Committee for Risk Assessment

RAC

Annex 1

Background document

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

Thiacloprid (ISO)

EC Number: N/A

CAS Number: 111988-49-9

CLH-O-0000001412-86-54/F

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

Adopted

12 March 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: THIACLOPRID

EC Number: **N/A**
CAS Number: **111988-49-9**
Index Number: **None allocated**

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Version number: **1** **Date:** **December 2013**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Thiacloprid***
EC number:	***N/A***
CAS number:	***111988-49-9***
Annex VI Index number:	***Not allocated***
Degree of purity:	***The active substance as manufactured has a concentration range of > 97 to 100%, with a typical purity of > 98.95%. The typical purity used in studies is > 97 %.***
Impurities:	***There are 9 process impurities; of these, the major impurity is present in a concentration range of ≥ 0.34% and ≤ 1.16%, with a typical concentration of 0.6%; the remainder are individually present at ≤ 0.1%. During the reviews under Directive 91/414/EEC and Directive 98/8/EC, none of the impurities were identified as contributing towards classification. Further information is provided in the IUCLID.***

1.2 Harmonised classification and labelling proposal

Table 2: Current Annex VI entry and the proposed harmonised classification

	CLP Regulation	***Directive 67/548/EEC (Dangerous Substances Directive; DSD)***
Current entry in Annex VI, CLP Regulation	*Not listed*	*Not listed*

Current proposal for consideration by RAC	*Acute Tox. 3; H301* *Acute Tox. 4; H332* *Carc. 2; H351* *Repr. 2; H361f* *Aquatic Acute 1; H400* *Aquatic Chronic 1; H410* *Aquatic Acute 1; H400 Acute M factor: 100* *Aquatic Chronic 1; H410 Chronic M factor: 100*	*T; R25* *Xn; R20* *Carc. Cat 3; R40* *Repr. Cat 3; R62* *R50/53* *N; R50/53* *Cn ≥ 0.25% = N, R50/53* *0.025% ≤ Cn <0.25% = N, R51-53* *0.0025% ≤ Cn <0.025% = R52-53*
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	*Acute Tox. 3; H301* *Acute Tox. 4; H332* *Carc. 2; H351* *Repr. 2; H361f* *Aquatic Acute 1; H400* *Aquatic Chronic 1; H410* *Aquatic Acute 1; H400 Acute M factor: 100* *Aquatic Chronic 1; H410 Chronic M factor: 100*	*T; R25* *Xn; R20* *Carc. Cat 3; R40* *Repr. Cat 3; R62* *R50/53* *N; R50/53* *Cn ≥ 0.25% = N, R50/53* *0.025% ≤ Cn <0.25% = N, R51-53* *0.0025% ≤ Cn <0.025% = R52-53*

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

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

					sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox 3; H301**	Not applicable	Not applicable	
	Acute toxicity - dermal	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
	Acute toxicity - inhalation	**Acute Tox 4; H332**	Not applicable	Not applicable	
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not applicable	data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.6.	Carcinogenicity	**Carc 2; H351**	Not applicable	Not applicable	
3.7.	Reproductive toxicity	**Repr 2; H361f**	Not applicable	Not applicable	
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic acute 1; H400** **Aquatic chronic 1; H410**	**Acute M factor: 100** **Chronic M factor: 100**	Not applicable	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification

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

Labelling:

Pictograms: GHS06, GHS08, GHS09

Signal word: Danger

Hazard statements: H301, H332, H351, H361f, H410

Precautionary statements: Not required as PS are not included in Annex VI

Proposed notes assigned to an entry:

None proposed.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Oxidising properties	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Flammability	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Other physico-chemical properties	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Thermal stability	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Acute toxicity	**T; R25** **Xn; R20**	Not applicable	Not applicable	
Acute toxicity – irreversible damage after single exposure	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Sensitisation	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Carcinogenicity	**Carc. Cat 3; R40**			
Mutagenicity – Genetic toxicity	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	**Repr. Cat 3; R62**	Not applicable	Not applicable	
Toxicity to reproduction – development	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	Not applicable	Not applicable	conclusive but not sufficient for classification
Environment	**R50/53**	**Cn ≥ 0.25% = N, R50/53** **0.025% ≤ Cn <0.25% = N, R51-53** **0.0025% ≤ Cn <0.025% = R52-53**	Not applicable	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

<u>Labelling</u>: <u>Indication of danger</u>: T, N

<u>R-phrases:</u> T:R25 Xn:R20-40-62 N:R50/53

<u>S-phrases:</u> S2- S13-S23-S36/37-S46-S60-S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Thiacloprid is a chloronicotinyl insecticide (nicotinergic agonist) that has been reviewed as a new active substance under both the Biocidal Products Directive (BPD) (98/8/EC) and Plant Protection Products Directive (PPP) (91/414/EEC). It was included into Annex I of the PPP Directive in 2004 and was listed in Annex I of the BPD Directive in 2009. Thiacloprid is not listed on Annex VI of CLP and has not previously been reviewed for harmonised classification and labelling.

