of action.

The fertility, gestation and rearing indices as well as gestation length and number of litters born were not changed by the treatment up to 10000 ppm in both generations. The insemination index was reduced at 10000 ppm (80% compared to 96, 100 and 92% at 0, 500 and 2500 ppm respectively). Of the five males not mating in this group, three were found to have no sperm. However, there were no biologically relevant effects on sperm parameters (epididymal sperm count, sperm motility and morphology and testicular spermatids counts). Five males at 2500 ppm were also found to be spermless (compared to one in controls and zero at 50 ppm). Given the lack of dose response, the fact sperm parameters at 1000 ppm were similar to controls and lack of a similar effect in F1 males, the occurrence of five or three spermless F0 males at 2500 or 10000 ppm is considered to be incidental.

A reduction (not statistically significant) in the total number of F1 pups was observed at 2500 ppm and 10000 ppm. However, this finding was considered to be secondary to the number of F0 males with 'no sperm' seen in these dose groups; litter size was unaffected by treatment. As noted above,

the incidence of 'no sperm' was not considered to be related to treatment in the absence of a dose-response relationship or similar findings in F1 males.

No other parameters were affected by treatment.

Table 24: Significant changes in litter parameters of F0 and F1 generations (means and partly ±SD)

Observation	Dietary concentration (ppm)			
	0	500	2500	10000
	F0→F1 Pups			
F0 Males No sperms-corresponds to female without implantations	1	0	5	3
Insemination index	96.0	100.0	92.0	80.0
Mean implantations [a)]	12.00 ±1.907	12.54 ±1.503	11.90 ±2.808	12.00 ±1.686
Mean prenatal loss [a)]	1.17 ±0.834	0.79 ±1.062	0.90 ±0.968	1.15 ±1.137
Number born	249	282	220	217
Number born dead	2	1	4	1
Live birth index	99.24	99.48	98.13	99.62
Number of litters	23	24	20	20
	F1→F2 Pups			
F1 Males No sperms-corresponds to female without implantations	0	0	0	0
Insemination index	92.0	100.0	100.0	100.0
Mean implantations [a)]	12.41 ±1.221	11.88 ±1.454	11.91 ±1.379	11.82 ±1.006
Mean prenatal loss [a)]	1.09 ±1.065	0.75 ±0.944	0.87 ±1.014	0.86 ±0.990
Number born	249	267	254	241
Number born dead	10	0**	1*	0**
Live birth index	95.52	100.00	99.64	98.64
Number of litters	22	24	23	22

[a)] Per litter.

* Statistically different from control, $p \leq 0.05$.

** Statistically different from control, $p \leq 0.01$.

Table 25: Individual litter size data (F1 and F2 Litters)

Female Number*	Numbers of pups born to each female							
	F1 Litters (dose Group ppm)				F2 Litters (dose Group ppm)			
	0ppm	500ppm	2500ppm	10000ppm	0ppm	500ppm	2500ppm	10000ppm
1	12	11	10	10 RVP	NI X	10	13	11
2	11	-SMBI X	10 RVP	13	11	12	9	11
3	0 SMIS X	11	12	13	13	11	11	13
4	10	11 RVP	1 RVP	14	11	10	10	13
5	9	14	- RNIS X♂	9	9	12	8 VPNS	10
6	-RNIS X♂	10	12	-RNIS X♂	12	9	12	11
7	11	14	10	13	9	13	10	12
8	12	12	13 RVP	10	15	10	12	NIS X
9	11	12	13	-NI X♂	12	13	11	10
10	12	13	10	11	12	12	12	11
11	10 VPNS	11 RVP	10	12	11	12	10	12
12	12 RVP	10 VPNS	10	-NI X	12	10 PDFK	12	10
13	11	13	12	10	10	9	10	11
14	12	13	10	9 VPNS	10	11 PDFK	12	11
15	4 RVP	11	-RNI X♂	13	11	9 PDFK	9	11
16	11	10	-NIS X♂	10	12	11	10	11
17	11	13	13	6	13	11	12	11
18	11	11 VPNS	14	-RNIS X	NI X	11	9 RVP	10
19	14	13	11	10	9 VPNS	7	RNIS X	12
20	11	8	13 RVP	6	11	13	12	10
21	12	13	12	11	NIS X	13	14	NIS X
22	10 VPNS	9	11	11	10 PDFK	13	NIS X	NIS X
23	10	13	13 VPNS	- NIS X♂	12	14	10	9
24	10	14 VPNS	- NIS X♂	12	12	11	12	11
25	12	12	- NIS X♂	14	12	RNIS X	14	10
Total numbers of pups	249	282	220	217	249	267	254	241

* Actual female identification numbers 101-200 for F0 females(F1 litters) and 201-400 for F1 females(F2 litters)

X Excluded from mean

NI Not inseminated

NIS No implantation sites

RNIS Re-mated no implantation sites

RVP Re-mated viable pups

SMBI Sac moribund before insemination (Those animals are sacrificed when moribund even before insemination took place, therefore in those animals no implantation sites could be counted)

SMIS Sac moribund implantation sites (i.e. Animals are sacrificed when moribund after insemination, therefore the implantation sites were counted)

VPNS Viable pups no sperm detected

PDFK Pups died female killed

4.10.1.2 Human information

There is no human information available.

4.10.2 Developmental toxicity

The potential for thiencarbazone methyl to cause developmental toxicity has been investigated in standard studies in rats and rabbits.

4.10.2.1 Non-human information

Table 26: Summary table of relevant developmental toxicity studies

Note: The NOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of the substance without further critical assessment.

<table>
<tr><th>Method</th><th>Results/Remarks</th><th>Reference</th></tr>
<tr><td>OECD TG 414
Gavage
Rat, Hsd Cpb: WU
25/dose
Days 6-19 of gestation
0, 50, 200 and 1000 mg/kg/day in 0.5% aqueous carboxy-methylcellulose
GLP</td><td>
<u>1000 mg/kg bw/day</u>
Maternal effects:
Marginal bodyweight loss (0.8%) over GD 6-7
Overall, ↓ absolute and corrected bodyweight gains (26% and 51% respectively compared to the control group) from GD 0-20.
↓ food consumption over GD 6-20 ranging from 14-23%

12 females with yellowish sediments in the urinary bladder (2 of which also had urethras filled with yellowish sediment and 1 of which had a pale kidney); 2 further females with unilateral dilated ureters with an inlet of yellowish sediment (in 1 of these, the caudal end of the kidney was also swollen with light brown discolouration).

Fetal effects:
↓ fetal weight (9%)
Incomplete or absent ossification of the distal phalanx digits, metacarpals, the 5th and 6th sternebrae and the sacral vertebral arches. (see table below)
↑ incidence of wavy ribs.

<u>200 mg/kg bw/day and 50 mg/kg bw/day</u>
Maternal effects:
No treatment related effects.

Fetal effects:
No treatment related effects.

<table>
<tr><th rowspan="2">Parameter</th><th colspan="5">Dose level (mg/kg bw/d)</th></tr>
<tr><th>0</th><th>50</th><th>200</th><th>1000</th><th>Historical</th></tr>
<tr><td></td><td colspan="5">Fetal findings: % fetal incidence [% litter incidence]</td></tr>
<tr><td>Unossified 5th right distal phalanx digits</td><td>16.1 [50.0]</td><td>26.0 [72.7]</td><td>21.9 [66.7]</td><td>33.9** [80.0]</td><td>0-15.2 [0-50.0]</td></tr>
<tr><td>Unossified 5th left distal phalanx digits</td><td>10.7 [45.0]</td><td>26.7** [68.2]</td><td>25.3** [66.7]</td><td>33.1** [80.0]</td><td>0-11.6 [0-50.0]</td></tr>
<tr><td>Unossified 5th right metacarpal</td><td>5.4 [25.0]</td><td>12.3 [45.5]</td><td>15.8* [58.3]</td><td>28.0** [80.0**]</td><td>2.5-15.1 [13.6-61.9]</td></tr>
<tr><td>Unossified 5th left metacarpal</td><td>9.8 [40.0]</td><td>13.7 [54.5]</td><td>19.9 [66.7]</td><td>31.4 [80.0]</td><td>3.3-18.2 [9.1-61.9]</td></tr>
<tr><td>Wavy ribs</td><td>1.8 [10.0]</td><td>2.1 [9.1]</td><td>3.4 [16.7]</td><td>10.2* [50.0*]</td><td>2.8-14.6 [10.0-42.9]</td></tr>
</table>
*significantly different to controls p≤0.05; **≤0.01
Historical control range: 15 studies performed 2002-2005

NOAEL (maternal toxicity): 200 mg/kg bw/day, based on the reduced weight gain and food consumption seen at the top dose level of 1000 mg/kg bw/day.

NOAEL (developmental toxicity): 200 mg/kg bw/day d, based on the slightly (but significantly) reduced mean fetal weight and the increased incidences of a number of skeletal variations seen at 1000 mg/kg bw/day.
</td><td>Report No. AT02339
Anon., 2005</td></tr>
</table>

EC B31 Gavage **Rabbit, New Zealand White,** 24 dose Days 6-28 of gestation 0, 50, 125 and 500 mg/kg/day in 0.5% aqueous carboxy-methylcellulose GLP	***Maternal effects:*** **<u>500 mg/kg bw/day</u>** 1 female killed on GD 15 due to marked bodyweight loss associated with no food intake from GD 8. Clinical signs consisted of no/few faeces and yellow sediment in the urine. ↑ incidence of dams with few faeces (10/25 c.f., 1/25 in controls), yellow sediment in the urine (all dams), and red traces under the tray (2/25 on GD 8 and/or 9). Mean bodyweight loss of 0.04 kg compared with a loss of 0.01 kg in the control group, between GD 6 and 8. Thereafter, mean bodyweight gain tended to be less compared with the controls, resulting in an overall significant ↓ in mean bodyweight gain of 45% by GD 29. Maternal corrected bodyweight change, was more pronounced (-0.29 kg) compared with the controls (-0.17 kg). ↓ Food consumption (11 and 19%) compared with the controls. The effect was most pronounced between GD 8 and 10. One female with white sediment in the kidney. One female with yellow sediment in the kidney and bladder One female with yellow sediment in the bladder. **125 mg/kg bw/day** Mean bodyweight loss of 0.03 kg compared with a loss of 0.01 kg in the control group, between GD 6 and 8 Yellow sediment in the urine (4/25)	Report No. SA 03350 Anon., 2006

50 mg/kg bw/day

Mean bodyweight loss of 0.02 kg compared with a loss of 0.01 kg in the control group, between GD 6 and 8

Fetal effects:

Finding	**Dose level** (mg/kg bw/d)			
	0	**50**	**125**	**500**
No of live fetuses	201	243	248	228
Mean No of live fetuses/litter	8.7 ± 2.4 (23)	10.1 ± 2.4 (24)	9.9 ± 2.6 (25)	9.5 ± 3.1 (24)
Mean fetal weight (g)	36.7 ± 5.9	32.5^{++} ± 6.5	34.7^{++} ± 6.2	32.2^{++} ± 7.1
	Fetal findings (% fetal incidence [% litter incidence])			
Runts	6.5 [34.8]	18.5 [58.3]	9.5 [36.0]	23.0 [62.5]
Ventricular septal defect	- -	- -	0.4 [4.0]	0.8 [4.2]
Misshapen thymus	4.0 [21.7]	2.3 [16.7]	4.9 [36.0]	5.0 [37.5]
Short innominate artery	1.5 [13.0]	1.0 [12.5]	0.9 [8.0]	2.2 [20.8]
25 pre-sacral, 6 lumbar vertebrae	- -	1.9 [8.3]	1.7 [8.0]	1.8 [8.3]
Hyoid incomplete ossification	15.1 [39.1]	18.6 [54.2]	11.4 [36.0]	20.4 [54.2]
Hyoid not ossified	0.9 [4.3]	2.7 [8.3]	2.8 [16.0]	3.6 [12.5]

Statistical analysis on mean fetal weights; ++: $\alpha = 0.01$

In addition 1 fetus from each of the 50 and 500 mg/kg bw/day had white sediment in the kidney.

NOAEL (maternal toxicity): 125 mg/kg bw/day, based on the clinical signs and the effects on body weight and food consumption seen at the top dose level of 500 mg/kg bw/day.

NOAEL (developmental toxicity): 125 mg/kg bw/day, based on the lower mean pup weights and the increased incidence of runts seen at the top dose level of 500 mg/kg bw/day

Rat Developmental Toxicity Study

At the top dose (1000 ppm), bodyweight loss occurred between GD 6-7 and overall absolute and corrected bodyweight gains were decreased (26% and 51% respectively compared to the control group). A slight decreased mean corrected bodyweight gain in the 200 mg/kg/day group was considered not to be a treatment-related effect since it was within the historical control range. At 1000 mg/kg bw/day there were increased incidences of urinary bladders, urethras and dilated ureters, each filled with yellowish sediment, in one case additionally with a pale kidney or a caudal end of one kidney swollen and light brown discoloured occurred at necropsy.

There were no treatment-related effects on the fertility rate, mean number of corpora lutea or pre/post-implantation loss. Further there were no effects on gestation rate (number of females with viable fetuses as a % of the number of females with implantations).

Treatment-related fetal effects were mainly limited to the 1000 mg/kg bw/day dose group. Findings were indicative of delayed skeletal ossification secondary to maternal toxicity, and included incomplete or absent ossification of the distal phalanx digits, metacarpals, the 5th and 6th sternebrae and the sacral vertebral arches. A significantly increased incidence of wavy ribs was also seen at 1000 mg/kg bw. The fetal incidence was clearly within the historical control range and the concurrent control value for this finding was noted to be unusually high (i.e., was outside the historical control range).

Isolated (but statistically significant) increases in the incidence of unossified 5th left distal phalanx digits were also seen at 50 and 200 mg/kg bw/day, but were not considered to be related to treatment in the absence of a dose-response relationship (i.e. fetal incidence of 10.7, 26.7, 25.3 and 33.1% and litter incidence of 45, 68.2, 66.7 and 80% at 0, 50, 200 and 1000 ppm respectively. The fetal and litter incidences of this finding in the concurrent control group were also at the high end of the historical control range (fetal incidence 0-11.6 and litter incidence 0-50%). A statistically significant increase in the incidence of unossified 5th right metacarpal at 200 mg/kg bw/day is also not considered to be treatment-related in the absence of similar treatment-related effects on other bones. Further the fetal incidence only marginally exceeds the historical control range whilst the litter incidence is within the historical control range.

Rabbit Developmental Study

One female (500 mg/kg/day) was killed for humane reasons on GD 15, following a marked loss in bodyweight from the commencement of treatment, associated with no food intake from GD 8 onwards. Clinical signs consisted of no/few faeces and yellow sediment in the urine. There were no macroscopic findings at autopsy.
Clinical signs noted at the top dose consisted of an increased incidence of dams with few faeces, yellow sediment in the urine, and red traces under the tray. At 125 mg/kg/day, treatment related signs were confined to yellow sediment in the urine.At 500 mg/kg/day, there was a mean bodyweight loss of 0.04 kg compared with a loss of 0.01 kg in the control group, between GD 6 and 8. Thereafter, mean bodyweight gain tended to be less at the high dose compared with the controls, resulting in an overall significant reduction in bodyweight gain of 45% by GD 29. Maternal corrected bodyweight change was more pronounced at 500 mg/kg/day (0.29 kg) compared with the controls (0.17 kg).
At 500 mg/kg/day, food consumption was reduced by between 11 and 19% compared with the controls. The effect was most pronounced between GD 8 and 10 where food consumption was reduced by 19%
At the top dose one female had white sediment in the kidney, one female had yellow sediment in the kidney and bladder and one female had yellow sediment in the bladder.
Mean fetal bodyweight for the combined sexes and for the individual sexes were lower in all three treatment levels, though not in a dose related manner. However, the total number of fetuses/group and mean number of live fetuses/litter were considerably higher in the treated groups compared with the controls. Once an adjustment was made to take this factor into account, statistically significant effects were confined to the high dose group.
The fetal (23%) and litter (62.5%) incidences of runts (defined as fetuses of weight <28 g) were clearly increased at the top dose level compared to the concurrent control values and historical control range (fetal incidence 5.2-16.3%; litter incidence 25.0-54.2%).

The single incidences of white sediment in the kidney in fetuses at 50 and 500 mg/kg bw/day are not considered to be clearly related to treatment in the absence of a dose-response relationship and findings at the intermediate dose level of 125 mg/kg bw/day. The high dose group fetus exhibiting this finding was not from the one female in this group with a similar finding (yellow sediment in the kidney).

The incidence of ventricular septal defect was higher in fetuses at 125 and 500 mg/kg bw/day However, values are clearly within the laboratory's historical control range for this finding and are therefore not considered to be clearly related to treatment (see table below)

The litter incidence of short innominate artery was slightly higher at 500 mg/kg bw/day, this value exceeds the laboratory's historical control range for this finding (12.5%). However it is notable that the concurrent control incidence also exceeds the historical control range and there is no dose response. Whilst there is an increase in the fetal incidence at the top dose, this is within the historical range and there is no dose response. The increased incidence seen at 500 mg/kg bw/day is therefore not considered to be treatment-related (see table below).

Table 27: Summary of findings in the rabbit developmental toxicity study

Finding	**Dose level** (mg/kg bw/day)				**Historical control data[a/b]**
	0	**50**	**125**	**500**	
	% fetal incidence [% litter incidence]				
Ventricular septal defect	-	-	0.4	0.8	mean fetal incidence 0.4% (range 0-1.3%)
	-	-	[4.0]	[4.2]	mean litter incidence 3.6% (range 0-12.5%)
Short innominate artery	1.5	1.0	0.9	2.2	mean fetal incidence 1.9% (range 1.0-3.9%)
	[13.0]	[12.5]	[8.0]	[20.8]	mean litter incidence 11.4% (range 9.1 – 12.5%).

[a]Total number of fetus examined 1250, total number of litter examined 139
[b]Data take from 6 studies conducted between 2000-2005

Higher incidences of incomplete and absent ossification of the hyoid centrum seen in all treated groups are not considered to be related to treatment as the values are within the laboratory's historical control range (fetal and litter incidences of 11.8-24.3% and 28.6-54.5%; 0.7-4.2% and 4.2-20.8% of litters respectively).

The litter incidence of (unilaterally or bilaterally) misshapen thymus was higher at 125 and 500 mg/kg bw/d, however values are clearly within the laboratory's historical control range for this finding (20.8-57.1%), and are associated with a low concurrent control value. The fetal incidences were comparable in all groups. These findings are not considered to be treatment-related.

4.10.2.2 Human information

There is no information available.

4.10.3 Other relevant information

4.10.4 Summary and discussion of reproductive toxicity

Fertility

In the 2-generation study in the rat, effects in parental animals were consistent with the mode of action of thiencarbazone-methyl and were associated with urolithiasis. Mortality, secondary to renal necrosis, was noted in one male at the top dose (10000ppm). Effects in other parental animals were less severe but were largely characteristic of histopathological changes in the urinary system. A reduction (not statistically significant) in the total number of F1 pups was observed at 2500 ppm and 10000 ppm, but this finding was considered to be secondary to the number of F0 males with no sperm observed in these dose groups (1, 0, 5 and 3 in the 0, 500, 2500 and 10000 ppm groups respectively). Litter size was not affected by treatment, and given the lack of dose response and lack of similar findings in the F1 males; this is not considered to be a treatment related effect. Overall, it is concluded that there is no evidence for effects on fertility.

Developmental toxicity

In the rat, maternal toxicity (including body weight loss GD 6-7 and overall decrease in bodyweight gain of 51% compared to controls) was noted in the high dose group of 1000 mg/kg bw/day. Developmental effects were limited to slightly reduced fetal weight and increased incidences of skeletal variations, indicative of delayed ossification, in this high dose group.

In the rabbit, maternal toxicity (mortality, body weight loss, overall decreased body weight gain of 45% compared to controls) was noted at the top dose level of 500 mg/kg bw/day. Lower pup weights and an increased incidence of runts were noted at this dose. An increased incidence of ventricular septal defect was noted in fetuses at 125 and 500 mg/kg bw/day. However, the incidence was found to be within the laboratory's historical control range and was not considered to be clearly related to treatment. An increased litter incidence of short innominate artery was also noted at 500 mg/kg bw/day and was outside of the historical control range of the laboratory. However, the incidence in the concurrent controls also exceeded the historical control range and there was again no dose response. Further, the fetal incidence was within the historical control range of the laboratory and only slightly exceeded the value seen in concurrent controls. Overall, this finding is not considered to be treatment related.

4.10.5 Comparison with criteria

Fertility

In a standard two generation study in rats, there were no treatment related effects on reproductive performance, fertility or parturition at concentrations of up to 10000ppm thiencarbazone-methyl. Therefore the criteria for classification for effects on fertility are not met.

Development

In the rat, effects were limited to slightly reduced fetal weight and increased incidences of skeletal variations (indicative of delayed ossification), which occurred at maternally toxic doses. In the rabbit, developmental effects were limited to lower pup weights and an increased incidence of runts which again occurred at maternally toxic doses. These findings are considered to non-specific secondary consequences arising from maternal toxicity. Consequently, the criteria for classification for effects on development are not met.

4.10.6 Conclusions on classification and labelling

Not Classified – conclusive but not sufficient for classification

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The effect of thiencarbazone-methyl on fertility and sexual function was assessed based on the results of the 2-generation study in rats.

In the 2-generation study in rats (25/sex/dose) performed according to OECD TG 416 (Report No AT03180), thiencarbazone-methyl was given to the F0 parental generation in diet at concentrations of 0, 500, 2 500 or 10 000 ppm throughout premating, mating, gestation, and lactation periods. After weaning of the F1 generation, at 4 weeks of age, selected weanlings were maintained in the same dietary groups through maturation, mating, gestation, and lactation. The final necropsy took place when the F2 offspring were weaned at 4 weeks of age. The achieved doses in mg/kg bw/d in the 0, 500, 2 500 or 10 000 ppm groups of rats, as calculated during the pre-mating phase, were in the F0 generation: 46, 245, 946 mg/kg bw/d in males and 56, 264 or 968 mg/kg bw/d in females; in F1 generation: 50, 261 or 992 mg/kg bw/d in males and 68, 353 or 1 284 mg/kg bw/d in females.

Parental toxicity:

Effects at the high dose level (10 000 ppm) were associated with urolithiasis: death of one male (F1) at this dose level was secondary to renal necrosis (no F0 animals or F1 females died at this dose level). Findings in other parental animals in this dose group were less severe, but were largely limited to histopathological changes in the urinary system characteristic to urolithiasis. Slight reductions in weight gain and food consumption and increased kidney weight (in females) were also suspected to be related to urolithiasis.

Oestrus cycle length and periodicity:

Results from the evaluation of vaginal smears performed at the end of pre-mating phases indicated that there were no biologically relevant effects on the oestrous cycle up to 10 000 ppm in F0 or F1 rats.

Sperm measurements:

There were no biologically relevant effects on sperm parameters (epididymal sperm count, sperm motility and morphology, or testicular spermatids counts) in F0 or F1 rats at 10 000 ppm. The occurrence of five and three spermless F0 males at 2 500 and 10 000 ppm, respectively, was considered as incidental, since the effect was not dose-dependent (1, 0, 5 and 3 in the 0, 500, 2 500 and 10 000 ppm groups, respectively), and the sperm parameters were not affected in other animals of these groups or in F1 generation males.

