Committee for Risk Assessment
RAC

Opinion
proposing harmonised classification and labelling
at EU level of

Thiacloprid (ISO); {(2Z)-3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide

EC Number: N/A
CAS Number: 111988-49-9

CLH-O-0000001412-86-54/F

Adopted

12 March 2015

15 March 2015

CLH-O-0000001412-86-54/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 harmonized classification and labelling (CLH) of:

Chemical name: **Thiacloprid (ISO); {(2Z)-3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide**

EC Number: **N/A**

CAS Number: **111988-49-9**

The proposal was submitted by **Ireland** and received by RAC on **05 December 2013.** 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 **04 February 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 March 2014**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Stephanie Vivier**

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 harmonized classification and labelling was adopted on **12 March 2015** 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	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current entry										
Dossier submitters proposal		thiacloprid (ISO); {(2Z)-3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	-	111988-49-9	Carc. 2 Repr. 2 Acute Tox. 4 Acute Tox. 3 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H301 H400 H410	GHS06 GHS08 GHS09 Dgr	H351 H361f H332 H301 H410		M=100 M=100	
RAC opinion		thiacloprid (ISO); {(2Z)-3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	-	111988-49-9	Carc. 2 Repr. 1B Acute Tox. 4 Acute Tox. 3 STOT SE3 Aquatic Acute 1 Aquatic Chronic 1	H351 H360FD H332 H301 H336 H400 H410	GHS06 GHS08 GHS09 Dgr	H351 H360FD H332 H301 H336 H410		M=100 M=100	
Resulting Annex VI entry if agreed by COM		thiacloprid (ISO); {(2Z)-3-[(6-chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	-	111988-49-9	Carc. 2 Repr. 1B Acute Tox. 4 Acute Tox. 3 STOT SE3 Aquatic Acute 1 Aquatic Chronic 1	H351 H360FD H332 H301 H336 H400 H410	GHS06 GHS08 GHS09 Dgr	H351 H360FD H332 H301 H336 H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

No classification was proposed by the Dossier Submitter (DS) for physical hazards based on the negative results in standard tests for explosivity (EEC-method A14) and flammability (EEC-method A10). Additionally, it was mentioned that thiacloprid did not liberate any flammable gases in contact with water (EEC-method A12), did not exhibit any pyrophoric properties (EEC-method A13) and did not show self-heating properties (no self-ignition according to the EEC-method A16).

The DS stated that according to its chemical structure, thiacloprid is considered to have no oxidizing properties: it does not contain chemical groups typical for oxidizing agents and it is regarded as incapable in reacting exothermically with a combustible material such as powdered cellulose.

Comments received during public consultation

No specific comments were received, but three Member States (MSs) provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

Based on negative results in standard studies, RAC supported the proposal of DS not to classify thiacloprid for physical hazards.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify thiacloprid in category 3 for acute toxicity via oral route (Acute Tox. 3; H301). Identified LD_{50} values ranged from 177 to 444 mg/kg in female rats. One OECD TG 401 study in rats indicated an LD_{50} of 444 mg/kg in females and of 836 mg/kg in males. Another acute toxicity study showed an LD_{50} of 396 mg/kg in female and 621 mg/kg in male rats.
In one acute neurotoxicity study in rats the LD_{50} was found to be 177mg/kg (only females tested).

The DS proposed a classification in Category 4 for acute toxicity via inhalation route (Acute Tox. 4; H332) based on a single acute inhalation toxicity study in the rat conducted according to the OECD TG 403 (CAR A6.1.3 (1996)), which resulted in an LC_{50} of 1.2 mg/L in females.

The DS proposed not to classify for acute toxicity via the dermal route since no mortality or clinical signs of toxicity were observed at the tested dose of 2000 mg/kg in an acute dermal toxicity study in the rat conducted in compliance with an OECD TG 402 (CAR A6.1.2 (1996b)) (LD_{50} >2000 mg/kg).

Comments received during public consultation

One MS indicated its support for the proposal on this specific endpoint and three MSs provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

For acute toxicity via oral route, the lowest LD_{50} obtained from an acute neurotoxicity study, conducted according to a US-EPA guideline, was 177 mg/kg in females, which is within the range of 50-300 mg/kg meeting the CLP criteria for Acute oral toxicity Category 3. The second lowest LD_{50} obtained in acute standard tests was close to this range (396 mg/kg) and therefore supports this classification. In conclusion, RAC supports the DS's proposal to classify thiacloprid as Acute Tox. 3; H301.

The LC_{50} obtained in an acute inhalation toxicity standard test was 1,2mg/L which is within the range of 1-5 mg/L for dusts meeting the CLP criteria for Acute Tox. 4; H332. In conclusion, RAC supports the DS's proposal to classify thiacloprid as Acute Tox. 4; H332.

The LD_{50} obtained in an acute dermal toxicity standard test was above the cut-off value for classification (2000 mg/kg) according to the CLP criteria. RAC supports the DS proposal not to classify thiacloprid for acute dermal toxicity.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

No classification for STOT SE was proposed by the DS since there were neither human data to provide information on this end point nor any clear evidence of any specific toxic effects on any target organ or tissue from the animal data available.

Clinical signs of toxicity observed after single exposures to thiacloprid were transient in nature and they were considered to be non-specific signs of general acute toxicity.

In the acute inhalation study, clinical signs included bradypnoea, dyspnoea, laboured breathing, rales and red encrustations around snout and nose. However, these clinical signs were considered insufficient by the DS to regard the substance as a respiratory irritant since these signs were considered as common observations during acute inhalation studies.

Comments received during public consultation

Three MSs provided their general support for the CLH proposal and on this endpoint, one MS suggested to consider a classification for STOT SE (1 or 2) for effects on the nervous system since the clinical signs were considered as severe (poor reflexes, impaired motor and locomotor activity, spastic gait, tremor). Some of these clinical signs were observed without co-occurring other effects or mortality, the effects were considered as transient (up to 6 days post exposure) and consistent with the mode of action of the substance (selective nicotinic acetylcholine receptor agonist), and although the effects were not reported in longer-term studies, this could be explained by applying different routes of administration than in the acute toxicity studies (gavage/inhalation versus diet).

Assessment and comparison with the classification criteria

Respiratory tract irritation
No human data was available. In animal studies, the breathing pattern showed some concentration-dependent respiratory distress (bradypnea, dyspnea, rales, labored breathing),

starting at 0.48 mg/L in the acute inhalation study. This dose did not cause lethality but there were some signs of general toxicity including significantly reduced body weight (no detailed results were given in the CLH report) and hypothermia. Additionally, red encrustations around snout and nose and chromodacryorrhea were observed (no information on the dose-responses were provided in the CLH report), which indicated a general alteration in the condition of the animals. Necropsy findings revealed reddish coloured lungs and red foci in the decedents but not in the animals killed at scheduled sacrifice. In a 4-week study, the highest test concentration was reduced because of respiratory distress but also because of a significantly reduced body weight. At the new top concentration of 0.143 mg/L, the similar respiratory effects co-occurred again with hypothermia and reduced body weight. At necropsy, there were no effects in female lung weights (females were more sensitive to the lethal effect than males) whereas some effects were observed in males, but no dose-response relationship or histopathological findings indicative of any histological alterations in the respiratory tract were reported. Thus, RAC concludes that the reported clinical signs on breathing pattern are insufficient to justify classification and RAC supports the conclusion of the DS that no classification for respiratory tract irritation is warranted. The neurotoxic potential of thiacloprid is assessed by RAC below.

Neurotoxicity

In the standard acute toxicity studies in rats, clinical signs associated with effects on nervous system were reported, including prostration, poor reflexes, decreased/reduced motility, apathy, spastic gait, spasmodic state, convulsions and tremor. These effects occurred concurrently with other signs of toxicity (diarrhea/constipation, piloerection, bradypnea, dyspnea, lacrimation, hypothermia, 'significantly reduced body weight' in the inhalation study (no data details were provided in the CLH report), but they were not associated with any necropsy findings or changes in brain weight.

Thiacloprid was also tested in several acute neurotoxicity studies in rats via oral route:

- In a dose range-finding study (CAR A6. 9 (1997)), tremors, decreased activity and repetitive chewing movements were found at lethal doses. Clinical signs that were slight and reversible within 24 h were reported also at sub-lethal doses. These consisted of slight repetitive chewing movements in males at 35 mg/kg, and of slight tremors and repetitive chewing movements in both sexes at 85 mg/kg. Other signs of toxicity had resolved by day 7.
- In the main neurotoxicity study (CAR A6. 9 (1997)), no lethality was observed up to the highest tested dose of 109 mg/kg. Clinical signs included tremors, decreased activity, ataxia, dilated pupils, ptosis and reduced body temperature, which occurred on the day of treatment and was typically resolved by day 1, and by day 5 at the latest. Few animals showed these signs at 22 mg/kg but toxicity was reported to be severe at the top dose of 109 mg/kg. Also reversibly decreased motor activity was reported in females at all doses (27%, 41%* and 71%* at 22, 53 and 109 mg/kg, respectively, being statistically significant from 53 mg/kg), and at 109 mg/kg in males.
- In an additional acute neurotoxicity study (CAR A6.9 (1998)), female rats were dosed with 3.1 mg/kg and 11 mg/kg which were below the lowest dose of the above mentioned main study. Slight decreases in motor and locomotor activities were observed at the top dose.

All described effects were considered as non-specific by the DS. RAC acknowledges that most of ***these effects are common in acute toxicity studies (e.g. tremors, decreased motility or reactivity,*** poor reflexes and prostration). The effects are considered as transient since clinical signs were reversible within 24 hours and other effects resolved by day 7. In addition, the effects were not observed in a 13-week neurotoxicity study, in which the same parameters were tested at the same doses as tested in the acute neurotoxicity studies. According to the CLP Regulation, transient functional changes are not considered relevant for classification for STOT SE Category 1 or 2.

Category 3 covers 'transient effects' occurring after single exposure, specifically respiratory tract irritation and narcotic effects. Classification in Category 3 is primarily based on human data which was not available for thiacloprid. According to the CLP criteria, also narcotic effects that are observed in animal studies and that may include lethargy, lack of coordination, loss of righting

reflex and ataxia can justify classification of substances for narcotic effects in Category 3. These narcotic effects were observed in animal studies on thiacloprid. STOT SE and acute toxicity are independent of each other, and STOT SE will be considered where there is clear evidence for specific organ toxicity especially in the absence of lethality. In standard acute studies on thiacloprid, neurotoxic effects occurred at doses causing general toxicity and they were likely related to the toxicity that resulted in death of the animals. However, available information from several acute neurotoxicity studies showed transient and significant neurotoxic effects (e.g. decreased motor activity) at non-lethal doses and in the absence of any other effects (from 11 mg/kg in females, 10-fold below doses causing lethality), and these effects are considered by RAC to fulfil the criteria for classification as STOT SE 3 according to CLP.

