Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine

EC Number: 428-650-4
CAS Number: 153719-23-4

CLH-O-0000006724-70-01/F

Adopted

5 December 2019

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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CLH-O-0000006724-70-01/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: **thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine**

EC Number: **428-650-4**

CAS Number: **153719-23-4**

The proposal was submitted by **France** and received by RAC on **12 December 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

France 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 **21 January 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 March 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

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 **5 December 2019** by **consensus**.

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

	Index No	Chemical name	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	613-267-00-9	thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine	428-650-4	153719-23-4	Acute Tox. 4* Aquatic Acute 1 Aquatic Acute 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		M=10	
Dossier submitters proposal	613-267-00-9	thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine	428-650-4	153719-23-4	**Retain** Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 **Add** Flam. Sol. 1 Repr. 2	**Retain** H302 H400 H410 **Add** H228 H361	**Retain** GHS07 GHS09 **Add** GHS02 GHS08 **Modify** Dgr	**Retain** H302 H410 **Add** H228 H361		**Retain** M=10 **Add** oral: ATE = 800 mg/kg bw M=10	
RAC opinion	613-267-00-9	thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine	428-650-4	153719-23-4	**Retain** Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2	**Retain** H302 H400 H410 **Add** H361fd	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H302 H410 **Add** H361fd		**Retain** M=10 **Add** oral: ATE = 780 mg/kg bw M=10	
Resulting Annex VI entry if agreed by COM	613-267-00-9	thiamethoxam (ISO); 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine	428-650-4	153719-23-4	Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H400 H410	GHS07 GHS08 GHS09 Wng	H361fd H302 H410		oral: ATE = 780 mg/kg bw M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Thiamethoxam is a neonicotinoid insecticide used in plant protection products.

The substance has a current Annex VI entry. During the renewal assessment of thiamethoxam under Reg. (EU) 1107/2009, the Rapporteur Member State proposed to reconsider harmonized classification for selected hazard classes.

RAC evaluation of flammable solids

Summary of the Dossier Submitter's proposal

The DS proposed classification with Flam. Sol. 1 based on an A.10 test they interpreted as positive.

Comments received during public consultation

Comments were received from 2 MSCAs, both in support of the DS's proposal.

Assessment and comparison with the classification criteria

According to the CLP regulation, classification in this hazard class should be based on a result of the UN Test N.1. The EC A.10 test is very similar to the UN Test N.1 except that the former is unable to distinguish between Category 1 and 2 due to lack of a wetted zone in the test design.

According to the draft Renewal Assessment Report under Reg. EU 1107/2009) Vol. 3 – B.2 (AS), the test was conducted twice. First, thiamethoxam was tested strictly following the EC A.10 method, including the use of a non-combustible base plate made from glass. The result was negative. The second, "extended" test using a fibreboard base plate was positive. The test substance propagated combustion over 100 mm in 40 seconds, which is below the criterion of 45 seconds. RAC notes that both the UN Test N.1 and the EC A.10 test clearly specify that the base plate must be non-combustible. Pyrolysis products from the fibreboard (a wood-based material) are likely to have contributed significantly to the combustion of the test substance in the second test. RAC considers the test using fibreboard to be unsuitable for classification purposes.

Therefore, RAC proposes **no classification** for flammable solids based on the negative result obtained.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute oral toxicity

Summary of the Dossier Submitter's proposal

In the current Annex VI entry the substance has a minimum classification with Acute Tox. 4*; H302. Two acute oral toxicity studies are available, one in the rat and one in the mouse. The

LD_{50} values from both studies are in the range of 300 < LD_{50} ≤ 2 000 mg/kg bw, confirming the current classification and allowing removal of the asterisk.

As to the ATE, the DS proposed 800 mg/kg bw, a rounded value based on the lowest LD_{50} of 783 mg/kg bw for male mice and taking into account the other available LD_{50} values, i.e. 964 mg/kg bw for female mice and 1 563 mg/kg bw for rats.

Comments received during public consultation

Comments were received from 3 MSCAs. All 3 MSCAs supported classification in Category 4. 2 MSCAs supported the rounded ATE of 800 mg/kg bw while 1 MSCA preferred the unrounded value of 783 mg/kg bw.

Assessment and comparison with the classification criteria

Both acute oral toxicity studies were conducted according to OECD TG 401 and under GLP. The rat LD_{50} was 1 563 mg/kg bw (the same value for both sexes). The mouse study (strain CD-1) yielded LD_{50} values of 783 mg/kg bw for males, 964 mg/kg bw for females and 871 mg/kg bw for combined sexes. The CLH report also mentions a mouse micronucleus test (strain Tif:MAGf) where 7 out of 13 females died at 1 250 mg/kg bw. All this information is consistent with Category 4.

The Guidance on the application of the CLP criteria generally recommends choosing the lowest ATE in the most sensitive appropriate species tested. RAC agrees with the DS to base the ATE on the lowest available LD_{50} of 783 mg/kg bw (male mice). In previous cases LD_{50} values were usually not rounded for the purpose of ATE setting. On the other hand, RAC notes that rounded values are easier to use and that unrounded values may give an impression of precision that in fact does not exist (the 95 % confidence interval for the LD_{50} in male mice is 619-1 000 mg/kg bw). RAC therefore proposes to classify thiamethoxam as **Acute Tox. 4; H302 with an ATE of 780 mg/kg bw**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Reproductive toxicity of thiamethoxam had been investigated in two 2-generation studies in the rat, in two prenatal developmental toxicity (PNDT) studies, one in the rat and one in the rabbit, and in a developmental neurotoxicity study (DNT) in the rat. Information on effects on reproductive organs was also obtained from repeat dose toxicity studies in rats, mice and dogs.

The DS proposed a classification for reproductive toxicity without sub-categorisation based on the following findings:

- Testicular tubular atrophy in the 90-day and 1-year dog studies, occurring in the absence (1-year study) and presence (90-day study) of slight general toxicity.
- Testicular tubular atrophy (dose-dependent increase in the incidence and severity) in F1 males of the first 2-generation study (1998), occurring in the absence of general toxicity.
- Delayed preputial separation, significantly increased incidence of germ cell loss/disorganization and Sertoli cell vacuolation, significantly reduced testicular sperm count at 3 doses and reduced sperm velocity in F1 males of the second 2-generation study (2004), occurring in the presence of slight general toxicity (increased incidence of hyaline

change in renal tubules, no effect on terminal bw in F1). Preputial separation was delayed also in the DNT study.
- Effects on brain weight and morphometric changes in the offspring in the DNT study, occurring in the presence of moderate maternal toxicity (reduced maternal bw gain of 12 %).

The DS did not consider the observed effects sufficient for classification in Category 1B because fertility and reproductive performance were not affected in the available studies. The other effects on prenatal development (mainly variations) were only observed at doses causing marked maternal toxicity whereas the delayed preputial separation and brain effects were observed in either the absence or presence of only slight maternal toxicity.

The DS proposed not to specify 'f' or 'd' as the effects in F1 males such as testicular atrophy, reduced sperm count and delayed preputial separation were considered to fall within classification criteria for both fertility and developmental toxicity. Thus, the classification proposal was Repr. 2; H361.