Reproductive performance:

The fertility, gestation and rearing indices as well as gestation length and number of litters born were

not changed by the treatment up to 10 000 ppm in either generation (the table below). The insemination index was reduced in F0 at 10 000 ppm (80 % compared to 96, 100 and 92 % at 0, 500 and 2 500 ppm, respectively). The reduced insemination index in 10 000 ppm F0 males of 80 % did not lie within the laboratory's historical control range (88.9-100 %; mean 97.7 %) reported for 16 studies. However, out of the five males not mating in this group, three were those observed with 'no sperm'. This finding was not considered to be related to the treatment in the absence of similar findings in males of the F1 generation. Litter size was not affected by the treatment.

***Table**: Reproductive performance (means ±SD).*

	F0 Generation				**F1 Generation**			
	Dietary concentration (ppm)							
Observation	0	500	2 500	10 000	0	500	2 500	10 000
Insemination index	96.0	100.0	92.0	80.0	92.0	100.0	100.0	100.0
Fertility index	100.0	100.0	87.0	100.0	95.7	96.0	92.0	88.0
Gestation index	95.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Gestation length (days)	22.11 ±0.567	21.89 ±0.459	21.67 ±0.900	21.89 ±0.583	21.86 ±0.478	21.67 ±0.482	21.52 ±0.750	22.09 ±0.526
Co-housed females	25	24	25	25	25	25	25	25
Matings until day 0 p.c.	3.8 ±4.17	3.8 ±4.05	4.9 ±4.19	2.4 ±2.12	4.2 ±3.49	4.3 ±4.08	4.0 ±2.30	3.7 ±2.62
	F0 Males				F1 Males			
Number co-housed	25	24	25	25	25	25	25	24
Intercurrent deaths	0	0	0	0	0	0	0	1
	F0 Females				F1 Females			
Number co-housed	25	24	25	25	25	25	25	25
Number fertile	24	24	20	20	22	24	23	22
Intercurrent deaths	0	1	0	0	0	0	0	0
Number of litters 1)	23	24	20	20	22	24	23	22

Rearing index	100.0	100.0	95.0	100.0	95.5	87.5	100.0	100.0

Conclusion:

Based on the results obtained in this study, the DS concluded that thiencarbazone-methyl did not affect sexual function and fertility at concentrations in diet of up to 10 000 ppm and no classification was warranted for this endpoint.

Developmental toxicity

There were three studies in the CLH report on developmental toxicity; the 2-generation study in accordance with the OECD TG 416 on rats (Report No AT03180), and a prenatal toxicity study in accordance with the OECD TG 414 on rats (Report No AT02339) and on rabbits (Report No SA03350).

The DS concluded that there were no adverse effects on pre- or postnatal development of the offspring in the 2-generation study on rats (Report No AT03180) performed according to OECD TG 416. A statistically insignificant reduction in the total number of F1 pups was observed at 2 500 ppm and 10 000 ppm. However, this finding was considered to be secondary to the number of F0 males with 'no sperm' seen in these dose groups, which was considered to be not related to the treatment in the absence of a dose-response relationship or similar findings in F1 males. In addition, the litter size was unaffected by the treatment (the table below).

***Table**: Significant changes in litter parameters of F0 and F1 generations (means and partly ±SD).*

Observation	**Dietary concentration (ppm)**			
	0	**500**	**2 500**	**10 000**
	F0 parental animals/F1 pups			
Spermless males	1	0	5	3
Insemination index	96.0	100.0	92.0	80.0
Mean implantations[a)]	12.00 ± 1.907	12.54 ± 1.503	11.90 ± 2.808	12.00 ± 1.686
Mean prenatal loss[a)]	1.17 ± 0.834	0.79 ± 1.062	0.90 ± 0.968	1.15 ± 1.137
Number born	249	282	220	217
Number born dead	2	1	4	1
Live birth index	99.24	99.48	98.13	99.62
Number of litters	23	24	20	20
	F1 parental animals/F2 pups			
Spermless males	0	0	0	0
Insemination index	92.0	100.0	100.0	100.0
Mean implantations[a)]	12.41 ± 1.221	11.88 ± 1.454	11.91 ± 1.379	11.82 ± 1.006
Mean prenatal loss[a)]	1.09 ± 1.065	0.75 ± 0.944	0.87 ± 1.014	0.86 ± 0.990

Number born	249	267	254	241
Number born dead	10	0**	1*	0**
Live birth index	95.52	100.00	99.64	98.64
Number of litters	22	24	23	22

a) Per litter.
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01.

In **the developmental toxicity study on rats (Report No AT02339)** performed according to OECD TG 414, thiencarbazone-methyl was given by gavage to pregnant female rats (25 animals/dose) on 6-19 days of gestation at doses of 0, 50, 200 and 1 000 mg/kg bw/d.

Maternal toxicity:

No mortality up to 1 000 mg/kg bw/d was observed during the study. The DS reported body weight loss between GD 6 and 7 at 1 000 mg/kg bw/d, related to the decrease in food consumption. Overall (GD 0-20) absolute and corrected body weight gains were statistically significantly decreased (26 and 51 %, respectively) at 1 000 mg/kg bw/d as compared to the control. At 200 mg/kg bw/d, the marginally decreased mean corrected body weight gain was considered not to be treatment-related, because the value was within the HCD range for the rat strain.

Developmental toxicity:

The mean numbers of corpora lutea, live foetuses per litter, pre-implantation losses and implantation sites in the different groups did not differ significantly. Foetal weight was statistically significantly decreased by 9 % at 1 000 mg/kg bw/d as compared to the control group. No effect on foetal weight was observed at lower dose groups.

The total number of foetuses or litters with malformations was unaffected by treatment.

***Table**: Findings in the rat developmental toxicity study*.

Parameter	Dose level (mg/kg bw/d)				
	0	50	200	1 000	HCD
Maternal weight gain Day 6-7	2.5	3.0	1.9	-0.8	-
	Foetal findings: % foetal incidence [% litter incidence]				
Renal pelvis dilated	5.9 [35.0]	7.4 [63.6]	12.7* [79.2*]	8.9 [60.0]	0.8-13.3 [10.0-75.0]
Ureter dilated	3.2 [30.0]	4.9 [45.5]	5.1 [41.7]	6.7 [50.0]	0-5.6 [0-47.4]
Unossified 5th right distal phalanx digits	16.1 [50.0]	26.0 [72.7]	21.9 [66.7]	33.9** [80.0]	0-15.2 [0-50.0]

Unossified 5th left distal phalanx digits	10.7 [45.0]	26.7** [68.2]	25.3** [66.7]	33.1** [80.0]	0-11.6 [0-50.0]
Unossified 5th right metacarpal	5.4 [25.0]	12.3 [45.5]	15.8* [58.3]	28.0** [80.0**]	2.5-15.1 [13.6-61.9]
Unossified 5th left metacarpal	9.8 [40.0]	13.7 [54.5]	19.9 [66.7]	31.4 [80.0]	3.3-18.2 [9.1-61.9]
Wavy ribs	1.8 [10.0]	2.1 [9.1]	3.4 [16.7]	10.2* [50.0*]	2.8-14.6 [10.0-42.9]

*significantly different to controls $p \leq 0.05$; **≤ 0.01. Historical control range: 15 studies performed 2002-2005

Treatment-related foetal effects were mainly limited to the 1 000 mg/kg bw/d dose group. Findings were indicative of delayed skeletal ossification secondary to maternal toxicity, and included incomplete or absent ossification of the distal phalanx digits, metacarpals, the 5th and 6th sternebrae and the sacral vertebral arches. A statistically significantly increased incidence of wavy ribs was also seen at 1 000 mg/kg bw/d. The foetal incidence was clearly within the historical control range and the concurrent control value for this finding was noted to be unusually low (1.8), being outside the historical control range.

Isolated (but statistically significant) increases in the incidence of unossified 5th left distal phalanx digits seen at 50 and 200 mg/kg bw/d were not considered to be related to the treatment in the absence of a dose-response relationship (foetal incidence of 10.7, 26.7, 25.3 and 33.1 % and litter incidence of 45, 68.2, 66.7 and 80 % at 0, 50, 200 and 1 000 ppm, respectively). The foetal and litter incidences of this finding in the concurrent control group were also at the high end of the historical control range (foetal incidence of 0-11.6 and litter incidence of 0-50 %). A statistically significant increase in the incidence of unossified 5th right metacarpal at 200 mg/kg bw/d was considered not to be treatment-related in the absence of similar treatment-related effects on other bones.

The apparently increased incidences of dilated renal pelvis and ureter in the treated groups compared to the control were not considered to be related to the treatment in the absence of statistical significance and/or a clear dose-response relationship.

The DS concluded that in the rat, developmental effects were limited to slightly reduced foetal weight and increased incidences of skeletal variations, which occurred at maternally toxic doses. Therefore, the criteria for classification for effects on development were considered not to be met.

In **the developmental toxicity study in rabbits (Report No SA03350)** performed according to OECD TG 414, thiencarbazone-methyl was given by gavage to pregnant female rabbits (25 animals/dose) on 6-28 days of gestation at doses of 0, 50, 125 and 500 mg/kg bw/d.

Maternal toxicity:

At 500 mg/kg bw/d, one female was killed for humane reasons on GD 15 following a marked loss in body weight and poor food consumption. Clinical signs comprised of no/few faeces and yellow sediment in the urine. No macroscopic findings were observed at autopsy. At this dose level, treatment-related clinical signs consisted of an increased incidence of dams with few faeces, yellow sediment in the urine of all dams and red traces under the tray of 2/25 females. There was a mean body weight loss of 0.04 kg compared to a loss of 0.01 kg in the control group between GD 6 to 8.

Thereafter, mean body weight gain tended to be lower in the high dose group compared to the controls. Maternal corrected body weight change was more pronounced at 500 mg/kg bw/d (-0.29 kg) compared to the controls (-0.17 kg), the effect being statistically significant. At the top dose, the mean food consumption was reduced throughout treatment by between 11 and 19 %, compared with the controls, the effect being most pronounced between GD 8 and 10. At autopsy, one female had white sediment in the kidney, one female yellow sediment in the kidney and urinary bladder and one female yellow sediment in the bladder.

At the dose of 125 mg/kg bw/d, treatment-related clinical signs in the dams consisted of yellow sediment in the urine noted in 4/25 females. No other maternal parameters were affected. At 50 mg/kg bw/d no treatment-related maternal or foetal findings were noted.

Developmental toxicity:

The total number of foetuses per group and the mean number of live foetuses per litter were higher in the treated groups than in the controls. Mean foetal body weight for the combined sexes and for the individual sexes were lower in all three treatment levels, though not in a dose-related manner. Once an adjustment was made to take into account the increased number of foetuses in treated groups, statistically significant effects ($p < 0.05$) were only confined to female foetuses at 500 mg/kg bw/d, where there was an 8 % reduction in body weight in comparison with the controls (the table below).

***Table**: Findings in the rabbit developmental toxicity study*

Parameters		**Dose levels (mg/kg bw/d)**			
		0	**50**	**125**	**500**
Number of live foetuses per litter	Mean ± SD (N)	8.7 ± 2.4 (23)	10.1 ± 2.4 (24)	9.9 ± 2.6 (25)	9.5 ± 3.1 (24)
Number of live foetuses		201	243	248	228
Foetal weight (g)[a]	Mean ± SD (N)	36.7 ± 5.9 (201)	32.5^{++} ± 6.5 (243)	34.7^{++} ± 6.2 (248)	32.2^{++} ± 7.1 (228)
Male foetal weight (g)[a]	Mean ± SD (N)	36.9 ± 5.7 (96)	32.3 * ± 6.5 (128)	34.9 ± 6.3 (133)	32.5 * ± 7.1 (121)
Female foetal weight (g)[a]	Mean ± SD (N)	36.4 ± 6.0 (105)	32.8 * ± 6.7 (115)	34.5 ± 6.0 (115)	31.9 * ± 7.1 (107)
Adjusted mean weight estimate (g)[b]		34.99	33.13	35.08	32.68
Adjusted mean male weight estimate (g)[b]		35.23	33.46	35.17	32.96

Adjusted mean female weight estimate (g)[b]	35.01	32.68	35.10	32.27 *

a: Statistical analysis on mean foetal weights; ++: p = 0.01 with Dunn test; *: p = 0.05 with Dunnett test
b: Statistical analysis on adjusted mean foetal weight using the total number of live foetuses per litter as covarianc
*: p = 0.05 with Dunnett test

There were no treatment-related external malformations, anomalies, or variant findings at any dose levels. The foetal and litter incidence of the ventricular septal defect was higher in foetuses at 125 and 500 mg/kg bw/d; the foetal incidence being 0.4 % and 0.8 % and the litter incidence being 4.0 % and 4.2 %, respectively. However, these values were clearly within the laboratory's historical control range for this finding (foetal incidence 0-1.3 %; litter incidence 0-12.5 %) and were therefore considered not to be clearly related to the treatment. The litter incidence of short innominate artery (20 %) was slightly higher at 500 mg/kg bw/d as compared to the control, and this value exceeded the laboratory's HCD range for the effect (up to 12.5 %). However, it was considered notable that the concurrent control incidence (13 %) also exceeded the HCD range and there was no dose-response. Whilst there was also an increase in the foetal incidence at the top dose, this was within the HCD range and there was no dose-response. The increased incidence seen at 500 mg/kg bw/d was therefore considered not to be treatment-related (the table below).

***Table**: Incidences of ventricular septal defect and short innominate artery.*

Finding	**Dose level (mg/kg bw/d)**				**Historical control data***
	0	**50**	**125**	**500**	
	% foetal incidence [% litter incidence]				
Ventricular septal defect	-	-	0.4	0.8	mean foetal incidence 0.4 % (range 0-1.3 %)
	-	-	[4.0]	[4.2]	mean litter incidence 3.6 % (range 0-12.5 %)
Short innominate artery	1.5	1.0	0.9	2.2	mean foetal incidence 1.9 % (range 1.0-3.9 %)
	[13.0]	[12.5]	[8.0]	[20.8]	mean litter incidence 11.4 % (range 9.1-12.5 %).

*= Data take from 6 studies conducted between 2000-2005; Total number of foetus examined 1 250, total number of litter examined 139

The number of runt foetuses (body weight < 28.0 g) was increased at 500 mg/kg bw/d, where the mean percentage of foetuses classified as runts was 23 % and the percentage of litters affected was 62.5 %, compared to 6.5 % and 34.8 %, respectively, in the concurrent control group. The historical control range for the foetal incidence was 5.2-16.3 % and for the litter incidence 25.0-54.2 %. This effect could be related to lower food consumption and lower body weight gain of dams at this exposure level.

No treatment-related, statistically significant, and dose-dependent skeletal malformations, anomalies, or variant findings were observed at any dose level.

The DS concluded that in the rabbits, developmental effects were limited to lower pup weights and to

an increased incidence of runts which occurred at maternally toxic doses. Therefore, the criteria for classification for effects on development were considered not to be met.

Comments received during public consultation

One MSCAs supported the DS's proposal to not classify thiencarbazone-methyl for reproductive toxicity noting that the data were not sufficient to warrant classification.

One MSCAs noted that care should be given for the interpretation of the significance of abnormal penis in the 18-month mouse study, together with the spermless males (F0) in the rat 2-generation study. The DS responded that the slightly increased incidence of abnormal penis as compared to the concurrent control was not associated with any intrinsic histopathological findings in the 18-month carcinogenicity study in mice. The effect was associated with chronic ulcerative dermatitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination, and therefore it was considered not to be related to the finding of no sperm in the F0 generation. In the 2-generation study in rats, the incidence of F0 males with no sperm was not dose-related and not statistically significant. Furthermore, this finding was not observed in F1 males exposed to thiencarbazone-methyl. In this study thiencarbazone-methyl did not affect any sperm parameter (epididymal sperm count, sperm motility and morphology, or testicular spermatids counts), and therefore the occurrence of spermless males in F0 generation was considered to be unrelated to the treatment.

Assessment and comparison with the classification criteria

In the 2-generation study in rats, thiencarbazone-methyl did not affect sexual function and fertility, and therefore the CLP criteria are not met.

In the prenatal developmental toxicity studies in rats and in rabbits, thiencarbazone-methyl at the highest dose induced a slightly reduced foetal weight, an increased incidence of runts and an increased incidences of skeletal variations in the presence of clear maternal toxicity. These developmental effects are considered as secondary non-specific consequences of maternal toxicity.

Therefore, taking into account the available evidence coming from the reliable animal studies RAC is of the opinion that thiencarbazone-methyl **does not warrant classification for adverse effects on sexual function and fertility, or on development**.

4.11 Other effects

No further information. All information relevant to the human health classification and labelling assessment of thiencarbazone-methyl is presented above.

5 ENVIRONMENTAL HAZARD ASSESSMENT

In the pesticide DAR (under Directive 91/414/EEC), as well as in the following sections of this CLH Report, thiencarbazone-methyl is often tested and referred to by its development code of 'BYH 18636'. Degradants/metabolites of thiencarbazone-methyl are similarly referred to as: 'BYH 18636-carboxylic acid', 'BYH 18636-sulfonamide', etc.

Environmental fate and ecotoxicological data have been presented in the thiencarbazone-methyl DAR for a number of environmental degradants of the parent substance. Aquatic toxicology data for these are tabulated briefly in Appendix I. Due to their predominantly low toxicity to aquatic organisms (no greater than parent) all of the major aquatic degradants of thiencarbazone-methyl are not considered further in relation to the classification of the parent substance.

All of the environmental fate and behaviour and ecotoxicological studies reported below are considered to be fully reliable for the purposes of hazard classification, any significant deviations from respective guidelines are noted in the individual study evaluations. Full details of any studies are included in the thiencarbazone-methyl DAR.

5.1 Degradation

Table 28: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis as a function of pH. OECD Guideline 111	pH 4: DT_{50} 118 d (20 °C), 50 d (25 °C) pH 7: DT_{50} n.a. (20 °C), 146 d (25 °C) pH 9: DT_{50} n.a. (20 °C), 153 d (25 °C)	To GLP. DT_{50}s extrapolated beyond duration of the study. Values at 50 °C not included but notably shorter.	Haas, M., Sneikus, J. (2005)
EU FOCUS kinetics	Single first order (SFO) DT_{50} at 25°C = 139 days for pH 9	Recalculation of the above study	Hammel, K. (2007)
Direct aqueous photolysis - to SETAC and US EPA: Subdivision N, Section 162-1 guidelines	Mean photolytic half-life in the test = 90.6 days at pH 7 and 25 °C. Projected half-life in Phoenix (Arizona, USA) = 333 solar summer days; in Athens (Greece) = 516 solar summer days	To GLP	Sneikus, J. (2005)
Direct phototransformation in water to German UBA and ECETOC guideline	At pH 4, 7 and 9 and 25 °C No phototransformation reported; half-life would be > 1 year	To GLP Indirect mechanisms of enhanced photo-degradation in natural water not considered	Heinemann, O. (2004)
Ready biodegradability to EEC Method C.4-D and OECD Guideline	0 % degradation after 28 days	To GLP	Weyers, A. (2006)

301 F	Not readily biodegradable		
Water/sediment simulation study (anaerobic) US EPA, Subdivision N, Section 162-3	After 123 days in the dark at mean 20.8 °C: Mean primary degradation DT_{50} for two radiolabels in total system = 7.6 days; mineralization was minimal at ≤1.5 % AR by 123-days	To GLP Only single sand sediment system tested	Arthur, E. *et al*. (2007)
Water/sediment simulation study (aerobic) US EPA, Subdivision N, Section 162-4	After 120 days in the dark at 20 °C: The primary degradation DT_{50} in both total water/sediment systems was a maximum 29 days; mineralization reached 7.6-13.4 % AR by 120-days	To GLP Sandy loam and loamy sand sediment systems tested	Henk, F., Haas, M. (2005)
EU FOCUS kinetics	Degradation SFO DT_{50} in each water/sediment whole system was: Sandy loam system = 21.9 days Loamy sand system = 31.3 days	Recalculation of the above aerobic study	Hammel, K. (2007)

5.1.1 Stability

5.1.1.1 Aqueous hydrolysis

Study 1 (Haas, M., Sneikus, J., 2005)

A study has been submitted on the abiotic aqueous hydrolysis of thiencarbazone-methyl (company code BYH 18636). The study was conducted to GLP and in accordance with OECD Guideline No. 111 (as well as similar US EPA, Canadian PMRA and Japanese test guidelines).

The hydrolytic degradation of thiencarbazone-methyl was investigated at three different temperatures (20, 25 and 50 °C) in sterile buffer solutions at pH 4, 7 and 9 using two different radiolabels, [dihydrotriazole-3-^{14}C] and [thiophene-4-^{14}C]-BYH 18636. Radiochemical purity was > 99 % for all batches. Samples were taken at regular points up to 6 days after application at 50 °C and up to 30 days after application at 20 and 25 °C. Samples were analysed directly without extraction, clean-up, or sample concentration, using liquid scintillation counting (LSC) and high performance liquid chromatography (HPLC) at each sampling point and thin layer chromatography (TLC) at selected sampling points.

Thiencarbazone-methyl was hydrolytically unstable under acidic, neutral and alkaline conditions at 20 °C (only tested at pH 4). At 25 °C, the half-life of BYH 18636 was 50 days at pH 4 and approximately 150 days at pH values of 7 and 9. At these pH, the half-life decreased with increasing temperature, although there was no clear trend with respect to pH. At all pH values tested, the major degradation products were BYH 18636-MMT and BYH 18636-sulfonamide (minor metabolite at pH 9) formed by cleavage of the molecule. The concentration of BYH 18636-MMT increased towards the end of incubation at all pH values tested, whereas BYH 18636-sulfonamide was degraded further, especially under alkaline conditions. Total recoveries at all pH and temperatures were >>90 % (mean of two labels).

In contrast to the behaviour in non-sterile environmental test systems, abiotic hydrolysis was not considered a relevant route of degradation of thiencarbazone-methyl in the aquatic environment,

especially under more extreme acidic or alkaline conditions. Hydrolytic degradation followed single first-order kinetics (R^2 ranged from 0.922 to 0.988) and DT_{50}s are given below in Table 29. They are of uncertain accuracy because they are extrapolated well beyond the duration of the study.

Table 29: Single first-order hydrolysis half-life (DT_{50}) of thiencarbazone-methyl in aqueous solution

pH	DT50 (d) at a temperature of:		
	20 °C	25 °C	50 °C
4	118	50	1.83
7	n.a.	146	3.90
9	n.a.	153	2.95

n.a. not analysed

Based on the temperature correlation and already high DT_{50} relative to CLP triggers at 20 °C, conversion of degradation rates to 12 °C (as suggested by ECHA guidance) is not included as the result would only be higher still. The hydrolytic degradation observed in this study was further analysed in accordance with FOCUS degradation kinetics guidance in a separate study evaluated below.

Study 2 (Hammel, K., 2007)

The hydrolytic degradation of thiencarbazone-methyl and some of its degradants was kinetically evaluated based on results of the above laboratory study (Haas, M., Sneikus, J., 2005) following EU FOCUS kinetics. These re-calculations were not subject to GLP.

The re-evaluation only considered the results at 25 °C and pH 9 to utilise the maximum information on transformation products and because certain degradants only occurred at this pH. The label-specific measured data were equally weighed (weighting factor 1). Non-detected values were either set to 0.5 LOD, zero or excluded from the analysis. The test at 50 °C was not kinetically re-evaluated because this temperature is environmentally not relevant. The hydrolytic degradation of thiencarbazone-methyl was considered to follow single first-order kinetics.

The chi^2 values for parent were 1.0 and 0.9 % (based on label A and B data respectively) and the rate constant parameter was significantly different from zero. Radiolabel-specific fits for thiencarbazone-methyl resulted in very similar half-lives (148 and 130 days). These differences were small so that mean values are given in the following table:

Table 30: Re-calculated single first-order hydrolytic half-life (DT_{50}) of thiencarbazone-methyl and certain degradants in sterile aqueous solution at 25 °C and pH 9 (according to FOCUS kinetics)

Substance	DT_{50} (days)
BYH 18636 (thiencarbazone-methyl)	139
BYH 18636-sulfonamide	11
BYH 18636-sulfonamide-carboxylic acid	stable
BYH 18636-MMT	stable

It was noted that the DT_{50} for parent thiencarbazone-methyl (139 d) was extrapolated well beyond the duration of the study (i.e. 30 d) and was therefore subject to a degree of uncertainty. A proposed hydrolysis degradation pathway is included in Annex 1 Figure 1.