RAC concludes that the effects observed in the acute neurotoxicity studies on thiacloprid fulfil the CLP criteria for classification as STOT SE 3; H336 (for narcotic effects).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The skin irritation potential of thiacloprid (97.3% purity) had been tested in three male New Zealand White rabbits in accordance with the OECD TG 404 (CAR A.6.1.4 (1995c)). Thiacloprid caused slight (grade 1) erythema in all three animals which was reversible by 72 hours. This response did not meet the CLP criteria for classification as a skin irritant and therefore DS did not propose any classification for this hazard class.

Comments received during public consultation

No specific comments were received, but three MSs provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

In a standard OECD TG 404 test, thiacloprid caused slight (grade 1) reversible erythema in all three animals tested. The score was below the cut-off value for classification (2 animals out of 3 with a mean score ≥ 2.3) and RAC agrees with DS that thiacloprid does not meet the classification criteria for skin corrosion/irritation.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The severe eye damage/eye irritation potential of thiacloprid (97.3% purity) had been tested in three male New Zealand White rabbits in accordance with the OECD TG 405(CAR A.6.1.4 (1995c)). No corneal or iridial lesions were evident. Conjunctival redness (grade 1) and swelling (grade 1 and 2) were observed in all animals at the 1- and 24-hour observation points. The average score for the three animals was 0.6 for conjunctival redness and 0.6 for chemosis at 24 hours. All ocular lesions had resolved by 48 hours post application. The DS did not propose to classify thiacloprid for serious eye damage/eye irritation.

Comments received during public consultation

No specific comments were received, but three MSs provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

In a standard OECD TG 405 test, thiacloprid caused only mild and transient eye irritation characterised by conjunctival redness and swelling for which the maximal average score per animal was 0.6. As such, RAC agrees with the DS that thiacloprid does not meet the CLP classification criteria for eye irritation (average score for conjunctival redness ≥ 2, and/or conjunctival oedema ≥ 2, in at least 2 of 3 tested animals calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material and which fully reverses within an observation period of 21 days) or for serious eye damage.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

In a standard Magnusson and Kligman Guinea pig maximisation test, 10 test animals were treated with intradermal injections of thiacloprid (purity 97.3%) (0.1 ml) at 5 %, by topical induction (0.5 ml) at 50 %, and challenge at 25% (CAR A.6.1.5 (1996)). Skin reactions (grade 1) occurred in 1/10 animals and were observed at both 48 and 72 hours after the challenge. This is below the response of 30% required for classification, and the DS did not propose to classify thiacloprid as a skin sensitiser.

Comments received during public consultation

No specific comments were received, but three MSs provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

The skin sensitization potential of thiacloprid (97.3% purity) had been tested in a standard Guinea Pig Maximisation Test. Skin reactions occurred in 1/10 treated animals after intra-dermal induction with a 5% solution and RAC agrees with the DS not to classify thiacloprid for skin sensitisation.

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

Summary of the Dossier submitter's proposal

Thiacloprid had been studied extensively in standard GLP/OECD TG compliant studies involving repeated oral exposure of rats and mice for up to two years and of dogs for up to one year. Exposure via the inhalation and dermal routes had been studied in rats for up to 28 days.
After repeated oral and inhalation exposure, the main target organs in rats, mice and dogs were the liver and the thyroid. The liver was also the target organ after dermal administration.

Liver/Thyroid
In rats and dogs, the liver effects at all doses and study durations were associated with adaptive changes and consisted of weight increases, induction of hepatic enzymes, hypertrophy and cell proliferation, with some minor histopathological changes to the hepatic cytoplasm that were probably secondary to the enzyme induction. Similar hepatic adaptive responses were reported in mouse studies with durations from 14 to 90 days, together with increased lipid content from the high dose of 765 mg/kg bw/d. Additionally, more serious histopathological changes (degeneration, fatty change, necrosis) were reported in a two-year mouse study, but only from 234 mg/kg bw/d.

The thyroid effects consisted of organ weight increases (rats), changes in the thyroid hormone levels (rats, dogs), follicular epithelial hypertrophy and follicular cell hyperplasia (rats) and were suggestive of adaptive rather than toxic effects. No effects on the thyroid were reported in mice.

The DS concluded that although there were changes in some clinical chemistry parameters (liver enzymes and thyroid hormones) at dose levels relevant for classification, these were indicative of increased liver/thyroid activity as the result of adaptive changes and, in those studies that included a recovery period, were reversible. Such adaptive responses constituted a normal biochemical or physiological response, were not considered as consistent or significant adverse changes in clinical biochemistry, haematology or urinalysis parameters and did not indicate classification according to the DS.

Neurotoxicity
Prolonged oral administration of thiacloprid to female rats for two years resulted in retinal atrophy from 3.3 mg/kg bw/d, degenerative myelopathy and degeneration of the lens from 33.5 mg/kg bw/d (20/50 at 33.5 mg/kg bw/d and 30/50 at 69.1 mg/kg bw/d). When the oral guidance value was adjusted from a 90-day study to one of 24-months' duration, a value of 12.5 mg/kg bw/d was obtained, which was below the dose at which degenerative myelopathy and degeneration of the lens were reported. However, a chronic exposure was assumed to be necessary to induce this effect since in the rat 90-day neurotoxicity study, no neurotoxic, motor or locomotor effects were recorded up to the maximum tested dose of 115 mg/kg bw/d. Additional consideration by DS on these effects was that some of the findings associated with the degenerative myelopathy were known to occur in aged rats and could be exacerbated by xenobiotics and the degeneration of the lens was present in many control animals (15/50). No adverse histological findings occurred in the neurological system of mice at doses up to 875 mg/kg bw/d during a two-year study. Therefore, these two effects were not considered as significant functional changes in the central or peripheral nervous systems and did not provide a sufficient basis according to DS to justify the classification of thiacloprid for repeated dose toxicity.
Other effects
Increases in the weights of prostate (associated with hypertrophy of the glandular epithelium) and uterus in dogs and of adrenal glands in mice were reported, but since there was no evidence of organ dysfunction, these organ weight changes did not justify classification according to the DS.

Overall, the DS did not propose to classify thiacloprid for STOT RE.

Comments received during public consultation

Three MSs provided their general support for the CLH proposal.

One MS, however, suggested that classification for STOT RE be warranted based on retinal atrophy observed in the carcinogenicity study in rats from 3.3 mg/kg bw/day and on lens degeneration observed from 33.5 mg/kg bw/day. The retinal atrophy which occurred at a dose below the (adjusted) cut-off value for classification and was an effect that may cause blindness seemed to fulfil the CLP criteria 3.9.2.7.3.g; "Evidence of appreciable cell death (including cell degeneration and reduced cell numbers) in vital organs incapable of regeneration".

Assessment and comparison with the classification criteria

The repeated dose effects of thiacloprid had been studied in animals after oral, dermal and inhalation exposures with information available on the reversibility of effects following all routes of exposure.

Liver effects
a) Thiacloprid significantly induced hepatic enzymes in a dose-related and reversible manner. The hepatic enzymes assessed included CYP 450, GST, UDPGT, 7-ethoxycoumarin deethylase (ECOD), or N-demethylase (N-DEM), O-demethylase (O-DEM), aldrin epoxidase (ALD) and epoxide hydrolase (EH). Increases in enzyme induction levels occurred in rats

- from 49.5 mg/kg bw/day in a 14-day diet study (ECOD, ALD, EH, GLU-T – no details on the results were provided in the CLH report)
- from 28.6 mg/kg bw/day in a 90-day dietary study (up to 3-fold increase in CYP 450 and GST and 5-fold increase in UDPGT at the top dose of 123.2 mg/kg bw/day and a trend of an increased enzyme induction from the lowest dose of 1.9 mg/kg bw/day)
- from 25.2 mg/kg bw/day in a 2-year dietary study (up to 2-fold increase in CYP450, GST or UDPGT at the top dose of 69.1 mg/kg bw/day and a trend of an increase from the lowest dose of 1.2 mg/kg bw/day).

As compared to rats, the enzyme induction occurred at slightly higher doses after oral treatment in dogs (at 80 mg/kg bw/day for 70 days and at 35 mg/kg bw/day for 105 days) and mice (at 765 mg/kg bw/day for 14 days and at 542 mg/kg bw/day for 90 days). Hepatic enzyme induction was also noted at the top doses of both inhalation studies in rats (at 0.143 mg/L after 28 days and at 0.205 mg/L after 5 days) but was not reported in the dermal exposure study or in the 1-year dietary study in dogs. This enzyme induction was accompanied by an increase in liver weights (>10%) and by some histopathological changes in all species, rat being again the most sensitive species:

- The lowest dose at which a significant increase in liver weight was observed was 51.7 mg/kg bw/day (top dose) in male rats in a two-year dietary study. Liver weights were approximately > 20 % than in controls (similar increase was reported after 14 days at 60 mg/kg bw/day via gavage and at 187 mg/kg bw/day via diet). Mice and dogs appeared less sensitive than rats (in mice, increases of ca 10% were noted only at 546 mg/kg bw/day in a 2 year study). Some changes in clinical chemistry parameters related to liver were observed but were not consistently reported: in a 90-day study in rats, ASAT, ALAT, AP were increased from the lowest dose of 5 mg/kg bw/day (max 17%) and with a maximum of 65% at the top dose of 120 mg/kg bw/day (in females).
- Hepatocellular hypertrophy was the most common finding. In the 2-year diet study in rats, hypertrophy occurred from the dose of 2.5 mg/kg bw/day in males (at 50 ppm). The increased incidence was dose-related (0/50, 12/50, 44/50 and 49/50 at 25, 50, 500 and 1000 ppm, respectively), and reached 98% in males at the top dose of 51.7 mg/kg bw/day (1000 ppm). In females, the incidence of hypertrophy was 60% at 33.5 mg/kg bw/day (500 ppm) and up to 72% at 69.1 mg/kg bw/day (1/50, 0/50, 30/50 and 36/50 at 25, 50, 500 and 1000 ppm, respectively). In a study of shorter duration (14-day, via diet), hypertrophy occurred in rats treated with 49.5 mg/kg bw/day. In a 2-generation study, hepatocellular hypertrophy occurred from 22 mg/kg bw/day and in several one generation studies at 54-75 mg/kg bw/day.

Hepatocellular hypertrophy did not seem to be fully reversible as hypertrophy was not reversed in 3/9 males during the recovery period of a 90-day dietary study at a dose of 123.2 mg/kg (hypertrophy was observed in 2/10 females and in 9/10 males and reversed in the females and in 6 males) and in 2/5 males after dermal exposure at 300 mg/kg bw/day. Hepatocellular hypertrophy was often observed concomitant with cytoplasmic changes. No details on these changes were always provided but when given, they included 'eosinophillic cytoplasm with basophilic strands' (rats, oral), 'homogeneously structured cytoplasm' (rats, dermal) or pale perinulcear cytoplasm (dogs). Changes in the cytoplasm were observed in mice and dogs, but did not occur in rats after gavage or inhalation routes.