The hazards of thiacloprid have been assessed by the UK's Health and Safety Executive as part of the BPD and PPP regulatory programmes. These assessments were discussed and agreed by European technical committees under each review programme.

At the time of submission there are no registrations for this substance under REACH..

2.2 Short summary of the scientific justification for the CLH proposal

In accordance with Article 36 (2) of CLP, thiacloprid should now be considered for harmonised classification and labelling. As the substance is not listed on Annex VI of CLP this proposal covers all hazard classes. The proposal is based mainly on the information presented in Document IIA of the BPD assessment (attached to the IUCLID dossier).

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not currently listed on Annex VI of the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Not currently listed on Annex VI of the CLP Regulation.

2.4 Current self-classification and labelling

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

Acute Tox 4; H302,
Acute Tox 4; H332,
Carc 2; H351,
Aquatic Acute 1; H400,

Aquatic Chronic 1; H410.

2.4.2 Current self-classification and labelling based on DSD criteria

Xn; R20/22, Carc. Cat 3; R40, N, R50/53

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Thiacloprid is a chloronicotinyl insecticide (nicotinergic agonist) that has been reviewed under the Biocidal Products Directive (98/8 EC) for use as a wood preservative against wood destroying organisms such as termites and longhorn beetles. Thiacloprid has also been evaluated as a new active substance, for use as an insecticide on various outdoor and protected crops, in the context of Directive 91/414/EEC concerning the placing of plant protection products on the market.

In accordance with Article 36 (2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, thiacloprid should now be considered for harmonised classification and labelling.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

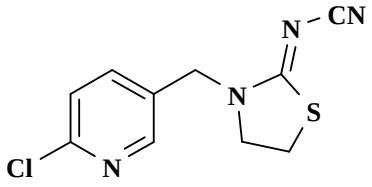
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	N/A
EC name:	Thiacloprid
CAS number (EC inventory):	
CAS number:	111988-49-9
CAS name:	Cyanamide,N-[3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]-,[N(Z)]-
IUPAC name:	(Z)-N-{3-[(6-Chloro-3-pyridinyl)methyl]-1,3-thiazolan-2-yliden}cyanamide
CLP Annex VI Index number:	Not allocated
Molecular formula:	$C_{10}H_9ClN_4S$
Molecular weight range:	252.73 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cyanamide,N-[3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]-,[N(Z)]-	98.95%	> 97% to ≤ 99.37%	

Current Annex VI entry: Not listed

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
All impurities are confidential	Process impurities are individually present at < 1.16%		

Current Annex VI entry: Not listed.

The impurities were thoroughly evaluated during the review under Directive 91/414/EEC and 98/8/EC and do not additionally impact on the classification proposed in this dossier.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The purity of the material tested is stated in the relevant sections of the dossier. During review under 91/414/EEC and 98/8/EC the tested material was considered to be equivalent to that identified above.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Yellow- brown solid	Reubke K.J (2001)	Purity 99.3%
Melting/freezing point	136°C	Krohn, J (1996)	EU, A1 Purity 99.3%
Boiling point	The substance decomposed at 270 °C before boiling	Krohn, J (1996)	OECD 103 (DTA/TGA) Purity 99.3%
Relative density	1.46 at 20 °C	Krohn, J (1996)	OECD 109 Purity 99.3%
Vapour pressure	8×10^{-10} Pa at 25 °C 3×10^{-10} Pa at 20 °C (extrapolated)	Krohn, J (1996)	OECD 104 Purity 99.7%
Surface tension	66 mN/m	Krohn, J (1996)	OECD 115 Purity 98.1%
Water solubility	184 mg/L at pH 7 and 20 °C	Krohn, J (1996)	OECD 105 Purity 99.3%
Partition coefficient n-octanol/water	1.26 at pH 7 and 20 °C 0.73 at pH 7	Krohn, J (1996) Gruener R (2001)	OECD 107 (Shake flask method) Purity 99.3% OECD 117 (HPLC Method) Purity 99%
Flash point	Not applicable since thiacloprid is a solid		
Flammability	Thiacloprid is not highly flammable, does not liberate gases in hazardous amounts in contact with water and has no pyrophoric properties.	Mix, K.H. (1995) Mix, K.H. (1995) Mix, K.H. (1995)	EU, A10 EU, A12 EU, A13 Purity 97.5%
Explosive properties	Thiacloprid is not explosive	Mix, K.H. (1995)	EU, A14 Purity 97.5%
Self-ignition temperature	No self ignition occurred.	Mix, K.H. (1995)	EU, A16 Purity 97.5%
Oxidising properties	Examination of the chemical structure of thiacloprid establishes	Mix, K.H. (1995)	