5.1.1.2 Aqueous photolysis

Study 1 (Sneikus, J., 2005)

Data are available on the direct photolysis of thiencarbazone-methyl (company code BYH 18636) in aqueous solution (Sneikus, J.; 2005). The study was conducted in accordance with GLP to SETAC-Europe guideline: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, March 1995, Section 10 (Aqueous Photolysis) - as well as US EPA: Subdivision N, Section 162-1 and similar Canadian guideline.

Photodegradation of thiencarbazone-methyl (radiochemical purity: > 99 % (dihydrotriazole label), > 98 % (thiophene label) was studied in sterile aqueous buffer solution at pH 7 and at 25 °C. The test vessels were irradiated by simulated natural sunlight (xenon lamp, ®Suntest). Irradiation was conducted continuously over 9 days with sampling by LSC followed by HPLC and/or TLC at 0, 1, 2, 5, 6, 7 and 9 days of irradiation. Dark controls were investigated after 5 and 9 days. No significant radioactivity was lost from the vessels or during processing (radioactivity recoveries of 97.9-102.8 %).

Three photodegradation products were formed and increased during the irradiation period. BYH 18636-sulfonamide, BYH 18636-MMT and BYH 18636-triazolinone-carboxamide were formed at maximum fractions of 5.2 %, 8.3 % and 1.2 % of AR, respectively, at the end of the irradiation period. $^{14}CO_2$ accounted for a maximum of 0.1 % of the applied radioactivity at study termination, therefore mineralisation was minimal. The photodegradation of thiencarbazone-methyl followed single first order kinetics. The mean values of both radiolabels were used for determination. Thiencarbazone-methyl was almost stable under dark conditions and degraded slightly under intensive light exposure. Of the applied thiencarbazone-methyl, 95.6 % and 91.2 % remained undegraded as the thiophene and dihydrotriazole labels, respectively. The mean photolytic half-life in the test was 90.6 d (extrapolated). The half-life under environmental conditions was projected to be 333 solar summer days at Phoenix (Arizona, USA) and 516 solar summer days at Athens (Greece). The UV-spectrum of thiencarbazone-methyl dissolved in water showed nearly no overlap between the UV absorption at $\lambda = 290$ nm and the spectral range of sunlight and the filtered xenon light used in the experiment.

Study 2 (Heinemann, O., 2004)

A study has been submitted to try and determine the quantum yield and environmental half-life of thiencarbazone-methyl through direct photodegradation in water (Heinemann, O., 2004). The study was conducted in accordance with GLP and to a German UBA guideline 'Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, (1992-12) as well as an ECETOC polychromatic light source test method.

The absorption spectra of thiencarbazone-methyl (99.3 % pure) were measured in the wavelength range of 290 to 500 nm in aqueous 0.01 mol/L buffer solutions of thiencarbazone-methyl (acetate pH 4, phosphate pH 7 and borate pH 9) including some acetonitrile. Acetonitrile was added due to low water solubility.

The absorption of light by thiencarbazone-methyl in buffered solutions terminated at approximately 282 nm and did not extend into the range of wavelengths relevant for the environment. No photodegradation was investigated because thiencarbazone-methyl did not absorb above 282 nm. Consequently, no quantum yield of direct phototransformation in water could be determined. Even if a quantum yield of 1 were assumed, the environmental photo-transformation half-life would be longer than one year. Direct interactions of thiencarbazone-methyl in aqueous solution with sunlight in the troposphere were not considered likely, although indirect mechanisms of enhanced photodegradation in natural water were not considered.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not submitted or included as actual test data are available.

5.1.2.2 Screening tests

The ready biodegradability of thiencarbazone-methyl (tested as BYH 18636, 96.5 % pure) was determined according to GLP and to EEC Method C.4-D 'Manometric Respirometry Test' which is essentially the same as OECD Guideline 301 F (Weyers, A.; 2006). A 100 mg/L solution of thiencarbazone-methyl in a mineral medium was inoculated with activated sludge originating from a waste water plant treating predominantly domestic sewage and incubated for 28 days under aerobic conditions.

The mixture was stirred in a closed respirometer flask at a constant temperature (22 ± 2 °C) for up to 28 days. The consumption of oxygen was determined and evolved carbon dioxide was absorbed in a solution of potassium hydroxide. The amount of oxygen taken up was expressed as a percentage of theoretical oxygen demand (ThOD) or chemical oxygen demand (COD). Sodium benzoate (99 % pure) was used as a reference compound.

Thiencarbazone-methyl showed 0 % degradation after 28 days, while the reference compound showed 83 % degradation after 14 days. Thiencarbazone-methyl was, therefore, considered to be 'not readily biodegradable'.

5.1.2.3 Simulation tests

Water/sediment studies (anaerobic)

The anaerobic biotransformation of radiolabelled thiencarbazone-methyl (tested as BYH 18636, radiochemical purity: >99 %) was studied in a pond water/sediment system (Arthur, E. *et al*., 2007). The study was conducted to GLP and in accordance with US EPA, Subdivision N, Section 162-3 and similar Canadian guidelines.

The study was carried out in natural water/sediment systems from Clayton, North Carolina, US for 123 days in the dark at 25 ± 1 °C. The characteristics of the sediment and the corresponding supernatant water are summarized in Tables 31 and 32, respectively. The sediment/water ratio was 1:3. The kinetics test systems consisted of an Erlenmeyer flask containing 50 g (dry weight) sieved sediment and 150 mL pond water treated at a target concentration of 0.0075 µg/mL. The test systems were pre-incubated under the projected test conditions (i.e. at 25 ± 1 °C in the dark) for 10 days in order to equilibrate. Eight sampling intervals were conducted over a period of 123 days at 0, 4, 7, 14, 28, 63, 91, and 123 days post treatment. Following accelerated solvent extraction thiencarbazone-methyl residues in water and sediment were analysed by HPLC coupled to a ^{14}C detector. Identification of thiencarbazone-methyl and major degradates was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) as well as by GC-MS. Temperature was not maintained throughout the study at 25 ± 1 °C as measurements ranged from 19.8-22.3°C (mean 20.8 °C, however this is not considered to have substantially affected the result. The pH in the total system ranged from 6.0 to 6.6. From the redox potential and accompanying measurements of the oxygen, it can be concluded that the water and sediment were anaerobic throughout the entire study.

Table 31: Physico-chemical characteristics of the sediment phase

Parameter	Clayton
Geographic Location	Clayton (Johnston County (NC) Latitude N35° 38.754' Longitude: W078° 25.622'
Soil Taxonomic Classification (USDA) Sand (2000 - 50 µm) Silt (<50 - 2 µm) Clay (<2 µm)	Sand 90 8 2
pH in 1:1 Soil:Water ratio pH in 0.01 M $CaCl_2$	6.5 5.4
Organic Matter [%]	2.4
Organic Carbon [%]	1.4
Soil Microbial Activity [cells/g soil]	2.61×10^8 (initial) 1.90×10^8 (final)
Cation Exchange Capacity	4.4 meq/100g
Total Nitrogen [%]	0.095
Total Phosphorus [mg P/kg]	63

Table 32: Physico-Chemical characteristics of the water phase

Parameter	Clayton
pH	6.4
Hardness ($CaCO_3$/L)	31
Total organic carbon (TOC) (ppm)	8.8
Dissolved organic carbon (DOC) (ppm)	6.5
Total nitrogen (ppm)	5.4
Redox potential E_h [mV]	-55.8 (initial) -41.4 (final)
Oxygen Concentration (mg/L)	0.0 (initial) 0.5 (final)
Biomass (cells/ mL water)	2.13 x 10^7 (initial) 1.54 x 10^7 (final)

For the dihydrotriazole label, the total material balance in the water/sediment system was 99.7 ± 2.3 % (mean ± SD) of the applied amount. The mean percent of applied radioactivity recovered at day 123 in water, extractable and unextractable from sediment was 65.9 % (±0.9), 21.7 % (±0.0), and 7.1 % (± 0.5), respectively. Extractable [^{14}C] residues in sediment increased from 4.4% at day 0 to 28.9 % at day 7 and then declined to 21.7 % at the end of the study (day 123). Non-extractable [^{14}C] residues in sediment increased from 2.4 % at day 14 to 7.1 % of the applied amount at day 123. At the end of the study, 0.9 % of the applied radioactivity was present as CO_2, and volatile organic compounds were below the limit of detection.

The concentration of [dihydrotriazole-3-^{14}C] thiencarbazone-methyl in water decreased from 96.5 % at day 0 to 3.6 % of the applied amount at day 28, and was below the limit of quantitation beyond day 28. The concentration of thiencarbazone-methyl in sediment increased from 4.4 % at day 0 to 13.8 % of the applied amount at day 4, declined to 1.6 % by day 28, and was below the LOQ beyond day 28. On day 123 at study termination, 21.7 % of the applied radioactivity was partitioned from water to sediment (sum of extractable and non-extractable in sediment).

For the thiophene label, the total material balance in the water/sediment system was 98.8 ± 2.4 % (mean ± SD) of the applied amount. The mean percent of applied radioactivity recovered at day 123 in water, extractable and unextractable from sediment was 74.0 % (±0.7), 23.7 % (±1.0), and <LOQ, respectively. Extractable [^{14}C] residues in sediment increased from 7.1 % at day 0 to 29.6 % at day 7 and the declined to 23.7 % at the end of the study (day 123). Non-extractable [^{14}C] residues in sediment ranged from 1.7 %, to 3.1 % between days 7 and 63, and were below the LOQ for all other intervals. At the end of the study, 1.1 % of the applied radioactivity was present as CO_2, and volatile organic compounds were below the limit of detection.

The concentration of [thiophene-^{14}C] thiencarbazone-methyl in water decreased from 91.6 % at day 0 to 2.6 % of the applied amount at day 28, and was below the limit of quantitation beyond day 28. The concentration of thiencarbazone-methyl in the sediment increased from 7.1% at day 0 to 16.0 % of the applied amount at day 4, declined to 0.8 % by day 28, and was below the LOQ beyond day 28. On day 123 at study termination 23.7 % of the applied radioactivity was partitioned from water to sediment (sum of extractable and non-extractable in sediment).

The major products detected were thiencarbazone-methyl itself, with a maximum concentration of 51.7 % on day 14, and BYH 18636-MMT, with a maximum concentration of 18.7 % on day 63, both of which declined by the end of the study. Additionally, major transformation products BYH 18636-NMT, with a maximum concentration of 68.4 % on day 123, and BYH 18636-sulfonamide carboxylic acid, with a maximum concentration of 95.2 % on day 123, were also

observed. There was an additional minor metabolite identified, BYH 18636-sulfonamide, with a maximum concentration of 6.1 % on day 7, and declined to the end of the study.

Recovery of $^{14}CO_2$ from both test systems accounted for a maximum of 1.5 % (DAT-7) and 1.2 % (DAT-7) for the dihydrotriazole label and thiophene label test systems respectively. Non-extractable residues accounted for a maximum of 9.4 % (DAT-63) and 3.1 % (DAT-14) for the dihydrotriazole and thiophene label systems respectively. The mean first-order DT_{50}s, reflecting both dissipation and degradation of parent thiencarbazone-methyl in the anaerobic water and primary degradation in the total system were calculated as 6.5 and 7.6 days, respectively. The DT_{90} of thiencarbazone-methyl in anaerobic water and total system were 21.4 and 25.2 days, respectively. A summary of kinetics analyses is shown in Table 33.

Table 33: The mean first-order degradation rates calculated for thiencarbazone-methyl in anaerobic water and total system.

Matrix	Radiolabel[A]	First Order, $C(t) = C(0) \times e^{-kt}$			DT_{90} (days)
		Half-life (days)	k day^{-1}	R^2	
Water	A	6.5	0.1067	0.997	21.6
	B	6.4	0.1085	0.998	21.2
	Mean	6.5			21.4
Entire System	A	7.7	0.09018	0.991	25.5
	B	7.5	0.09239	0.996	24.9
	Mean	7.6			25.2

[A] A = dihydrotriazole radiolabel; B = thiophene radiolabel

The study conclusion was that upon entering natural surface water thiencarbazone-methyl will be eliminated from the supernatant water via translocation into the sediment, as well as via degradation. The mean degradation DT_{50} for thiencarbazone-methyl in the total system was 7.6 days.

Water/sediment studies (aerobic)

Study 1 (Henk, F., Haas, M., 2005)

The aerobic biotransformation of radiolabelled thiencarbazone-methyl (tested as BYH 18636, radiochemical purity: >99 %) was studied in two static water/sediment systems (Henk, F., Haas, M., 2005). The study was conducted to GLP and in accordance with US EPA, Subdivision N, Section 162-4 and similar Canadian guidelines.

The study was carried out in natural water/sediment systems from Hoenniger Weiher, Germany (water: pH 6.7; sediment: texture sandy loam, pH 5.3, organic carbon 4.0 %) and Clayton, North Carolina, US (water: pH 5.7; sediment: texture loamy sand, pH 5.8, organic carbon 2.2 %), respectively. The full characteristics of the sediment and the corresponding supernatant water are summarized in Tables 34 and 35. Thiencarbazone-methyl was applied at a rate of 4.5 µg/L and 45 µg/L using [dihydrotriazole-3-^{14}C] - and [thiophene-4-^{14}C]-labelled test item. The water/sediment systems were incubated for a maximum of 120 days in the dark at 20 ±1 °C. The sediment/water ratio used was approximately 1:3 (v:v). The test systems for the two concentrations

consisted of 30 glass incubation flasks, each, attached with traps for collection of $^{14}CO_2$ and volatile organics. Samples were analysed at 0, 1, 3, 7, 14, 30, 59, 92, and 120 days of incubation. After separation from the sediment by decanting, the water samples were directly analysed by TLC or (after concentration with a rotary evaporator) by HPLC. The sediment samples were extracted at room temperature, twice with acetonitrile/water 2:1 (v:v), followed by an extraction with acetonitrile. Transformation products were identified by co-chromatography with authentic reference compounds and by LC-MS/MS.

Table 34: Physico-chemical characteristics of the sediments used

	System Hoenniger	System Clayton
Geographic location	Wipperfuerth, Northrhine-Westfalia, Germany	Clayton, North Carolina, USA
Latitude and longitude	N 51°08.213' E 007°27.140'	N 35°38.754' W 078°25.622'
Type of aquatic system	meso-/oligotrophic	oligotrophic
Taxonomic classification	loam	sand
Textural class [USDA]	sandy loam	loamy sand
Sand (2000-50 µm); (%) Silt (50-2 µm); (%) Clay (< 2 µm); (%)	52 42 6	82 16 2
pH: in 1:1 Soil:Water ratio pH in 0.01 M $CaCl_2$	5.3 5.0	5.8 5.2
Organic matter (%)	6.90	3.79
Organic carbon (%)	4.0	2.2
Microbial activity (mg CO_2/(h × kg of dry sediment)) Initial (at date of sampling) Final (at latest processing date)	 33 16	 14 10
Cation exchange capacity (meq Ba^{2+}/100 g sediment)	8.6	7.0
Total nitrogen (% N)	>0.292	>0.118
Total phosphorous (mg P/kg dry matter)	>730	>167
$CaCO_3$ (%)	0.3	< 0.1
Water content (%)	61.5	37.6
Redox potential (mV)	158	-60

Table 35: Physico-chemical characteristics of the water used

	System Hoenniger	System Clayton
Temperature at sampling (°C)	4.9	0.6
pH at sampling	6.7	5.7
Hardness (grad DH)	3.7	2.7
Electrical conductivity		
Oxygen concentration (mg/L) Initial (at date of sampling) Final (at latest processing date)	 10.0 7.5	 6.9 8.9
Dissolved organic carbon, DOC (mg C/L)	n.d.	n.d.
Total organic carbon, TOC (mg C/L)	8	< 2
Total nitrogen (mg N/L)	>2920	>1180
Total phosphorous (mg P/L)	>730	>167
Redox potential (mV) Initial (at date of sampling) Final (at latest processing date)	 218 205	 300 218
Biomass (mg microbial C/100 g)	n.a.	n.a.

n.a. not analysed, n.d. not detected

Results are mainly reported for the 1× application rate and the mean of the two labels. Only the maximum formation data refers to both rates (1× and 10×) and both labels. The total material balance in the water/sediment system was 103.6 ± 3.4 % for the Hoenniger test system and 101.4 ± 3.4 % of the applied amount for Clayton. The radioactivity in the water of Hoenniger decreased from 103.4 % at day 0 to 34.9 % at the end of the incubation period. The radioactivity in the water of Clayton decreased from 98.6 % at day 0 to 44.3% at study termination. Extractable radioactive residues in the Hoenniger sediment increased from 2.1 % of the applied radioactivity at day 0 to 22.1 % already at day 14. Extractable radioactive residues in the Clayton sediment increased from 5.7 % of the applied radioactivity at day 0 to approximately 22 % at day 14. Non-extractable [^{14}C]-residues in the Hoenniger sediment increased from <0.1 % at day 0 to 45.4 % of the applied radioactivity at study termination (day 120) and from 0.9 % to 30.0 % at study termination for the Clayton sediment.

The concentration of thiencarbazone-methyl in water decreased from approximately 100 % of the applied amount at day 0 to no longer determined at study termination in the Hoenniger system and from about 100 % at day 0 to 6.7 % at study termination in the Clayton system. In the Hoenniger sediment the concentration of thiencarbazone-methyl increased from <0.1 % at day 0 to 19.7 % of the applied amount at day one but was no longer detected after 92 days. In the Clayton sediment the amount increased from <0.1 % at day 0 to 13.2 % at day three and was no longer detected at study termination (day 120). Test systems, which were treated with [dihydrotriazole-3-^{14}C]-thiencarbazone-methyl showed higher bound residues than those which were treated with the thiophene label. The formation of $^{14}CO_2$ was detected from 30 days after application in both test systems. The amount of $^{14}CO_2$ steadily increased to values of up to 7.6 % AR in the Hoenniger system and 13.4 % in the Clayton system at the study termination (mostly in the two sediments). Organic volatile compounds were negligible in all samples. The radioactivity found in the PU traps amounted to 0.5 % AR for both systems at study termination. The concentration of CO_2 evolved from the dihydrotriazole label was consistently higher than that evolved from the thiophene label.

The major transformation products detected in water were the BYH 18636-sulfonamide-carboxylic acid with a maximum concentration in the Hoenniger system of 45.6 % observed at study termination, followed by the BYH 18636-carboxylic acid 24.0 % on day 30 (mean of both labels). BYH 18636-MMT accounted for 12.2 % at day 92, all detected in the Hoenniger-system. The respective maximum concentrations in the Clayton system were 29.1 % for the BYH 18636-sulfonamide-carboxylic acid (day 59), 24.6 % for the BYH 18636-carboxylic acid (day 30, mean of both labels), and 24.9 % for BYH 18636-MMT at day 92. BYH 18636-dicarboxy-sulfonamide was detected at 18.9 % at day 120, only in the water of the Clayton system.

In both sediments, major metabolites (> 10 % AR) already identified in the water layer were detected at maximum concentrations of 21.3 % (BYH 18636-sulfonamide-carboxylic acid), 13.0 % (BYH 18636-carboxylic acid) in the Hoenniger system, and 10.1 % (BYH 18636-carboxylic acid) in the Clayton system, respectively. Minor degradates were present transiently throughout the study period and unidentified radioactivity reached no more than 4.0 % in either whole system.

In both water/sediment systems, thiencarbazone-methyl was lost from the water body via movement/dissipation into the sediment. It also underwent degradation to five main metabolites and limited total metabolism to $^{14}CO_2$ plus non-extractable residues. Thiencarbazone-methyl and its metabolites are mineralized in water/sediment systems but not quickly enough to be considered rapidly degradable. The degradation DT_{50} in both of the total water/sediment systems was a maximum 29 days. Although degradation was evaluated in this report it was re-evaluated in more detail in another study (Hammel, K., 2007) according to FOCUS kinetics and based on a

simultaneous fit to the whole pathway not only to the degradation or dissipation of thiencarbazone-methyl alone. Results are presented below.

Study 2 (Hammel, K., 2007)

The dissipation and degradation of thiencarbazone-methyl and its main degradants was investigated by kinetic evaluation of the two aerobic water-sediment systems (Hoenniger and Clayton) - as considered above in the study by Henk and Haas (2005). The evaluation by Hammel, K. (2007) followed EU FOCUS (2005[1]) kinetics and considered trigger and modelling endpoints used for pesticide assessment. GLP was not applicable.

The study analysed degradation in the total system and the dissipation in single phases (water or sediment). Because the results of the two treatments (1× and 10×) were very similar, the 10× samples were included in the analyses as additional replicates. Results from the two radiolabels were available for thiencarbazone-methyl and BYH 18636-carboxylic acid. A separate fit was made for each label, however the values obtained differed only marginally and the mean values for both labels were selected. All experimental data sets and all single data points were weighted equally (weighting factor 1). Non-detected values were either set to 0.5 LOD, zero or excluded from the analysis, according to FOCUS guidelines. The goodness of fit was assessed by visual inspection and an error criterion based on a chi-square (χ^2) significance test. A single-sided T-test was used to identify the probability that a parameter is not significantly different from zero.

Although other kinetic fit models were considered, in all cases the simple first order model (SFO) proved to be the appropriate kinetic model to describe degradation of the parent substance. Single phase evaluations (i.e. for water and sediment separately) were performed based on decline, i.e. dissipation, from each compartment. Dissipation half-lives in water were only available for parent thiencarbazone-methyl and BYH 18636-carboxylic acid). Since CLP is more concerned with degradation, these are not presented further. No reliable degradation or dissipation rates could be calculated for BYH 18636-MMT and BYH 18636-dicarboxy-sulfonamide due to the absence of a clear decline phase. However, whole system degradation DT_{50} and DT_{90} values are available for thiencarbazone-methyl and other degradants - see Table 36. Degradation plots of the parent substance in each test system and a proposed degradation pathway are also shown in the following Figures.

Table 36: SFO degradation parameters for the total system

Compound	Hoenniger		Clayton	
	DT_{50} (days)	DT_{90} (days)	DT_{50} (days)	DT_{90} (days)
Thiencarbazone-methyl *	21.9	72.7	31.3	103.8
BYH 18636-carboxylic acid *	29.1	96.6	32.4	107.7
BYH 18636-sulfonamide[#]	23.3	77.4	6.5	21.5
BYH 18636-sulfonamide-carboxylic acid[#]	142.0	471.6	33.9	112.7
BYH 18636-MMT	n.i.	n.i.	n.i.	n.i.
BYH 18636-dicarboxy-sulfonamide	n.i.	n.i.	n.i.	n.i.