More severe signs of liver toxicity were observed in long-term studies. In a two-year dietary study in mice, at the same dose that caused hypertrophy (no data on enzyme induction was provided in the CLH report), increased fat storage (fatty changes; 15/50 and 21/50 in males at 234 mg/kg bw/day and the top dose of 564.4 mg/kg bw/day, respectively, vs 3/50 in controls), and liver degeneration were noted (males only: 5/50 and 16/50 at 234 and 564.4 mg/kg bw/day, respectively, vs 0/50 in controls). Also necrosis was reported at the top dose (31/50 at 546.4 mg/kg bw/day vs 5/50 in controls in males; 25/50 at 872.5 mg/kg bw/day vs 15/50 in controls in females). In rats, increased incidences of mixed eosinophilic/clear cell foci were noted (at the same dose causing enzyme induction, hypertrophy and cytoplasmic changes) in males at 2.5 mg/kg bw/day (5/50 vs 1/50 in controls) and above (up to 22/50) and in females at the top dose 69.1 mg/kg bw/day (10/50 vs 6/50 in controls) in a 2-year dietary study. However, no neoplastic lesions were observed in these studies.In conclusion on liver effects, the most sensitive

reported effect was the dose-related reversible enzyme induction with changes in liver weights and small histopathological changes with no evidence of liver dysfunction. RAC agrees with the DS that these effects were adaptative responses to increased metabolic need due to treatment and they should not trigger classification. More severe signs of toxicity, such as necrosis and liver degeneration, occurred only after chronic exposure at dose levels (234.1 mg/kg bw/day in the 2-year study in mice) above the cut-off value for classification (i.e. 12.5 mg/kg bw/day for a 2-year oral study). RAC agrees with DS that thiacloprid should not be classified for repeated dose toxicity based on liver effects.

*Thyroid*Histopathological changes were noted in the thyroid of rats after oral, inhalation and dermal routes but not in dogs or mice. The most common finding was an increased incidence of hypertrophy or increased mitotic index within the follicular epithelium.

Hypertrophy was reported at 2.5 mg/kg bw/day in males and at 33.5 mg/kg bw/day in females in the 2-year diet study. The incidence in males was from 44 % to 68 % at the top dose level of 51.7 mg/kg bw/day, compared to 24 % in controls and in females up to 46 % compared to 12% in controls. In this study, hypertrophy was also observed in both males and females at the one-year interim sacrifice at 500 ppm (25.2 and 33.5 mg/kg bw/day in males and females, respectively). Colloid alteration and pigment were also significantly increased. Additionally, follicular cell hyperplasia was also noted at the top dose level in 6 % of females compared to 0 % in controls and increase in TSH was reported (Cf. below).

An increase in the mitotic rate was reported in a 14-day gavage study at 120 mg/kg bw/day. Similar findings were also reported following a 14-day diet study: increase in the mitotic rate from 49.5 mg/kg bw/day in males (80% vs 20% in controls) and 100% at the top dose of 187 mg/kg bw/day. At the top dose, also hypertrophy of the follicular epithelium occurred at an incidence of 100 % in males. In this study, increase in TSH was reported (Cf. below).

Hypertrophy was also noted in both sexes in a dermal study at 1000 mg/kg bw/day (reversible in females but not fully reversible in males), in a 28-day inhalation study at 0.143 mg/L, as well as in an oral 2-generation study (at 22 mg/kg bw/day) and in several one-generation studies (at 54-75 mg/kg bw/day).

Effects on TSH concentrations were also reported. In the 14-day rat diet study, a 35% increase in TSH concentrations was reported in females at the top dose of 2000 ppm (187.2 mg/kg bw/day). In a 2-year rat diet study, there were increases in TSH levels in males at all time-points and in females at some time points at the top dose (69.1 mg/kg bw/day in females and 51.7 mg/kg bw/day in males) being a trend in males at all time points (max 146% at week 26) and statistically significant in females at some time points (up to 135% increase in females at week 105, 58% in males at that time point). In these 2 studies, no effects on T3/T4 were reported although some changes in those were reported in other studies. In a 90-day study in rats, a transient increase in T3 and T4 were reported in males: at week 3, T3 was increased at all doses in a dose-related manner from 14% at 1.9 mg/kg bw/day and up to 42% at the top dose of 123.2 mg/kg bw/day whereas T4 was increased 12% at the top dose only. At week 12, increase (30%) in T3 was noted only at the top dose. In dogs, increases in T3 and decreases in T4 were reported in 2 studies (at 65.7 mg/kg bw/day in a 70-day study and at 35 mg/kg bw/day in a 105-day study), with no other effects on thyroid (no changes in TSH, no hypertrophy).

A 66 % increase in thyroid weight was also observed in a 90-day dietary study in rats at 123.2 mg/kg bw/day (a 25% increase after the recovery period). This effect was not associated with hypertrophy and hypertrophy was consistently reported in other studies despite an absence of effect on thyroid weight.
In the thyroid gland, the main reported effects were dose-related increases in mitotic index and/or hypertrophy of the follicular epithelium associated with some effects on TSH levels. The observed effects are not considered as adverse and RAC agrees with the DS that classification of thiacloprid for repeated toxicity based on thyroid effects is not warranted. Some neoplastic lesions were also observed in long-term studies and these will be discussed in section on carcinogenicity.

Other main effects

In the two-year oral rat study (CAR A6.5/6.7 (1998; amended 2007)), in females, statistically significantly increased retinal atrophy occurred from 3.3 mg/kg bw/day and degeneration of the lens (cataract) occurred from 33.5 mg/kg bw/day. The increases were dose-related for retinal atrophy (20/50, 24/50*, 25/50*, 32/50* vs 21/50 in controls with a statistical significance (*) from 3.3 mg/kg bw/day) and for lens degeneration (18/50, 16/50, 20/50*, 30/50* vs 9/50 in controls with a statistical significance (*) from 33.5 mg/kg bw/day). Increased incidences of degenerative myelopathy were also reported in both sexes. It consisted of radiculoneuropathy of the spinal cord with degeneration of sciatic nerve together with a related atrophy and degeneration of skeletal muscle (from 25.2 and 33.5 mg/kg bw/day in males and females, respectively). These effects are considered by RAC as severe and, in females the retinal atrophy started at doses below the guidance value for classification (adjusted value of 12.5 mg/kg bw/day). However, incidences of the retinal atrophy were reported to be within the historical control range at low doses and exceeded the historical control range at the top dose only (64% for retinal atrophy vs 18-60% in 9 studies from 1993) which was a dose where some general toxicity was also seen (mainly reduced body weight of 21%, liver effects). No historical control data was provided in the CLH report for lens degeneration, but for both effects it is emphasized that a low survival in the female control group (22/50 survivors at the end of the study) may have influenced the statistical analysis, which is supported by the fact that no such findings were reported in males (incidence of 21/50 in control males and 9/50 in control females for lens degeneration). An increase in incidences of degenerative myelopathy was observed in treated rats but morphology and severity of the lesions were similar to controls and to the changes described in literature for this common age-related lesion. The incidence was reported at completion of the 2-year study but not at the 1-year interim sacrifice which indicates that thiacloprid may not have changed the onset for the occurrence of this age-related pathology. The increase in incidence occurred at doses above the value for classification and no other neurotoxicity findings were reported in subchronic and neurotoxicity studies. RAC agrees with the DS that these findings are not supporting a classification for STOT RE.

In mice, incidence and severity of vacuolization of the adrenal X-zone was increased in all females that received dietary thiacloprid for either 90 days or 2 years, often leading to hypertrophy, and with severity increasing with dose. Also in the 90-day study, adrenal weights were increased (up to 42%) from 139 mg/kg bw/day. The toxicological relevance of these changes is not clear (the X-zone naturally degenerates although generally it regresses earlier, and its function is not well-known). RAC agrees with the DS that in the absence of other signs, these effects are not sufficient to trigger a classification for repeated toxicity.

Effects were also seen in the prostate and uterus of dogs, with large increases in weights (and hypertrophy of prostate epithelium). RAC agrees with the DS that these findings are not considered as serious damage and do not fulfil criteria for classification.

In conclusion, RAC agrees with the DS that thiacloprid does not fulfil the criteria for classification as STOT RE.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In a battery of *in vitro* genotoxicity studies conducted according to, or similar to, internationally accepted test guidelines including two Ames tests, one chromosome aberration test (Chinese hamster fibroblasts V79) and two gene mutation tests (HPRT/V79 cells and unscheduled DNA synthesis test in rat hepatocytes), thiacloprid did not cause gene mutations or chromosome aberrations. In addition, thiacloprid did not induce micronuclei in somatic cells in an *in vivo* mouse micronucleus test. It was concluded by the DS that no classification for mutagenicity was warranted according to the CLP Regulation.

Comments received during public consultation

No specific comments were received for this endpoint, but three MSs provided their general support for the CLH proposal.

Assessment and comparison with the classification criteria

Mutagenic properties of thiacloprid were negative in several *in vitro* assays (see above) and in one *in vivo* micronucleus assay in mice. RAC supports the conclusion of the DS that classification of thiacloprid for germ cell mutagenicity is not warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS proposed to classify Thiacloprid as Carc. 2. Oral administration of thiacloprid to rats and mice for two years resulted in increased incidences of three types of tumours: malignant uterine tumours and benign thyroid tumours in rats as well as benign ovarian tumours in mice. The tumour findings indicated that thiacloprid has a carcinogenic potential. Additional factors were also taken into account when assessing the overall level of concern and for making the decision on the category of classification.

Carcinogenicty data summary

Thyroid tumours in rats

In the 2-year rat study (CAR A6.5/6.7 (1998)), carried out according to the OECD TG 453, thiacloprid was administered through the diet at doses of 0, 25, 50, 500 or 1000 ppm. Thiacloprid induced benign follicular cell adenomas in male Wistar rats with statistical significance (*) at the two highest doses:

- Incidence of 2% (1/50 vs 0/50 in controls) at 50 ppm – eq. to 2.5 mg/kg
- Incidence of 10% (5/50* vs 0/50 in controls) at 500 ppm - eq. to 25 mg/kg
- Incidence of 16% (8/49* vs 0/50 in controls) at 1000 ppm – eq. to 52 mg/kg.

The mean historical control incidence was 1.6% (range 0-5%). One adenoma (1/10) was also observed at 1-year interim sacrifice at the top dose.

Only a slight increase was reported in females (0/50, 1/50, 1/50, 1/50, 2/48), just outside the historical control range of 0-2% (mean 0.8%).

Thyroid tumours in mice

No thyroid tumours were observed in a 2-year study in mice.

Uterine tumours in rats

In the 2-year rat study (CAR A6.5/6.7 (1998)), carried out according to OECD TG 453, thiacloprid was administered through the diet at doses of 0, 25, 50, 500 or 1000 ppm. Thiacloprid induced tumours (out of 50 animals) in the uterus as follows:

- Malignant adenocarcinoma: 6, 3, 3, 14, 18;
- Benign adenoma: 0, 0, 1, 1, 2;
- Malignant adenosquamous carcinoma: 0, 0, 0, 1, 2.

The incidence in malignant adenocarcinoma was 28% at 500 ppm (25 mg/kg) and 36% at 1000 ppm (52 mg/kg) vs. 12% in the control. The statistical analysis showed a positive trend but the analysis by pair was negative, and the tumours were therefore reported as not statistically significant by the authors. However, the incidence in the control group was high when compared to the two lowest doses and the incidences at 500 and 1000 ppm were above the historical control range of 0-24% (mean 6.6%).