	that it does not contain any chemical groups typical for oxidizing agents. Thus the active substance can be regarded as incapable of reacting exothermically with a combustible material such as powdered cellulose.		
Granulometry	Not available		
Dissociation constant	Thiacloprid has no acid or basic properties in aqueous solutions. It is therefore impossible to specify dissociation constants of the active ingredient in water.	OECD 112 Krohn, J (1996)	

2 MANUFACTURE AND USES

2.1 Manufacture

Thiacloprid is manufactured in the EU and is also formulated into plant protection and biocidal products within the EU.

2.2 Identified uses

The predominant use in the EU is as an insecticidal plant protection product in the form of foliar spray applications for professional use. It is mainly applied against sucking insects and beetles in arable crops (primarly oilseed rape). A small proportion is formulated in ready-to-use formulations for the control of sucking insects on ornamental plants in the garden.

Thiacloprid is also marketed as a biocidal active for use in wood preservatives. The products are used as manufacturing concentrates for primers or stains that can then be applied to wood constructions by industrial, professional and non-professional users. They can also be used industrially in the protection of wood or wood based construction products from wood destroying insects.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to Table 9			

3.1 Explosivity

In a standard study (Mix, 1995), thiacloprid was found not to exhibit any explosive properties. No classification for explosivity is proposed.

3.2 Flammability

In standard studies (Mix, 1995) thiacloprid was found to be non-flammable, it did not exhibit any pyrophoric properties and did not liberate any flammable gases in contact with water.

No classification for flammability is proposed.

3.3 Oxidising potential

Examination of the chemical structure of thiacloprid establishes that it does not contain any chemical groups typical for oxidizing agents. Thus the active substance can be regarded as incapable of reacting exothermically with a combustible material such as powdered cellulose.

No classification for oxidising properties is proposed.

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.

4 HUMAN HEALTH HAZARD ASSESSMENT

Presented below is the key information pertinent to determining a classification position based on the UK's review of thiacloprid under Dir 98/8/EC. The test substance used in all of the following studies has been judged to be equivalent to the thiacloprid covered by this proposal.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Thiacloprid is well absorbed (100 %) following single and repeated oral exposure and single inhalation exposure, with approximately 10 % becoming systemically available following a single dermal application. Thiacloprid is extensively metabolised following oral dosing, the main metabolic pathways being glycine conjugation and monohydroxylation of the thiazolidine ring followed by glucuronidation. Subsequent distribution of thiacloprid and its metabolites is widespread. Elimination is rapid via both the urine and faeces. There are no marked gender-related differences in absorption, distribution, metabolism or excretion. There is no information to inform on any quantitative or qualitative differences that may exist between species. The toxicokinetic information available suggests that bioaccumulation in tissues is not a concern.

4.1.2 Human information

None available.

4.1.3 Summary and discussion on toxicokinetics

See section 4.1.1.

4.2 Acute toxicity

The acute toxicity of thiacloprid has been investigated in a number of studies.