The DS proposed no classification for effects on or via lactation. The only potentially relevant effect was reduced pup body weight during late lactation (PND 14 and 21) in generational studies. However, as pups already begin eating the diet in this period, the body weight reduction was not considered to be linked to lactation.

Comments received during public consultation

Comments were received from 5 MSCAs and 1 manufacturer.

One MSCA supported Category 2 without specification for fertility or development and no classification for effects on or via lactation. The MSCA pointed out the lack of maternal toxicity at the top dose in the 2-generation studies and questioned the top dose selection, explaining that an effect on female reproductive function cannot be excluded.

Another MSCA supported Category 2 but proposed to specify 'development' in the hazard statement since fertility and reproductive performance were not affected and the reduction in sperm count in one of the generational studies was not clearly dose dependent. As to the brain effects in the DNT study, the MSCA mentioned that most of the changes in morphometric measurements were within the historical control range.

Two other MSCAs supported classification "at least" in Category 2, one of them specifically indicating a need for a discussion about Category 1B, due to effects on several parameters related to male reproduction in two species starting in some cases from relatively low doses.

One MSCA expressed preference for Category 1B, additionally pointing out effects on ovaries, both in a 90-day mouse study and in a 90-day dog study. The MSCA was of the opinion that effects on reproduction in general were observed from rather low doses in several species in both sexes and in the absence of other toxic effects.

Regarding the ovarian findings, the DS replied that reduced weight, atrophy and reduced numbers of corpora lutea were indeed observed from 3 500 ppm (626 mg/kg bw/d) with concomitant general toxicity. However, no such effects were observed in an 18-month study at doses up to 2 500 ppm (479 mg/kg bw/d).

As to classification, the DS maintained their proposal for Category 2, reiterating that reproductive performance was not affected.

Industry proposed no classification and provided detailed analysis of the available studies. Their main arguments for no classification are summarised as follows:

- Sperm parameters (2-generation study, 1998, F1 generation): The decrease in testicular sperm count was not dose-related, with only one value being outside the historical control range. Thus, the testicular sperm counts are likely to reflect normal biological variation. Although sperm velocities were reduced at the top dose, the values remained well within the historical control range.
- Delayed preputial separation (developmental neurotoxicity study; 2-generation study, 2004): A delay by at least 2 days in the absence of body weight reduction is generally considered toxicologically significant. The developmental neurotoxicity study reported a delay by 1.5 days in conjunction with a significant reduction in body weight. Based on the small magnitude of delay associated with the bodyweight effects, this effect is considered to be of no toxicological relevance. The second 2-generation study reported a delay by only 1 day without statistical significance.
- Histopathological findings in the testes of rats (2-generation study, 1998, F1 generation; 2-generation study, 2004, F1 generation): The increased incidence of germ cell loss/disorganisation +/– Sertoli cell vacuolation in the second study was of low severity, with approximately 0.4 % of tubules affected at the top dose, and did not lead to reduction in testicular weight. The testicular atrophy was minimal to mild in both studies, barely exceeded background variability and had no effect on reproductive function or spermatogenesis. Thus, the histopathological findings were not considered adverse.
- Testicular findings in dogs (90-day study; 1-year study): Dogs were sexually immature at the start of the studies and systemic toxicity caused an overall developmental delay, leading to lower testicular weight and immature histopathological appearance of the testes.
- Effects on brain weight and brain morphometric measurements (developmental neurotoxicity study): These findings are secondary to lower pup bodyweights at birth, which are in turn secondary to reduced maternal food consumption (fc reduced by up to 15 %). The relevance of body weight for the assessment of brain weight is apparent from scatter graphs on the brain weight vs body weight. While the adult brain is relatively insensitive to changes in body weight, published literature indicates that the developing brain is affected by deficits in maternal nutrition, food consumption and body weight. There were no histopathological changes in the brain and no changes in the investigated functional and neurobehavioural parameters.

The DS gave a detailed response to industry's comment, and retained their original classification proposal.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Two 2-generation studies are available for thiamethoxam, both conducted according to OECD TG 416 and under GLP. Both studies were performed in the same rat strain and with the same batch of the test substance but in different laboratories. The more recent study (2004) was conducted according to the current (2001) version of OECD TG 416. The older study (1998) followed the older (1981) version and therefore did not investigate some parameters, such as puberty onset, required by the current test guideline.

In addition, certain findings from the developmental neurotoxicity study in rats (GLP, OECD TG 426) and repeated dose studies in dogs and mice are also considered in the assessment.

<u>2-generation study in the rat (1998)</u>

The top dose was 2 500 ppm (ca. 160/200 mg/kg bw/d in m/f, respectively). Parental toxicity at the top dose was limited to mild effects on body weight and food consumption in males (bw reduced by up to 8 % as compared to controls). Treated males also showed increased incidence

of hyaline change in renal tubules related to α2u-globulin nephropathy. No general/parental toxicity was observed in top dose females. According to the study report, the top dose selection was based on a 90-day study and on a 1-generation range-finding study.

In the 90-day rat study a dose of 5 000 ppm caused body weight reduction by 19 % as compared to controls and renal lesions in males but no general toxicity in females. In the 1-generation range-finding study, males were exposed for 4 weeks (2-week premating, 2-week mating and post-mating periods) and females for ca. 7 weeks (2 weeks premating, 3 weeks gestation, 2 weeks lactation) to dietary concentrations of up to 4000 ppm. Terminal body weights of males and females were reduced by 5% and 6% respectively compared to controls at the top dose. It is noted that the exposure duration in this range-finding study was considerably shorter than that in the full 2-generation study.

The top dose selection in the main study is considered acceptable for males given the marked body weight reduction at 5000 ppm in the 90-day study. However, RAC is of the opinion that a dose higher than 2500 ppm would have been well-tolerated by females. Therefore, this 2-generation study may not provide sufficient information about the potential of the substance to cause adverse effects on female sexual function and fertility and adverse developmental effects on the offspring.

The study design included two matings per generation and a 10 weeks premating period. Selected F1a young animals were used to produce F2a and F2b offspring. No effect on reproductive performance was observed in any generation. There was no effect on sperm parameters in P males (no reliable data are available for F1 males). A 9 % reduction in absolute testes weight and increased incidence of testicular tubular atrophy were seen in F1 generation. Detailed results are presented in the table below (for testicular tubular atrophy the table shows the data from re-examination by the Pathology Working Group).

2-generation study (1998): testes weight and incidence of testicular tubular atrophy in F1 males

Dose (ppm)	**0**	**10**	**30**	**1 000**	**2 500**
Dose (mg/kg bw/d)	0	0.6	1.8	61	158
Terminal bw (g)	582	593	578	575	573
Testes weight, absolute (g)	4.48	4.42	4.24	4.41	4.08*
No. of animals examined	30	30	30	30	30
Incidence of testicular tubular atrophy, total	6	10	13*	21**	18*
– grade 1	6	7	9	8	10
– grade 2		3	4	8	5
– grade 3				1	1
– grade 4				2	1
– grade 5				2	1

* statistically significant difference from control, $p \leq 0.05$

For comparison, incidence of testicular tubular atrophy in controls of 5 reference studies performed by the same laboratory and examined by the same Pathology Working Group ranged from 3 to 11 out of 30 males (average grade 1.2 to 2.8). This shows that control values are exceeded in terms of incidence but not necessarily in terms of severity from 1 000 ppm. The dose-response relationship is not very clear and obscured by the large interval between 30 ppm

and 1 000 ppm. However, when testicular weights are taken into account, the effect is likely to be most pronounced at the top dose.