*Arithmetic mean of two label-specific fits

[#] Although illustrative of degradation rates and retained, the fits for either one or both Hoenniger or Clayton systems were subsequently rejected by the pesticide RMS due to the relatively poor visual and statistical fit and variable data or due to the absence of a clear decline phase.

n.i. not identifiable by SFO kinetics - for BYH 18636-MMT degradation was best described by FOMC with a DT_{50} of 385 days in Hoenniger and 214 days days in an alternative Anglerweiher sediment

[1] FOCUS (2005). "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Report of the FOCUS Working Group on Degradation Kinetics. EC Document Reference Sanco/10058/2005 version 1.0

Figure 1: Plot of SFO model for the total system residues of thiencarbazone-methyl in the Hoenniger system

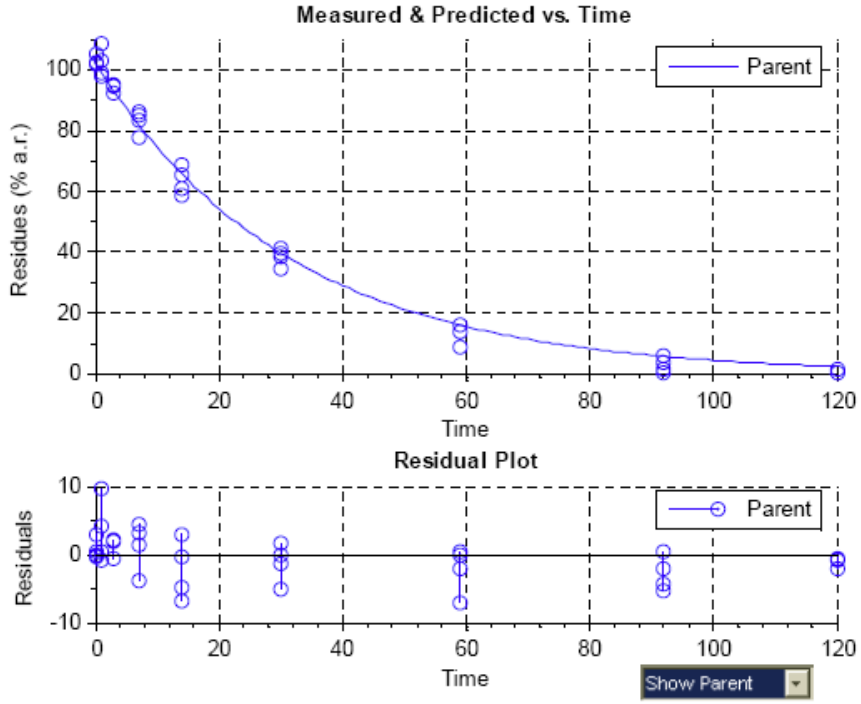


Figure 2: Plot of SFO model for the total system residues of thiencarbazone-methyl in the Clayton system

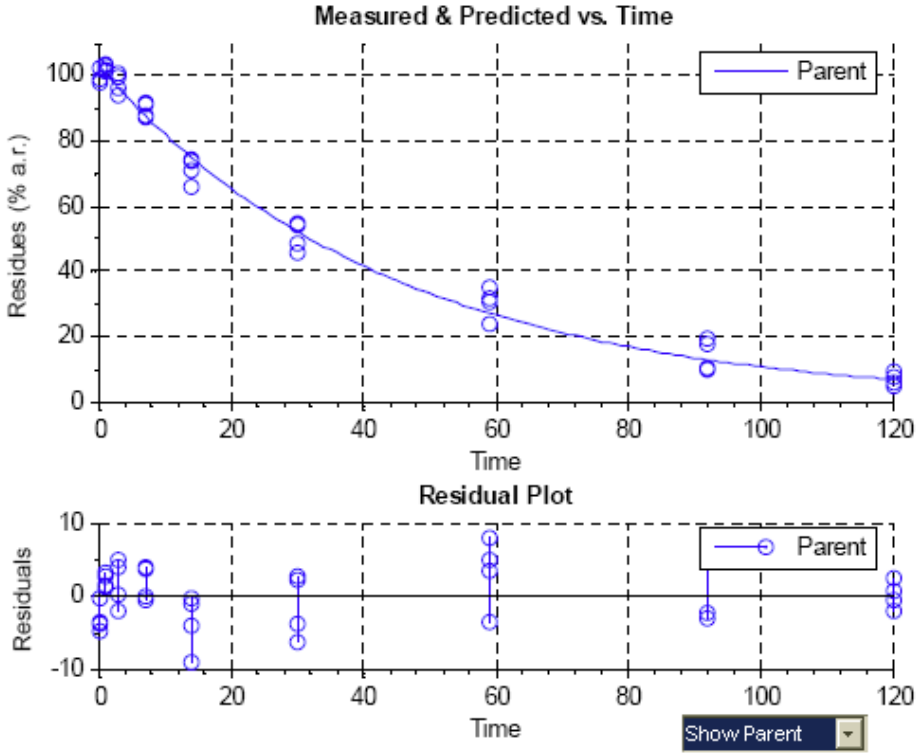
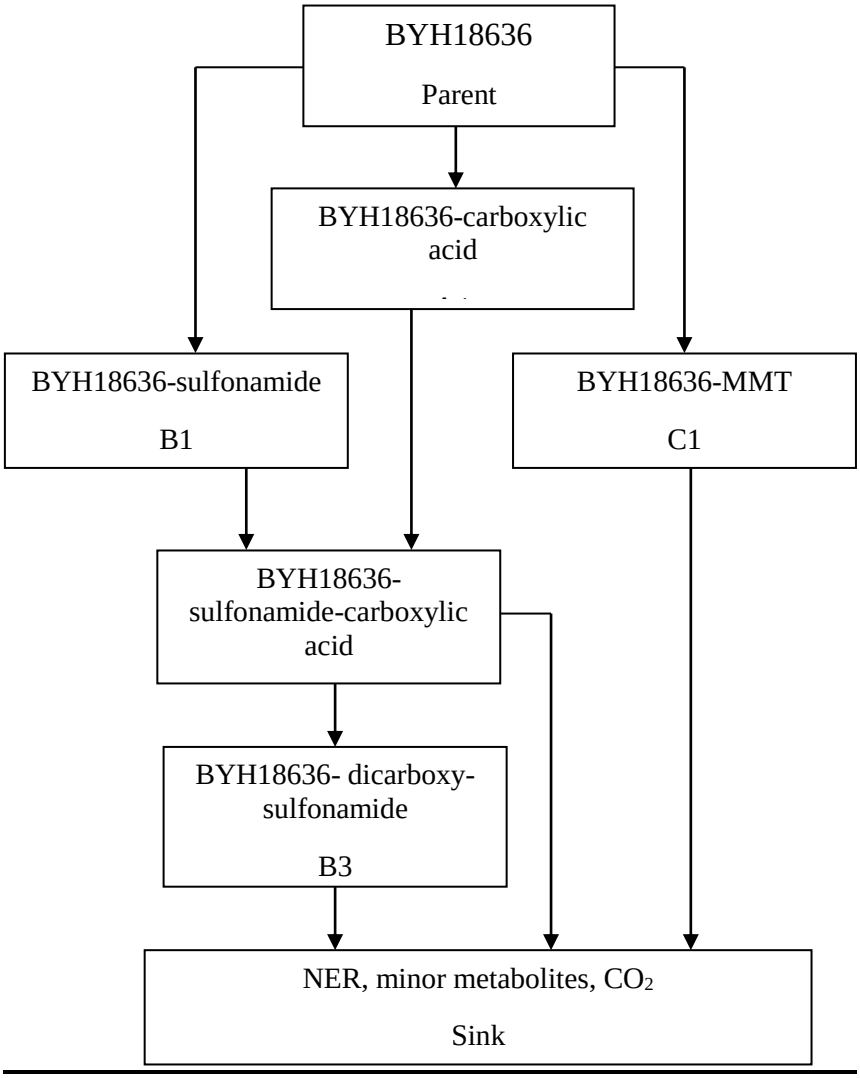


Figure 3 Proposed degradation pathway of thiencarbazone-methyl in aerobic water/sediment



5.1.3 Summary and discussion of degradation

Thiencarbazone-methyl is slowly hydrolysed under sterile acidic, neutral and alkaline conditions. At 25 °C, the hydrolytic half-life (DT_{50}) of thiencarbazone-methyl was 50 days at pH 4 and about 150 day at pH values of 7 and 9. At 20 °C and pH 4, the half-life was 118 days and it was 1.8 days at 50 °C, so the half-life decreased with increasing temperature. At all pH values tested, the major degradation products were BYH 18636-MMT and BYH 18636-sulfonamide, formed by cleavage of the molecule. BYH 18636-carboxylic acid was a minor hydrolysis product.

Solar radiation under environmental conditions does not significantly contribute to the degradation of thiencarbazone-methyl in aqueous solutions. The photodegradation half-life of thiencarbazone-methyl was 91 days under experimental conditions (Xenon lamp, Sun Test) extrapolated to 333-516 solar summer days under environmental conditions approximating to southern Europe. Direct phototransformation in buffered water was considered to contribute only to a minor extent to the degradation of thiencarbazone-methyl in the environment.

Thiencarbazone-methyl showed 0 % degradation after 28 days at 22 °C in an OECD 301 F ready biodegradation test with activated sewage sludge. A degradation half-life was not calculated (not the intention of this study) however, it was determined that thiencarbazone-methyl can be considered to be 'not readily biodegradable'.

The degradation of thiencarbazone-methyl was investigated in two dark water/sediment systems under aerobic conditions at 20 °C. Thiencarbazone-methyl rapidly partially partitioned to the sediment (13.2 - 19.7 % AR before day 3, reaching a maximum occurrence of 26.1 %) where it was also degraded and was no longer detected after 92 - 120 days. Thiencarbazone-methyl degraded in the whole system with half-lives of 21.9 to 31.3 days. Degradants BYH 18636-carboxylic acid, BYH 18636-MMT, BYH 18636-sulfonamide-carboxylic acid and BYH 18636-dicarboxy sulphonamide were found at levels above 10 % AR in the water phase. Degradants BYH 18636-carboxylic acid and BYH 18636-sulfonamide-carboxylic acid were also found at levels above 10 % AR in the sediment. For these degradants the whole system DT_{50}s ranged from 23.3 days for BYH 18636-sulfonamide, 32.4 days for BYH 18636-carboxylic acid, 142.0 days for BYH 18636-sulfonamide-carboxylic acid and, in a separate study, 184 days for BYH 18636-MMT (SFO, 385 days for FOMC). Non-extractable residues at the end of the study (120 days) were 30.0 - 45.4 % AR. Mineralization reached 7.6 - 13.4 % AR at the end of the experiments.

In addition to the standard aerobic experiments, an anaerobic water/sediment study on one sediment system (at 25 °C) is available in the dossier. In this study the metabolite BYH 18636-NMT, not identified in any of the aerobic studies, was identified at levels above 10 % AR in both the water and sediment phases. Thiencarbazone-methyl again dissipated quickly from the water phase (no longer being detected after 28 days) and reached a maximum of 30 % AR sediment at day 7. Full mineralization was minimal, with ≤1.5 % AR as CO_2 by the end of the 123-day study. Non-extractable residues throughout the study were ≤9.4 %. Although anaerobic degradation is not usually considered for classification, the mean first-order degradation rate of thiencarbazone-methyl calculated (for completeness) in the whole system was 7.6 days.

Temperature correction of degradation half-lives from available study results to 12 ˚C (based on the Arrhenius equation) was not conducted. This is because at 20-25 ˚C it is already clear that thiencarbazone-methyl would not be degraded in whole aquatic systems such that a degradation half-life <16 days (corresponding to >70 % degradation within 28 days) would be achieved. This is supported by the ready biodegradation test which indicates degradation rates would only increase at a lower temperature. In addition, the available data on the main degradants of thiencarbazone-methyl also indicates that there would not be full mineralisation and ecotoxicological data on these compounds is not sufficient to conclude that they would not themselves be classified (see Annex 1).

Overall, the degradation information does not provide sufficient data to show that thiencarbazone-methyl is ultimately degraded (mineralised) within 28 days or undergoes primary degradation to non-classifiable degradants with half-lives < 16 days. Consequently, thiencarbazone-methyl is considered to be 'not rapidly degradable' for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

An experimental study has been submitted on the adsorption and desorption of thiencarbazone-methyl in five soils (Fliege, R.; 2003). The study was conducted to GLP and to OECD Guideline No. 106 and EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate: 163-1 as well as a similar Canadian guideline.

Freundlich adsorption and desorption constants of thiencarbazone-methyl (tested as BYH 18636 with radiochemical purity: ≥ 98 %) were determined in batch equilibrium experiments with five soils using labelled test substance, [thiophene-4-^{14}C]-thiencarbazone-methyl. The soils had different pH, organic carbon contents and clay contents, three soils originated from Germany and two from USA; full details are given in Table 37. Five concentrations of the test item were evaluated for each soil, covering a concentration range of approximately 1.0 to 0.01 mg/L, i.e. two orders of magnitude. Optimal soil-to-solution ratios were derived from a pre-test and ranged from 1:1 to 1:5 (w/w) for the different soils. Plateau times allowing establishment of an equilibrium partitioning in the adsorption and desorption steps were 48 hours per step, based on results of the pre-test.

Table 37: Characteristics of soils used for adsorption/desorption of thiencarbazone-methyl

Soil code	**AXXa**	**AIII**	**SLS**	**HCB**	**SSC**
Origin	**Monheim / Northrhine-Westfalia / Germany**	**Monheim / Northrhine-Westfalia / Germany**	**Hattersheim / Hessen / Germany**	**Grand Forks / North Dakota / USA**	**Stilwell / Kansas / USA**
Textural class [USDA]	Sandy loam	Silt loam	Silt loam	Silt loam	Silty clay
Textural analysis [USDA]					
Sand [2000-50 µm] (%)	72.4*	36.9*	23.2	19	3.0*
Silt [50-2 µm] (%)	22.6*	51.1*	54.0	62	51.7*
Clay [<2µm] (%)	5.0*	12.0*	22.8	19	45.3*
pH (water)	6.9	7.6	8.3	7.6	5.7
pH ($CaCl_2$)	6.3	6.8	7.5	7.4	4.8
Organic carbon content (%)	1.47	0.88	1.30	4.1	1.15
Organic matter** (%)	2.53	1.51	2.24	7.05	1.98
CEC (meq/100 g soil)	10.3	9.8	36.9	26.0	23.1
Particle density (g/mL)	2.5*	2.55*	n.days.	n.days.	2.54*
Bulk density (g/mL)	n.days.	n.days.	n.days.	0.90	1.11*

(*) Texture data marked by an asterisk refers to historical data for the sampling site. All other data refers to a recent analysis (<6 months old) of the actual soil batch used within this study
(**) % organic matter = % organic carbon × 1.72

Suspensions of the soils in 0.01 M $CaCl_2$ were agitated in a shaker for 48 hours in the dark at 20 ± 1 °C. The suspensions were then centrifuged. For the desorption experiment the supernatants from the adsorption experiment were decanted, and a corresponding volume of fresh $CaCl_2$ solution was added. After agitation for again 48 hours and centrifugation, the supernatants were analysed by LSC and by HPLC.

The mean total recovery of applied radioactivity (value per soil series) per soil series ranged from 91.2 % to 94.0 % (range 89.9 % to 96.7 %). No significant amount of radioactivity dissipated from

the test containers or was lost upon processing. The test substance also did not significantly degrade in control or soil samples within the experimental timescale.

All adsorption coefficients in were determined according to Freundlich and are thus called K_F (in some other reports they are termed K_d). Organic carbon normalised adsorption coefficients were calculated from K_F and are in fact $K_{F,OC}$ values, although they are just termed K_{OC}. The adsorption behaviour of thiencarbazone-methyl was accurately described for all soils by the Freundlich equation. The adsorption constants K_F(ads) of the Freundlich isotherms ranged from 0.40 to 6.23 mL/g. The Freundlich exponents 1/n were below 1.0 for all soils (0.886 to 0.932). The adsorption coefficient K_F(ads) was normalized for the organic carbon content of the soil, in order to obtain K_{OC} values. The K_{oc}(ads) derived from the Freundlich equation varied between 43 and 190 mL/g for the adsorption step. The desorption constants K_F(des) of the Freundlich isotherms ranged from 1.71 mL/g (soil AIII) to 9.62 mL/g (soil HCB). Desorption K_{OC}(des) values ranged from 145 mL/g to 363 mL/g (see Table 38 for details).

Freundlich exponents (1/n) below 1.0 for all of the soils indicated a favoured soil sorption at lower test substance concentrations. The desorption constants K_F(des) of thiencarbazone-methyl were about 1.5 to 4 times higher than the respective adsorption constants, indicating an enhanced sorption of the test substance once adsorbed to the soil. Except for organic carbon content, no correlation of K_F of thiencarbazone-methyl with other soil physico-chemical properties was noticed.

Table 38: Adsorption and desorption of thiencarbazone-methyl

Soil	Soil type	Adsorption			Desorption		
		K_F b) [mL/g]	1/n a)	K_{OC} [mL/g]	K_F b) [mL/g]	1/n a)	K_{OC} [mL/g]
AXXa	sandy loam	0.64	0.899	43	2.13	0.953	145
AIII	sandy loam	0.40	0.886	46	1.71	0.943	194
SLS	silt loam	0.88	0.917	68	2.60	0.940	200
HCB	silt loam	6.23	0.897	152	9.62	0.908	235
SSC	silty clay	2.18	0.932	190	4.17	0.955	363
arithmetic mean		2.07	0.906	100	4.05	0.940	227

a) 1/n was rounded to 3 significant figures rather than 4 in the original report
b) called K_d in the original report but values were evaluated according to Freundlich and are therefore called K_F

In summary, the potential mobility in soil of thiencarbazone-methyl was assessed by batch adsorption/desorption studies in five soils. The degradants BYH 18636-carboxylic acid, BYH 18636-MMT, BYH 18636-sulfonamide, BYH 18636-sulfonamide-carboxylic acid and BYH 18636-triazolinone-carboxamide were also investigated in other studies not considered here. According to the results of these studies, thiencarbazone-methyl may be considered to exhibit ‘high’ to ‘very high’ mobility in soil; its degradants also indicated ‘medium’ to ‘very high’ mobility.

5.2.2 Volatilisation

The vapour pressure of thiencarbazone-methyl (8.8×10^{-14} Pa at 20 °C (see Section 1.3, Table 8) and the calculated Henry’s constant of 4.77×10^{-13} Pa m^3 mol^{-1} in water at pH 3.9 and 20 °C (Smeykal, H., 2005) indicate that thiencarbazone-methyl is non-volatile. Consequently, there was no need to study volatility under laboratory conditions.

5.2.3 Distribution modelling

None submitted or required

5.3 Aquatic Bioaccumulation

Table 39: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference, inc. dossier ref.
OECD 107 and EC A.8 shake flask method	Partition coefficient n-octanol/water (log K_{ow}) = -0.13 at pH 4 -1.98 at pH 7 -2.14 at pH 9	To GLP Thiencarbazone-methyl, purity: 99.3 %	Mühlberger, B., Eyrich, U.; 2005 M-248402-01-1

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The octanol/water partition coefficient (log K_{ow}) for thiencarbazone-methyl ranges from -0.13 at pH 4, to -1.98 at pH 7 and -2.14 at pH 9 (OECD 107 shake flask study by Mühlberger, B. and Eyrich, U.; 2005 - see Section 1.3, Table 8). This very low log K_{ow} indicates a low potential to bioaccumulate.

The thiencarbazone-methyl pesticide DAR (Section IIA 8.2.6 and Table 8.2-1) also includes log K_{ow} values for principal degradants of thiencarbazone-methyl and all have low ≤ 1.1, mostly < 0.

5.3.1.2 Measured bioaccumulation data

No experimental study available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The Log K_{ow} for thiencarbazone-methyl is -1.98 at pH 7, this (and values at other pH) is less than the trigger value of 4 given in the CLP Regulation. No experimental fish BCF study is available but overall, a low bioaccumulation potential is predicted for thiencarbazone-methyl.

5.4 Aquatic toxicity

Table 40: Summary of relevant information on aquatic toxicity of thiencarbazone-methyl

Test organism	**Test guideline and type**	**Duration**	**LC/EC$_{50}$ mg a.s./L**	**NOEC mg a.s./L**	**Reference Inc. dossier ref. & study code**
Fish					
Oncorhynchus mykiss (rainbow trout)	OECD Guideline 203 static	96 h acute	**> 104^{mm}**	104^{mm}	Anon, 2005a IIA 8.2.1.1/01 EBGSM014
Lepomis macrochirus (bluegill sunfish)	OECD Guideline 203 static	96 h acute	> 107^{mm}	107^{mm}	Anon, 2005b IIA 8.2.1.2/01 EBGSM013
Cyprinodon variegatus (sheepshead minnow)	OECD Guideline 203 static	96 h acute	> 106^{mm}	106^{mm}	Anon, 2005c IIA 8.11.1/01 EBGSM011
Pimephales promelas (fathead minnow)	OECD Guideline 210 ELS flow-through	35 d chronic	-	**4.8^{mm}**	Anon., 2006 IIA 8.2.4/01 EBGSP013
Aquatic invertebrates					
Daphnia magna	OECD Guideline 202 static	48 h acute	>98.6^{mm}	98.6^{mm}	Banman & Lam, 2005d IIA 8.3.1.1/01 EBGSM007
Crassostrea virginica	OPPTS Guideline 850.1025 (draft) and FIFRA 72-3 flow-through	96 h acute	>100^{mm}	4.6^{mm}	Cafarella, 2006 IIA 8.11.1/02 EBGSP010
Chironomous riparius	OECD 202 static *C. riparius* larvae exposed in aqueous phase	48 h acute	>100^{nom}	100^{nom}	Bruns, 2006 IIA 8.5.1/01 EBGSP037
Americamysis bahia	OPPTS Guideline 850.1035 and FIFRA 72-3 flow-through	96 h acute	**>94^{mm}**	94^{mm}	Putt, 2006a IIA 8.11.1/03 EBGSP011

Test organism	**Test guideline and type**	**Duration**	**LC/EC$_{50}$ mg a.s./L**	**NOEC mg a.s./L**	**Reference Inc. dossier ref. & study code**
Americamysis bahia	U.S. EPA FIFRA Guideline 72-4 (1982) flow-through	28 d chronic	-	5.9^{mm}	Putt, 2006b IIA 8.11.1/04 EBGSP004
Daphnia magna	OECD Guideline 211 semi-static	21 d chronic	-	**3.54^{mm}**	Kern & Lam, 2007 IIA 8.3.2.1/01 EBGSM008
Algae					
Pseudokirchneriella subcapitata (freshwater green alga)	OECD Guideline 201 semi-static	96 h acute & chronic	**72 h E$_r$C$_{50}$ = 1.017^{mm}**	**72 h NOE$_r$C = 0.0307^{mm}**	Kern *et al.*, 2005 IIA 8.4/02 EBGSM001
Navicula pelliculosa (freshwater diatom)	OECD Guideline 201 semi-static	96 h acute & chronic	72 h E$_r$C$_{50}$ = 64.0^{mm}	72 h NOE$_r$C = 51.6^{mm}	Kern *et al.*, 2005 KIIA 8.4/04 EBGSM015
Anabaena flos-aquae (freshwater blue-green alga)	OECD Guideline 201 semi-static	96 h acute & chronic	72 h E$_r$C$_{50}$ = 9.15^{mm}	72 h NOE$_r$C = 2.7^{mm}	Kern & Lam, 2006a IIA 8.4/05 EBGSP012
Skeletonema costatum (marine diatom)	OECD Guideline 201 semi-static	96 h acute & chronic	72 h E$_r$C$_{50}$ >114^{mm}	72 h NOE$_r$C = 114^{mm}	Christ & Lam, 2006 IIA 8.11.1/05 EBGSM017
Aquatic macrophytes					
Lemna gibba G3	OECD Guideline 221 semi-static	7 d acute & chronic	**7 d E$_r$C$_{50}$ = 0.00131^{mm}**	**7 d NOE$_r$C = 0.00021^{mm}**	Kern & Lam, 2006b IIA 8.6/01 EBGSM016
Myriophyllum spicatum	Non-guideline static (14 d exposure period (+14 d recovery not reported here)	14 d acute & chronic	**14 d E$_r$C$_{50}$ = 0.00094^{mm}**	14 d NOE$_r$C = 0.00031^{mm}	Christ & Lam, 2007b IIA 8.6/03 EBGSP077 and recalculation by Bruns & Solga, 2013
Potamogeton pectinatus	Non-guideline static (14 d exposure period (+14 d recovery not reported here)	14 d acute & chronic	14 d E$_r$C$_{50}$ = 0.0053^{mm}	**14 d NOE$_r$C = 0.000075^{mm}**	Hoberg, 2007 IIA 8.6/04 EBGSP086

Bold values indicate most sensitive acute/chronic endpoints for each group considered for classification.