Table 11: Summary table of relevant acute toxicity studies

Method	LD_{50}	Remarks	Reference
Oral Rat (5/sex/dose) 62.5 – 1000 mg/kg[1] Purity 97.3 % OECD 401	836 mg/kg (males) 444 mg/kg (females)	Deaths occurred 2-8 days after treatment. Mortality was observed in 0/5, 1/5 and 3/5 females at 100, 300 and 500, mg/kg respectively and in 0/5, 1/5 and 4/5 males at 300, 700 and 1000 mg/kg respectively. Clinical signs of toxicity at 100 mg/kg and above included piloerection, decreased motility, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnoea and dyspnoea.	CAR A6.1.1 (1996a)
Oral Rat (5/sex/dose) 100-5000 mg/kg[1] Purity 98.3 %	621 mg/kg (males) 396 mg/kg (females)	Clinical signs were seen at all dose levels and included decreased motility and reactivity, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnoea and dyspnea, diarrhoea.	CAR A6.1.1 (1995a)

No guideline stated			
Oral Rat acute neurotoxicity study Range finding: (5/sex/dose) 27 - 526 mg/kg[2] Main study: (12/sex/dose) 22 - 109 mg/kg[2] US-EPA guideline	177 mg/kg (calculated from 100% mortality at 244 mg/kg and 0% mortality at 109 mg/kg)	All rats died within 24 hours of receiving either 244 or 526 mg/kg. Clinical observations prior to death included tremors, decreased activity, repetitive chewing movements, cool-to-touch body, dilated pupils and clear lacrimation. No mortality at 109 mg/kg. Clinical signs from 22 mg/kg included incoordination, tremor, decreased activity, dilated pupils, ptosis and reduced body temperature. At 109 mg/kg impaired motor and locomotor activity were also observed in males.	CAR A6. 9 (1997)
Oral Rat acute neurotoxicity study (12/sex/dose) 0, 3.1, 11 mg/kg[2] US-EPA guideline	Not observed	No deaths and no clinical signs of toxicity at any dose.	CAR A6.9 (1998)
Inhalation Rat (5/sex/dose) 0, 0.08, 0.48, 1.5 or 2.5 mg/l for 4 hours, aerosol (MMAD approx. 3µm in lower exposure groups, <10µm in high-dose group) Purity 97.2 % OECD 403	> 2.5 mg/l (male) 1.2 mg/l (female)	No deaths occurred in males. Clinical signs of systemic toxicity observed up to day 6 post exposure included concentration-dependent bradypnoea, dyspnoea, rales, prostration, mydriasis, chromodacryorrhea, tremor, reduced motility, apathy, un-groomed hair, hypothermia and piloerection.	CAR A6.1.3 (1996)
Dermal Rat (5/sex/dose) 2000 mg/kg Purity 97.3 % OECD 402	> 2000 mg/kg	There were no deaths and no clinical signs of toxicity or local skin reactions.	CAR A6.1.2 (1996b)

[1]- Vehicle was 2 % Cremophor EL in demineralised water: [2]- Vehicle was 0.5 % methylcellulose and 0.4 % Tween 80 in deionised water

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In four studies that investigated the acute oral toxicity of thiacloprid, LD_{50} values that ranged between 177 and 444 mg/kg (in females) were identified.

4.2.1.2 Acute toxicity: inhalation

A single acute inhalation study resulted in the identification of an LC_{50} of 1.2 mg/l in female rats.

4.2.1.3 Acute toxicity: dermal

In an acute dermal study, no deaths occurred at the tested dose of 2000 mg/kg.

4.2.1.4 Acute toxicity: other routes

No information.

4.2.2 Human information

No information.

4.2.3 Comparison with criteria

The oral LD_{50} can be identified as 177-444 mg/kg. In the acute neurotoxicity range-finding study, the dose-response curve was steep. As the lowest value lies within the range (50-300 mg/kg) for classification as Acute Tox. 3; H301(Toxic if swallowed) under the CLP Regulation, it is proposed to use this value for the classification . The next lowest value, 396 mg/kg, also lies close to the range for Acute Oral Tox. 3.

This oral LD_{50} also lies within the range (20-200 mg/kg) for classification as T;R25 under Directive 67/548/EEC.

The inhalation LC_{50} of 1.2 mg/l lies within the range (1-5 mg/l) for classification as Acute Tox. 4; H332 (Harmful if inhaled) under the CLP Regulation.

The inhalation LC_{50} also lies within the range (1-5 mg/l/4h) for classification as Xn;R20 under Directive 67/548/EEC.

The dermal LD_{50} lies above the classification cut-off of 2000 mg/kg under both the CLP Regulation and Directive 67/548/EEC; therefore no classification is proposed.

4.2.4 Conclusions on classification and labelling

CLP Regulation:	**Acute Tox. 3; H301** **Acute Tox. 4; H332**
Directive 67/548/EEC:	**T;R25** **Xn;R20**

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.