The historical control incidence for adenosquamous carcinoma was not known.

Ovarian tumours in mice

In the 2-year mouse study (CAR A6.7 (1998)), carried out according to the OECD TG 451, thiacloprid was administered through the diet at doses of 0, 30, 1250, or 2500 ppm. Thiacloprid

induced ovarian tumours in mice in the highest dose groups of 1250 and 2500 ppm (475.3 and 872.5 mg/kg, respectively).
The increase in benign ovarian luteomas was as follows: 0/47, 1/48, 5/49 (10.2%), 5/47 (10.6%). No statistical significance was reached but incidences were above historical control ranges at the 2 highest doses (from the laboratory: occurrence in 6/29 studies with incidences of 2, 2, 2, 2, 4 and 6.25% with the mean of 0.64%; from the NTP: occurrence in 3/927 animals, i.e. 0.3%).
In one mouse at the top dose a malignant tumour was observed. The combined incidence of tumours was thus 6/47, i.e. 12.8%.
The total number of tumours found at necropsy was provided with no statistical difference between treated and control animals. However in the controls more malignant than benign tumours occurred.

The DS concluded the thyroid follicular cell tumours in rats to be a consequence of prolonged hormone perturbation on the thyroid-pituitary (HPT) axis following the RIVM strategy (report N°601516009) to assess the thyroid follicular cell tumours in rats:

1. Thiacloprid was not genotoxic;
2. Thiacloprid induced disturbance of the HPT axis at or below the doses at which tumour induction occurred.
    - Evidence for a (histo)pathological sequence of events characteristic of prolonged thyroid stimulation in repeated-dose rat studies: increased thyroid weight, mitotic rate and follicular cell hypertrophy;
    - Evidence for sustained alterations in circulating hormones (TSH + T3 or T4): increased TSH was recorded in the two-year rat study, and temporary alterations in T3/T4 were reported in a 90-day study.
    - Additional information or experimental evidence on the mode of action: thiacloprid had no direct inhibitory effect on thyroid peroxidase (TPO) according to a specific in vitro study on hog thyroid extract. However, from repeated toxicity studies, thiacloprid had shown to induce liver enzymes (CYP P450, PROD, BROD and UDP-glucuronyl transferase). Substances were thought to induce thyroid tumours through a perturbation of the thyroid-pituitary axis as a consequence of liver enzyme induction since liver enzyme induction in rats led to an enhanced metabolism and excretion of thyroid hormones and a consequent prolonged thyroid stimulation by increased production of TSH. Humans were stated to be less sensitive to this mechanism of action because of the reservoir of thyroid hormone that is bound to thyroxine binding globulin.

The DS concluded that the thyroid tumours in rats were of low relevance for humans and based the classification proposal on the two other types of tumours (uterine and ovarian tumours).

The increased incidences in uterine tumours in rats and ovarian tumours in mice were observed together with severe general toxicity (reduced bodyweight of 15-20% in rats and histopathological degenerative changes in both species) and therefore according to the DS the tumours had a more doubtful relevance for carcinogenicity in humans. The tumours were mainly benign and not statistically significant. A prolonged perturbation of sex hormones was proposed by the DS to be the most likely mechanism of action at least for the uterine tumours. The absence of evidence for a genotoxic mechanism of action (but rather a hormonal disturbance) lowers the level of concern for humans. Besides, the tumours were species-specific: mice did not exhibit uterine tumours and ovarian tumours were not observed in rats. Based on the above considerations, classification in Category 2 was judged the most appropriate by the DS.

Comments received during public consultation

Three MSs provided their general support for the CLH proposal.
In addition, for this endpoint, three specific comments were received.
One MS agreed with the proposed classification as Carc. 2 based on (i) increased incidences in uterine and thyroid tumours in rats at dose levels where severe toxic effects were observed and (ii) incidences in benign ovarian tumours in mice (iii) the target organs were different in the two studies conducted in two different species. None of the findings was statistically significant but at the same time they were above the historical control ranges.

Two comments received were in disagreement with the classification proposal: one MS argued for a more severe classification (Carc. 1B), whereas industry was in favour of no classification.

1. Industry requested a review on the mode of action (MoA) for uterine adenomacarcinoma in rat and ovarian luteoma in mouse. The proposed MoA involved a decrease in secretion of prolactin, and industry argued that it was not relevant to humans. This MoA was proposed for uterine tumours in rats and it was supposed to be identical for ovarian tumours in mice. In support of this, a review of the thiacloprid rodent carcinogenicity studies regarding increased incidences of tumours in the female genital tract was provided and it was also reported that additional mechanistic studies were planned.
2. The MS proposed Carc. 1B since development of different types of tumours was observed in two species, the substance seemed to have an intrinsic potential to alter the hormonal secretion pattern in at least two species and it could thus not be excluded that a tumorigenic response could occur also in humans, although the affected tissue may be different.
   Further to this, the MS provided a different opinion from the DS on other aspects as well, including that the systemic toxicity could not explain the tumours observed as there was no toxicity in mice. In addition, since both benign and malign uterine tumours (rat) and ovarian tumours (mice) were observed, benign tumours were supposed to progress to malignancy as in rats the frequency of malignant adenocarcinomas was higher than the frequency of benign adenoma.

Assessment and comparison with the classification criteria

Carcinogenic potential of thiacloprid has been shown with increased incidences of three types of tumours: malignant uterine tumours and benign thyroid tumours in rats, ovarian tumours in mice (mainly benign but with one malignant at top dose). Based solely on a combination of benign and malignant neoplasms in two species, a classification as Category 1B could be argued. However, there were several additional factors that were considered by RAC when assessing the overall level of concern.

- Progression to malignancy

The uterine tumours occurring in rats were malignant, while in mice the ovarian tumours were benign. One malignant ovarian tumour was reported in mice, which could be an indication of the potential of the tumour to progress to malignancy. According to the Guidance on the application of CLP Criteria, some benign tumours may have the potential to progress to malignant tumours and therefore any indication that the observed tumours have the potential to progress to malignancy may increase the level of concern. However this single malignant tumour in mice is considered by RAC as marginal indication of progression to malignancy.

- Confounding effects of excessive toxicity

The malignant uterine tumours in rats occurred at doses with reduced body weight gains (15% at mid dose and 20 % at the high dose in comparison to controls) as well as increased incidence and severity in age-related degenerative myelopathy (characterised by radiculoneuropathy, sciatic nerve degeneration and subsequent skeletal muscle atrophy), retinal atrophy and lens degeneration. RAC concludes that these effects were not signs of severe systemic toxicity and that the MTD was not exceeded.
The ovarian tumours did not co-occur with general toxicity.

- Single or both species

Two species were responsive but the target organ was uterine in rats and ovary in mice. In addition, it was noted that enlarged uterus in dogs was reported after a dietary exposure to thiacloprid for 105 days (increase in absolute weight of 32, 26 and 71% at 8.89, 34.7, 65.3 mg/kg bw/d, respectively) indicating that the uterus might be the target organ in two species (although no effect was reported on the dog uterus in the 1-year study, the dose level was lower with a maximum of 33.8 mg/kg bw/d). Thyroid tumours did not occur in mice.

- Mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression

Thiacloprid was not shown to be genotoxic.

According to the EU specialised experts (1999), referred to in the Guidance on the application of CLP Criteria, classification for thyroid tumours was not recommended for non-genotoxic substances causing thyroid tumours via clearly established mechanisms disturbing thyroid-pituitary axis with low or medium potency and when other mechanisms were excluded. However, in cases where this mechanism could be clearly established and the substance would have high potency, or the mechanism was unknown, or there was insufficient data on the mechanism, Category 2 for these tumours was recommended by EU specialised experts. For thyroid tumours, RAC concludes that the sequence of events for the MoA proposed by the DS, i.e. an induction of the UDP glucuronyltransferase (UDPGT) leading to decrease in serum T4 and T3 levels and a compensatory increase in TSH that would in turn result in thyroid hyperplasia and tumours, was not fully demonstrated. In the 2-year study in rats, UDPGT induction was reported at the two highest doses (only measured at week 54), hypertrophy was statistically significantly increased at three highest doses and thyroid follicular cell adenoma was reported at the two highest doses at week 107 but T4 and TSH levels did not change significantly (measured at weeks 26, 53 and 105) and thyroid follicular cell hyperplasia was not dose-related. Furthermore, an increase in T3 (week 3 and week 12) and T4 (week 3) was reported in a 90-day study in rats (CAR A.6.4.1, 1997) and no decrease was observed in other studies. Besides, other MoAs (endocrine disruption) were not fully excluded for these thyroids tumours. RAC therefore concludes that the relevance of the observed thyroid tumours to humans cannot be excluded in this particular case.

According to the DS, a long-term perturbation of steroid sex hormones, as a result of thiacloprid targeting ovarian follicles, and in particular the elevated oestradiol levels suggesting a shift to oestrogen dominance, was a plausible MoA in the uterine and ovarian tumour formation.

During public consultation industry proposed a new MoA for uterine and/or ovarian tumours related to decreased prolactin levels (hypoprolactinemia). This MoA was proposed not to be relevant for humans. Industry also communicated its intention to perform new studies with the aim to test this hypothesised MoA. Later on, industry communicated that they had no new data which would confirm the proposed new MoA for the uterine adenocarcinoma in rat and the ovarian luteoma in mice. RAC therefore concluded that the classification proposal for carcinogenicity, based on tumours observed after long-term treatment with thiacloprid, should be discussed based on the original MoA as proposed by DS.

Several studies on the MoA were available. An initially hypothesized MoA involved thiacloprid-mediated induction of hepatic aromatase, which catalyses the conversion of androstenedione to oestrone and testosterone to oestradiol, resulting in elevated plasma oestradiol concentrations. A prolonged stimulation of the uterus by oestradiol was proposed to lead to increased uterine tumour formation. Increased hepatic aromatase activity was reported in a study in 1998. However, further studies (2009) on the induction of hepatic aromatase activity provided negative results, leading to the conclusion that the first study was not specific for the aromatase. However, despite these negative results on aromatase, a member of the cytochrome P450 superfamily, hepatic enzyme induction was one the most sensitive effects of thiacloprid in repeated toxicity studies, and CYP 450 induction may have contributed to the alterations in circulating hormone levels, leading to overstimulation of the sensitive organs and tumours in them. Additionally, thiacloprid was shown to influence the steroid biosynthesis *in vitro* by inducing the enzyme that metabolizes testosterone to androstenedione, a precursor to oestradiol (1998a).