5.4.1 Fish

Studies have been submitted on the acute/short-term toxicity of technical thiencarbazone-methyl to three fish species: rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*) and the marine species sheepshead minnow (*Cyprinodon variegatus*). These were all conducted according to OECD test guideline 203 (and similar). A study on the chronic/long-term toxicity of technical thiencarbazone-methyl to fish has also been submitted on one species fathead minnow (*Pimephales promelas*) according to OECD guideline 210 (fish early life stage). These studies were all conducted to GLP and are considered to be reliable for the purposes of hazard classification. Further details of each test are summarised below.

5.4.1.1 Short-term toxicity to fish

Study 1 (Anon 2005a)

In a 96-hour acute toxicity study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed under static conditions to technical thiencarbazone-methyl (96.3 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 203 as well as OPPTS Guideline 850.1075, FIFRA 72-1, Dir. 92/69/EEC and ASTM Standard E729 (1996). Based on preliminary range-finding data it was run as a limit test with one nominal treatment level of 100 mg thiencarbazone-methyl/L in addition to a water control group (no solvent vehicle was required). This corresponded to a mean measured test concentration of 104 mg/L, which was within 80-120 % of nominal, however the biological results were based on this mean measured concentration.

The test consisted of three randomised replicates of 10 animals in glass aquaria containing 18 L of test medium. The study was conducted at a temperature of 12.0 to 13.5 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 623 to 1018 lux. The pH range was 7.5 to 8.0 and dissolved oxygen was 7.8 to 8.7 mg/L (77 to 84 % saturation). Fish were not fed during the test and were observed for survival (mortality) and sublethal behavioural effects at 4, 24, 48, 72 and 96 hours.

No treatment related effects (mortality or symptoms of toxicity) were seen in any of the test replicates through the course of the study. Therefore the 96-hour mean measured LC_{50} for thiencarbazone-methyl to rainbow trout was determined to be >104 mg/L, the single limit concentration tested.

Study 2 (Anon., 2005b)

In a 96-hour acute toxicity study, juvenile bluegill sunfish (*Lepomis macrochirus*) were exposed under static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (i.e. no major deviations) with OECD Guideline 203 as well as OPPTS Guideline 850.1075, FIFRA 72-1, Dir. 92/69/EEC and ASTM Standard E729 (1996). Based on preliminary range-finding data it was run as a limit test with one nominal treatment level of 100 mg thiencarbazone-methyl/L in addition to a water control group (no solvent vehicle was required). This corresponded to a mean measured test concentration of 107 mg/L, which was within 80-120 % of nominal, however the biological results were based on this mean measured concentration.

The test consisted of three randomised replicates of 10 animals in glass aquaria containing 18 L of test medium. The study was conducted at a temperature of 21.2 to 22.4 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 775 to 980 lux. The pH range was 7.5 to 8.3 and

dissolved oxygen was 5.9 to 8.4 mg/L (67 to 94 % saturation). Fish were not fed during the test and were observed for survival (mortality) and sublethal behavioural effects at 4, 24, 48, 72 and 96 hours.

No treatment related effects (mortality or symptoms of toxicity) were seen in any of the test replicates through the course of the study. Therefore the 96-hour mean measured LC_{50} for thiencarbazone-methyl to bluegill sunfish was determined to be >107 mg/L, the single limit concentration tested.

Study 3 (Anon., 2005c)

In a 96-hour acute toxicity study, juvenile sheepshead minnow (*Cyprinodon variegatus*) were exposed under static conditions to technical thiencarbazone-methyl (96.3 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 203 as well as OPPTS Guideline 850.1075, FIFRA 72-3. Based on preliminary range-finding data it was run as a limit test with one nominal treatment level of 100 mg thiencarbazone-methyl/L in addition to a water control group (no solvent vehicle was required). This corresponded to a mean measured test concentration of 106 mg/L, which was within 80-120 % of nominal, however the biological results were based on this mean measured concentration.

The test consisted of three randomised replicates of 10 animals in glass aquaria containing 30 L of test medium. The study was conducted at a temperature of 21.4 to 22.9 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 816 to 1043 lux. The pH range was 7.5 to 7.9 and dissolved oxygen was 6.1 to 7.3 mg/L (77 to 90 % saturation), salinity was 17 parts per 1000 (‰). Fish were not fed during the test and were observed for survival (mortality) and sublethal behavioural effects at 4, 24, 48, 72 and 96 hours.

No treatment related effects (mortality or symptoms of toxicity) were seen in any of the test replicates through the course of the study. Therefore the 96-hour mean measured LC_{50} for thiencarbazone-methyl to sheepshead minnow was determined to be >106 mg/L, the single limit concentration tested.

5.4.1.2 Long-term toxicity to fish

In a 35-day early life stage toxicity study (Anon., 2006), fathead minnow (*Pimephales promelas*) were exposed under flow-through conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 210 as well as OPPTS Guideline 850.1400 EPA-FIFRA 72-4 and ASTM Standard E729 (2002). The nominal concentrations (mean measured in brackets) were: control (<0.10), 0.63 (0.60), 1.25 (1.13), 2.50 (2.58), 5.00 (4.80) and 10.0 (10.8) mg thiencarbazone-methyl/L. Only a water control was used as no solvent vehicle was required. The mean measured test concentrations range was 90 % to 108 % with all individual measurements within 83 to 120 % of nominals. The biological results were presented as mean measured concentrations.

The test consisted of four randomised replicates of 35 animals in glass aquaria containing 30 L of test medium. The study was conducted at a temperature of 24.8 to 25.5 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 668 to 907 lux. The pH range was 7.5 to 8.0 and dissolved oxygen was 6.0 to 7.5 mg/L (73 to 91 % saturation). Fish were fed during the test and were observed for survival (mortality) and sublethal behavioural effects at 4, 24, 48, 72 and 96 hours.

Fathead minnow eggs starting at <24 hours old were observed for hatch rate. Hatched fish were thinned down to 20 young fish/replicate and were assessed for abnormal behaviour, physical changes, mortality and growth (length, dry weight). Observations for sublethal effects and survival were made daily, hatching observations were made daily during the hatching phase. Growth determinations were made at the end of the exposure. The endpoints for each tested parameter are presented below.

Table 41: Results from fish early life stage toxicity test with fathead minnow exposed for 35-days to technical thiencarbazone-methyl

Parameter	NOEC endpoint (mm)		LOEC endpoint (mm)	
Alevin survival (day 5)	NOEC	10.8 mg a.s./L	LOEC	>10.8 mg a.s./L
Fry survival (day 35)	NOEC	4.8 mg a.s./L	LOEC	10.8 mg a.s./L
Percent hatch	NOEC	10.8 mg a.s./L	LOEC	>10.8 mg a.s./L
Time to hatch	NOEC	10.8 mg a.s./L	LOEC	>10.8 mg a.s./L
Growth (length and weight)	NOEC	10.8 mg a.s./L	LOEC	>10.8 mg a.s./L
Morphological and behavioural effects	NOEC	10.8 mg a.s./L	LOEC	>10.8 mg a.s./L

mm = based on mean measured test concentrations

For most of the parameters tested, no treatment-related effects were noted. Percent survivorship at Day 35 was determined as (# of fish on Day 35/# of fish at thinning) * 100. The percent fry survival on Day 35 ranged between 85.0 - 95.2 % for the controls. Survivorship for replicates in some treatment groups was less than 85 %, specifically, for the 0.60 mg a.s./L one replicate at 75.0 %, for the 1.13 mg a.s./L group one replicate at 70.0 %, one replicate with 70.0 % survivorship in the 4.80 mg a.s./L group, and two replicates in the 10.8 mg a.s./L group with 75.0 and 76.2 % survivability. The result from the 10.8 mg a.s./L treatment group was reported as a statistically significant effect.
The 35-day exposure to thiencarbazone-methyl technical resulted in a mean measured NOEC of 4.80 mg a.s./L based on fry survival.

5.4.2 Aquatic invertebrates

Acute/short-term toxicity data are available for thiencarbazone-methyl on three aquatic invertebrate species, *Daphnia magna*, eastern oysters (*Crassostrea virginica*) and mysid shrimp (*Americamysis bahia*). Chronic/long-term data are also available on *Daphnia magna* and mysid shrimp. These studies were all conducted according to GLP and to standard guidelines and they are considered reliable for use in hazard classification. Summaries are provided below.

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1 (Banman, C.S., Lam, C.V., 2005d)

In a 48-hour acute toxicity study, *Daphnia magna* (neonates, <24 hours old) were exposed under static conditions to technical thiencarbazone-methyl (96.3 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 202 as well as OPPTS Guideline 850.1010, FIFRA 72-2 and an ASTM Standard (2002). Based on preliminary range-finding data it was run as a limit test with one nominal treatment level of 100 mg thiencarbazone-methyl/L in addition to a water control group (no solvent vehicle was required). This corresponded to a mean measured test concentration of 98.6 mg/L, which was within 80-120 % of nominal, however the biological results were based on this mean measured concentration.

The test consisted of four randomised replicates of 5 animals. The study was conducted at a temperature of 19.2 to 19.9 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 481 to 549 lux. The pH range was 8.1 to 8.3 and dissolved oxygen was 8.0 to 8.3 mg/L (90 to 93 % saturation). *Daphnia* were not fed and test solutions were not aerated during the test. The daphnids were observed for immobilisation and sublethal behavioural effects at 4, 24 and 48 hours.

No treatment related effects were seen in any of the test replicates through the course of the study. Therefore the 48-hour mean measured EC_{50} for thiencarbazone-methyl to *Daphnia magna* was determined to be > 98.6 mg/L, the single limit concentration tested.

Study 2 (Cafarella, M. A., 2006)

In a 96-hour acute toxicity study, eastern oysters (*Crassostrea virginica*) were exposed under flow-through conditions to technical thiencarbazone-methyl (95.7 % pure). The study was conducted to GLP and in accordance (no major deviations) with OPPTS Guideline 850.1025 (draft) and FIFRA 72-3. Reduction of shell deposition was used as the indicator of toxicity. Oysters were exposed to five nominal test concentrations of 2.6, 6.4, 16, 40 and 100 mg thiencarbazone-methyl/L and a dilution water control (no solvent vehicle was required). Mean measured concentrations were 1.6, 4.6, 12, 49 and 100 mg/L. This corresponded to analytical recoveries ranging from 62 to 120 % of nominals, therefore the biological results were based on mean measured concentrations.

The test consisted of two randomised replicates of 20 animals in aquaria containing 18 L of test medium. The flow to each aquarium (75 mL/minute) provided approximately 6.0 solution volume replacements every 24 hours in order to provide a 90 % solution replacement rate of approximately 9 hours. The dilution water used during this study was filtered natural seawater and was prepared daily by adjusting the salinity to 20 to 21‰ with laboratory well water.

The study was conducted at a temperature of 19.0 to 22.0 °C using a photoperiod of 16 hours light/8 hours dark. The pH range was 7.4 to 7.9 and dissolved oxygen was 5.3 to 7.5 mg/L (> 60 % saturation). During the exposure, the oysters received supplemental feedings of algae (*Tetraselmis maculata*). Visual observations for abnormalities (excessive mucous production or lack of faecal production) were made at test initiation and at each subsequent 24 hour interval.

No mortality or abnormalities were observed at any of the treatment levels tested. Growth among dilution water control oysters at test termination averaged 4.1 mm, which is within the guideline range as well as the historical range at the laboratory. After 96 hours exposure, the 1.6, 12, 49, and 100 mg a.s./L (measured) concentrations resulted in reduced shell deposition of 2, 11, 9, and 12 %, respectively. At the 4.6 mg a.s./L test concentration there was no percent reduction relative to the control but oysters in this group exhibited a positive response compared to the control.

To provide an estimate of the NOEC, a Williams' test was conducted on the shell growth data which determined a significant reduction at treatment levels ≥ 12 mg a.s./L when compared to the control. The NOEC was therefore a mean measured 4.6 mg a.s./L. Since no concentration tested resulted in ≥ 50 % reduction in shell growth, the 96-hour EC_{50} value was empirically estimated to be > 100 mg a.s./L, the highest mean measured concentration tested.

Study 3 (Putt, A., 2006a)

In a 96-hour acute toxicity study, mysid shrimp (*Americamysis bahia*) ≤ 24 hours old, were exposed under flow-through conditions to technical thiencarbazone-methyl (95.7 % pure). The study was conducted to GLP and in accordance (no major deviations) with OPPTS Guideline 850.1035 and FIFRA 72-3. Survival was used as the indicator of toxicity. Mysids were exposed to five nominal test concentrations of 13, 22, 36, 60 and 100 mg a.s./L, corresponding to mean measured concentrations of 10, 23, 34, 56 and 94 mg a.s./L. There was also a dilution water control (no solvent vehicle was required). Analytical recoveries ranged from 80 to 100 % of nominals, however the biological results were based on mean measured concentrations.

The test consisted of two randomised replicates of 10 juvenile animals in glass jars containing 0.74 L of test medium. Test solutions were delivered to the exposure vessels (200 mL/vessel/cycle) at an approximate rate of 5.9 solution volume replacements per day in order to provide a 90 % test solution replacement rate of approximately 9.0 hours. The study was conducted using a photoperiod of 16 hours light/8 hours dark and a light intensity of 880 to 11000 lux. Temperature was 24.0 to 26.0 °C apart from slight deviation in one replicate but this was not considered to have affected the results. The pH range was 7.9 to 8.0 and dissolved oxygen was maintained at > 60 % saturation. The dilution water used had a salinity range of 20 to 21‰. Mysids were fed brine shrimp (*Artemia salina*) nauplii, *ad libitum*, twice daily. Biological observations (e.g., abnormal behaviour or appearance) were made at test initiation and at 24 hour intervals. Mortality was defined as lack of movement after gentle prodding with a glass pipette.

No treatment related effects were seen in any of the test replicates through the course of the study. Therefore the 96-hour mean measured EC_{50} for thiencarbazone-methyl to *Americamysis bahia* was determined to be > 94.0 mg/L, the single limit concentration tested.

Study 4 (Bruns, E; 2006)

In a 48-hour acute toxicity study, *Chironomus riparius* midge larvae (first instar, <2-3 days old) were exposed under static conditions to technical thiencarbazone-methyl (95.7 % pure). The study was conducted to GLP but not to any specified guideline, it was based on OECD 202 (1984) and a revised OECD draft proposal from 2004. The study was conducted in water only with no sediment phase. It was also a limit test at a single nominal concentration of 100 mg thiencarbazone-methyl/L. There were six control and treatment replicates at this concentration. Lethality and the occurrence of symptoms were recorded and evaluated after 48 hours of exposure.

The study was conducted at a temperature of 20.7 to 21.5 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 500 to 1000 lux. The pH range was 7.3 to 8.3 and the dissolved oxygen range was 8.4 to 8.5 mg/L (94 to 98% saturation). Once, directly after insertion of the larvae into the test vessels, a small amount (0.01 mL) of an aqueous fish food suspension was added to each test beaker.

Control mortality did not exceed 10%. The analytical findings of thiencarbazone-methyl in the freshly prepared Day 0 and in the aged test media on Day 2 showed mean measured concentrations of 103% relative to the nominal concentration. Due to the high recoveries at the beginning and end of the exposure period, results were based on the nominal concentration.

No mortality or treatment related adverse effects were seen in any of the test replicates through the course of the study. Therefore, the 48-hour nominal EC_{50} for thiencarbazone-methyl to *Chironomus riparius* was determined to be > 100 mg/L, the single limit concentration tested.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1 (Kern, M.E. and Lam C.V., 2007)

In a 21-day chronic toxicity study, *Daphnia magna* (neonates, <24 hours old) were exposed under semi-static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 211 as well as OPPTS Guideline 850.1300 (draft), FIFRA 72-4(b) and an ASTM Standard (2002). The exposure concentrations were nominal (mean measured in brackets) concentrations of control (<0.29), 3.13 (3.54), 6.25 (6.97), 12.5 (13.7), 25.0 (27.2), 50.0 (56.6) and 100 (111.7) mg thiencarbazone-methyl/L. There was only a water control group, no solvent vehicle was required. This corresponded to a mean measured test concentrations of 109 % to 113 %, which was within 80-120 % of nominal, however the biological results were based on mean measured concentrations.

Three replicates were used for sublethal and survival effects assessment with 5 organisms per replicate (multiple organism beakers). Ten replicates were used for sublethal, survival, reproduction and growth effects assessment with one organism per replicate (single organism beakers).

The study was conducted at a temperature of 19.6 to 20.3 °C using a photoperiod of 16 hours light/8 hours dark and a light intensity of 477 to 637 lux. The pH range was 8.1 to 8.6 and dissolved oxygen was 7.6 to 10.7 mg/L (84 to 118 % saturation). Daphnids were fed daily with green algae (*Pseudokirchneriella subcapitata*) and blended flaked fish food. Parameters measured were sublethal effects, survival (immobilization), time to first brood release, reproduction (neonates per adult reproductive day) and growth (length and dry weight at study termination). Observations for sublethal effects and survival were made daily, reproductive output (neonates counts) occurred at the time of first brood release and on Monday, Wednesday and Friday thereafter up to the day of termination. Growth determinations were made at the end of the exposure.

No mortality was observed in the control group which had an average of 21.8 neonates produced per adult reproduction day. No apparent dose-response effects were observed for adult survival or sublethal effects even though the adult body length, dry weight, and reproduction were statistically different from the control in some of the treatment levels. A summary of results for each key parameter is presented below:

Table 42: Reproduction and growth of *Daphnia magna* exposed for 21 days to technical thiencarbazone-methyl

Mean measured concentration (mg a.s./L)	Mean % survival of reproductive adults	Time to first brood	Neonates / adult reproduction day	Adult body length	Adult dry weight
Control (0.0)	100	7	21.8	5.20	1.683
3.54	100	7	19.1	5.06	1.701
6.97	100	7	18.7	5.02*	1.362*
13.7	100	7	16.5*	4.89*	1.292*
27.2	100	7	15.0*	4.90*	1.534
56.6	100	7.3	16.2*	5.02*	1.527
111.7	100	7	19.9*	5.04*	1.606

*statistically significant effect ($P < 0.05$)

In the DAR the pesticide evaluator proposed the following key endpoints for the chronic toxicity of thiencarbazone-methyl to *Daphnia magna*:

21-day adult survival NOEC: 111.7 mg a.s./L

21-day reproduction NOEC: 6.97 mg a.s./L

21-day adult body length NOEC: 3.54 mg a.s./L

21-day adult body dry weight NOEC: 3.54 mg a.s./L

All endpoints were based on mean measured concentrations.

The overall 21-day mean measured NOEC for thiencarbazone-methyl to *Daphnia magna* was determined to be 3.54 mg/L based on adult length and weight.

Study 2 (Putt, A., 2006b)

In a 28-day chronic toxicity study, mysid shrimp (*Americamysis bahia*) ≤ 24 hours old, were exposed under flow-through conditions to technical thiencarbazone-methyl (95.7 % pure). The study was conducted to GLP and in accordance (no major deviations) with U.S. EPA FIFRA Guideline 72-4 (1982) and 'The standard guide for conducting life-cycle toxicity test with saltwater mysids' (ASTM, 1994). Mysids were exposed to five test concentrations of nominal 5, 10, 20, 40 and 80 mg a.s./L (corresponding to mean measured concentrations of 5.9, 11, 21, 41 and 83 mg a.s./L) and a dilution water control, no solvent vehicle was required. Analytical recoveries ranged from 100 to 120 % of nominals, however the biological results were based on the mean measured concentrations.

The test initially consisted of two randomised non-paired replicates of 30 juvenile mysids per replicate. The volume within the retention chambers was between 920 to 1540 mL. Solution volume in the pairing chambers, subsequently used to house paired sexually mature male and female mysids (at day 14) fluctuated from 470 to 790 mL. During each cycle of the diluter system ≈1000 mL of

exposure solution was delivered to each replicate test vessel at a rate of approximately 8.4 aquarium volume additions per day, providing a 90 % test solution replacement rate of approximately 7.0 hours. The study was conducted using a photoperiod of 16 hours light/8 hours dark and a light intensity of 720 to 970 lux. Temperature was 25.0 to 26.0 °C. The pH range was 8.0 to 8.3 and dissolved oxygen was maintained between 83 to 102 % of saturation. The artificial dilution seawater had a salinity range of 18 to 21‰. Mysids were fed nutrient enriched brine shrimp (*Artemia salina*) nauplii twice daily then every other day after pairing. After pairing, the number of dead males and females, the number of offspring per individual female and any abnormal appearance or behaviour was recorded. Observations were made daily throughout the study. Dead parental mysids and offspring were recorded, removed and discarded when observed during the test. Mortality was defined as lack of movement after gentle prodding with a glass pipette.

At test termination, all mysids were sacrificed dried and separated into male and female groups for each replicate exposure level. Individual body length to the nearest 0.1 mm was determined. Male and female mysids were then oven dried and placed in a desiccator. Individual total dry body weight to the nearest 0.01 mg was determined. Individual lengths and weights of all surviving males and females were recorded separately for each replicate of each concentration and the control.

Adult survival, cumulative number of offspring produced per female per reproductive day, average total body length and average dry weight were used as the indicators of toxicity. Results for each parameter and treatment group are presented in detail in the thiencarbazone-methyl DAR. No significant reduction in mysid survival or number of offspring per female was seen in any of the treatment levels tested compared to the control data. A Dunnett's Test determined a significant difference in average dry body weight among males exposed to 11, 21 and 83 mg/L when compared to the control. Significant effects on average total body length were also determined amongst males and females at 11 mg/L and above. The effects in these treatment groups were slight but were statistically significant according to the methods and level of probability ($p < 0.05$) used by the study author. The effects for these parameters are tabulated below.

Table 43: Effects on body length of mysids exposed for 28 days to technical thiencarbazone-methyl

Mean Measured Concentration (mg a.s./L)	Average Total Body Length (mm)			
	Males	Significance	Females	Significance
Control	7.4		7.5	
5.9	7.3		7.5	
11	7.1	+	7.3	+
21	7.2	+	7.2	+
41	7.0	+	7.3	+
83	7.0	+	7.2	+

Sign.: + Significantly reduced compared to the control, based on Dunnett's Test

Table 44: Effects on body weight of mysids exposed for 28 days to technical thiencarbazone-methyl

Mean Measured Concentration (mg a.s./L)	Dry Body Weight (mg)			
	Males	Significance	Females	Significance
Control	1.06		1.35	
5.9	0.98		1.38	
11	0.92	+	1.34	
21	0.95	+	1.45	
41	0.97		1.44	
83	0.95	+	1.40	

Sign.: + Significantly reduced compared to the control, based on Dunnett's Test

Overall, based on effects on body weight and length, a chronic 28-day mean measured NOEC of 5.9 mg a.s./L was determined for *Americamysis bahia* exposed to thiencarbazone-methyl.

5.4.3 Algae and aquatic plants

Thiencarbazone-methyl is a herbicide and data are available on two algal species, the green alga *Pseudokirchneriella subcapitata* and the blue-green alga *Anabaena flos-aquae*, as well as the freshwater diatom *Navicula pelliculosa* and saltwater diatom *Skeletonema costatum*. Thiencarbazone-methyl was also tested on the aquatic macrophyte *Lemna gibba*. These studies were all conducted according to GLP and generally according to respective guidelines and they are considered reliable for use in hazard assessment.