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

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

There was no clear evidence of any specific toxic effects on a target organ or tissue. Clinical signs of toxicity were observed after single exposures to thiacloprid but were transient in nature and are considered to be unspecific signs of general acute toxicity (refer to section 4.2). Respiratory tract irritation is discussed in section 4.4.3. There are no human data to provide information on this end point. No classification as STOT SE under Regulation CLP is proposed.

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).

4.4 Irritation

4.4.1 Skin irritation

Thiacloprid's potential to cause skin irritation has been tested in rabbits.

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit, New Zealand White 3 males OECD 404	Average scores at 24, 48, 72 hours were Erythema: 1, 1, 0 Oedema: 0, 0, 0	No classification	CAR A.6.1.4 (1995c)

4.4.1.1 Non-human information

The skin irritation potential of thiacloprid (purity 97.3%) has been tested in a standard combined skin and eye irritation study in three male New Zealand White rabbits. Very slight erythema of the skin (grade 1) occurred in all three rabbits tested but all skin reactions had resolved by 72-hours post-application.

4.4.1.2 Human information

No information

4.4.1.3 Comparison with criteria

Thiacloprid caused only slight (grade 1), reversible erythema and swelling of the skin in 2 animals, which is below the response required (mean score of 2.3 or more) for classification as a skin irritant under both the CLP Regulation and Directive 67/548/EEC.

4.4.1.4 Conclusions on classification and labelling

CLP Regulation:	**No classification**
Directive 67/548/EEC:	**No classification**

4.4.2 Eye irritation

Thiacloprid's potential to induce eye irritation has been investigated in rabbits.

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit, New Zealand White, 3 males OECD 405	Average scores at 24, 48, 72 hours were Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctiva redness: 0.6, 0, 0 Conjunctiva chemosis: 0.6, 0, 0	No classification	CAR A.6.1.4 (1995c)

4.4.2.1 Non-human information

The eye irritation potential of thiacloprid (purity 97.3%) has been tested in a combined skin and eye irritation study. No corneal or iridial lesions were evident. Conjunctival redness (grade 1) and swelling (grade 1 and 2) were seen in all animals at the 1 and 24 hour observation points. The average score over the 3 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.

4.4.2.2 Human information

No information.

4.4.2.3 Comparison with criteria

Thiacloprid caused only mild, transient eye irritation characterised by conjunctival redness and swelling where the average score did not reach above 0.6. This observation does not meet the appropriate criteria for classification (average score for redness ≥ 2.5; oedema ≥2; iris lesion 1-1.5; corneal opacity 2-3) under Directive 67/548/EEC; nor does it meet the classification criteria (average score for iritis ≥ 1, and/or corneal opacity ≥ 1, and/or conjunctival redness ≥ 2, and/or conjunctival oedema ≥ 2, in at least 2 of 3 tested animals) for irritation under the CLP Regulation.

4.4.2.4 Conclusions on classification and labelling

CLP Regulation:	**No classification**
Directive 67/548/EEC:	**No classification**

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

The respiratory tract irritation potential of thiacloprid has not been directly investigated in animals. In an acute toxicity study via the inhalation route (section 4.2), clinical signs seen after exposure to 0.48 mg/l (4 h) thiacloprid included bradypnoea, dyspnoea, rales, red encrustations around snout and nose. However, these signs are considered as common observations during acute inhalation studies and do not indicate a potential for thiacloprid to cause respiratory tract irritation. In the available repeat dose inhalation studies (5- and 28-days exposure, 6 h per day; CAR A6.3.3, 1995; 1998; section 4.7.1.2), a few common, non-specific signs of toxicity (bradypnea, laboured breathing), typical of those seen following repeat inhalation exposure, were noted. In the 5-day study it was concluded that thiacloprid (0.205 mg/l, 6h/day) had ‘a minor potential to act as an upper respiratory tract irritant’ although ‘conclusive signs of respiratory irritation (e.g. serous discharge from nose) had not been observed at any time’ (CAR A6.3.3, 1995). In addition, no changes in lung weights or macroscopic changes on the lungs were noted (no histopathology data are available). Microscopy of the respiratory tract in the 28-day study did not reveal any treatment-related findings (CAR A6.3.3, 1998). On balance, there is limited evidence from animals that thiacloprid has a minimal potential to cause respiratory system irritation and therefore no classification is proposed.

4.4.3.2 Human information

No information.

4.4.3.3 Conclusions on classification and labelling

CLP Regulation:	**No classification**
Directive 67/548/EEC:	**No classification**

4.5 Corrosivity

Thiacloprid did not lead to full thickness or irreversible damage to the skin when tested for skin and eye irritation and therefore does not meet the criteria for classification as corrosive.