2-generation study in the rat (2004)

This study employed the same top dose as the first 2-generation study (1998), i.e. 2 500 ppm. General toxicity in top dose males was similar to that in the first study: a reduction in body weight (by up to 10%, only in the F0 generation) and increased incidence of hyaline change in renal tubules. No toxicity was observed in females. Due to low dosing, this study is not considered sufficiently informative about the reprotoxic potential of the substance in females and about adverse developmental effects on the offspring.

No effect on reproductive performance was observed in either generation. A number of findings in F1 generation related to male reproductive organs were discussed in the CLH report and are summarised in the table below: delayed preputial separation, increased testicular and epididymal weight, increased incidence of germ cell loss/disorganisation and Sertoli cell vacuolation, reduced testicular sperm count, increased epididymal sperm count and reduced sperm velocity. No effect on these parameters was observed in the P generation.

The germ cell loss/disorganisation in the 2004 study is likely to represent the same entity as testicular tubular atrophy in the 1998 study because germ cell loss/disorganisation is one of the key features of testicular tubular degeneration/atrophy (Creasy *et al.*, 2012) and the strain and the top dose were identical in both studies.

The table also shows results of two control studies (RR0942, denoted HCD 1; RR0943, denoted HCD 2) run in parallel with the main study in order to provide information on the background variability of the strain used since the Tif:RAIf strain was not frequently used by the performing laboratory.

2-generation study (2004): findings related to reproductive organs in F1 males							
Dose (ppm)	**0**	**20**	**50**	**1 000**	**2 500**	**HCD 1**	**HCD 2**
Dose (mg/kg bw/d)	0	1.2	3.0	62	156		
Day of preputial separation; (±SD)	47.7 (±2.9)	47.3 (±2.0)	47.2 (±3.6)	46.7 (±2.3)	48.7 (±1.9)	47.1	47.2
Body weight at PS (g)	174	176	169	171	178	179	182
Body weight on PND 22 (g)	36.8	38.7	38.5	36.9	35.8		
Terminal bw (g)	464	477	469	467	458	469	483
Testes weight, abs. (g); (±SD)	3.89 (±0.29)	4.15* (±0.32)	4.02 (±0.39)	4.13* (±0.33)	4.19** (±0.46)	4.01	4.01
Epididymides weight, abs. (g); (±SD)	1.58 (±0.12)	1.63 (±0.13)	1.62 (±0.14)	1.66 (±0.14)	1.66* (±0.18)	1.63	1.67
No. of animals examined, main study	26	26	26	26	26		
Incidence of germ cell loss/disorganization, with or w/o Sertoli cell vacuolation (only one testis examined)[b]	3 (2 ±, 1 +)	1 (1 +)	1 (1 ±)	3 (3 ±)	15** (14 ±, 1 ++)		

No. of animals examined, satellites	14	14	14	14	14		
Incidence of germ cell loss/disorganization, with or w/o Sertoli cell vacuolation, unilateral[b]	1 (1 ±)	4 (3 ±, 1 +)	2 (2 ±)	3 (3 ±)	0		
Incidence of germ cell loss/disorganization, with or w/o Sertoli cell vacuolation, bilateral[b]	1 (1 +)	0	0	1 (1 ±)	5 (4 ±, 1 +)		
Testicular sperm count, per testis (millions); (±SD)	87 (±22)	93 (±23)	70** (±19)	63** (±16)	74* (±18)	Range[a]: 69–103	
Epididymal sperm count, per cauda epid. (millions); (±SD)	153 (±38)	153 (±37)	163 (±53)	168 (±49)	192** (±37)	Range[a]: 137–170	
Sperm velocity: straight line velocity (µm/s); (±SD)	71.6 (±5.6)	71.8 (±6.0)	72.3 (±5.2)	71.3 (±7.4)	67.6* (±6.0)	Range[a]: 64.9–76.7	
Sperm velocity: curvilinear velocity (µm/s); ±SD	305 (±17)	297 (±20)	302 (±17)	298 (±21)	290** (±18)	Range[a]: 278–316	
Sperm velocity: average path velocity (µm/s); ±SD	124 (±8)	122 (±8)	123 (±8)	120 (±8)	116** (±8)	Range[a]: 113–131	

Statistically significant difference from control: *, p≤0.05; **, p≤0.01

[a] The range includes the controls from both generations of the current study (i.e. including the P and F1 generation) and both generations of the two additional control studies (HCD 1 and HCD 2). Individual control mean values are provided under 'Supplemental information'.

[b] Severity grades: ±, minimal; +, slight; ++, moderate

While there are a number of findings related to male reproductive organs and sperm parameters in the F1 generation, the main evidence for a treatment-related effect comes from the histopathology, namely from the statistically significantly increased incidence of germ cell loss at the top dose of 2 500 ppm. The severity was mostly 'minimal' and, on average, 10 tubules per section were affected per affected animal (study report, Appendix H), which represents a relatively small portion (in the order of 0.4 % according to the study report) of the tubular cross sections examined. The changes in sperm parameters were still within normal variation. The delay in puberty onset in males was slight and not statistically significant, so its relation to treatment would be debatable should this finding be considered in isolation. The testicular and epididymal weights were only slightly increased.

Developmental neurotoxicity study in the rat

The animals were dosed from GD 7 to LD 22, the top dose was 4 000 ppm (equivalent to ca. 300 mg/kg bw/d during gestation). Maternal toxicity was limited to body weight reduction by 5-10 % compared to controls (–5%, –7%, –8%, –10% and –6% on GD 15 (unadjusted), LD 1, 8, 15 and 22, respectively). The top dose was chosen on the basis of a preliminary study, where a dose of

5 000 ppm was reported to cause maternal toxicity (not further specified in the study report of the main study) and reduced pup weight at birth and afterwards.

The only finding related to sexual function and fertility was a statistically significant delay in preputial separation by approx. 1 day. The delay was associated with reduced body weight (by ca. 10 %), so the finding may at least partly be attributed to a general developmental delay. However, some contribution of a specific MoA cannot be excluded considering the delay of approx. 1 day at 2 500 ppm in the 2-generation study (2004) observed in the absence of an effect on body weight. There was no effect on vaginal opening.