Data are available from three additional studies on aquatic macrophytes (covering *Lemna* and four other plant species). These are from non-standard 'higher tier' recovery studies based on the *Lemna* OECD 221 test guideline and were conducted to GLP. Whilst the information on recovery has not been used, the effects during the initial exposure phase may be considered relevant for hazard classification and so they have been included here.

Full details of all studies are provided in the thiencarbazone-methyl DAR but summaries are given below.

5.4.3.1 Toxicity to algae/diatoms

Study 1 (Kern, M.E., Banman, C.S. and Lam, C.V., 2005)

In a 96-hour toxicity study, green algae *Pseudokirchneriella subcapitata* were exposed under static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 201 (1984, 2004 draft), FIFRA Guideline 123-2 (1982) and OPPTS Guideline 850.5400 (1996 draft). Algae were exposed to six nominal test concentrations of 31, 63, 125, 250, 500 and 1000 µg a.s./L, corresponding to mean measured concentrations of 30.7, 61.1, 125, 251, 506 and 1024 µg a.s./L. There was also a dilution water control (no solvent vehicle was required). Mean measured concentrations were 97 to 102 % of nominals, which is within 80 to 120 %, however the biological results were based on mean measured concentrations. There were three replicates per test concentration and control with an initial cell density of 1 x 10^4 cells/mL. The study was conducted using a photoperiod of 24 hours light and a light intensity of 3875 to 4682 lux. Temperature was 24.5 to 24.9 °C.

The pH range was 7.4 to 10.1. An increase in the pH of the control medium by more than 1.5 pH units during the test is considered a deviation from the guideline. From a starting pH of 7.4-5, deviations beyond amount were seen in the control and lowest four test concentrations where Day-4 pH values were 10.0, 10.1, 10.1, 10.1, 10.1 and 9.5 respectively. Measurements of pH were only made at test initiation and termination (96-hours) and are not available at 72 hours. It is not uncommon for pH to increase in algal tests (mostly due to uptake of inorganic carbon and nitrate) and this was seen more in the control and lower concentrations where growth was greatest. The main validity criteria were all met however; There was at least a 16-fold exponential increase in cell density over 0-72 hours (actual ≈ 148-fold); the mean coefficient of variation for section-by-section specific growth rates in the control did not exceed 35 % (actual 19.5 %) and the coefficient of variation of average specific growth rates between control replicates did not exceed 7 % over 0-72 hours (actual 1.3 %). Overall therefore, the pesticide RMS did not consider the pH deviation likely to have substantially affected the results at 72-hours.

Cell density was determined daily. Observation parameters were growth rate (NOEC and EC_{50}) at 72 hours and standing crop, cumulative biomass and growth rate (NOEC and EC_{50}) at 96 hours. For *Pseudokirchneriella subcapitata* the 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be 1017 µg thiencarbazone-methyl/L (1.017 mg/L), with a 72-hour NOE_rC value of 30.7 µg thiencarbazone-methyl/L (0.0307 mg/L). The 96-hour E_rC_{50} was greater than the highest concentration tested (1024 µg/L) with a 96-hour NOE_rC value of 125 µg/L. All endpoints were based on mean measured concentrations. In line with normal practice under CLP, the 72-hour rather than 96-hour values will be used for hazard classification.

Study 2 (Kern, M.E., Roberts, J.A. and Lam, C.V., 2005)

In a 96-hour toxicity study, the freshwater diatom *Navicula pelliculosa* was exposed under static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 201 (1984, 2004 draft), FIFRA Guideline 123-2 (1982) and OPPTS Guideline 850.5400 (1996 draft). Diatom cells were exposed to six nominal test concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./L, corresponding to mean measured concentrations of 3.11, 6.16, 12.1, 24.1, 51.6 and 101 mg a.s./L. There was also a dilution water control (no solvent vehicle was required). Mean measured concentrations were 96 to 103 % of nominals, which is within 80 to 120 %, however the biological results were based on mean measured concentrations.

There were three replicates per test concentration and control with an initial cell density of 1 x 10^4 cells/mL. The study was conducted using a photoperiod of 24 hours light and a light intensity of 3907 to 4564 lux. Temperature was 23.8 to 24.5 °C. The pH range measured at days 0 and 4 was 7.1 to 8.7 for the control and nominal 3.13, 6.25, 12.5 and 25 mg a.s./L test levels. Initial pH values for the 50 and 100 mg a.s./L nominal test levels were 6.3 and 4.2, respectively. Initial pH values were reduced with higher test concentrations. This was thought to be an effect of the test item since initial media (prior to dosing) was prepared as a batch with a pH of 7.5. Ending pH values on day 4 for the 50 and 100 mg a.s./L solutions were 8.6 and 4.2, respectively. Although appropriate adjustments to the pH should have been considered, endpoints at 72-hours would have been less affected and pH did not appear to be a growth limiting factor. The pesticide RMS did not, therefore, consider this to have substantially affected the results.

Cell density was determined daily. Observation parameters were growth rate (NOEC and EC_{50}) at 72 hours and standing crop, cumulative biomass and growth rate (NOEC and EC_{50}) at 96 hours.

The main validity criteria relating to at least a 16-fold exponential increase in cell density over 0-72 hours, a mean coefficient of variation for section-by-section specific growth rates in the control not exceeding 35 % and the coefficient of variation of average specific growth rates between control replicates not exceeding 7 % over 0-72 hours, were all met.

For *Navicula pelliculosa* the 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be 64.0 mg thiencarbazone-methyl/L, with a 72-hour NOE_rC value of 51.6 mg thiencarbazone-methyl/L. The 96-hour E_rC_{50} was 59.3 mg/L, with a 96-hour NOE_rC value also of 51.6 mg/L. All endpoints were based on mean measured concentrations. In line with normal practice under CLP, the 72-hour rather than 96-hour values will be used for hazard classification.

Study 3 (Kern, M.E. and Lam, C.V., 2006a)

In a 96-hour toxicity study, the blue-green algae *Anabaena flos-aquae* was exposed under static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 201 (1984, 2004 draft), FIFRA Guideline 123-2 (1982) and OPPTS Guideline 850.5400 (1996 draft). Algae were exposed to six nominal test concentrations of 0.31, 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L, corresponding to mean measured concentrations of 0.33, 0.63, 1.25, 2.70, 5.49 and 11.2 mg a.s./L. There was also a dilution water control (no solvent vehicle was required). Mean measured concentrations were 93 to 117 % of nominals, which is within 80 to 120 %, however the biological results were based on mean measured concentrations.

There were three replicates per test concentration and control with an initial cell density of 1 x 10^4 cells/mL. The study was conducted using a photoperiod of 24 hours light and a light intensity of 1959 to 2207 lux. Temperature was 23.3 to 24.3 °C. The pH range was 7.3 to 8.6. Cell density was determined daily. Observation parameters were growth rate (NOEC and EC_{50}) at 72 hours and standing crop, cumulative biomass and growth rate (NOEC and EC_{50}) at 96 hours.

The main validity criteria relating to at least a 16-fold exponential increase in cell density over 0-72 hours and the coefficient of variation of average specific growth rates between control replicates not exceeding 7 % over 0-72 hours, were all met. The mean coefficient of variation for section-by-section specific growth rates in the control should also not exceed 35 % and in this study it was 21 %.

For *Anabaena flos-aquae* the 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be 9.15 mg thiencarbazone-methyl/L, with a 72-hour NOE_rC value of 2.70 mg thiencarbazone-methyl/L.

The 96-hour E_rC_{50} was 8.92 mg/L with a 96-hour NOE_rC value of 0.63 mg/L. All endpoints were based on mean measured concentrations. In line with normal practice under CLP, the 72-hour rather than 96-hour values will be used for hazard classification.

Study 4 (Christ, M. T. and Lam, C.V., 2006)

In a 96-hour toxicity study, the saltwater diatom *Skeletonema costatum* was exposed under static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 201 (1984, 2004 draft), FIFRA Guideline 123-2 (1982) and OPPTS Guideline 850.5400 (1996 draft). Diatom cells were exposed to six nominal test concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./L, corresponding to mean measured concentrations of 3.47, 6.94, 14, 28, 57 and 114 mg a.s./L. There was also a dilution water control (no solvent vehicle was required). Mean measured concentrations were 110 to 114 % of nominals, which is within 80 to 120 %, however the biological results were based on mean measured concentrations.

There were three replicates per test concentration and control with an initial cell density of 1 x 10^4 cells/mL. The study was conducted using a photoperiod of 24 hours light and a light intensity of 3875 to 4715 lux. Temperature was 19.5 to 20.3 °C. The pH range was 7.3 to 8.6. Cell density was determined daily. Observation parameters were growth rate and cumulative biomass (NOEC and EC_{50}) at 72 and 96 hours.

The main validity criteria relating to at least a 16-fold exponential increase in cell density over 0-72 hours, a mean coefficient of variation for section-by-section specific growth rates in the control not exceeding 35 % and the coefficient of variation of average specific growth rates between control replicates not exceeding 7 % over 0-72 hours, were all met.

For *Skeletonema costatum* the 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be >114 mg thiencarbazone-methyl/L, with a 72-hour NOE_rC value of 114 mg thiencarbazone-methyl/L (the highest concentration tested). The 96-hour E_rC_{50} was also >114 mg /L with a 96-hour NOE_rC value also of 114 mg/L. All endpoints were based on mean measured concentrations. In line with normal practice under CLP, the 72-hour rather than 96-hour values will be used for hazard classification (although the same in this case).

5.4.3.2 Toxicity to aquatic plants

Study 1 (Kern, M.E. and Lam, C.V., 2006b)

In a 7-day toxicity study, the aquatic macrophyte *Lemna gibba* G3 (duckweed) was exposed under semi-static conditions to technical thiencarbazone-methyl (96.5 % pure). The study was conducted to GLP and in accordance (no major deviations) with OECD Guideline 221 (2004 draft), FIFRA Guideline 123-2 and OPPTS Guideline 850.4400 (1996 draft). Duckweed plants were exposed to six nominal test concentrations of 0.082, 0.205, 0.512, 1.28, 3.20 and 8.00 µg a.s./L, corresponding to mean measured concentrations of 0.086, 0.209, 0.542, 1.26, 3.06 and 7.70 µg a.s./L. There was also a dilution water control (no solvent vehicle was required). Mean measured concentrations were 96 to 106 % of nominals, which is within 80 to 120 %, however the biological results were based on mean measured concentrations.

There were three replicates per test concentration and control. The study was conducted using a photoperiod of 24 hours light and a light intensity of 4919 to 5630 lux. Temperature was 24.2 to

24.9 °C. The pH range was 7.5 to 8.7. Growth was determined by frond counts on days 0, 3, 5 and 7 and frond dry weights from day 0 and day 7. At test initiation, 3 replicates of 3 plants and 12 fronds (representing the control fronds) were dried and then weighed to determine the growth rate based on dry weight at study termination. On day 7, after the frond count was completed, the plants from each replicate were dried and weighed to determine the dry weight. The biological parameters measured at day 0, 3, 5 and 7 during the test were assessed visually or on balance:

- Fronds: frond counts, growth rate, cumulative biomass (NOEC and EC_{50})
- Biomass: dry weights and growth rate for dry weights (NOEC and EC_{50})

The doubling time of frond number in the control group during the 7 day test was 1.67 days or approximately 40 hours which meets the acceptability criteria of 2.5 days in the OECD guideline (2004).

For *Lemna gibba* the 7-day mean measured EC_{50} value for growth rate (E_rC_{50}), based on frond number, was determined to be 1.31 µg thiencarbazone-methyl/L (0.00131 mg a.s./L), with a 7-day mean measured NOE_rC of 0.21 µg thiencarbazone-methyl/L (0.00021 mg a.s./L) also based on frond number. Plant endpoints other than growth rate are not normally used for hazard classification under CLP so these have not been included.

Additional aquatic plant studies

Three other aquatic macrophyte studies are included in the thiencarbazone-methyl DAR, these are non-standard 'higher tier' recovery studies but they will each be considered for their reliability and relevance for hazard classification. They were based on the *Lemna* test guideline, OECD 221, conducted to GLP and included exposure and then recovery phases in clean water or untreated growth media. Summaries are included below:

Study 2 (Christ, M.T. and Lam, C.V., 2007a)

In this GLP recovery study on *Lemna gibba* the objective was to determine the recovery potential of exponentially growing *Lemna*, following a 7-day static exposure period to thiencarbazone-methyl (96.5 % pure).

The exposure phase consisted of three replicates of aquatic plants exposed to nominal concentrations of 0 (control), 1.0, 2.2, 4.8, 10.6 and 22.4 µg a.s./L in growth media for a 7-day period. All treatments had 12 fronds per triplicate. The recovery phase consisted of two 7-day intervals. In the first recovery phase, 12 fronds were transferred from each exposure vessel into freshly prepared growth media. In the second recovery phase, 12 fronds were transferred from the control and 2.2 µg/L treatment level from the first recovery phase. Test vessels were placed in an environmental chamber under continuous light at 6674 to 8159 lux with a test temperature of 25 ±2°C over the 21-day study period.

The Day 0 (exposure) measured concentrations were 0.90, 2.09, 4.38, 9.83 and 21.1 µg/L, representing 90 to 95% of nominal. The Day 7 (exposure) measured concentrations for the corresponding test concentrations were 0.66, 1.53, 3,19, 7.04 and 13.9 µg/L, representing 62 to 70% of nominal. Despite Day-7 levels dropping below 80% of nominal, all reported toxicity values were based on the nominal concentrations of thiencarbazone-methyl rather than mean measured. The control frond growth corresponded to a doubling factor of 2.1 days which met the OECD acceptability criteria of <2.5 days.

Only the initial exposure phase endpoints are potentially relevant for hazard classification; however these were not specifically determined. It is reported that at the nominal 1.0 and 2.2µg/L treatments the corresponding percent growth inhibition values relative to the control were 34% and 79%, respectively, so the 7-day E_rC_{50} would be between these concentrations, however no regression analysis was done. The study report states that the percent inhibition values correspond to the expected dose:response effects and this result would be broadly consistent with that from Study 1 above on *Lemna gibba* (Kern, M.E. and Lam, C.V., 2006b). An overall study nominal NOEC of 1.0 µg/L was reported but this was based only on visual phytotoxic effects on *Lemna*.

Since it is not clear whether accurate mean measured growth rate E_rC_{50} and NOE_rC values can be determined separately for the 7-day exposure period, this additional *Lemna* recovery study is not relied on for classification purposes.

Study 3 (Christ, M.T. and Lam, C.V., 2007b - and recalculation by Bruns & Solga, 2013)

In this recovery study on *Myriophyllum spicatum* the objective was to determine the dose-response effect of thiencarbazone-methyl (96.5 % pure) on the species over a 14-day static exposure and then 14-day recovery period in clean water. The EC_{25} and EC_{50} for the most sensitive endpoint in the exposure phase were determined through measurements of plant growth - as plant length, dry weight or sectional specific growth rate (rate of change in growth with time) relative to the controls. The recovery phase only analysed for plant length growth rate. The NOEC was determined based on growth rate and on visual phytotoxic symptoms. Since measurements were only taken at the beginning and end of each period, 7-day endpoints are not determinable.

The test system consisted of four replicate aquaria per treatment. Each replicate contained 4 plants per control and treatment. All plants were grown in individual beakers filled with an artificial sediment. The rooted aquatic plants were submerged in the aquaria and exposed to nominal water concentrations of 0 (control), 0.13, 0.32, 0.8, 2.0 and 5.0 µg a.s/L for an initial 14-day exposure period. The test solutions were not renewed as it was stated that the test compound was stable in the test system (although subsequent analysis did not support this view). The recovery phase exposed the remaining plants to the identical test system with the exception that all test solutions were replaced by clean test water at start of the 14-day interval and this was renewed once after 7 days. All test vessels were maintained under artificial lighting with a photoperiod of 16 hours light: 8 hours dark at 9050 to 12970 lux. The test temperature was 23 °C ± 3°C over the 28-day study duration, with a mean water pH of 8.4.

The Day 0 (exposure) initial measured concentrations were 0.20, 0.45, 0.91, 2.4 and 5.7 µg/L which ranged from 114 to 154% of nominal. The Day 6 and Day 7 measured concentrations ranged from 94 to 100% of nominal; the Day 11 measured concentrations ranged from 84% to 115% of nominal and the Day 14 measured concentrations ranged from 61% to 85% of nominal. Due to the adsorption/degradation of the test material in the test system, the reported toxicity values were originally based on the Day 0 measured water concentrations only.

The 14-Day E_rC_{50} (specific growth rate as plant length) was originally calculated to be 1.2 µg thiencarbazone-methyl/L (0.0012 mg a.s./L) during the initial exposure phase. The 14-Day NOE_rC based on plant length growth rate was determined to be 0.45 µg thiencarbazone-methyl/L (0.00045 mg a.s./L). Endpoints were based on initial measured test concentrations not mean measured. This study was conducted prior to validation of a specific *Myriophyllum* guideline (e.g. OECD TGs 239/238 with/without sediment) and it is not clear how well the validity criteria (established for *Lemna*) were met in this study - although it appeared well conducted, according to GLP. Given a

lack of standard guideline, the inclusion of sediment and variable water phase exposure concentrations, there is uncertainty regarding the relevance of the original results for classification purposes.

However, during the registration process of thiencarbazone-methyl as a new pesticidal active substance, the Applicant recalculated the shoot length endpoint for *Myriophyllum spicatum* (14-day exposure phase) based on mean measured concentrations and considering varying start lengths of the shoots (Bruns & Solga, 2013). The mean measured concentrations of thiencarbazone-methyl in the water phase over 14 days were determined to be 0.15, 0.31, 0.67, 1.85 and 4.4 µg/L. This re-inspection of the Christ and Lam, 2007b study report revealed that the original endpoints had been calculated from data on final shoot lengths after 14 days of exposure without considering varying start lengths of the shoots and duration of the exposure period, respectively. For this reason, the originally reported endpoints cannot be regarded as 'growth rate' (i.e. E_rC_x) endpoints.

The subsequent recalculation resulted in revised growth rate endpoints based on mean measured concentrations in the water phase during the exposure phase. The revised 14-day E_rC_{50} was determined to be 0.94 µg a.s./L (0.00094 mg a.s./L). This endpoint was accepted and included in the EU agreed List of Endpoints for thiencarbazone-methyl (cf. EFSA Journal 2013;11(7):3270). A NOE_rC was not recalculated but the 14-day E_rC_{25} was determined to be 0.5 µg a.s./L. A visual re-inspection of the data in Bruns & Solga, 2013 reveals that statistically significant growth effects were seen at a mean measured 0.67 µg a.s./L and above, so the revised 14-day NOE_rC would be 0.31 µg a.s./L (0.00031 mg a.s./L) - which equates with the initial measured NOE_rC of 0.45 µg a.s./L originally reported in Christ and Lam, 2007b.

Although there are concerns over the lack of an agreed protocol at the time of the original study and the inclusion of sediment, the revised endpoint calculations for *Myriophyllum* are based on mean measured concentrations in the water phase as well as on growth rate - and so the eMSCA considers them to be reliable and potentially relevant for hazard classification.

Study 4 (Hoberg, J.R., 2007)

In a comparative toxicity study, three aquatic macrophytes, Elodea (*Elodea canadensis*), Sago or Fennel Pondweed (*Potamogeton pectinatus*) and Water Mint (*Mentha aquatica*) were exposed to thiencarbazone-methyl (95.7 % pure) for 14 days and observed for their ability to recover from any adverse effects over a subsequent 14-day recovery period in clean water.

For each species 4 replicates, each containing 12 plants were established for each treatment level and the control. All plants were grown in individual pots containing artificial sediment. Nominal test concentrations were: control, 0.33, 1.1, 3.7, 12 and 41 µg a.s./L for *Elodea canadensis* and for *Mentha aquatica*; control, 0.10, 0.33, 1.1, 3.7 and 12 µg a.s./L for *Potamogeton pectinatus*. During the exposure phase the mean measured concentrations in the water phase were <0.054 (control), 0.23, 0.82, 2.7, 9.7 and 31 µg a.s./L for *Elodea canadensis*; <0.022 (control), 0.20, 0.56, 2.0, 5.6 and 19 µg a.s./L for *Mentha aquatica* and <0.016 (control), 0.075, 0.26, 0.95, 3.1 and 10 µg a.s./L for *Potamogeton pectinatus.*

The test solutions were not renewed. The recovery phase exposed the remaining plants to the identical test system with the exception that all test solutions were replaced by clean test water at the start of the 14-day interval and renewed once after 3 days. Environmental conditions were:
Water temperature: 18 - 27°C for Elodea and Sago Pondweed; 13 - 28°C for Water Mint.
Photoperiod: 16 hours light/8 hours dark; light intensity: 4100 to 27,700 lux (381 to 2570 footcandles) for Elodea and Sago Pondweed, 5400 to 21,000 lux (500 to 1950 footcandles) for Water Mint. pH: Elodea: 7.9 - 8.5; Sago Pondweed: 7.9 – 8.4;Water Mint: 7.9 – 8.2.

Shoot length, growth rate based on shoot length, shoot dry weight and growth rate based on shoot dry weight, No-Observed-Effect Concentration (NOEC), Lowest-Observed-Effect Concentration (LOEC) and EC_{25} and EC_{50} values following 14 days of exposure and NOEC and LOEC values for the 14-day recovery phase were determined. Endpoints after 7-days were not calculated.

Minimal growth was attained over the 14-day exposure phase for Elodea and Water Mint, consequently any potential effects of thiencarbazone-methyl on these two species were difficult to determine. A tentative water phase EC_{50} for these species was empirically estimated to be >10 µg a.s./L (>0.01 mg/L). However, a better concentration-response was observed with Sago Pondweed (*Potamogeton pectinatus*) shoot length and growth rate during the 14-day exposure phase, providing a mean measured EC_{50} value of 5.3 µg thiencarbazone-methyl/L (0.0053 mg/L) for shoot length growth rate. The shoot length growth rate data are shown in the table and figure below:

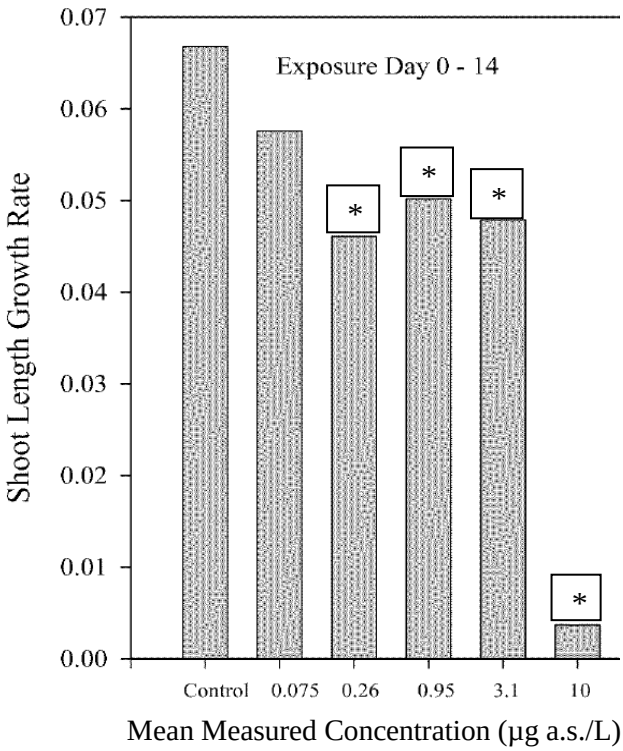
Table 45: Growth rates (based on shoot length) of sago pond weed (*Potamogeton pectinatus*) plants exposed to thiencarbazone-methyl during the 14-day exposure period.