4.5.1 Conclusions on classification and labelling

CLP Regulation:	**No classification**
Directive 67/548/EEC:	**No classification**

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.

4.6 Sensitisation

4.6.1 Skin sensitisation

The skin sensitisation potential of thiacloprid has been investigated in a guinea pig test.

Table 14: Summary table of relevant skin sensitisation studies

Method	Doses	Results	Reference
OECD 406 (GPMT) Guinea pig	*Induction* 5% intra-dermal 50% topical *Challenge* 25% Formulated in 2 % Cremophor	Positive results in: 1/10 thiacloprid 0/10 vehicle-only controls Conclusion: non-sensitiser	CAR A.6.1.5 (1996)

4.6.1.1 Non-human information

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%. Skin reactions (grade 1) occurred in 1/10 animals and were observed at both 48 and 72 hours after challenge. Sensitisation did not occur around a naïve area of skin in thiacloprid-induced animals. Contemporary positive control data were available in which 2-mercaptobenzothiazole produced the expected responses.

4.6.1.2 Human information

No information available.

4.6.1.3 Comparison with criteria

In a standard Magnusson and Kligman guinea pig maximisation test, thiacloprid led to skin

sensitisation in only 1/10 animals tested. This is below the response required in 30%[1] of animals tested for classification under both Directive 67/548/EEC and the CLP Regulation.

4.6.1.4 Conclusions on classification and labelling

CLP Regulation:	**No classification**
Directive 67/548/EEC:	**No classification**

4.6.2 Respiratory sensitisation

There is no available information on the potential of thiacloprid to induce respiratory sensitisation.

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.

4.7 Repeated dose toxicity

Thiacloprid has been studied extensively in standard GLP/OECD-compliant studies involving repeated oral treatment of rats and mice for up to two years, and for up to one year in dogs. Exposure via the inhalation and dermal routes has been studied in rats for up to 28-days.

Substances are classified for repeated dose toxicity when serious damage ('clear functional disturbance or morphological change which has toxicological significance') is seen following repeated or prolonged exposure below guidance values provided in the classification criteria. In this report, there is therefore a focus on whether serious damage is induced by thiacloprid and, if so, whether the doses at which effects are seen merit classification.

[1] In an adjuvant study, for example the Magnusson and Kligman Guinea pig maximisation test.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

There are five studies available: two of 14-days' duration, two 90-day studies, and a 2 year-study.

Table 15: Summary table of relevant oral repeated dose toxicity studies (14 days, rats)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily gavage 14 days Wistar rats: 3/sex/group Non-GLP	0, 5, 10, 20, 60, 120 mg/kg/day Purity: 98.3%	No deaths occurred; male and female body weights and associated food intake were decreased at 60 and 120 mg/kg/day. Plasma aspartate transaminase (ASAT), alanine transaminase (ALAT), alkaline phosphatase (AP) increased at 120 mg/kg/day (up to 65 % above controls). Increased relative liver weights of approximately 20 % and 40 % were seen in males and females at 60 and 120 mg/kg/day, correlating with a slight untypical structure of the hepatocellular cytoplasm. Hepatic enzymes were induced at all doses. Increased zonal cell proliferation in the liver of females at 120 mg/kg. Increased mitotic rate in thyroids (males) at 120 mg/kg. No effects on TSH, T3 or T4. Reduced thymus weights at 60 mg/kg and above. NOAEL = 20 mg/kg/day; LOAEL = 60 mg/kg/day [CAR A.6.3.1 1995b]
Diet, dietary, 14 days Wistar rats: 5/sex/group GLP	0, 25, 100, 500 or 2000 ppm Males: 0, 2.5. 11.2, 49.5 or 187.6 mg/kg/day Females: 0, 2.3, 9.8, 49.5, or 187.2 mg/kg/day mg/kg/d equivalents calculated from actual food intake Purity: 98.6%	No deaths occurred. Decreased terminal body weights of up to 11 and 24 % at 500 and 2000 ppm; also food intake reduced by up to 17 % and 37 % at 500 and 2000 ppm. Liver and thyroid were the only organs examined by histopathology. Small increases in liver weight at 500 and 2000 ppm; increased incidence of distinct lobulation of the liver in 4/5 males at 2000 ppm and in 1/5 females each at 25, 100, 500 ppm, and in 2/5 females at 2000 ppm; hepatocyte hypertrophy with slight cytoplasmic changes at 500 ppm and above. Hepatic enzyme induction at 500 ppm and above: (including ECOD, ALD, EH, GLU-T). No effects on thyroid weight. The frequency of increased follicular epithelial mitotic rate was increased significantly in males at 500 and 2000 ppm, and hypertrophy of the follicular epithelium was seen only in males (100 %) at 2000 ppm. Slightly increased TSH at 2000 ppm (female only). No treatment related effects on T3, T4 or thyroxin-binding capacity (TBC). Dose related increased cholesterol statistically significant at 100 ppm and above (male) and 2000 ppm (female). Increased bile acid and GGT at 2000 ppm (male + female). NOAEL = 9.8 mg/kg/day; LOAEL = 49.5 mg/kg/day (approx) [CAR A.6.3.1 (1996c)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