Developmental neurotoxicity study: preputial separation				
Dose (ppm)	**0**	**50**	**400**	**4 000**
Dose (mg/kg bw/d)	0	4.3	35	299
Day of PS; (±SD)	44.9 (±0.9)	45.6* (±0.8)	45.1 (±0.9)	46.4** (±1.3)
Body weight on PND 1 (g)	6.1	5.9	6.0	5.7**
Body weight on PND 43 (g)	212	210	212	191
Body weight on PND 50 (g)	275	269	273	249
Body weight at PS (g)	230	233	231	221**

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

<u>90-day dog study</u>

The top dose of 2 500 ppm had to be reduced after 2 weeks to 2 000 ppm (51 mg/kg bw/day in females) due to body weight loss especially in females. Still, females showed a marked body weight reduction compared to controls until the end of the study (terminal bw 26 % lower than in controls, cumulative bw gain 0.5 kg vs 2.9 kg in controls), indicating that the top dose was in excess of MTD for this sex. The toxicity in males was less pronounced but not negligible (cumulative bw gain 1.9 kg vs 3.0 kg in controls).

The animals were 5 to 7 months old at the beginning of the study. The age of the animals at the end of the study was 8 to 10 months, which is approximately the age when Beagle dogs normally reach sexual maturity.

3 out of 4 top dose females were found to have immature ovaries (bilateral). The 1 non-affected animal was that with the highest body weight gain (1.5 kg). RAC considers the immaturity of ovaries in this study as a secondary non-specific consequence of developmental delay caused by severe general toxicity, and as such not relevant for classification for reproductive toxicity. No changes in the ovary were observed at 1500 ppm in a 1-year study in the absence of significant general toxicity.

90-day dog study: ovarian findings					
Dose (ppm)	**0**	**50**	**250**	**1 000**	**2 500/2 000**
Dose (mg/kg bw/d)	0	1.8	9.3	34	51
No. of animals examined	4	4	4	4	4
Initial body weight (kg)	7.5	7.3	7.5	7.6	7.3
Terminal body weight (kg)	10.4	9.8	10.3	10.7	7.8*

Body weight gain (kg) [individual data]	2.9	2.5	2.8	3.1	0.5* [-0.4; -0.2; 1.0; 1.5]
Ovarian weight, abs. (g)	0.84	0.66	0.70	0.71	0.54*
Ovarian weight, rel. (‰)	0.087	0.072	0.073	0.074	0.076
Immature ovary	0	0	0	0	3
Immature uterus	0	0	0	0	2

* Statistically significant difference from control, p<0.05

All top dose males showed reduced spermatogenesis and spermatic giant cells, and testicular weight was reduced by 43%. These findings may be indicative of immaturity (cf. Goedken *et al.*, 2008; Creasy *et al.*, 2012) although some contribution of direct testicular toxicity cannot be excluded as a slight increase in testicular atrophy was also observed in a 1-year study. As to the mode of action behind the delayed puberty onset, general developmental delay is considered by RAC plausible (body weight gain was reduced by 36%), but a direct effect on puberty onset cannot be excluded given the slight delay in preputial separation seen in F1 rats (2-generation study, 2004; developmental neurotoxicity study).

90-day dog study: testicular findings					
Dose (ppm)	**0**	**50**	**250**	**1 000**	**2 500/2 000**
Dose (mg/kg bw/d)	0	1.6	8.2	32	55
No. of animals examined	4	4	4	4	4
Initial body weight (kg)	8.3	8.4	8.6	8.7	8.7
Terminal body weight (kg)	11.3	11.7	11.4	11.6	10.6
Body weight gain (kg)	3.0	3.3	2.8	2.9	1.9
Testicular weight, abs. (g)	16.5	14.8	14.6	15.6	9.4*
Tubular atrophy	0	0	0	0	1 (1 ++)
Spermatogenesis reduced	0	1 (1 +)	0	0	4 (1 +, 2 ++, 1 +++)
Spermatic giant cells	1 (1 +)	1 (1 ++)	0	1 (1 +)	4 (3 +, 1 ++)

* Statistically significant difference from control, p<0.05

Severity scores: +, minimal (slight); ++, moderate; +++, marked

1-year dog study

No remarkable effect on body weight was observed at the top dose of 1 500 ppm (42/45 mg/kg bw/d m/f). Males showed a slightly increased incidence and severity of tubular atrophy from 750 ppm (see the table below). Testicular weight was statistically non-significantly reduced by 16 % at the top dose. There was no correlation between testicular weights and histopathological findings at the level of individual animal data.

1-year dog study: histopathological findings in the testes					
Dose (ppm)	**0**	**25**	**150**	**750**	**1 500**
Dose (mg/kg bw/d)	0	0.7	4.1	21	42
No. of animals examined	4	4	4	4	4
Testicular weight, abs. (g)	19.1	20.5	19.8	20.7	16.1
Tubular atrophy (severity grade, 1-5)	1 (1)	1 (1)	1 (3)	2 (2,2)	2 (2,2)

Severity grades: 1, minimal; 2, slight; 3, moderate

90-day mouse study

Increased incidence of ovarian atrophy manifested by reduced number of corpora lutea was observed from 3500 ppm (626 mg/kg bw/d) in this study. The severity at 3500 ppm was mostly slight but a more pronounced effect was observed at the top dose of 7000 ppm (1160 mg/kg bw/d), see the table below. Absolute ovarian weight was reduced by 20% and 44% at 3500 and 7000 ppm, respectively. There was no general toxicity at 3500 ppm and the body weight was reduced only about 10% as compared to controls at the top dose. No effect on ovaries was observed in an 18-month mouse study at 2 500 ppm (479 mg/kg bw/d).

90-day mouse study: ovarian findings						
Dose (ppm)	**0**	**10**	**100**	**1 250**	**3 500**	**7 000**
Dose (mg/kg bw/d)	0	2.0	19	231	626	1 160
No. of animals examined	10	10	10	10	10	10
Body weight week 13 (g)	31.8	30.4	30.6	31.1	30.5	29.2
Body weight gain (g)	6.1	5.4	4.9	5.6	4.9	3.8
Carcass weight (g)	30.1	30.8	30.6	31.9	30.3	25.6*
Ovaries weight absolute (mg)	48.1	50.1	41.3	43.2	38.7	31.6*,t^-
Ovaries weight relative (‰)	1.60	1.63	1.35	1.35	1.29t^-	1.24t^-
Ovarian atrophy	0	0	1 (1 +)	1 (1 +)	5 (4 +, 1 ++)	10 (6 +, 4 ++)

* statistically significant difference from controls (Lepage's test, $p < 0.01$)

t^- statistically significant negative trend from the control group to the respective dose group (Jonckheere's test, $p < 0.01$)

Severity scores: +, minimal (slight); ++, moderate

Conclusion on classification for fertility and sexual function

Findings related to fertility were observed in all three species investigated: rat, mouse and dog. RAC is of the opinion that the testicular tubular atrophy in F1 rats of the 2-generation study (1998), germ cell loss/disorganisation in F1 rats of the 2-generation study (2004) and the testicular tubular atrophy in the 90-day mouse study are sufficient to collectively trigger

classification in Category 2 for adverse effects on fertility and sexual function. The slight increase in testicular tubular atrophy in the 1-year dog study is considered by RAC as additional supportive evidence for classification.

RAC considers that the potential of the substance to adversely affect female sexual function and fertility has not been sufficiently investigated due to low dosing in the 2-generation studies.