A modification of hormone levels with an alteration of the oestrogen/progesterone (E/P) ratio (a trend to shift to oestrogen dominance) was reported in several repeated dose studies.
After a 4-day exposure to a dose of 60 mg/kg via gavage (11 weeks old rats),

- Evaluation 2 and 8 hours after the last dose: plasma progesterone concentration was significantly increased after 2 (70%) and 8 (54 %) hours and no oestradiol was measured (possible technical problems) (2009a);

- Evaluation 24 hours after the last dose, progesterone was increased by 74% and oestradiol slightly increased (28%, not statistically significant) (2009b);
- Evaluation after 2, 8 and 24 hours after the last dose: progesterone was significantly increased after 8 (57%) and 24 (81%) hours and no significant changes in oestradiol concentrations (2009c) were measured.
- After a 28-day exposure, an increase in plasma oestradiol concentration was reported: in a study in young rats (7 weeks old), estradiol was increased by 65% at 75 mg/kg bw/day and 60% at 107.7 mg/kg bw/day while progesterone concentration was only marginally increased (2009d); in a study in 72-week old rats (2009e), oestradiol concentration was also increased by 19% at 31.5 mg/kg bw/day (apparent decrease in progesterone levels but high inter-animal variability confounded the interpretation of the data).

The E/P ratio has been shown to be modified by thiacloprid in a recent one-generation study (2011c) as there was a 10-fold increase in the E/P ratio between GD 20 and 22 after thiacloprid treatment vs a 5-fold increase in the non-treated controls and in other non-treated rats between GD 19 and the onset of labour (Fang, 1996 cited in CLH report). In the 2-year carcinogenicity study in mice, an increased incidence of ovarian cysts was reported at the 2 highest doses (16/50, 15/49, 19/50, 22/48 and 24/50 at 0, 25, 50, 500 and 1000 ppm) and at the interim kill, a glandular hyperplasia of the uterus was reported (1/10, 0/10, 2/10, 4/10 and 4/10 at 0,25, 50, 500 and 1000 ppm). These findings are consistent with the activity of estradiol on these tissues and therefore support the proposed effect of thiacloprid on E/P ratio. In an uterotrophic assay (2007) in the immature rat (19 days old), thiacloprid exposure (3 days, 70 mg/kg s.c.) did not cause changes in the uterine weight or morphology indicating that thiacloprid does not have an oestrogenic effect on the uterus.

In vitro investigations also provided supportive information on the effects of thiacloprid on sex hormones (2010a, 2010b). Treatment with thiacloprid at 50, 100, 500 µM for 24 or 48 hours on wistar rat follicles (isolated from young, 7-week old females) produced a clear and consistent increase in progesterone at 24 and 48 hours (dose of 500 µM). In an *in vitro* study investigating steroidogenic effect of thiacloprid on human adrenal cells (H295R), thiacloprid induced a concentration-dependent inhibition of testerone secretion at 24 and 48 hours and an increase in progesterone secretion at 24 hours (not at 48 hours) on H295R cells at 50,100 and 500µM.

Thus, the mechanistic studies indicate that thiacloprid interfered *in vitro* with sex hormone biosynthesis and caused changes in sex hormone secretion by adrenal cells and preantral follicles. Alterations in circulating sex hormone levels were also reported in rats in *in vivo* studies, which may have been caused by liver enzyme induction by thiacloprid. The effect on oestradiol dominance was further supported by induction of ovarian cysts and glandular hyperplasia of uterus in the 2-year carcinogenicity study in mice as well as by negative uterotrophic assay.

According to the Guidance on the application of CLP Criteria, the existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs) may lead to a downgrading of a Category 1 to Category 2 classification. Although the definite MoA has not been clearly demonstrated, RAC concludes that a hormonal imbalance is a plausible MoA. Additionally, RAC concludes that a genotoxic MoA action can be excluded.

Based on the mechanistic information on tumour formation, RAC agrees with DS that classification of thiacloprid for Carcinogenicity Category 2 is justified.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Fertility

A one-generation dose range-finding study, a 2-generation study and several phased studies all conducted in the rat were available. DS proposed to classify thiacloprid as a suspected human reproductive toxicant for adverse effects on sexual function and fertility (Repr. 2; H361f) based on dystocia that occurred in several studies in rats at moderate doses and had serious toxicological consequences for dams and pups.

The DS proposed a classification in Category 2 rather than in Category 1B because of the following arguments:

1. A biologically plausible explanation for thiacloprid's mode of action for dystocia was a disturbance of the hormonal regulation of the maintenance of pregnancy and onset of parturition by progesterone and oestrogen. The uterine and ovarian tumours observed in rats and mice were coherent with this imbalance of sex steroid hormones. Although the available data generally supported this mode of action, it was acknowledged by the DS that hormone levels were variable throughout the day and therefore difficult to assess, and that large inter-animal variability in the levels was common. There were some indications of hormonal changes but in the absence of concrete evidence in terms of cause and effect, the proposed mode of action was regarded as speculative. However, it was proposed that the control of pregnancy and parturition was highly species specific, and in humans pregnancy and parturition were not controlled by a simple chain of events unlike in many other species. Instead, it was assumed than in humans there were multiple paracrine/autocrine events, foetal hormonal changes and overlapping maternal/foetal control mechanisms that triggered parturition. As a result, the decrease or absence of a single component was assumed to be compensated by changes in other pathways. A mode of action that involved disturbance of the normal progesterone and oestradiol levels during late gestation and parturition were therefore suggested to be of reduced concern for adverse effects on parturition in humans.

2. Adverse effects on parturition were only recorded at doses of thiacloprid that were maternally toxic in ways that were unrelated to the proposed mode of action (liver toxicity, reduced body weights compared with controls, pallor, hypoactivity). The parturition problems did not occur at maternally non-toxic doses.

3. The incidence of adverse effects on parturition was rather low: the overall mean incidence of dystocia in high-dose groups was 6.7 % and it occurred with maternal toxicity.

Development

Two developmental gavage studies conducted according to OECD TG 414 were available: one in rat and one in rabbit. In addition, there was one neurotoxicity study.

In development toxicity studies, increased post-implantation losses, total litter resorptions, decreases in foetal weight, increased incidences of skeletal variations and retardations in ossifications and delays in sexual maturation were seen in thiacloprid treated groups. However, all these effects occurred only in conjunction with maternal toxicity seen as reduced maternal body weight and food consumption, and were therefore judged as likely to be secondary non-specific consequences of maternal toxicity. Increases in stillbirths, decreased pup viability indices and pup weights reported in fertility studies were also concluded to be related to maternal toxicity and were not considered as relevant for classification for adverse effects on development.

As a conclusion, since no teratogenic effects in the absence of maternal toxicity were observed in the studies in rats and rabbits the DS did not propose classification of thiacloprid for developmental toxicity.

Comments received during public consultation

Three MSs provided their general support for the CLH proposal, one MS proposed no classification, one MS proposed that Category 1B should be considered and one was in agreement with Category 2 for adverse effects on sexual function and fertility.

Category 2 was supported by this MS since thiacloprid induced dystocia, a finding that was associated with only slight general maternal toxicity (decreased bodyweight, increase in liver weight and hepatocyte hypertrophy), and therefore it was to be assumed that effects on fertility were not secondary consequences of this toxicity.

The MS proposing no classification stated that dystocia was not considered to be a direct effect but a consequence of some other toxicity or condition that required prolonged exposure at high doses. Dams that exhibited dystocia also showed minimal to moderate liver necrosis which was not reported in the dams who delivered successfully. In addition, dystocia was observed in rats in several studies, but only when the substance was given in the feed and not when high toxic doses where given by gavage near parturition. Dystocia was restricted to a few dams in each study, and there was an inconsistency in this effect for the F0 and the F1 generations in the two-generation study. Dystocia was also considered as not relevant for humans because parturition is induced differently in rats and humans.

Category 1B was proposed by one MS because the data did not convincingly show that the observed effects were secondary consequences of maternal toxicity with only slight reductions in body weights. Some effects were not considered to be consequences of dystocia. An increase in the number of stillbirths in F1 and F2 generations were observed while dystocia was not noted in F2 females. Also, an increased number of stillbirths was observed in the one-generation (dose range-finding) study with no dystocia. It was also considered that more discussion was needed before the proposed mode of action could be concluded to lower the concern for humans since its biological consequences were not known.

Assessment and comparison with the classification criteria

Assessment of sexual function and fertility

In the 2-generation study (CAR A6.8.2 (1997)), performed according to a procedure similar to OECD TG 416, SD (Sasco) rats were exposed to thiacloprid at 0, 3.7, 22 or 43 mg/kg bw/day. The main finding was dystocia leading to death in P0 parental females (not in F1) at the end of gestation (GD 23-24): 4/30 dams at 22 mg/kg (13%) and 3/30 at top dose (10%). Parturition started in 3 dams with dystocia delivering some but not all pups, but it did not start in the remaining 4 dams in which all pups were found dead *in utero*. No information was available on to which dose groups these dams belonged.
Maternal effects observed from 22 mg/kg bw/day were minimal and consisted of increased absolute and relative liver weight (increase in absolute liver weight around 20% in both F0 and F1) and hypertrophy and of increased absolute and relative thyroid weight and follicular hypertrophy. At the top dose, mean body weights were 5-9% lower in F0 and F1 generations than in controls on GD20. These effects were not specifically pronounced in females with dystocia although dams that had suffered from dystocia showed pallor, wet or stained perineal areas, red vaginal discharge as well as red foci and liver necrosis at necropsy.

Dystocia had not been previously observed in the range-finding study, performed according to a procedure similar to OECD TG 415 (CAR A6.8.2 (1995)), in which Sprague-Dawley (Crl:CD BR) rats were exposed to thiacloprid at 100, 400, 1600 ppm (estimated to be approximately 117 mg/kg bw/day). However, the small group size could have reduced the likelihood of dystocia being observed in this study which was conducted on a slightly different strain of rats than in the actual 2-generation study (CAR A6.8.2 (1997). At the top dose, dams showed a significantly decreased body weight gain as compared to controls from the pre-mating day 0 to day 28 (-48%) but the decrease in body weight gain was minimal from GD0 to GD21 (-20%). At the top dose, the

mean body weight was 10% lower than in controls on the pre-mating day 28 and on GD0 and 13% lower than in controls on GD20.

Another one-generation study in rats (CAR A6.10 (1998a)) was conducted to further investigate the potential of thiacloprid to induce dystocia. Sprague-Dawley (Sasco) rats were tested at doses of 2, 23 and 75 mg/kg bw/day. At the top dose, 3/28 (10%) dams died from dystocia during late gestation; two of them after a partial delivery of pups and one with no signs of parturition. Clinical signs in these dams were consistent with difficult labour, but no gross pathological findings were reported in these animals. One additional dam died on GD24 without signs of labour, one dam died on test day 40 before mating and another dam died on test day 134 being sperm-positive but without implants. Other maternal toxicity at the top dose included clinical signs of suffering with paleness, labored breathing and hypothermia during late gestation. The body weight gain was decreased by 16% over the gestation period, and the mean body weight was maximally 10% lower than in controls during the gestation. Also a 21 and 31% increase in the absolute and relative liver weight, respectively, and a 18 and 33 % increase in the absolute and relative thyroid weight, respectively, were noted.