The 14-day studies demonstrated that rats showed an adaptive response to repeated dosing with thiacloprid.

In the first study, in which gavage administration was employed, increased relative liver weights in males and females at 60 and 120 mg/kg/day thiacloprid, accompanied by hepatic enzyme induction, demonstrated an adaption to cope with an increased metabolic load. At the higher dose, increased cell proliferation in the livers of females was further evidence of this. In males, increased mitotic rate in the thyroid at 120 mg/kg/d might also have been linked to this adaptive response.

In the second study, in which thiacloprid was administered via the diet, there was a focus on the liver and the thyroid. At approximately 50 mg/kg/d and 190 mg/kg/d, there were again modest increases in liver weight, which were accompanied by signs of hypertrophy and induction of hepatic enzymes. There were also signs of increased mitosis and hypertrophy in the thyroid at these doses.

There was no evidence of severe liver or thyroid toxicity in either of these studies.

Table 16: Summary table of relevant oral repeated dose toxicity studies (90 days, rats)

Method	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Diet 90 days (+35 days recovery) Wistar rats, 10 /sex/group GLP	0, 25, 100, 400 or 1600 ppm. Males: 0, 1.9, 7.3, 28.6 or 123.2 mg/kg/day Females: 0,2, 7.6, 35.6 or 160.6 mg/kg/day Purity: 98.6%	No animals died or showed clinical signs of toxicity; decreased body weight (up to 16 %) at 1600 ppm. The effect on body weight diminished with time during the recovery period. Food intake was not affected, although water intake was slightly reduced in males at 1600 ppm. No evidence of severe toxicity at any dose level. Increased mean absolute liver weights in males only (5%) at 400 ppm, and 21 % and 17 % at 1600 ppm, for males and females respectively. Moderate hepatocyte hypertrophy with cytoplasmic changes in 9/10 males and 2/10 females at 400 ppm and in all animals at 1600 ppm. Hepatocellular hypertrophy was not reversible in 3/10 males of the 1600 ppm recovery group. Significant reversible induction of hepatic cytochrome P450 and UDPGT in males and females at 400 and 1600 ppm. Dose-related increase in thyroid weight from 25 ppm in males, but only statistically significant at 1600 ppm (by 66 %; 25 % above controls after recovery). Also in males: T3 concentrations were increased in all dose groups at week 3, but only at 1600 ppm in week 12 (by 30%); T4 concentrations slightly increased at 400 and 1600 ppm at week 3, but not week 12. Effects reversible during recovery. No effects in females. At 90 days: decreased clotting time, increased creatine and increased cholesterol (up to 85 %) at 1600 ppm only. Urinalysis showed an increase in sodium and calcium at 1600 ppm in males during weeks 3, 11/12 and 17, although there was no histopathological sign of damage to the kidney. NOAEL = 7 mg/kg/day; LOAEL = 30 mg/kg/day (approx) [CAR A.6.4.1, 1997]
Diet, ad libitum Fischer rats 12/sex/group GLP	0,50, 400 or 1600 ppm Males: 0, 2.94, 24.2 or 101 mg/kg/day Females:	Study focussing on potential neurotoxicty. No deaths or clinical signs of toxicity; decreased body weights at 1600 ppm only, maximal values in comparison to controls: male decreased 12 % (day 7) remaining within 10% of controls for the remainder of the study, female decreased 6 % (day 7), recovering to values similar to controls thereafter. No signs of neurotoxicity or effects on motor or locomotor activity at 50 ppm and above.