Mean measured concentration (µg a.s./L)	Mean Day 0-7 growth rate based on shoot length (SD)	Day 7 percent Reduction	Mean Day 0-14 growth rate based on shoot length (SD)	Day 14 percent Reduction
Control	0.1025 (0.0044)	NA	0.0668 (0.0053)	NA
0.075	0.0947 (0.0313)	7.6	0.0576 (0.0054)	14
0.26	0.0640 (0.0036)	38	0.0461 (0.0069)*	31*
0.95	0.0733 (0.0167)	28	0.0502 (0.0072)*	25*
3.1	0.0651 (0.0140)	36	0.0479 (0.0059)*	28*
10	-0.0121 (0.0201)	120	0.0037 (0.0096)*	94*

NA = Not Applicable

* Significantly reduced compared to the control based on Dunnett's Test ($p < 0.05$)

Figure 4 Growth rates (based on shoot length) for sago pond weed (*Potamogeton pectinatus*) plants during the 14-day exposure period to thiencarbazone-methyl



* Significantly reduced compared to the control based on Dunnett's Test ($p < 0.05$)

The NOEC value for shoot length growth rate from the exposure phase was determined to be a mean measured 0.075 µg a.s./L (0.000075 mg/L). An E_rC_{25} was also determined for *Potamogeton* since the concentration-response at 0.075 to 3.1 µg a.s./L was fairly flat and a 25 % reduction in plant growth was considered by the study authors not to be ecologically relevant; this was a mean measured 0.81 µg/L (0.00081 mg/L). Recovery NOECs were also determined for each species but these are not considered relevant for classification.

There are some concerns over reliance on this study for hazard classification since it was not conducted to a standard guideline or established validity criteria for these species. Plants were also cultured potted in artificial sediment. However, the study appeared well conducted and was in accordance with GLP. All endpoints were based on mean measured concentrations in the water phase during the initial exposure period and a clear concentration-response was seen for Sago Pondweed, *Potamogeton pectinatus*. Its EC_{50} endpoints were also included in the EFSA peer review Conclusion and agreed List of Endpoints for thiencarbazone-methyl (algal/plant NOECs are not used for pesticide risk assessment). The endpoints for this species are therefore considered reliable and potentially relevant for classification purposes.

5.4.4 Other aquatic organisms (including sediment)

Some of the higher aquatic plant studies were conducted using sediment, however these are evaluated above. A study was submitted on the midge *Chironomus riparius*, however this was an acute study on first instar larvae in the water phase only and is evaluated in Section 5.4.2.1.

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

Abiotic and biotic degradation

Thiencarbazone-methyl is only slowly degraded via hydrolysis and photolysis in aquatic systems. The substance showed 0 % degradation after 28 days in an OECD 301 F ready biodegradation test and it is considered to be 'not readily biodegradable'.

In whole aerobic natural water/sediment systems, thiencarbazone-methyl rapidly but partially partitioned to the sediment where it degraded. However, the whole system half-life (primary degradation) was 21.9 to 31.3 days and mineralization accounted for only 7.6-13.4 % AR at the end of the study.

Temperature correction of degradation half-lives to 12˚C was not conducted. However, in studies at 20-25˚C it is already clear that thiencarbazone-methyl would not be degraded in whole aquatic systems such that a degradation half-life <16 days (corresponding to >70 % degradation within 28 days) would be achieved. Overall, the degradation data do not provide sufficient information to show that thiencarbazone-methyl is ultimately degraded (mineralised) within 28 days or undergoes primary degradation to non-classifiable degradants with half-lives <16 days. Consequently, thiencarbazone-methyl is considered 'not rapidly degradable' for the purpose of hazard classification under CLP.

Bioconcentration

The Log K_{ow} for thiencarbazone-methyl is -1.98 at pH 7, this (and values at other pH) is less than the trigger value of 4 given in the CLP Regulation. No experimental fish BCF study is available. Overall, a low bioaccumulation potential is predicted for thiencarbazone-methyl.

Aquatic toxicity

As well as information on the parent substance, toxicity data on algae and aquatic plants are also available on the main degradants of thiencarbazone-methyl (see Annex 1, Tables 2 & 3) which indicate they are less toxic than the parent substance. Therefore degradants are not considered further in relation to the classification of thiencarbazone-methyl.

Acute aquatic hazard:

Reliable acute toxicity data are available on thiencarbazone-methyl for fish, invertebrates, algae and aquatic plants (see summary Table 40). Fish and invertebrates showed low sensitivity with acute L/EC_{50} values around or greater than 100 mg/L. As expected for this herbicide, algae and aquatic plants are the most acutely sensitive groups. The most sensitive algal/diatom species tested was *Pseudokirchneriella subcapitata* with a 72-hour mean measured E_rC_{50} of 1.017 mg/L. However, a study on the aquatic macrophyte *Lemna gibba* gave a lower 7-day mean measured E_rC_{50} of 0.00131 mg/L.

Non-standard recovery studies (14-day exposure phase and subsequent recovery phase in clean media) are also available on other aquatic macrophytes including reliable endpoints for *Myriophyllum spicatum* and *Potamogeton pectinatus.* For *Myriophyllum* a 14-day mean measured E_rC_{50} was calculated (following revision) to be 0.00094 mg thiencarbazone-methyl/L during the exposure phase. For *Potamogeton* a 14-day mean measured E_rC_{50} during the exposure phase was calculated to be 0.0053 mg thiencarbazone-methyl/L. Whilst these additional plant studies were not conducted to a standard guideline specific for these species and they also used artificial sediment,

they were otherwise well conducted to GLP and considered reliable. All endpoints were based on mean measured concentrations in the water phase during the initial exposure period only and concentration-responses were seen.

It is noted for pesticide risk assessment that a geometric mean EC_{50} of 0.00135 mg/L was calculated for all reliably tested macrophytes, however this included endpoints other than growth rate (like yield or biomass) which are not normally used for classification. Section 4.1.3.2.4.3 of ECHA's Guidance on the Application of the CLP Criteria (2012), suggests that a geomean may be used for classification where four or more acceptable endpoints are available for the same species and it is not advised to combine tests from different species within a taxonomic group. In the case of thiencarbazone-methyl there are only three plant endpoints on different species and over different timescales (7 and 14 days), so a geomean will not be used for classification.

If the standard 7-day mean measured E_rC_{50} of 0.00131 mg/L for *Lemna gibba* is relied on, then this is in the acute classification range >0.001 to ≤0.01 mg/L and thiencarbazone-methyl would be classified as: Aquatic Acute 1: H400 with an Acute M-factor of 100.

The lowest acute E_rC_{50}, however, is 0.00094 mg/L for *Myriophyllum spicatum*. Although it was not conducted to a standard guideline, 14 days is now the typical duration for studies on slower growing *Myriophyllum*. The study was also static and included sediment but the E_rC_{50} was based on mean measured concentrations in the water phase, and so the eMSCA considers it potentially suitable for hazard classification. This *Myriophyllum* E_rC_{50} is in the range >0.0001 to ≤0.001 mg/L and therefore, using this endpoint, thiencarbazone-methyl would be classified as:
Aquatic Acute 1: H400 with an Acute M-factor of 1000.

Chronic aquatic hazard:

Reliable chronic toxicity data are available on thiencarbazone-methyl for fish, invertebrates, algae and aquatic plants (see summary Table 40). The chronic study on fish was a 35-day ELS study on fathead minnow (*Pimephales promelas*), for invertebrates chronic 21- and 28-day studies are available respectively on *Daphnia magna* and *Americamysis bahia*. Thiencarbazone-methyl again showed low toxicity to fish and invertebrates with chronic NOEC values all greater than 1 mg/L. Algae and aquatic plants were the most chronically sensitive groups. The most sensitive algal/diatom species tested was *Pseudokirchneriella subcapitata* with a 72-hour mean measured NOE_rC of 0.0307 mg/L. However, a study on the aquatic macrophyte *Lemna gibba* gave a lower 7-day mean measured NOE_rC of 0.00021 mg/L. If this standard 7-day mean measured NOE_rC for *Lemna* is relied on, then this is within the range >0.0001 to ≤0.001 mg/L and thiencarbazone-methyl would be classified as: Aquatic Chronic 1: H410 with a Chronic M-factor of 100.

Additional non-standard recovery studies on aquatic plants gave a 14-day mean measured NOE_rC during the exposure phase for *Myriophyllum spicatum* of 0.00045 mg thiencarbazone-methyl/L, which is in the same classification range as the endpoint for *Lemna gibba*. The study on *Potamogeton pectinatus* gave a 14-day NOE_rC during the exposure phase of 0.000075 mg/L, which indicates it may be more chronically sensitive than *Lemna*. This study also included sediment but again the endpoint was based on mean measured concentrations in the water phase. Like *Myriophyllum*, *Potamogeton* is slower growing than *Lemna* and so the fact that the endpoint is over 14 rather than 7 days may not be important. Significant differences in shoot length growth rate were only seen at 14 and not 7 days. It is noted however, that although these differences were statistically significant, the concentration-response around the LOEC for *Potamogeton* was quite

flat (see Fig. 4 above). An E_rC_{25} also determined for this species was 0.00081 mg/L and within the same range as the NOE_rC for *Lemna* (an E_rC_{10} was not determined). If this NOE_rC for *Potamogeton* is used for classification then it is within the range >0.00001 to ≤0.0001 mg/L and therefore, since thiencarbazone-methyl is also considered 'not rapidly degradable', it would be classified as: **Aquatic Chronic 1: H410 with a Chronic M-factor of 1000**.

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

It is proposed to consider the lowest reliable and most sensitive acute and chronic endpoints available for classification and thus propose the more conservative acute and chronic M-factors (1000 in each case). However, the views of the RAC are sought on the suitability of the additional plant studies and their endpoints for classification purposes.

Aquatic Acute category 1; H400: Very toxic to aquatic life

Acute M-factor = 1000

Aquatic Chronic category 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 1000

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Thiencarbazone-methyl (BYH 18636) is a herbicide which does not have an existing entry in Annex VI of CLP. The Dossier Submitter (DS) concluded that the substance was not rapidly degradable and had no potential to bioaccumulate. The DS proposed to classify the substance as Aquatic Acute 1: H400, based on a 14-day E_rC_{50} of 0.00094 mg/L for the aquatic macrophyte *Myriophyllum spicatum*. This value is in the range > 0.0001 to ≤ 0.001 mg/L giving an acute M-factor of 1 000. The proposed chronic classification was Aquatic Chronic 1: H410, based on a 14-day mean measured NOE_rC of 0.000075 mg/L for the aquatic macrophyte *Potamogeton pectinatus*. This value was in the range > 0.00001 to ≤ 0.0001 mg/L giving a chronic M-factor of 1 000 for a non-rapidly degradable substance.

Degradation

Aqueous hydrolysis was investigated in a study conducted according to GLP and in accordance with OECD TG 111. Thiencarbazone-methyl was hydrolytically unstable under acidic, neutral and alkaline conditions at 20 °C (only tested at pH4). At 25 °C, the half-life was 50 days at pH 4 and approximately 150 days at pH values of 7 and 9. At all pH values tested, the major degradation products were BYH 18638-MMT and BYH 18636-sulfonamide. The concentration of BYH 18636-MMT increased towards the end of

incubation at all pH values tested, whereas BYH 18636-sulfonamide was degraded further, especially under alkaline conditions. A recalculation of single first-order hydrolytic half-life according to FOCUS kinetics gave DT_{50s} of 139 and 11 days for thiencarbazone-methyl and BYH 18636-sulfonamide, respectively. The degradation products BYH 18636-sulfonamiden-carboxylic acid and BYH 18636-MMT were stable. The DT_{50} for thiencarbazone methyl was extrapolated well beyond the duration of the study (i.e. 30 days) and was therefore subject to a degree of uncertainty.

The study on direct photolysis of thiencarbazone-methyl in sterile aqueous buffer solution at pH 7 and at 25 °C was conducted in accordance with GLP according to SETAC-Europe guideline: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, March 1995, Section 10 (Aqueous Photolysis) - as well as US EPA: Subdivision N, Section 162-1 and a similar Canadian guideline. Three photodegradation products were formed and increased during the irradiation period. BYH 18636-sulfonamide, BYH 18636-MMT and BYH 18636-triazolinone-carboxamide were formed at maximum fractions of 5.2 %, 8.3 % and 1.2 % of AR, respectively, at the end of the irradiation period. $^{14}CO_2$ accounted for a maximum of 0.1 % of the applied radioactivity at study termination, therefore mineralisation was minimal. The mean photolytic half-life in the test was 90.6 days (extrapolated). The half-life under environmental conditions was projected to be 333 solar summer days in Phoenix (Arizona, USA) and 516 solar summer days in Athens (Greece). In a direct phototransformation in water test performed according to GLP and German UBA and ECETOC guidelines, no phototransformation was reported at pH 4, 7 and 9 at 25 °C, indicating that the half-life would be > 1 year.

The ready biodegradability of thiencarbazone-methyl was determined according to GLP and following EEC Method C.4-D 'Manometric Respirometry Test'. Thiencarbazone-methyl showed 0 % degradation after 28 days, while the reference compound sodium benzoate showed 83 % degradation after 14 days. Thiencarbazone-methyl was, therefore, considered to be not readily biodegradable.

The aerobic biotransformation of thiencarbazone-methyl was studied in two dark static water/sediment systems under aerobic conditions at 20 °C. The study was conducted to GLP and in accordance with US EPA, Subdivision N, Section 162-4 and similar Canadian guidelines. The study was carried out in natural water/sediment systems from Hoenniger Weiher, Germany and Clayton, North Carolina, US. Mineralisation reached 7.6 – 13.4 % AR by study termination after 120 days. The major transformation products detected in water were the BYH 18636-sulfonamide-carboxylic acid, BYH 18636-carboxylic acid and BYH 18636-MMT in both systems. BYH 18636-dicarboxy-sulfonamide was detected only in the water of the Clayton system. In both water/sediment systems, thiencarbazone-methyl was lost from the water body via movement/dissipation into the sediment (at 14 days 50.6 % AR in water, 9.2 % AR in sediment and 58.0 % AR in water, 10.3 % AR in sediment in Hoenninger and Clayton systems, respectively; (DAR, Volume 3, Annex B.8, April 2012). It also underwent degradation to metabolites and limited total metabolism to $^{14}CO_2$ plus non-extractable residues. Thiencarbazone-methyl and its metabolites were mineralized in water/sediment systems but not quickly enough to be considered rapidly degradable. The degradation DT_{50} in both of the total water/sediment systems was a maximum 29 days. Degradation was re-evaluated in another study according to FOCUS kinetics. The simple first order model (SFO) DT_{50}s for the water/sediment whole system were 21.9 days and 31.3 days for Hoenniger and Clayton systems, respectively. The DS concludes that degradation information does not provide sufficient data to show that thiencarbazone-methyl is ultimately degraded (mineralised)

within 28 days or undergoes primary degradation to non-classifiable degradants. Consequently, thiencarbazone-methyl is considered to be 'not rapidly degradable' for the purpose of classification and labelling.

Bioaccumulation

No BCF study is available. The octanol/water partition coefficient (log K_{ow}) for thiencarbazone-methyl ranges from -0.13 at pH 4, to -1.98 at pH 7 and -2.14 at pH 9 in an OECD TG 107 study performed according to GLP. This very low log K_{ow} indicates a low potential to bioaccumulate.

Aquatic toxicity

***Table**: Relevant information on aquatic toxicity of thiencarbazone-methyl*

Test organism	Test guideline and type	Duration	LC/EC_{50} mg/L	NOEC/EC_x mg/L
Fish				
Oncorhynchus mykiss	OECD TG 203, GLP static, limit test	96 h acute	> 104[mm] no effects	104[mm]
Lepomis macrochirus	OECD TG 203, GLP static, limit test	96 h acute	> 107[mm] no effects	107[mm]
Cyprinodon variegatus	OECD TG 203, GLP static, limit test	96 h acute	> 106[mm] no effects	106[mm]
Pimephales promelas	OECD TG 210, GLP ELS flow-through	35 d chronic	-	4.8[mm] [mm] 83-120 % of nominal
Aquatic invertebrates				
Daphnia magna	OECD TG 202, GLP static, limit test	48 h acute	> 98.6[mm] no effects	98.6[mm] [mm] 80-120 % of nominal
Crassostrea virginica	OPPTS Guideline 850.1025 (draft) and FIFRA 72-3, GLP flow-through	96 h acute	> 100[mm] < 50 % reduction in shell growth	4.6[mm] estimated [mm] 62-120 % or nominal
Americamysis bahia	OPPTS Guideline 850.1035 and FIFRA 72-3, GLP flow-through	96 h acute	> 94[mm] no effects at the highest tested concentration	94[mm] [mm] 80-100 % of nominal
Chironomous riparius	OECD TG 202,	48 h acute	> 100[nom]	100[nom]

	GLP static, limit test -aqueous phase, no sediment		no effects	
Daphnia magna	OECD TG 211, GLP semi-static	21 d chronic	-	3.54[mm] [mm] 109-113 % of nominal
Americamysis bahia	U.S. EPA FIFRA Guideline 72-4 (1982), GLP flow-through	28 d chronic	-	5.9[mm] [mm] 100-120 % or nominal
Algae				
Pseudokirchneriella subcapitata	OECD TG 201, GLP semi-static	96 h acute and chronic	72 h E_rC_{50} = 1.017[mm]	72 h NOE_rC = 0.0307[mm] [mm] 97-102 % of nominal
Navicula pelliculosa	OECD TG 201, GLP semi-static	96 h acute and chronic	72 h E_rC_{50} = 64.0[mm]	72 h NOE_rC = 51.6[mm] [mm] 96-103 % of nominal
Anabaena flos-aquae	OECD TG 201, GLP semi-static	96 h acute and chronic	72 h E_rC_{50} = 9.15[mm]	72 h NOE_rC = 2.7[mm] [mm] 93-117 % of nominal
Skeletonema costatum	OECD TG 201, GLP semi-static	96 h acute and chronic	72 h E_rC_{50} > 114[mm]	72 h NOE_rC = 114[mm] [mm] 110-114 % of nominal
Aquatic macrophytes				
Lemna gibba G3	OECD TG 221, GLP semi-static	7 d acute and chronic	7 d E_rC_{50} = 0.00131[mm] frond number	7 d NOE_rC = 0.00021[mm] frond number [mm] 96-106 % of nominal
Myriophyllum spicatum	Non-guideline (based on OECD TG 221), partly GLP static recalculated results	14 d acute and chronic	**14 d E_rC_{50} = 0.00094[mm]**	14 d NOE_rC = 0.00031[mm] [mm] 84-115 % of nominal
Potamogeton pectinatus	Non-guideline (based on OECD TG 221), GLP static	14 d acute and chronic	14 d E_rC_{50} = 0.0053[mm]	**14 d NOE_rC = 0.000075**[mm] [mm] 75-87 % of nominal

[mm] mean measured concentrations

Values leading to the acute and chronic classification in **bold.**

Acute aquatic toxicity

There were three reliable acute toxicity studies in fish available. They were run as limit tests with one nominal treatment level of 100 mg of thiencarbazone-methyl/L. No treatment-related effects were seen in any of the tests. The lowest 96 h mean measured LC_{50} was < 104 mg/L.

There was data from four aquatic invertebrate studies. Studies with *Daphnia magna* and *Chironomus riparius* were limit tests with one nominal treatment level of 100 mg of thiencarbazone-methyl/L. No treatment-related effects were seen. In the study with eastern oysters (*Crassostrea virginica*), reduction of shell deposition was used as the indicator of toxicity. Oysters were exposed to five nominal test concentrations of 2.6, 6.4, 16, 40 and 100 mg thiencarbazone-methyl/L. Mean measured concentrations were from 62 to 120 % of nominals. No mortality or abnormalities were observed at any of the treatment levels tested. After 96 hours exposure, the 1.6, 12, 49, and 100 mg a.s./L concentrations (measured) resulted in reduced shell deposition of 2, 11, 9, and 12 %, respectively. At the 4.6 mg a.s./L test concentration there was no percent reduction relative to the control but oysters in this group exhibited a positive response compared to the control. Since no concentration tested resulted in ≥ 50 % reduction in shell growth, the 96-hour EC_{50} value was empirically estimated to be > 100 mg a.s./L, the highest mean measured concentration tested. In a study with mysid shrimp (*Americamysis bahia*), mysids were exposed to five nominal test concentrations of 13, 22, 36, 60 and 100 mg a.s./L, corresponding to mean measured concentrations of 10, 23, 34, 56 and 94 mg a.s./L. No treatment-related effects were seen in any of the test replicates through the course of the study. Therefore, the 96-hour mean measured EC_{50} for thiencarbazone-methyl to *Americamysis bahia* was determined to be > 94.0 mg/L, which is the lowest LC/EC_{50} value for aquatic invertebrates.

There were four studies available on algae. All of the studies were 96-hour studies but in line with previous cases, the 72-hour data was used for hazard classification. In a 96-hour study, *Pseudokirchneriella subcapitata* was exposed to six nominal test concentrations of 31, 63, 125, 250, 500 and 1 000 µg a.s./L, corresponding to mean measured concentrations of 30.7, 61.1, 125, 251, 506 and 1 024 µg a.s./L. Observation parameters were growth rate at 72 hours and standing crop, cumulative biomass and growth rate at 96 hours. The mean measured 72-hour E_rC_{50} value was determined to be 1.017 mg thiencarbazone-methyl/L. The freshwater diatom *Navicula pelliculosa* was exposed to thiencarbazone-methyl in a 96-hour study. Diatom cells were exposed to six nominal test concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg/L, corresponding to mean measured concentrations of 3.11, 6.16, 12.1, 24.1, 51.6 and 101 mg/L. There were problems with the pH value at higher test concentrations. Ending pH values on day four for the 50 and 100 mg/L solutions were 8.6 and 4.2, respectively. Endpoints at 72-hours would have been less affected and pH did not appear to be a growth-limiting factor. This was not considered to have substantially affected the results. Observation parameters were growth rate at 72 hours and standing crop, cumulative biomass and growth rate at 96 hours. The 72-hour mean measured E_rC_{50} value was determined to be 64.0 mg thiencarbazone-methyl/L.

In a 96-hour toxicity study, the blue-green algae *Anabaena flos-aquae* was exposed to thiencarbazone-methyl. Algae were exposed to six nominal test concentrations of 0.31, 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L, corresponding to mean measured concentrations

of 0.33, 0.63, 1.25, 2.70, 5.49 and 11.2 mg a.s./L. Observation parameters were growth rate at 72 hours and standing crop, cumulative biomass and growth rate at 96 hours. The 72-hour E_rC_{50} value was determined to be 9.15 mg thiencarbazone-methyl/L. *Skeletonema costatum* was exposed to thiencarbazone-methyl. Diatom cells were exposed to six nominal test concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./L, corresponding to mean measured concentrations of 3.47, 6.94, 14, 28, 57 and 114 mg a.s./L. The 96 hour percent growth inhibition for cell density for the 3.5, 6.9, 14, 28, 57 and 114 mg a.s./L treatments were -1, 4, 21, 7, 0 and 13 %, respectively (DAR, Volume 3, Annex B.9, April 2012). For *Skeletonema costatum*, the 72-hour mean measured E_rC_{50} value was determined to be > 114 mg thiencarbazone-methyl/L (the highest concentration tested). The lowest acute toxicity value for algae was an E_rC_{50} of 1.017 mg/L for *Pseudokirchneriella subcapitata*.