A further study (CAR A6.10 (1998c)) was conducted to investigate if thiacloprid could induce dystocia after a short-term exposure during late gestation. Sprague-Dawley (Sasco) rats were exposed to thiacloprid by gavage at doses of 17.5, 35 and 60 mg/kg bw/day on GD18-21. These doses were selected based on the previous gavage study (CAR A6.10 (1998d)) that had showed severe maternal toxicity at 100 mg/kg. In CAR A6.10 (1998c), no dystocia was reported, but an early parturition was observed as "numerous" dams were reported to have delivered already on GD21 in the high dose group. Additionnally, because at the two highest doses, maternal lethality (1/27, 0/9, 7/29, 8/25 at 0, 17, 35 and 60 mg/kg/d) occurred again during late gestation (between GD 20 and 24), dystocia may not have been observed because of maternal death before labour. In RAC's view this study emphasizes the late gestation as a sensitive window for thiacloprid exposure-caused toxicity. In addition to lethality, mean body weights at 35 and 60 mg/kg bw/day were significantly lower (-14%) than in the control group on GD21 and there was a marked decrease in bw gain in all dose groups (weight loss at 35 and 60 mg/kg/d) from GD18 to GD21. Significant reductions in food intake were reported from GD18 to GD21 at all doses but it was more pronounced at the two highest doses (decreased by 85-94%). Clinical signs of toxicity included hypoactivity, chromorhinorrhoea and clear vaginal discharge from 35 mg/kg bw/day.

The CAR A6.10 (1998b) study was performed to further investigate the physiological mechanism of thiacloprid-induced dystocia in Sprague-Dawley (Sasco) rats from 10 weeks pre-mating until different time points from GD13 to parturition. One dam (1/30) in the dosed group (75 mg/kg bw/day) died because of dystocia on GD22 with 3 pups born and 12 in utero. This dam had shown no clinical signs of toxicity and its terminal body weight was higher than the group mean. Three other dams in the dosed group died on or before GD 15, but there was no relation to parturition. One of these dams was not pregnant, and another was suspected to have pregnancy toxaemia (not related to thiacloprid administration). There were no treatment-related effects on uterine electrophysiology, cervical extensibility, cervical collagen content or uterine alpha adrenergic receptor concentration, and microscopy did not reveal any effects on the uterus or cervix.

In a one-generation study (CAR 6.10 (1998; and 1998b)) with focus on steroid hormones, Sprague-Dawley (Sasco) rats were exposed to thiacloprid at 61 mg/kg bw/day. Dystocia occurred in 2 out of 12 dams (16%) in the group exposed from the pre-mating period to parturition. No clinical signs were noted. Significantly decreased body weight gains were reported during pre-mating and gestation periods but no further details were provided. The oestradiol and LH levels were statistically significantly increased in treated groups sacrificed after 9 $\pm$ 1 weeks of premating period and on lactation day 2 (including the dams suffering from dystocia). However, in the two animals suffering from dystocia, the LH levels were not increased as compared to animals that did not have dystocia, and in one of them the serum estradiol level was 212% of the group mean whereas in the other, the oestradiol level (37.5 pg/mL) was below the group mean (48.7 and 19.4 pg/mL in the group mean and control mean, respectively). Progesterone was statistically significantly increased in the treated group sacrificed on lactation day 2 (that included the dystocic dams) as compared to controls, but the levels in dystocic animals were not raised as

compared to animals that delivered normally. However, the values showed a considerable inter-individual variability.

Parturition was camera-recorded in a one-generation study (2011c) in which blood samples were taken prior to necropsy. Additional blood was sampled to study the oestradiol and progesterone levels on GD 20, 21 and 22 in a satellite group in order to establish a causal relationship between effects on hormone levels and dystocia. Thiacloprid was administered to Sprague-Dawley (Sasco) female rats at 0 or 60.9 mg/kg bw/day from at least 10 weeks prior to pregnancy over the pre-mating phase and at 0 or 54 mg/kg during the gestation phase. Dystocia was reported in 3/26 (11%) treated dams; one delivered 12 pups in 266 minutes while the mean time of delivery for treated animals was 105.6 (+/- 42.9) minutes, one dam was found dead on GD24 after the delivery (no blood sample was taken) having one dead pup in the uterus, and one dam showed clinical signs indicative of pain and difficult parturition (piloerection, reddish soiled anogenital region, reduced motor activity) and was killed during parturition on GD23. At necropsy, there was a marked uterus prolapse and three live pups in the uterine horn. As regards the general toxicity, there were no clinical signs and only a slight reduction in food consumption and body weight gain, as well as an increased weight and hypertrophy of liver and thyroid in treated animals as compared to controls. Some stress-related signs such as aggression and/or resistance to handling were identified. Modifications in hormone levels were found, albeit not statistically significant. Oestradiol was decreased on GD21 and GD22 as compared to that on GD20 in controls (27, 21, 22 pg/mL on GD 20, 21, 22, respectively) while it increased non-linearly in treated animals (20.2, 39.5, 28.8 pg/mL on GD 20, 21, 22, respectively).The oestradiol/progesterone ratio was increased 10 fold from GD20 to GD22 in treated animals in the satellite group vs 5 fold in controls but the change in the ratio was not statistically significant. Still, in the dystocic dam, the progesterone concentration was increased (455%) as compared to the group mean while a decrease in progesterone is expected at the time of parturition.

Data on dystocia as well as an assessment of general toxicity in different rat strains in historical controls and in rats treated with xenobiotics were provided, and they were compared to the data on thiacloprid. The incidence of thiacloprid-induced dystocia was 13 out of 192 (a mean of 6.7%) when counted for all Sprague-Dawley rats tested for thiacloprid during 1995-1998 by Bayer. This is above the incidence 6/635 dams (0.94%) for the same strain of rats treated by Bayer with other substances and showing maternal toxicity during 1988 - 1997.as well as above the incidence (11/906) in the historical control for the same strain of rats during the same period.
Conclusion
As highlighted by the DS, dystocia was consistently observed in studies and had serious consequences leading to death of dams. It occurred at doses around 54-75 mg/kg bw/d in one-generation studies and from 22 mg/kg bw/d in the two-generation study. The incidence of dystocia after thiacloprid treatment was rather low but above historical control and above the incidences for the same strain of rats treated with other substances.

Dystocia occurred in dams at doses causing maternal effects. RAC concludes that although maternal death was observed in some studies at doses causing dystocia, dystocia did not occur with severe maternal toxicity in all studies, and therefore RAC concludes that dystocia should not be considered solely as a secondary non-specific consequence of maternal toxicity. For instance, in the two-generation study, maternal effects consisted of decreased mean body weight (max 9%) during the pre-mating and gestation periods and of increased liver and thyroid weights which are not representing severe maternal toxicity according to RAC. Additionally, CLH report-containing data from rats treated with other xenobiotics did not show any influence of maternal toxicity on the incidence of dystocia, which supports the RAC conclusion that dystocia should not be considered solely as a secondary non-specific consequence of maternal toxicity.

An alteration in sex hormone levels and specifically in the E/P ratio was proposed to be the MoA for dystocia. RAC notes that changes in sex hormones were reported in several study reports but these alterations were considerably variable between individuals and no specific or consistent alterations were observed in dystocic dams. RAC concludes that a causal relationship for this MoA in the induction of dystocia has not been demonstrated and the available evidence for the

proposed MoA and for its non-relevance to humans is not sufficient to raise a doubt of human relevance of the observed effect, i.e. dystocia, either.

According to the CLP criteria for classification in Category 1B *"data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate*."

RAC concludes that the data provides clear evidence of an adverse effect, i.e. dystocia, of thiacloprid, which is considered not to be a secondary non-specific consequence of maternal toxicity. In addition, there is no robust data on the MoA to conclude that the effect is not relevant to humans or to raise doubt about the human relevance. Therefore, RAC concludes that Cat. 1B is justified. As any effect that has the potential to interfere with parturition should be considered as an adverse effect on sexual function and fertility and as dystocia appeared only in studies having exposure already during the pre-mating period, RAC agreed to classify thiacloprid for adverse effects on sexual function and fertility rather than on development.

Assessment of development

Two main studies were available on development. In the OECD TG 414 study (1997, amended by 2000), Wistar rats were exposed to thiacloprid at 2, 10 or 50 mg/kg bw/day from day 6 to day 19 post coitum. At the highest dose of 50 mg/kg bw, the main developmental findings were as follows: total resorption was reported in one dam, increased post-implantation loss (21.3% vs 7.2% in controls), decreased foetal weight (15%) and increased skeletal retardation. The total number of skeletal malformations was increased (6.3% vs 2.2% in controls and 4.48% in the historical control from 21 studies conducted from 1994 to 1999). This increase was due to multiple malformations (such as a cleft palate and a short mandible) in a single foetus and to an increased incidence of limb bone dysplasia (described as shortened, thickened and kinked bones) that was within the historical control range (8/270 foetuses (3%) in 6 litters (20.7%) vs 1/321 foetuses (0.3%) in the control and 0-3.45% foetuses (23.07% litters) in the historical control). At this dose level of 50 mg/kg bw/day, maternal toxicity consisted of significantly decreased food consumption (64% at the beginning of the treatment on days 6-11 post coitum, and 14% on days 11-16 post coitum), decreased body weight gain from day 6 to day 19 post coitum (45%) and lower mean body weight (9-11% on days 9, 14 and 20 post coitum) as compared to controls.

In the other OECD TG 414 study (CAR A6.8.1 (1996)), rabbits were exposed to thiacloprid at 2, 10 or 45 mg/kg bw/day from day 6 to day 28 *post coitum*. At the highest dose of 45 mg/kg, there were 2 abortions, 3 total litter resorptions (out of 24 females with implantations), a decreased foetal bodyweight (21%), an increased incidence of skeletal variations indicative of reduced or delayed ossification as well as a marginally increased incidence of supernumerary 13^{th} rib. Also an increased incidence of forelimb arthrogryposis, flexure of the limb in carpal joint (4,4% vs 2% in controls) was reported. This effect was indicated as common in this strain (the finding was among the historical control range (0-5.6%), and it has been described in literature as a consequence of restricted foetal movement. At the same dose, there was also an alteration in the sex ratio (decreased number of males). Maternal effects at the top dose of 45 mg/kg bw/day included significantly decreased food consumption (76 and 20% lower than in controls between days 6-11 and between days 24-29 post coitum, respectively), weight loss in the beginning of treatment (113 g vs 17 g in controls from day 6 to day 11 post coitum), decreased body weight gain as compared to controls from day 6 to day 28 post coitum (+5.4 g vs +154 g in controls), and decreased mean body weight (6%) as compared to controls on day 29 post coitum. Alopecia was also reported.

In a study designed specifically to investigate developmental neurotoxicity (2001), there was a decrease in pup weight and a delay in sexual maturation, but no signs of developmental neurotoxicity.

Effects during gestation (Malformations and variations, post-implantation losses)

In the developmental studies, some malformations were reported at the top dose but they remained among the historical control ranges. In addition, forelimb arthrogryposis is common in the tested rabbit strain and has been described in literature as a consequence of restricted foetal movement. Based on the incidences of the findings RAC concludes that the malformations are chance findings and skeletal variations are of minimal toxicological relevance.