Adverse effects on development

Two PNDT studies are available, one in the rat and one in the rabbit. Both have been conducted under GLP and according to OECD TG 414 and both are negative regarding developmental toxicity. Likewise, no developmental effects were observed in the two generational studies discussed in the fertility section. However, the developmental neurotoxicity study reported some effects on brain weight and morphometry that are considered relevant for classification.

Rat PNDT study

Maternal toxicity at the top dose of 750 mg/kg bw/d consisted of clinical signs (hypoactivity, piloerection, regurgitation of the test substance), reduced food consumption (by 35 % over the dosing period) and moribund condition (1 animal was killed for humane reasons). Developmental toxicity at this dose was limited to reduced foetal weight (by 9 %) and reduced ossification. No developmental toxicity was apparent at the lower dose of 200 mg/kg bw/d.

Rabbit PNDT study

The top dose of 150 mg/kg bw/d induced severe maternal toxicity including 3 deaths (1 spontaneous, 2 animals killed moribund) and markedly reduced food consumption (by 58 % during the dosing period). Developmental toxicity at the top dose consisted of increased post-implantation loss (46 % vs 21 %), reduced foetal body weight (by 15 %) and increased incidence of fused sternebrae 3 and 4 (5 foetuses vs 0 in the control). Developmental effects at the top dose are not considered relevant for classification as maternal mortality exceeded 10 % and there was no increase in malformations. No developmental effects were observed at the lower dose of 50 mg/kg bw/d.

Developmental neurotoxicity study in the rat

Statistically significant reductions in brain weight and size of some brain regions were observed at the top dose of 4 000 ppm (299 mg/kg bw/day) in the presence body weight reductions. The table below summarizes the body and brain weight data together with selected morphometry parameters.

Developmental neurotoxicity study: absolute brain weight and selected morphometry parameters (in brackets % reduction compared to control)					
Dose (ppm)	**0**	**50**	**400**	**4 000**	**HCD[a]**
Dose (mg/kg bw/d)	0	4.3	35	299	
PND 12, males					
Terminal body weight (g)	23.5	24.8	24.8	20.7 (-12%)	
Brain weight (g)	1.15	1.16	1.13	1.10* (-4%)	1.03–1.16 Mean 1.11
PND 12, females					
Terminal body weight (g)	24.0	22.9	23.0	20.5 (-15%)	
Brain weight (g)	1.11	1.09	1.10	1.06* (-5%)	1.01–1.12 Mean 1.08

PND 63, males					
Terminal body weight (g)	369	364	355	342 (-8%)	342–382 Mean 359
Brain weight (g), post-perfusion	2.03	2.01	2.00	1.93* (-5%)	1.89–2.11 Mean 1.99
Dorsal cortex thickness, level 4 (mm)	1.53			1.36** (-11%)	1.11–1.53 Mean 1.36
Dorsal cortex thickness, level 5 (mm)	1.40			1.32 (-6%)	1.19–1.41 Mean 1.32
Thalamus width, level 4 (mm)	8.98			8.39** (-7%)	8.27–8.86 Mean 8.59
Thalamus width, level 5 (mm)	8.11	8.04	7.93	7.49** (-8%)	7.41–7.98 Mean 7.78
Hippocampus width overall, level 5 (mm)	1.55	1.54	1.61	1.45* (-6%)	1.31–1.54 Mean 1.47
PND 63, females					
Terminal body weight (g)	228	220	218	207 (-9%)	205–232 Mean 218
Brain weight (g), post-perfusion	1.89	1.89	1.82*	1.80* (-5%)	1.75–1.92 Mean 1.82
Dorsal cortex thickness, level 4 (mm)	1.41			1.29 (-9%)	1.16–1.43 Mean 1.33
Dorsal cortex thickness, level 5 (mm)	1.41	1.39	1.35	1.33** (-6%)	1.19–1.34 Mean 1.29
Thalamus width, level 4 (mm)	8.46	8.51	8.73*	8.01** (-5%)	8.19–8.71 Mean 8.41
Thalamus width, level 5 (mm)	7.88	7.65	7.74	7.28** (-8%)	7.18–7.72 Mean 7.57
Hippocampus width overall, level 5 (mm)	1.55			1.46* (-6%)	1.34–1.58 Mean 1.46

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Missing morphometry values for 50 ppm and 400 ppm: Not analysed in the main study, analysed in a supplemental study and were processed several years after the main study. As variations between groups processed early (high-dose and control) versus those processed later (low-dose and mid-dose) could reflect a processing artefact, the values from the supplemental study are not included in the table.

[a] 11 studies conducted by the same laboratory in the same strain within 3 years of the present study (the present study started 03/2002, the HCD covers studies starting 10/2001–10/2004); the current study not included.
The ranges are ranges of control means per study (i.e. range for 11 mean values), the mean is a mean of means from the individual studies.

As to brain weights, scatter plots of the historical control data provided by industry during the public consultation (see 'Supplemental information') indicate a correlation between body weight and brain weight on PND 12. The regression equations predict a brain weight reduction of 4 % for top dose males and 5 % for top dose females, which are exactly the values observed in the study. Thus, the observed reductions in brain weight at the top dose on PND 12 can be attributed to reduced body weight.

The correlation between brain weight and body weight on PND 63 is apparently weaker. If the correlation is taken as significant (statistical significance of the slope is not reported in the

position paper), the observed reductions are slightly larger than the predicted reductions (males: predicted 3 %, observed 5 %; females: predicted 2 %, observed 5 %), which suggests a contribution of a direct effect on the brain. The brain weights at the top dose are still within the HCD range. This does not mean that the statistically significant brain weight reductions at the top dose are not treatment-related, but indicates a relatively low magnitude of the effect.

While there seems to be a weak correlation between brain weight and body weight on PND 63, there is no correlation between morphometry measurements and body weight on PND 63 (based on a limited analysis of the individual data from the current study and HCD means). Therefore, the observed differences in morphometry have to be taken as such, indicating a direct effect on the brain. The top dose values were mostly within the HCD range (where applicable).

No effects were found on histopathological examination or in functional and neurobehavioural tests (FOB, motor activity, auditory startle, Y-shaped water maze with one escape ladder). However, these tests may not be sufficiently sensitive for detection of subtle but adverse changes in brain function. Specifically, the Y-maze test in its basic setup (no alteration of arms) is not a very difficult task for rats and thus not very sensitive (cf. discussion of T-maze in the OECD guidance document no. 43). The study report does mention alteration of arms.

Conclusion on classification for development

RAC considers the changes in brain morphometry and brain weight observed following pre- and early postnatal exposure of rats in the developmental neurotoxicity study to constitute limited evidence of developmental toxicity, **warranting classification in Category 2**.

Classification for developmental toxicity is further supported by testicular findings in F1 rats in both 2-generation studies (discussed in the fertility section); no such effects were seen in F0 males, so the testicular tubular atrophy / germ cell loss/disorganization in F1 males is apparently a result of prenatal and/or early postnatal exposure.

Adverse effects on or via lactation

The first 2-generation study (1998) reported reductions in pup body weight by less than 11%, mostly towards the end of the lactation period when the pups already feed on maternal diet. No significant effect on pup body weight was found in the second 2-generation study (2004) using the same top dose (2 500 ppm, 156 mg/kg bw/day).