In a 7-day toxicity study, the aquatic macrophyte *Lemna gibba* G3 was exposed to thiencarbazone-methyl. Duckweed plants were exposed to six nominal test concentrations of 0.082, 0.205, 0.512, 1.28, 3.20 and 8.00 µg a.s./L, corresponding to mean measured concentrations of 0.086, 0.209, 0.542, 1.26, 3.06 and 7.70 µg a.s./L. Growth was determined by frond counts on days 0, 3, 5 and 7 and frond dry weights from day 0 and day 7. The biological parameters measured at day 0, 3, 5 and 7 during the test were assessed visually or on balance. For fronds frond counts, growth rate and cumulative biomass were observed. For *Lemna gibba* the 7-day mean measured E_rC_{50} value based on frond number, was determined to be 0.00131 mg thiencarbazone-methyl/L.

In a recovery study on *Myriophyllum spicatum*, the objective was to determine the dose-response effect of thiencarbazone-methyl on the species over a 14-day static exposure and then a 14-day recovery period in clean water. The EC_{25} and EC_{50} for the most sensitive endpoint in the exposure phase were determined through measurements of plant growth. Since measurements were only taken at the beginning and end of each period, 7-day endpoints are not determinable. The test system consisted of four replicate aquaria per treatment. Each replicate contained 4 plants per control and treatment. All plants were grown in individual beakers containing artificial sediment. The rooted aquatic plants were submerged in the aquaria and exposed to nominal water concentrations of 0 (control), 0.13, 0.32, 0.8, 2.0 and 5.0 µg a.s/L for an initial 14-day exposure period. The test solutions were not renewed as it was stated that the test compound was stable in the test system (although subsequent analysis did not support this view). This study was conducted prior to validation of a specific *Myriophyllum* guideline (e.g. OECD TGs 239/238 with/without sediment) and it was not clear how well the validity criteria (established for *Lemna*) were met. Given a lack of standard guideline, the inclusion of sediment and variable water phase exposure concentrations, there was uncertainty regarding the relevance of the original results for classification purposes. However, during the registration process of thiencarbazone-methyl as a new pesticidal active substance, the applicant recalculated the shoot length endpoint for *Myriophyllum spicatum* (14-day exposure phase) based on mean measured concentrations and considering varying start lengths of the shoots. The mean measured concentrations of thiencarbazone-methyl in the water phase over 14 days were determined to be 0.15, 0.31, 0.67, 1.85 and 4.4 µg/L. This re-inspection of the original study report revealed that the original endpoints had been calculated from data on final shoot lengths after 14 days of exposure without considering varying start lengths of the shoots and duration of the exposure period, respectively. For this reason, the originally reported endpoints could

not be regarded as 'growth rate' endpoints. The subsequent recalculation resulted in revised growth rate endpoints based on mean measured concentrations in the water phase during the exposure phase. The revised 14-day E_rC_{50} was determined to be 0.00094 mg/L. This endpoint was accepted and included in the EU agreed List of Endpoints for thiencarbazone-methyl (cf. EFSA Journal 2013;11(7):3270). Although there were concerns over the lack of an agreed protocol at the time of the original study and the inclusion of sediment, the revised endpoint calculations for *Myriophyllum* were based on mean measured concentrations in the water phase as well as on growth rate - and so the eMSCA considered them to be reliable and potentially relevant for hazard classification.

There was also a non guideline comparative toxicity study available. Three aquatic macrophytes, Elodea (*Elodea canadensis*), Sago or Fennel Pondweed (*Potamogeton pectinatus*) and Water Mint (*Mentha aquatica*) were exposed to thiencarbazone-methyl for 14 days. Minimal growth was attained for Elodea and Water Mint, consequently any potential effects of thiencarbazone-methyl on these two species were difficult to determine. However, a better concentration-response was observed with Sago Pondweed (*Potamogeton pectinatus*) shoot length and growth rate and these results were considered in connection to hazard classification. In the study, 4 replicates (each containing 12 plants) were established for each treatment level and the control. All plants were grown in individual pots containing artificial sediment. Nominal test concentrations were: control, 0.10, 0.33, 1.1, 3.7 and 12 µg/L. During the exposure phase the mean measured concentrations in the water phase were <0.016 (control), 0.075, 0.26, 0.95, 3.1 and 10 µg a.s./L for *Potamogeton pectinatus.* The test solutions were not renewed. Water temperature was 18 – 27 °C, pH from 7.9 to 8.4, photoperiod used was 16 hours light/8 hours dark and the light intensity ranged from 4 100 to 27 700 lux (381 to 2 570 footcandles). Shoot length, growth rate based on shoot length, shoot dry weight and growth rate based on shoot dry weight were determined. Endpoints after 7-days were not calculated. The mean measured 14-day E_rC_{50} value for shoot length growth rate was 0.0053 mg/L of thiencarbazone-methyl. According to the DS, there are some concerns over reliance on this study for hazard classification since it was not conducted to a standard guideline or established validity criteria for these species. Plants were also cultured potted in artificial sediment. However, the study appeared well conducted and was in accordance with GLP. All endpoints were based on mean measured concentrations in the water phase during the initial exposure period and a clear concentration-response was seen for *Potamogeton pectinatus*. Its EC_{50} endpoints were also included in the EFSA peer review conclusion and agreed List of Endpoints for thiencarbazone-methyl (algal/plant NOECs are not used for pesticide risk assessment). The endpoints for this species were therefore considered reliable and relevant for classification purposes.

Chronic toxicity

There was one reliable chronic toxicity test on fish available. In the 35-day early life stage toxicity study (FELS), fathead minnow (*Pimephales promelas*) were exposed under flow-through conditions to thiencarbazone-methyl. For most of the parameters tested, no treatment-related effects were noted. For fry survival, the result from the 10.8 mg a.s./L treatment group was reported as a statistically significant effect. Consequently, the 35-day exposure to thiencarbazone-methyl resulted in a mean measured NOEC of 4.80 mg a.s./L based on fry survival.

There were two reliable chronic toxicity tests available for invertebrates. *Daphnia magna*

(neonates, < 24 hours old) were exposed to nominal thiencarbazone-methyl concentrations (mean measured in brackets) concentrations of control (< 0.29), 3.13 (3.54), 6.25 (6.97), 12.5 (13.7), 25.0 (27.2), 50.0 (56.6) and 100 (111.7) mg thiencarbazone-methyl/L. No apparent dose-response effects were observed for adult survival or sublethal effects even though the adult body length, dry weight, and reproduction were statistically different from the control in some of the treatment levels. The overall 21-day mean measured NOEC for thiencarbazone-methyl to *Daphnia magna* was determined to be 3.54 mg/L based on adult length and weight.

In the other study *Americamysis bahia* mysids were exposed to five test concentrations of nominal 5, 10, 20, 40 and 80 mg a.s./L (corresponding to mean measured concentrations of 5.9, 11, 21, 41 and 83 mg a.s./L). Adult survival, cumulative number of offspring produced per female per reproductive day, average total body length and average dry weight were used as the indicators of toxicity. No significant reduction in mysid survival or number of offspring per female was seen in any of the treatment levels tested compared to the control data. A significant difference was determined in average dry body weight among males exposed to 11, 21 and 83 mg/L when compared to the control. Significant effects on average total body length were also determined amongst males and females at 11 mg/L and above. Overall, based on effects on body weight and length, a chronic 28-day mean measured NOEC of 5.9 mg a.s./L was determined for *Americamysis bahia* exposed to thiencarbazone-methyl.

There were four studies available to assess chronic toxicity for algae. The chronic toxicity values for algae were taken from the same tests than the acute values and the studies are described more in detail under the heading Acute toxicity. In a *Pseudokirchneriella subcapitata* study, a 72-hour mean measured NOE_rC value of 0.0307 mg thiencarbazone-methyl/L was determined. A 72-hour mean measured NOE_rC value of 51.6 mg thiencarbazone-methyl/L was determined in the *Navicula pelliculosa* study. For *Anabaena flos-aquae* the mean measured 72-hour NOE_rC value was 2.70 mg thiencarbazone-methyl/L. In a *Skeletonema costatum* study, a mean measured 72-hour NOE_rC value of 114 mg thiencarbazone-methyl/L (the highest concentration tested) was determined. The lowest chronic toxicity value for algae was NOE_rC of 0.0307 mg/L for *Pseudokirchneriella subcapitata*.

The chronic toxicity values for macrophytes were taken from the same tests as the acute values. The studies are described more in detail under the heading Acute toxicity.

For *Lemna gibba* the 7-day mean measured NOE_rC based on frond number was 0.00021 mg thiencarbazone-methyl/L.

The results of the original *Myriopyllum spicatum* study were recalculated to derive an E_rC_{50}. A NOE_rC was not, however, recalculated but the 14-day E_rC_{25} was determined to be 0.0005 mg/L. A visual re-inspection of the recalculated data reveals that statistically significant growth effects were seen at a mean measured 0.00067 mg/L and above, so the revised 14-day NOE_rC would be 0.00031 mg/L which equates with the initial measured NOErC of 0.00045 mg/L originally reported. Although there were concerns over the lack of an agreed protocol at the time of the original study and the inclusion of sediment, the revised endpoint calculations for *Myriophyllum* were based on mean measured concentrations in the water phase as well as on growth rate - and so the eMSCA considered them to be reliable and potentially relevant for hazard classification.

In a comparative toxicity study with aquatic plants, the 14-day NOE_rC value for *Potamogeton pectinatus* shoot length growth rate was determined to be a mean

measured concentration of 0.000075 mg/L. An E_rC_{25} was also determined for *Potamogeton* since the concentration-response at 0.000075 to 0.0031 mg/L was fairly flat and a 25 % reduction in plant growth was considered by the study authors not to be ecologically relevant; this was a mean measured concentration of 0.00081 mg/L. The DS considers that the fact that the endpoint is over 14 rather than 7 days may not be important since like *Myriophyllum*, *Potamogeton* is slower growing than *Lemna.*

Comments received during public consultation

Five Member States (MS) agreed with the Dossier Submitter's proposal to classify thiencarbazone-methyl as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 with acute/chronic M-factors of 1 000. An MS agreed to use non-standard species *Myriophyllum spicatum* and *Potamogeton pectinatus* due to thiencarbozone-methyl being a herbicide. Another MS wanted a clarification on the variation of temperature in the *Potamogeton pectinatus* test. The DS could not see any temperature related effects in the test.

One MS agrees with the classification categories but disagreed with the M-factors. In their opinion the water/sediment studies with *Myriophyllum spicatum* and *Potamogeton pectinatus* can not be considered appropriate for classification purposes. The CLH Report does not mention if spiked water test design has been used. Furthermore, rapid partitioning of the substance from water to sediment was demonstrated in degradation studies. Altogether exposure via sediment (root uptake) cannot be excluded. The DS answered that in both studies the substance was applied by spiking the water phase. Although there was degradation or dissipation to sediment, this was not as rapid as might have been predicted from the physicochemical and environmental fate data. In the *Myriophyllum* study, the Day 11 measured concentrations in the water phase ranged from 84 % to 115 % of nominal and the Day 14 measured concentrations ranged from 61 % to 85 % of nominal. The majority of exposure during this period would have been via the water phase, or indeed the sediment pore water (concentrations in which are normally modelled to be similar to that in overlying water). The proportion of uptake from direct contact of roots with the substance adsorbed to sediment particles is unknown but the DS expects it to be relatively low in comparison with water phase uptake. The subsequent recalculation did also determine a growth rate E_rC_{50} based on mean measured concentrations in the water phase over the whole 14-day exposure period. Similarly in the study on *Potamogeton* the mean measured concentrations in the water phase over the 14 days initial exposure period were < 0.016 (control), 0.075, 0.26, 0.95, 3.1 and 10 µg a.s./L. These ranged from 75 to 86 % of the nominals. The 14-day NOE_rC was also based on the mean measured concentrations in the water phase. The DS saw that, because exposure seems to be predominantly via the water phase, the data from these studies could be used although there is some generally uncertainty over the use of studies involving sediment.

Assessment and comparison with the classification criteria

Thiencarbazone-methyl was hydrolytically unstable under acidic, neutral and alkaline conditions. The hydrolysis half-life for thiencarbazone-methyl was 50 days at pH 4 and approximately 150 days at pH values of 7 and 9 At 25°C. Photolysis did not significantly contribute to the degradation of thiencarbazone-methyl in aqueous solutions.

Thiencarbazone-methyl showed 0 % degradation after 28 days in the ready biodegradation test and is, thus, considered to be not readily biodegradable. The degradation DT_{50} in two natural water/sediment systems under aerobic conditions at 20°C was a maximum 29 days. Using the FOCUS kinetics the simple first order model DT_{50}s for the water/sediment whole system were 21.9 days and 31.3 days for Hoenniger and Clayton systems, respectively. Thiencarbazone-methyl and its metabolites are mineralized in water/sediment systems but not quickly enough to be considered rapidly degradable as degradation rates are greater than 16 days.

In conclusion, thiencarbazone-methyl fulfils the criteria for a 'not rapidly degradable' substance under CLP because it is not readily biodegradable, does not ultimately degrade in surface water simulation tests with a half-life of < 16 days and does not primary degrade biotically or abiotically in the aquatic environment with a half-life < 16 days. RAC agrees with the DS that thiencarbazone-methyl is 'not rapidly degradable' for classification purposes.

There is no BCF study available. The log K_{ow} ranges from -0.13 at pH 4, to -1.98 at pH 7 and -2.14 at pH 9, which are below the CLP trigger value of ≥ 4 and, thus, indicate a low potential to bioaccumulate.

According to the data presented in Annex 1 to the CLH Report the degradation products are far less toxic that thiencarbazone methyl itself.

The lowest toxicity values for thiencarbazone-methyl are from studies performed with non-standard species *Myriophyllum spicatum* and *Potamogeton pectinatus.* RAC agrees to use these results as the basis for classification. In both studies, the plants were rooted in sediment and studies were run for 14 days. The substance was applied by spiking the water phase. In the *Myriophyllum* study, the Day 11 measured concentrations in the water phase ranged from 84 % to 115 % of nominal and the Day 14 measured concentrations ranged from 61 % to 85 % of nominal. In the study on *Potamogeton*, the mean measured concentrations in the water phase over the 14 days ranged from 75 to 86 % of the nominals. Consequently, the majority of exposure during this period would have been via the water phase. RAC is of the opinion that the mean measured concentrations in the tests demonstrated sufficient aqueous exposure although presence of sediment in the test system added uncertainty to the results. RAC also agrees with the DS to use the 14-day results. The 14-day duration of the tests did not allow multiple generations as normally required for a chronic test and as such the endpoint is not equivalent with a standard algal or Lemna test. However, as the substance is a herbicide and shows severe effects, RAC agrees to consider the test results both for acute and chronic classification.

There are acute toxicity data available on fish, invertebrates, algae, *Lemna*[2] and two other aquatic macrophytes. The lowest acute aquatic toxicity value was a 14-day E_rC_{50} of 0.00094 mg/L for *Myriophyllum spicatum*[3] which fulfils the criteria for Aquatic Acute 1; H400, i.e. toxicity below 1 mg/L. The value is in the range of $0.0001 < L(E)C_{50} \leq 0.001$, thus giving an M-factor of **1 000**.

There were chronic data available on fish, invertebrates, algae, *Lemna* and two other

[2] floats on the surface on the water with a root hanging down into the water
[3] submerged, rooted aquatic plant

macrophytes. The lowest value was a 14-day NOE_rC value of 0.000075 mg/L for *Potamogeton pectinatus*[4] which fulfils the criteria for Aquatic Chronic 1; H410, i.e. toxicity below 0.1 mg/L for a non-rapidly degradable substance. The value is in the range 0.00001 < NOEC ≤ 0.0001, thus giving an M-factor of **1 000**.

Overall, RAC agrees with the DS proposal to classify thiencarbazone-methyl as **Aquatic Acute 1; H400 (M=1 000) and Aquatic Chronic 1; H410 (M=1 000)**.

[4] submerged, rooted aquatic plant

6 OTHER INFORMATION

No other relevant information.

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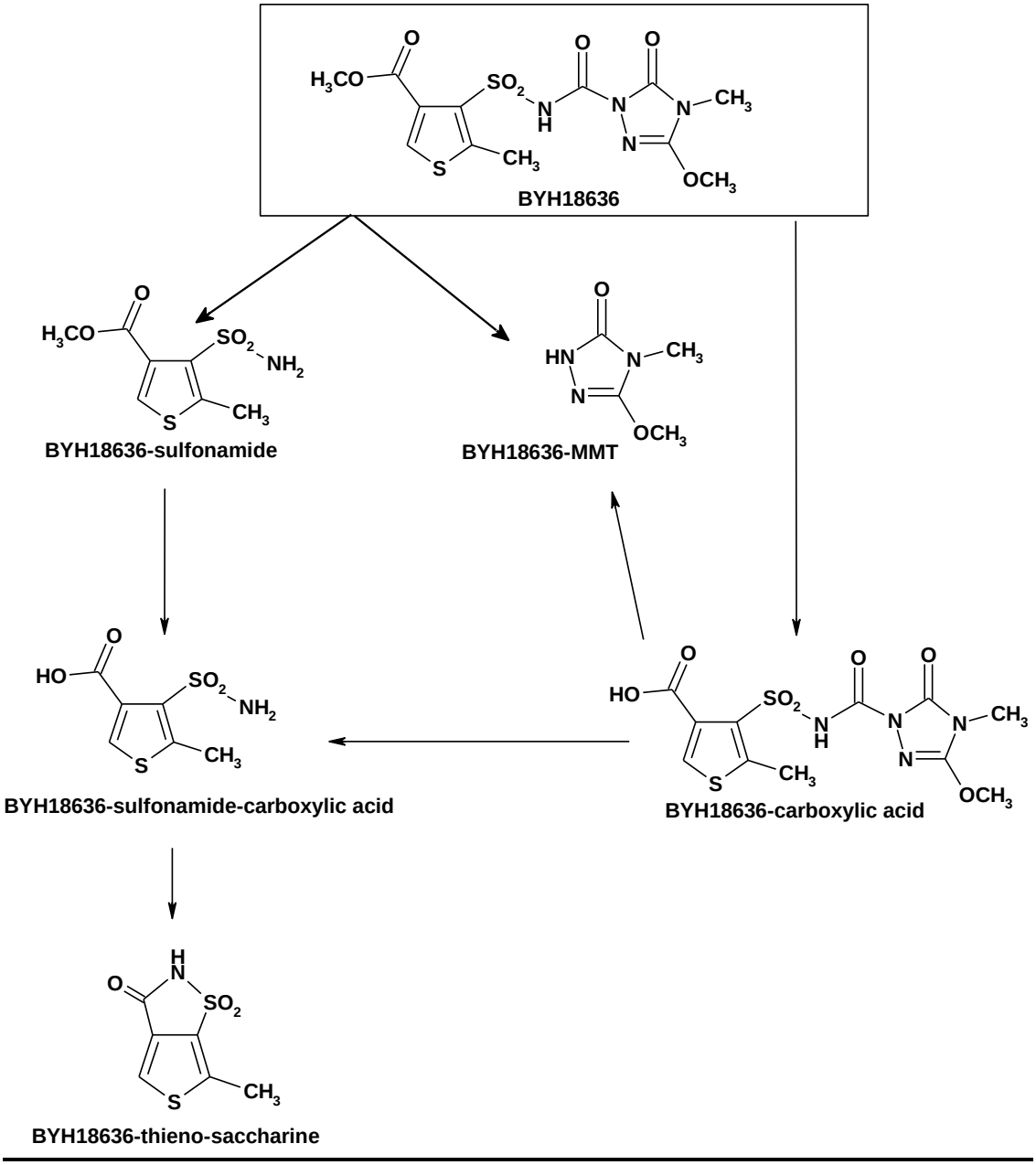
8 ANNEXES

Annex I - Environmental fate and ecotoxicological information on the degradation and degradation products of thiencarbazone-methyl

Annex II – Confidential references (separate document)

Annex 1 Environmental fate and ecotoxicological information on the degradation and degradation products of thiencarbazone-methyl

Figure 1 Proposed hydrolytic degradation pathway of thiencarbazone-methyl (BYH 18636) in aqueous solution (pH 4, 7, 9)



Thiencarbazone-methyl (BYH 18636) was hydrolysed to BYH 18636-MMT and BYH 18636-sulfonamide (Figure 1). At higher pH, BYH 18636-sulfonamide was further degraded to BYH 18636-sulfonamide-carboxylic acid and BYH 18636-thienosaccharine. BYH 18636-carboxylic acid was only detected in minor amounts.

Table 1 Major degradants of thiencarbazone-methyl (BYH 18636) found in soil and water/sediment systems

Compartment	Degradants of thiencarbazone-methyl
Soil	BYH 18636-carboxylic acid
	BYH 18636-MMT
	BYH 18636-sulfonamide
	BYH 18636-sulfonamide-carboxylic acid
Water/sediment	BYH 18636-carboxylic acid
	BYH 18636-MMT
	BYH 18636-sulfonamide-carboxylic acid
	BYH 18636-dicarboxy-sulfonamide

Summary of the aquatic toxicity of degradants of thiencarbazone-methyl (BYH 18636)

Table 2 Summary of toxicity of thiencarbazone-methyl degradants to fish and invertebrates

Test spp./substance	study type duration	L/EC50 mg/L	NOEC mg/L	Reference
***Oncorhynchus mykiss* (rainbow trout)**				
BYH 18636 – sulphonamide (M15)	static acute (96h)	> 98.3[mm]	50.2[mm]	Anon., 2005 IIA 8.2.1.3/01 EBGSP001-1
Daphnia magna				
BYH 18636 – sulphonamide (M15)	static acute (48h)	>100[nom]	100[nom]	Bruns, 2007 IIA 8.3.1.1/04 EBGSP087
Chironomous riparius				
BYH 18636 - carboxylic acid (M01)	static[1] acute (48h)	>100[nom]	100	Bruns, 2006 IIA 8.5.1/02 EBGSP079
BYH 18636 - sulphonamide-carboxylic acid (M03)	static[1] acute (48h)	>100[nom]	100	Bruns, 2006 IIA 8.5.1/03 EBGSP078

[1] *C. riparius* larvae exposed in aqueous phase

Table 3 Summary of toxicity of thiencarbazone-methyl degradants to algae and plants

Test spp./substance	**Study type**	**Endpoint mg/L**	**References**
Pseudokirchneriella subcapitata (FW green alga)			
BYH 18636 – sulphonamide (M15)	chronic (semi-static)	72h ErC50 = 1.61[mm] 72h EbC50 = 0.50[mm]	Banman & Lam, 2005 IIA 8.4/03 EBGSP003
Lemna gibba **G3**			
BYH 18636 - carboxylic acid (M01)	chronic, 7d, (static)	7d ErC50 = 3.54[mm] 7d EbC50 = 2.08[mm]	Banman & Lam, 2005 IIA 8.6/05 EBGSP019
BYH 18636 - sulphonamide-carboxylic acid (M03)	chronic, 7d, (static)	7d ErC50 >100[nom] 7d EbC50 >100[nom]	Dorgerloh, 2006 IIA 8.6/06 EBGSP042
BYH 18636 – sulphonamide (M15)	chronic, 7d, (static)	7d ErC50 = 90.5[mm] 7d EbC50 = 61.6[mm]	Christ & Lam, 2006 IIA 8.6/07 EBGSP029
BYH 18636 – MMT (M21)	chronic, 7d, (static)	7d ErC50 >95.7[mm] 7d EbC50 >95.7[mm]	Christ & Lam, 2007 IIA 8.6/08 EBGSP040
BYH 18636 - dicarboxy-sulfonamide (M25)	chronic, 7d, (static)	7d ErC50 >104[mm] 7d EbC50 >104[mm]	Christ *et al.*, 2007 IIA 8.6/09 EBGSP045

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