Post-implantations losses were reported at the top dose of the developmental studies both in rats (21% at 50 mg/kg bw/day vs 7.2% in controls) and rabbits (26% at 45 mg/kg bw/day vs 11% in controls). At these dose levels, decreases in maternal body weight/maternal body weight gain were reported. The OECD guidance document number 43 indicates that in the latest available feed restrictions studies (2005), severe decrease in body weight gain in rabbits up to body weight loss can result in reduced foetal weight, and alterations in ossification, and abortion (but no malformations) only occurred when feed was restricted to an amount that produced maternal body weight loss. In rats reductions in maternal gestational body weight gain of approximately 50% compared to ab lib fed rats only caused a reduction in foetal body weight, and up to a 15% maternal gestational body weight loss had no effect on embryo viability in rats, but induced minor changes in skeletal development (no malformations) were associated with any of the levels of maternal body weight reduction or loss. Consequently, RAC concludes that the post-implantations losses are not solely secondary non-specific consequences of maternal toxicity.

Effects around birth (stillbirths, pup viability, pups weights)

In the two-generation study, an increase in stillbirths in F1 (0.6, 4.4, 4.5 and 5.7% at 0, 3.7, 22 or 43 mg/kg bw/day) and F2 (2.9, 4.0, 2.5 and 5.8% at 0, 3.7, 22 or 43 mg/kg bw/day) was noted. This effect cannot be associated with dystocia since it was reported in P0 (4/30 at 23 mg/kg bw, 3/30 at 43 mg/kg bw), but not in F1. However, the increased incidence was not dose-related in F2. Signs of maternal effects in both generations observed were limited to reduced mean body weight gains as compared to the control over the whole treatment period, increased liver and thyroid weights and increased incidence of liver and thyroid hypertrophy. RAC is of the opinion that there is no evidence that the observed stillbirths in the two-generation study are secondary to maternal toxicity. No increase in stillbirths was noted in the dose range-finder study (3.1% at the top dose of 107 mg/kg bw/day), in which the increase in stillbirths was rather high in the control group (5.6%) (historical control range for rat stillbirths in one-generation range-finder studies was 0-1.6% according to information received from IND). However, seven animals per dose were tested in the dose range-finder study and the group size affects the robustness of the data. In the one-generation rat study (CAR A6.10 (1998a)), an increased incidence of stillbirths was again reported (incidences of 3.9%, 1.7%, 5.2%, 7.6% at 0, 2, 23 and 75 mg/kg bw/day, respectively). At the top dose, dystocia (leading to death) was observed in 3 dams and three additional maternal deaths occurred. Additional maternal effects consisted of paleness, laboured breathing and hypothermia, the body weight gain during gestation was decreased by 16% as compared to the control, and the mean body weight was maximally 10% lower than in the control during the gestation phase. According to the information received from IND, of the seven dams with stillbirths out of 20 pregnant dams in the high dose group, one showed clinical effects (lacrimation, malocclusion of upper incisors, ulcerated hard palate, missing upper and lower incisors, urine stain, paleness and vaginal discharge) and three dams had a reduced bodyweight. In the rat short-term study (CAR A6.10 (1998c)) with a gavage exposure during late gestation (GD 18-21), a clear increase in stillbirths was reported at 35 and 60 mg/kg bw/day (12.1 and 26.6%, respectively vs 1.6% in controls), but at two highest doses there was maternal lethality (7/29 and 8/25 dams at 35 and 60 mg/kg bw/day, respectively) during late gestation (GD 20-24). In the one generation video recording-study (2011) the incidences of stillbirths were 10.1% at 54 mg/kg bw/d and 2.4% in the control group. According to the report provided by IND, the clinical signs in dams with stillbirths in the dosed group consisted of reduced bodyweight and in 2/15 dams with stillbirths of hepatocellular hypertrophy and/or thyroid hyperplasia/hypertrophy. In the sub-acute plasma thiacloprid level-study (1998), the incidence of stillbirths was 20.8% in the treated group (75 mg/kg bw/d) and 11.1% in the control group. Dams with stillbirths had no significant clinical signs or effects on body weight. However, the group size affects the robustness of the data, and there were only 8 dams in the treated group and 5 dams

in the control group. Overall, RAC notes that the increase in stillbirths was reported in several studies but in some studies at doses causing also severe maternal toxicity (death) or with deficiencies in the study design/confounding factors. An increase in the number of stillbirths was observed in the two-generation study that was not considered to be secondary non-specific consequence of maternal toxicity.
The viability index (on post-natal day 4) in the two-generation study was reduced (82.8% vs 97.4% in controls) in F1 genetation at the top dose of 43mg/kg bw/day as a result of cannibalization by the dams, which may not have been treatment-related. It is also emphasized that if cannibalization occurred, pups might not have been strong enough to survive, and that there was also an increased number of weak pups and a decrease in pup weights on post-natal day 4. In the F2 generation, the viability index was only slightly reduced and not statistically significant (91.6 vs 93.9% in controls). However, a reduced pup viability index (21%) on day 4 of lactation was also reported at the top dose of 75 mg/kg bw/day in the one-generation study (CAR A6.10 (1998a)). Severe maternal toxicity was reported in that study (see above). However, in the range-finding study (1995) at the high dose of 117 mg/kg bw/day, an increase in pup deaths in F1 on post-natal day 4 (16 vs 3 in controls) resulting in a slightly lower viability index was observed, which was not associated with maternal toxicity. As compared to controls, the body weight gain of dams was reduced during gestation, but during the lactation phase, body weight gain was above controls and the mean body weight was normal at the end of the lactation phase (see above). Overall, RAC concludes that the effect on pup viability is thiacloprid-treatment related and that it cannot be explained by maternal toxicity.

In the two-generation study, at the top dose of 43 mg/kg bw/day, pup body weights were reduced in F1 and F2 from post-natal day 7 up to 15% by post-natal day 21 while they were not affected at birth. Although mean body weights of dams were lower than control during pre-mating and gestation, the mean body weights of dams were not affected during the lactation phase. Therefore the decrease in pup weights cannot be considered as secondary to maternal toxicity. Similarly, in the range-finder study (1995), while pup weights were not affected at birth, a decrease in body weight occurred from post-natal day 4 (17%) until post-natal day 35, and the body weight gain of dams during lactation phase was above the control and the mean body weight was normal at the end of lactation phase (post-natal day 21). In the study designed specifically to investigate developmental neurotoxicity (2001), there was a decrease in pup body weight that was regarded by DS as a secondary non-specific consequence of maternal toxicity, but no details were provided on the study. Overall, RAC notes that an effect on pup weight was observed after treatment with thiacloprid, and it cannot be explained by maternal toxicity.

Conclusion

RAC concludes that the findings provide clear evidence of thiacloprid having adverse effects on development (increases in post-implantation losses in developmental studies in two species, decreased pup viability and decreased pup weights in several one-generation studies and in the two-generation study) that are not considered secondary non-specific consequences of maternal toxicity. Overall, RAC concludes that classification of thiacloprid in Cat. 1B for adverse effects on development according to CLP criteria is warranted.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Thiacloprid is a chloronicotinyl insecticide. It is currently not listed in Annex VI of the CLP Regulation. As the substance is unlikely to bioaccumulate, is not rapidly degradable and is very toxic to aquatic organisms the DS proposed to classify the substance as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100). The basis for the acute classification is a 48-h EC_{50} of 0.0077 mg/L for *Ecdyonurus* sp. Larvae (the DS has confirmed that the scientific name used in the CLH

report is incorrect and the mayfly genus is *Ecdyonurus*). Because there is no chronic data for the same species the surrogate approach based on the same acute toxicity value is used for chronic classification.

Degradation

The results of a hydrolysis study following US EPA guidelines showed that thiacloprid is hydrolytically stable under acidic (pH 5), neutral (pH 7) and alkaline (pH 9) conditions. The DT50 is considered to be > 1 year at 25°C at environmentally relevant pH conditions.

There are two photodegradation studies using thiacloprid. The first study following US EPA guidelines showed the shortest DT50 of 79.7 days. On the basis of the two aqueous photolysis studies, thiacloprid is not expected to undergo significant photodegradation in the environment.

The ready biodegradability of thiacloprid was investigated in a Manometric Respirometry Test (OECD TG 301F) over a period of 28 days. Inoculum prepared using activated sludge from a domestic wastewater treatment was exposed to an initial test concentration of 102 mg active substance (a.s.)/L which was below the water solubility of 184 mg/L. Zero percent biodegradation of thiacloprid was observed by the end of the study showing that the substance is not readily biodegradable. Thiacloprid was shown not to inhibit the activated sludge microorganisms.
Aerobic water/sediment degradation of thiacloprid in pond and lake systems was assessed following BBA and SETAC methods in a GLP study. Flasks of untreated Hönniger pond water (artificially dammed pond in Germany; pH 7.2) and associated sandy silt loam sediment (pH 6.0, 3.8% organic carbon), and Lienden lake water (lake in an agricultural area in the Netherlands, pH 8.3) and associated sediment (pH 8.4, 0.39% organic carbon) were exposed to radiolabelled thiacloprid at approximately 0.120 mg a.s./L. Applied radioactivity (AR) in the supernatant water decreased rapidly and values of <2% AR were detected after 35 days of incubation. Thiacloprid was not detectable after 100 days. Thiacloprid degraded to form one major metabolite (M02), one minor metabolite (M30, maximum of 9.5% AR) and one unknown minor metabolite. The metabolites M02 and M30 were predominantly found in the aqueous phase in Lienden samples, but were more equally distributed between water and sediment in the Hönniger samples. No organic volatiles other than carbon dioxide were detected. Assuming first-order kinetics, the DT_{50} ranges 2.9-6.3 days for pond water and 10.6-10.8 days for lake water. The whole system DT50 ranges for pond and lake were 20.3-27.9 days and 10.7- 12.1 days, respectively. The ratio of water to sediment in the test was 9:1 although the recommended ration in the OECD TG 308 aquatic sediment simulation guideline is from 3:1 to 4:1.

The route and rate of thiacloprid degradation was investigated according to the methods BBA and US EPA using soil 'Howe' (Indiana, US, top 15 cm) and the rate of degradation was further investigated using three more soils: 'BBA 2.1' (Jockgrim, Germany, top 30 cm), 'BBA 2.2' (Hanhofen, Germany, top 30 cm) and 'Höfchen' (Burscheid, Germany, top 20 cm). The mean value of the experimental disappearance time (DT_{50}) for thiacloprid was estimated to be 2.33 days for all soils based on first order kinetics. Bound residues accounted for 21.7 - 29.9% AR, at the end of the study. Two metabolites, M02 and M30, occurred above 10% of the AR, with the amide derivative of thiacloprid (M02) shown to be the most abundant metabolite in all three soils investigated. M02 accounted to max. 66.4% AR after 30 days (Howe) and M30 accounted for max. 19.7% AR after 60 days (BBA 2.1). Mineralisation of thiacloprid, based on measured $^{14}CO_2$, was between 6.5 and 34% by the end of the test (100 days).

The DS concluded that although the degree of mineralisation was very low, significant primary degradation of thiacloprid was seen in a study using two aerobic water-sediment systems that contained a higher water:sediment ratio than is specified in the OECD TG 308. The parent substance rapidly dissipated to sediment as indicated by the peak in AR in sediments after three days. It is therefore more appropriate to consider the primary degradation DT_{50} for the whole system rather than water alone, and this varied between test systems from 10.7-12.1 and 20.3-27.9 days, depending on sediment type. Since only one of these is below 16 days, these data are not sufficient for thiacloprid to be considered as rapidly degradable (even if the degradants were not classifiable as environmentally hazardous). The aerobic soil simulation study indicated rapid primary degradation of thiacloprid, but not rapid ultimate degradation.