In the DNT study the pup body weight at 4 000 ppm (299 mg/kg bw/day) was reduced by 8 %, 12 %, 13 % and 15 % on PND 1, 5, 12 and 18, respectively. The magnitude of body weight reduction corresponding to the lactation period (ca. 6 %) is not considered sufficient for classification.

Overall, RAC agrees with the DS that classification for adverse effects on or via lactation is not warranted.

Overall conclusion on reproductive toxicity

Mainly based on (1) testicular tubular atrophy / germ cell loss/disorganisation in F1 rats, (2) ovarian atrophy in mice and (3) reduced size of certain brain regions (changes in weight and morphometry) in rat offspring exposed *in utero* and during the early postnatal period, RAC concludes to **classify thiamethoxam as Repr. 2; H361fd**. The effects on sexual function and fertility are supported by testicular effects seen in dogs.

RAC is of the view that **due to the low dosing in the 2-generation studies, the potential of the substance to adversely affect female sexual function and fertility has not been fully investigated**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Thiamethoxam is an insecticidal active substance used in Europe on various crops. Thiamethoxam has an existing entry in Annex VI of CLP with harmonised classification for environment as Aquatic Acute 1; H400, Aquatic Chronic 1; H410, with a generic M-factor of 10.

Overall, the dossier submitter (DS) concluded that thiamethoxam is 'not rapidly degradable', has low potential for bioaccumulation and proposed classification based on results from the most sensitive species group aquatic invertebrates:

Aquatic Acute 1 with an M-factor of 10, based on the lowest 48h EC_{50} value for invertebrates (*Cloeon sp.)* of 0.014 mg/L; and

Aquatic Chronic 1 with an M-factor 10, based on the lowest 30d NOEC value for invertebrates (*hironomus riparius*) of 0.0027 mg/L.

Degradation

Two studies have been conducted in order to investigate the hydrolytic stability of thiamethoxam (CGA293343) in sterile aqueous buffer solutions (pH 1, 5, 7 and 9) at different temperatures (25, 40, and 60°C). One additional study had also been conducted to investigate the hydrolytic stability of thiamethoxam's major soil metabolite CGA322704 (clothianidin). All three studies were in compliance with GLP, however some deviations from OECD guidelines were indicated by the DS related to the temperature (test performed at 60°C instead of 50°C) and to the pH (pH value of 5 was used instead of 4). Nevertheless, the DS considered that no significant impact on the study outcomes is expected and that the studies are still valid and acceptable.

Both studies, (OECD TG 111, Clark A., 1998c and OECD TG 111, Lowery E., 1996) indicated that thiamethoxam is stable at pH 1, 5 and 7 at 25°C (experimental). However, at pH 9 (25°C) thiamethoxam degraded with a DT_{50} of 4.2-8.4 days (respectively). At pH 9 (20°C), thiamethoxam degraded with a DT_{50} of 7.3-15.6 days (calculated).

The study with the major soil metabolite (OECD TG 111, Ulbrich, 1999) indicated that CGA322704 (clothianidin) is stable at pH 4, 5, 7 and 9 in the dark at 20°C under sterile conditions.

Two studies have been provided by the DS in order to estimate the photodegradation of thiamethoxam. Both were in compliance with GLP and follow US EPA 161-2 guidelines although no major deviations from equivalent guideline OECD TG 316 were observed by the DS and the studies were considered as acceptable.

The first study (Schwartz B., 1998b), showed a first order kinetic degradation for thiamethoxam in aqueous buffers solutions at pH 5, under photolytic conditions. A half-life of 3.1 days was calculated. In a non-irradiated control or hydrolytic conditions at pH 5, thiamethoxam did not significantly degrade.

In the second study (Sparrow K., 1997c), the half-life of thiamethoxam under photolytic conditions was 2.3 days. Thiamethoxam also did not significantly degrade under non-irradiated control or hydrolytic conditions at pH 5.

In a ready biodegradation study following OECD TG 301B, biodegradation of thiamethoxam was observed with 7% mineralisation by day 29 (Grade R, 1996). Therefore, thiamethoxam was considered as "not readily biodegradable".

According to an aerobic mineralization study (OECD TG 309, Hüben, 2015a), the mineralization rate and the rate and route of degradation of thiamethoxam was investigated in Heiminghausen natural lake water amended with 0.01 g/L suspended sediment. The mineralization was low (< 1.6% AR) in all systems tested. No adsorption on suspended sediments was observed. The best-fit DT_{50} range for thiamethoxam in natural sediment amended lake water was 87 to 96 days. There were no significant observed differences between the degradation rate of the low and the high dosed systems. Two metabolites were formed: CGA355190 reached a maximum of 36.56% after 61 days and metabolite NOA404617 reached a maximum of 8.8 % after 61 days.

The fate and behaviour of thiamethoxam in the aquatic environment has been investigated in two water/sediment studies (Adam, 1998a&b). No guideline was followed in both studies. However, only minor deviations from OECD TG 308 guidelines were observed. The two systems used in the studies had very similar pH values and sediment textures. The OECD guideline recommends that both textures should have silt contents different from at least 20%. However, the DS consider that no significant impact on the results is expected and that the studies are still valid and acceptable. In support of these studies, an additional study following OECD TG 308 has been conducted in order to determine the degradation behaviour of thiamethoxam in a water/sediment system, with a water and sediment pH < 7, under aerobic conditions (Kang, 2015). Kinetic analysis was also performed according to FOCUS kinetics guidance (Ford S., 2015h). The maximal amount of thiamethoxam observed in sediment was 36.6% AR after 8 days. The active substance mineralization was low: max. 12% AR after 100 days. Several metabolites were observed in whole water-sediment systems: CGA355190 (max 8.9% AR after 100 d), NOA407475 (max. 47.4% AR after 42 d) and NOA404617 (max. 8% AR after 48 d). Non-extractable residues and mineralization reached respectively a maximum of 22.2-51.3 % AR after 80-100 days and 11.96% AR after 100 days. No DT_{50} values have been considered as reliable and robust enough in water-sediment systems for the active substance thiamethoxam and its metabolites.

Overall, due to the results summarised above, the DS consider that thiamethoxam is not readily biodegradable and not degraded in the aquatic environment to a level > 70 % within a 28-day period. As a consequence, thiamethoxam was considered as not rapidly degradable, according to the CLP criteria.

Aquatic Bioaccumulation

No experimentally derived BCF was available. The experimentally derived Log K_{ow} of thiamethoxam was -0.13 at 25°C (Stulz, 1995b). For classification and labelling purposes a substance with Log K_{ow} < 4 may be considered unlikely to bioaccumulate in aquatic organisms. Therefore, DS concluded that thiamethoxam has a low potential for bioaccumulation.

Aquatic Toxicity

The relevant and most representative information for classification according to DS are summarised in the following tables and sections. All data refers to thiamethoxam as the test substance.

