Committee for Risk Assessment

RAC

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

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-244/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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: **Not assigned**

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

The proposal was submitted by **United Kingdom** and received by RAC on **7 September 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 November 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **12 January 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	thiencarbazone-methyl (ISO); methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl)carbonylsulfamoyl]-5- methylthiophene-3-carboxylate	-	317815-83-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H400 H410		M=1000 M=1000	
RAC opinion	607-RST-VW-Y	thiencarbazone-methyl (ISO); methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl)carbonylsulfamoyl]-5- methylthiophene-3-carboxylate	-	317815-83-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H400 H410		M=1000 M=1000	
Resulting Annex VI entry if agreed by COM	607-RST-VW-Y	thiencarbazone-methyl (ISO); methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl)carbonylsulfamoyl]-5- methylthiophene-3-carboxylate	-	317815-83-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H400 H410		M=1000 M=1000	

GROUNDS FOR ADOPTION OF THE OPINION

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**.

HUMAN HEALTH HAZARD EVALUATION

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} ≤ 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**.

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**.

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**.

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.**

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.

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)**.

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**.

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**.

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

	F0 Generation				F1 Generation			
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

Observation	Dietary concentration (ppm)			
	0	**500**	**2 500**	**10 000**
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 covariance; *: 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**.

ENVIRONMENTAL HAZARD EVALUATION

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, GLP static, limit test -aqueous phase, no sediment	48 h acute	> 100[nom] no effects	100[nom]
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*[1] and two other aquatic macrophytes. The lowest acute aquatic toxicity value was a 14-day E_rC_{50} of 0.00094 mg/L

[1] floats on the surface on the water with a root hanging down into the water

for *Myriophyllum spicatum*[1] 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} ≤ 0.001, thus giving an M-factor of **1 000**.

There were chronic data available on fish, invertebrates, algae, *Lemna* and two other macrophytes. The lowest value was a 14-day NOE_rC value of 0.000075 mg/L for *Potamogeton pectinatus*[2] 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)**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

[1] submerged, rooted aquatic plant
[2] submerged, rooted aquatic plant