The conclusion of the DS is that thiacloprid is not rapidly degradable.

Bioaccumulation

Thiacloprid was observed to be extensively metabolised in metabolism studies using rats. Although a lower rate of metabolism could be expected in fish, an aquatic bioaccumulation study has not been conducted, and it is assumed that thiacloprid in unlikely to bioaccumulate in fish. Based on the measured log K_{ow} values of 0.73 (OECD TG 117) and 1.26 (OECD TG 107) and evidence of extensive metabolism in rats, thiacloprid is considered to have a low bioaccumulation potential in aquatic organisms.

Toxicity

The substance is an insecticide. There is information on short-term toxicity for fish, several invertebrates (*Daphnia magna, Asellus aquaticus, Gammarus pulex, Ecdyonurus* sp., *Hyalella azteca*), algae and aquatic plant (*Lemna gibba*). There is information on long-term toxicity for fish, invertebrates (*Daphnia magna, Chironomus riparius*), algae (*Scenedesmus subspicatus*, *Pseudokirchneriella subcapitata*) and duckweed (*Lemna gibba*). There are also data available on acute toxicity of the degradation products M02 and M30 for fish, invertebrates and algae.

Lowest acute aquatic toxicity values for each trophic level.

Substance and purity	**Species**	**Test Guideline**	**Endpoint**	**Toxicity value mg/l**	**Conditions**
Thiacloprid (97.3%)	*Lepomis macrochirus*	OECD 203	96-h LC_{50}	25.2	Static Measured
Thiacloprid (99.2%)	***Ecdyonurus sp.***	**OECD 202**	**48-h EC_{50}**	**0.0077/ 0.006**(*	**Static Nominal/measured**
Thiacloprid (96.8%)	*Scenedesmus subspicatus*	OECD 201	72-h E_rC_{50}	96.7	Static Nominal
Thiacloprid (96.8%)	*Lemna gibba*	US EPA	15-d EC_{50} frond number	>95.4	Measured

(* measured value given in public consultation

The lowest acute nominal toxicity value is a 0.0077 mg/L for an invertebrate mayfly larvae *Ecdyonurus* sp. The result is based on mortality and immobilisation. Nominal concentrations were 0.004, 0.009, 0.019, 0.041 and 0.09 mg/L. Analysis indicated that measured concentrations were 75, 84, 90, 92 and 93% of nominal. As the mean measured concentration was 87% of nominal, nominal concentrations were used.

Lowest chronic aquatic toxicity values.

Substance and purity	**Species**	**Test Guideline**	**Endpoint**	**Toxicity value**	**Conditions**
Thiacloprid (97.3%)	*Oncorhynchus mykiss*	OECD 210	97-d NOEC growth	0.244 mg/L	Flow-through Measured
Thiacloprid (97.5%)	*Chironomus riparius*	BBA / OECD 219	28-d NOEC	0.0005 mg/L	Static Measured
Thiacloprid (97.4%)	*Daphnia magna*	OECD 202	21-d NOEC parent length	0.58 mg/L	Semi-static Measured
Thiacloprid (96.8%)	*Scenedesmus subspicatus*	OECD 201	72-h NOE_rC	32 mg/L	Static Nominal
Thiacloprid (96.8%)	*Lemna gibba*	US EPA	15-d NOEC	46.8 mg/L	Measured

The lowest chronic toxicity value is 0.0005 mg/L for a sediment dwelling larvae of the freshwater dipteran *Chironomus riparius* (see table above). The study was undertaken in accordance with GLP and using a BBA method similar to OECD 219 Sediment-Water Chironomid Toxicity Test Using Spiked Water. Each test container was filled with a 2 cm layer of artificial sediment and 20 cm reconstituted overlying water. Thiacloprid was introduced beneath the water surface and gently mixed to give initial nominal concentrations in the water fraction of 0.00032, 0.00056, 0.001, 0.0018, 0.0032, 0.0056 and 0.010 mg a.s./L. The measured test concentrations of three dose levels were 83 to 113% of nominal after one hour and consequently the results are based on nominal concentrations. However, the concentration of active substance in the water phase declined over the course of the study, with mean measured values of 64.5% on day 7 and 15.7% on day 28 compared to the initial nominal values. The average amount of active substance in the pore water also decreased over the course of the study. It was 3.4% of the nominal applied amount at day 0, 1.3% on day 7 and 0.1% on day 28. A NOEC for this study was not presented by the study author, however based on a delay in emergence and a slight reduction in the numbers emerged at 0.0018 mg a.s./L, the NOEC was considered to be 0.001 mg a.s./L based on nominal concentrations. For the biocides assessment the NOEC was recalculated to account for the loss of active substance from the water phase during the exposure period. This was done by determining the geometric mean for the test concentration 0.001 mg/L, using time 0 (nominal) and predicted concentrations on day 7 (64.5% of nominal) and 28 (15.7% of nominal). This gave a 28-d NOEC of 0.0005 mg/L which is considered suitable for classification purposes. However, there is some uncertainty how the organism were exposed to the active substance. The rapid dissipation of the substance to sediment observed in the aquatic simulation study suggests that organism exposure in the OECD TG 219 study may have been via sediment contact and ingestion as well as through the water phase and pore water.

A 21-day reproduction study was performed on thiacloprid according to OECD TG 202 and US EPA guideline 72-4 using *Daphnia magna* under static renewal conditions. The nominal concentrations tested were 0.10, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L. The determination of the test substance in the test medium showed that measured concentrations were above 80% of nominal and therefore results were based on mean measured concentrations. The lowest 21-d NOEC is 0.58 mg/L based on body length of parent animals.

Acute studies were performed for the three trophic levels with one major (M02) and one minor degradant (M30). The lowest acute toxicity values seem to be for *Hyalella azteca* and *Oncorhynchus mykiss* on M02 and M30, respectively. The acute toxicity for *Hyalella azteca* on M02 was 96h EC_{50} of > 47.6 mg/L and for *Oncorhynchus mykiss* >90.1 mg/L.

Comments received during public consultation

Comments were received from five MSs, all of which supported the environmental classification proposed by the DS. Some of them had comments related to test design, concentrations used and to the use of surrogate approach. One MS also had test data not presented in the CLH dossier.

One MS brought up the fact that in the OECD test guideline used in the acute *Ecdyonurus* test, the recommended number of animals is 20, preferably divided into four groups of five animals. In the test design used there were only 10 animals divided into 10 groups of 1 animal, weakening the statistical power which is sample size dependant. The EC_{50} tend to be higher in studies with smaller numbers of animals per dose group. However, *Ecdyonurus* is found to be the most sensitive species for acute aquatic toxicity. Furthermore, the measured concentration for the lowest dose tested (0.0004 mg/L) was < 80% of the nominal. Notwithstanding the fact that it will not affect the order of magnitude of the EC_{50} it would have been better to use the measured concentration. The DS acknowledged the comments but concluded that they do not affect the proposed classification and M-factors.

Another MS pointed out a few mistakes in the CLH report and informed that there is more data available. It was clarified by the DS that the acute toxicity value for *Hyalella azteca* of 0.0407 mg/L was used instead of 0.0245 mg/Ll because the former value was based on immobility which is a standard measure and the latter value on the number of 'floaters' at the surface in test vessel.

The acute EC_{50} value for *Ecdyonurus* sp. expressed as measured concentration is 0.006 mg/L compared to the nominal value of 0.0077 mg/L but this has no effect on classification. The additional data is for the saltwater mysid *Mysidopsis bahia*: 96-h LC_{50} in a flow-through system is 0.031 mg/L nominal and the 32-day NOEC in a flow-through system is 0.001 mg/Ll nominal. This information was not available to the DS at the time of writing the CLH report and they have not validated these results. However, this information would not affect the classification proposal.

One MS pointed out that the use of *Ecdyonurus* result, although not being a 'standard' test organism, for classification purposes is actually acceptable according to the ECHA Guidance on the application of CLP Criteria.

The use of the surrogate approach for chronic classification was not preferred by one MS. They thought that chronic classification should be based on the valid chronic chironomid study (NOEC = 0.0005 mg/L). The DS explained that they had used both the surrogate approach and the supporting chironomid study resulting in the same Chronic 1 classification and M-factor. Whilst the *Ecdyonurus* study is not ideal it was not used in the chronic tests and the surrogate approach should be considered. There are also uncertainties when interpreting the chironomid endpoint for classification. The DS stated that they prefer to take both approaches into account.

Assessment and comparison with the classification criteria

Degradation

The RAC agrees that thiacloprid is not rapidly degradable based on the results of the OECD 301F ready biodegradability test and the water/sediment study. The whole system DT_{50} for pond and lake water is 20.3-27.9 and 10.7-12.1 days. The water DT_{50} for pond and lake water is 2.9-6.3 and 10.6-10.8 days, respectively. Non-extractable residues increased to 22% AR for the pond system and 17% AR for the lake system, respectively. The carbon dioxide increased to 4% AR. The major degradation product M02 is classifiable for environment based on the substance being not rapidly degradable (mean DT50 in soil 69.5 days) and acutely harmful (96h EC_{50} of > 47.6 mg/L for *Hyalella azteca*).

Bioaccumulation

Based on the measured log Kow values of 0.73 and 1.29, RAC agrees that thiacloprid has a low bioaccumulation potential.

Toxicity

The substance is an insecticide. RAC agrees that the lowest acute toxicity value for *Ecdyonurus* sp. should be used for short-term classification. The measured value is 0.006 mg/L and the nominal value is 0.0077 mg/L both fitting to the range $0.001 < EC_{50} \leq 0.01$ mg/L. RAC agrees with the DS's proposal to use the surrogate approach for long-term classification because there is no chronic test data on the same species. There are adequate chronic toxicity data available for the three trophic levels. The lowest relevant chronic toxicity data is for the fish *Oncorhynchus mykiss* namely a 97-d NOEC for growth of 0.244 mg/L which would warrant Aquatic Chronic 2 classification. The acute toxicity values for fish are in the range 10-100 mg/L showing that fish is not likely to be the most sensitive species. The *Chironomus riparius* test is a sediment-water test where there is uncertainty about whether the organisms were exposed mainly via sediment rather than water and thus the result 0.0005 mg/L can only be used as supporting evidence to the surrogate approach.

Conclusion on classification

Acute

Thiacloprid fulfils the criteria for classification as Aquatic Acute 1, M=100 based on the lowest measured acute toxicity value of 0.006 mg/L (0.0077 mg/L as nominal) on mayfly larvae *Ecdyonurus* sp.

Chronic

Thiacloprid fulfils the criteria for classification as Aquatic Chronic 1, M=100 based on the surrogate approach, since the substance is not rapidly degradable, not likely to bioaccumulate and the lowest acute toxicity data is 0.006 mg/L.

ANNEXES:

Annex 1 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 comments (excluding confidential information).