Acute Aquatic toxicity

Test organism	Guideline, test method	Short-term result (endpoint)	Reference
Oncorhynchus Mykiss	OECD TG 203 (1992) 92/69/EEC C.1 FIFRA No. 72-1 (1989) / GLP	96h LC_{50} > 125 mg/L (mm)	Anonymous (1996a)
Asellus aquaticus	Not stated / GLP (generally could follow OECD TG 202)	48h EC_{50} = 0.084 mg/L (nom)	Ashwell and Dark (2002)
Cloeon sp.	No guideline available, so based on: OECD TG 202 (1984) 92/69/EEC Part C.2 (1992) OPPTS 850.1010 (1996) EPA 540/9-86-141 FIFRA No.: 72-2	**48h EC_{50} = 0.014 mg/L (nom)**	Knauer (2000)
Selenastrum capricornutum	OECD TG 201, 92/69/EEC Part C.3 (1992) / GLP	72h E_rC_{50} > 81.8 mg/L (mm)	Grade (1996a)
Lemna gibba	ASTM 1415-91 FIFRA No. 122-2 and 123-2 OECD 1996 Draft guideline for the Anabeana toxicity test OPPTS Draft proposal April 1996, GLP	7d E_rC_{50} > 90.2 mg/L (mm)	Grade (1998c)

All available studies were considered acceptable and reliable by the Rapporteur Member State for the risk assessment and are therefore used by the DS for classification, even if conditions of these studies do not fully comply with the current guidance or if there is no agreed testing guideline for non-standard test species, for example *Asellus aquaticus* or *Cloeon sp*. However, these studies comply with the current reliability and validity criteria for acute toxicity testing with *Daphnia magna*. Therefore, these studies were considered reliable and valid, and therefore relevant for classification by the DS.

Overall, based on these results the DS considered that the most sensitive group is aquatic invertebrates with an EC_{50} of 0.014 mg/L for *Cloeon sp.* and propose classification as Aquatic Acute 1, M=10.

Chronic Aquatic Toxicity

Test organism	Guideline, test method	Long-term result (endpoint)	Reference
Sheepshead minnow (Cyprinodon variegatus)	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 210 (draft): Fish, Early-Life Stage Toxicity Test (2013) US EPA Ecological Effects Test Guidelines, OCSPP 850.1400 (public draft): Fish Early Life-Stage Toxicity Test, Freshwater and Marine (1996) / GLP	28d NOEC = 1.7 mg/L (mm)	Anonymous (2015)
Oncorhynchus mykiss	U.S EPA 540/9-82-024 (1982), U.S EPA 540/9-86-138 (1986), ASTM Standard E1241-88 (1988) / GLP	88d NOEC = 20 mg/L (mm)	Anonymous (1997)
Daphnia magna	OECD TG 202 (1984), Revised draft OECD guideline 202 Part II (1996), FIFRA Guideline No 72-4 (1989) / GLP	21d NOEC = 100 mg/L (nom)	Neumann (1997b)
Mysidopsis bahia	US EPA Ecological Effects Test Guidelines, OCSPP 850.1350: Mysid Chronic Toxicity Test (1996)/ GLP	28d NOEC = 0.560 mg/L (nom)	Sayers (2015)
Chironomus riparius	OECD Proposal for Toxicity Test with Chironomidae, November (1997) Proposal for a BBA Guideline: Effects of Plant Protection Products on the Development of Sediment-Dwelling Larvae of Chironomus	**30d NOEC = 0.0027 mg/L (mm)**	Grade (1998b)

	riparius in a Water-Sediment System (1995) / GLP		
Selenastrum capricornutum	OECD TG 201 (1984) 92/69/EEC Part C.3 (1992) /GLP	72h NOE_rC = 81.8 mg/L (mm)	Grade (1996a)
Lemna gibba	ASTM 1415-91 FIFRA No. 122-2 and 123-2 OECD 1996 Draft guideline for the Anabeana toxicity test OPPTS Draft proposal April 1996 / GLP	7d NOE_rC = 90.2 mg/L (mm)	Grade (1998c)

All provided studies comply with the current reliability criteria and were considered as acceptable and reliable by the RMS for the risk assessment even if conditions of these studies do not fully comply with the current guidance. Any minor deviations from the guideline do not affect the reliability of the studies. They are therefore used by the DS for classification.

Overall, the DS considers that the most sensitive group is aquatic invertebrates with an NOEC = 0.0027 mg/L for *Chironomus riparius* and propose classification Aquatic Chronic 1, M=10 considering that thiamethoxam is not rapidly degradable.

Comments received during public consultation

Three MSs submitted comments on the environmental part of the DS's proposals. One of them agreed that thiamethoxam is "not readily biodegradable", however they pointed out that in the substance approval process under the biocidal product regulation, further information is available regarding degradation in soil and in water/sediment systems. In response, the DS noted that CLH report has been based on the data submitted in the frame of the EU PPP renewal dossier for thiamethoxam. During public consultation no additional data on degradation was provided. Consequently, RAC has evaluated available data which was provided in the dossier.

The second MS supported Aquatic Chronic 1 with M factor of 10. However, they mentioned that the surrogate approach using acute endpoints in the range 0.01 to 0.1 mg/L (including the most acutely sensitive endpoint and acute toxicity to *Chironomus* endpoint) should be noted. In response, the DS noted that the surrogate approach would also support the Aquatic Chronic 1, M=10 proposal.

The third MS supported the conclusion that thiamethoxam is considered not readily biodegradable and unlikely to have a potential for bioaccumulation, for classification purposes. They also agreed that the most sensitive group is invertebrates and that the key data for aquatic acute classification is from a non-guideline study using a species of mayfly, *Cloeon dipterum* with an EC_{50} of 0.014 mg/L. For aquatic chronic classification, the MS pointed out that the lowest value is from a study performed with midge larvae, *Chironomus riparius* with a NOEC value of 0.0027 mg/L. However, they indicated that the proposed NOEC is based on the measured geometric mean. Hence, the mean measured concentrations declined below the level of detection during the test and the concentrations of the test medium were analysed only three times during the test. Therefore, the actual NOEC is lower than that based on nominal concentrations. The DS agreed that the actual NOEC would be lower than that based on nominal concentrations and

therefore expressed the endpoint in terms of geomean measured concentrations. The DS also pointed out that surrogate approach would lead to the same classification and M-factor.

Assessment and comparison with the classification criteria

Degradation

In addition to the studies assessed in the CLH report, one more study on the photodegradation of thiamethoxam and one study with the major soil metabolite of thiamethoxam were included in the dossier (RAR Vol. 3 B8). These studies were not assessed in the CLH report by the DS but were taken into account by RAC as supplemental information. The photodegradation study was done in compliance with GLP and no major deviations from OECD TG 316 were observed. The results from the soil degradation study with thiamethoxam (Zetzsch C., 1997) showed that half-lives estimated for different seasons vary between 0.8 days in summer and 8 days in winter with an annual half-life of 1.2 days at 40°N and of 1.6 days at 50°N, respectively. The study with the metabolite (Rüdel, 1998) indicated that the half-life for major soil metabolite CGA322704 (clothianidin) referring to direct photolysis in natural sunlight for Northern latitude 52°N vary between 7.2 hours in summer and 8.5 days in winter.

Regarding photolysis, test results thiamethoxam seems to be primarily degraded with half-life <16 days, however information on photochemical degradation is difficult to use for classification purposes. The actual degree of photochemical degradation in the aquatic environment depends on local conditions (e.g. water depth, suspended solids, turbidity as well as seasonal influences). Photolytic degradation also led to formation of at least 22 - 25 components including clothianidin, which seems to be more toxic than the parent substance and meets the criteria for classification as hazardous to the aquatic environment (clothianidin has a current entry in Annex VI to the CLP regulation as Aquatic Acute 1 and Aquatic Chronic 1, M=10). Consequently, primary degradation via photolysis cannot be used to conclude that thiamethoxam is rapidly degradable.

Hence, RAC considers that thiamethoxam is not readily biodegradable and there is not sufficient information to show that thiamethoxam is ultimately degraded to a level > 70 % within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products.

In conclusion, RAC agrees with the DS that thiamethoxam should be considered as not rapidly degradable.

Aquatic Bioaccumulation

The experimentally derived Log K_{ow} of thiamethoxam -0.13 at 25 °C is well below the CLP trigger of ≥ 4. Therefore, RAC agrees with the DS's conclusion that thiamethoxam has a low potential for bioaccumulation.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for thiamethoxam and its metabolites for fish, aquatic invertebrates and algae included in the CLH dossier. However, as thiamethoxam is an insecticide, invertebrates are expected to be the most sensitive group for both acute and chronic toxicity. All relevant metabolites with exception of the major soil metabolite CGA322704 (clothianidin) are less toxic and are not considered as relevant in the further evaluation on classification. Although, RAC recognises that metabolite CGA322704 (clothianidin) is more acutely and chronically toxic than the parent substance. However, as clothianidin occurred in soil and not in aqueous media, RAC considers that clothianidin is not relevant for aquatic hazard classification purposes (see section "Supplemental information - In depth analyses by RAC").

Acute Aquatic Toxicity

Relevant acute toxicity data of thiamethoxam is available for a wide range of invertebrate species, including *Daphnia magna*, *Daphnia pulex*, *Mysidopsis bahia*, *Crassostrea virginica*, etc. (25 invertebrate species in total). Therefore, only aquatic invertebrate species with EC_{50} <1 mg/L (most sensitive) have been summarized in following table.

Test organism	**Guideline, test method**	**Short-term result (endpoint)**	**Reference**
Asellus aquaticus	Not stated / GLP	48h EC_{50} = 0.084 mg/L (nom)	Ashwell and Dark, 2002
Crangonyx pseudogracilis	Not stated / GLP	48h EC_{50} = 0.42 mg/L (nom)	Ashwell and Dark, 2002[b]
Ostracoda	GLP / based on OECD, EEC and EPA guidelines with modifications.	48h EC_{50} = 0.18 mg/L (nom)	Knauer, 2000d[a]
Chironomus riparius	GLP / FIFRA Series 72-2 (OECD TG 235)	48h EC_{50} = 0.035mg/L (nom)	Mank and Krueger, 1998[a]
Chironomus riparius	Not stated / GLP	48h EC_{50} = 0.045 mg/L (nom)	Ashwell and Dark, 2002[b]
Chironomus riparius	Not stated / GLP	48h EC_{50} = 0.071 mg/L (nom)	Pickervance et al., 2003 [c, d]
Cloeon dipterum	Not stated / GLP	48hr EC_{50} = 0.021 mg/L (nom)	Ashwell and Dark, 2002[b]
Cloeon dipterum	Not stated / GLP	48h EC_{50} = 0.044 mg/L (nom)	Pickervance et al., 2003 [c, d]
Cloeon sp.	No guideline available, so based on: OECD TG 202 (1984) 92/69/EEC Part C.2 (1992) OPPTS 850.1010 (1996) EPA 540/9-86-141 FIFRA No.: 72-2	**48h EC_{50} = 0.014 mg/L (nom)**	Knauer, 2000
Coenagrionidae	Not stated / GLP	48h EC_{50} = 0.98 mg/L (nom)	Ashwell and Dark, 2002[b]
Dytiscidae	Not stated / GLP	48h EC_{50} = 0.069 mg/L (nom)	Ashwell and Dark, 2002[b]
Dytiscidae	Not stated / GLP	48h EC_{50} = 0.047 mg/L (nom)	Pickervance et al., 2003 [c, d]

RAC acknowledges that some of the most sensitive species were not standard test species and there is no standardised testing guideline for these species. Nevertheless acute data are available for three different insect groups (mayfly, water beetle and midge) plus one crustacean species (water louse) and are in the same range for classification purposes. The substance is an insecticide, hence it is appropriate to consider the data even though not all of them are conducted to standard test guidelines. RAC recognises that most of studies comply with the current reliability and validity criteria for acute toxicity testing with *Daphnia magna* (equal to OECD TG 202) or with Sediment-Water Chironomid Toxicity Using Spiked Water test (OECD TG 219) and are relevant and reliable for classification.

Consequently, data are available for all three trophic levels, RAC agrees that the lowest acute endpoint for aquatic acute classification purpose is the 48h EC_{50} value for *Cloeon sp.* of 0.014 mg/L based on nominal concentrations.

Aquatic Chronic

RAC notes that the most sensitive invertebrate specie for aquatic **chronic** toxicity (*Chironomus riparius*) is not the most sensitive invertebrate species in invertebrate **acute** testing (*Cloeon sp.*). However, RAC also notes that in acute testing, toxicity to both species (*Chironomus riparius* and *Cloeon sp.*) were in same order of magnitude. Furthermore, RAC is of the opinion that the *Chironomus* tests are acceptable for classification purposes in this case because there is very little dissipation to sediment.

Hence, RAC gave preference to the available chronic toxicity data instead of using the surrogate approach. However, it should be noted that the surrogate approach would lead to the same aquatic chronic classification and M-factor.

Consequently, RAC agrees that lowest chronic endpoint for aquatic chronic classification purposes is the 30d NOEC value for *Chironomus riparius* of 0.0027 mg/L, based on geometric mean measured concentration. However, RAC would like to stress that if further aquatic chronic studies will became available with invertebrates (especially with *Cloeon*) the classification of thiamethoxam may require revision.

Conclusion on classification

Thiamethoxam is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC is of the opinion that thiamethoxam warrants classification as:

- **Aquatic Acute 1** based on an EC_{50} = 0.014 mg/L for *Cloeon sp.* As this acute toxicity value falls within the 0.01 < L(E)C_{50} ≤ 0.1 mg/L range, the **acute M-factor is 10**.
- **Aquatic Chronic 1** based on a NOEC = 0.0027 mg/L for *Chironomus riparius*. As this chronic toxicity value falls within the 0.001 < NOEC ≤ 0.01 mg/L range, the **chronic M-factor is 10.**

Additional references

Creasy *et al.* (2012) Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. Toxicologic Pathology 40:40S-121S

Goedken; Kerlin; Morton (2008) Spontaneous and age-related testicular findings in Beagle dogs. Toxicologic Pathology 36:465-471

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).