	0, 3,.41, 27.9 or 115 mg/kg/day Purity: 96.6-97.5%	No microscopic observations (tissues examined: skeletal muscle, peripheral nerves, eyes, optic nerves, and tissues from CNS). [CAR A6.9 (1997)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

In the first dietary study, rat body weight was significantly decreased and absolute liver weight was increased at approximately 125 mg/kg/day thiacloprid. There was also evidence of moderate hepatocellular hypertrophy with cytoplasmic changes and increased hepatic enzyme levels and activity. Transiently increased triiodothyronine (T3) and thyroxine (T4) levels were observed at 125 mg/kg/day, and this may have led to the increased thyroid weights seen at this top dose. There was no evidence of serious damage to the liver or thyroid at this dose. At about 30 mg/kg/day, there was also some evidence of effects in the liver and associated changes in the thyroid, but these were less prevalent and marked than at the top dose.

In the second dietary study, which focused on neurotoxicity parameters, there were no significant body weight changes that persisted throughout the 90-day treatment period. Small, transient decreases at approximately 100 mg/kg/day seem to have been related to decreased food intake. No signs of neurotoxicity or effects on motor or locomotor activity were seen at any of the dose levels.

Table 17: Summary table of relevant oral repeated dose toxicity studies (2 years, rats) – non-tumour findings

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Diet, ad libitum Wistar rats, 2-year: 50 males and 50 females per group Interim sacrifice; 10 males & 10 females (1 yr). GLP	0, 25, 50, 500 or 1000 ppm Males: 0, 1.2, 2.5, 25.2 or 51.7 mg/kg/day Females: 0, 1.6, 3.3, 33.5 or 69.1 mg/kg/day Purity 96.8 – 97.2 %	No effects on survival rates. In females only, decreased body weight at 500 and 1000 ppm: maximal difference from controls of 15 % between weeks 55 – 77 and remained above 10 % until termination (500 ppm); max. 21 % in week 69/71 (1000 ppm). In males, there was increased liver weight (20%) at 1000 ppm; and centrilobular hypertrophy, cytoplasmic changes in the hepatocytes (eosinophilic cytoplasm with basophilic strands) and eosinophilic/clear cell foci at 50, 500 and 1000 ppm. In females, similar histopathological changes were only seen at 500 and 1000 ppm. Hepatic enzyme induction (including cytochrome P450) seen from 25 ppm in males and 500 ppm in females. In the thyroid, follicular epithelial hypertrophy was increased in males from 50 ppm and in females from 500 ppm. Follicular cell hyperplasia seen at 1000 ppm in females only (3/50*, controls 0/50). No effect on T3/T4 at any time point, but plasma TSH consistently increased in males and females at 1000 ppm, and in males at 500 ppm at 26 weeks. Prevalence of skeletal muscle atrophy was increased in females at 500 and 1000 ppm and increased sciatic nerve degeneration was seen in males from 500 ppm and in females at 1000 ppm. Females also showed significantly increased incidences of radiculoneuropathy (31/50, 32/50, 32/50, 37/50, 39/50*), retinal atrophy (15/50, 20/50, 24/50*, 25/50* and 32/50**) and lens degeneration (9/50, 18/50, 16/50, 20/50** and 30/50**) at 1000 ppm, and from 50 ppm and 500 ppm, respectively. Not evident after 1 year. Decreased incidence of galactocele and lacteal cysts in the mammary glands of females, combined incidences at 0, 25, 50, 500 and 1000 ppm: 21/50, 18/50,

		14/50, 14/50 and 6/50. At two years, there was an increased incidence of ovarian cysts from 500 ppm: 16/50, 15/49, 19/50, 22/48, 24/50 at 0, 25, 50, 500 and 1000 ppm. At the one-year interim sacrifice, glandular hyperplasia of the uterus was observed: 1/10, 0/10, 2/10, 4/10, 4/10 at 0, 25, 50, 500 and 1000 ppm. NOAEL = 1.2 mg/kg/day (25 ppm) ; LOAEL = 2.5 mg/kg/day (50 ppm). [CAR A6.5/6.7 (1998; amended 2007)]

Statistical significance: p<=0.05 and p<=0.01**. NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.*

The most severe non-neoplastic toxicological findings in this two-year study were seen in females from 500 ppm (*circa* 33.5 mg/kg/day), including: degenerative myelopathy in the nervous system characterised by radiculoneuropathy, sciatic nerve degeneration and subsequent skeletal muscle atrophy (Bomhard, 1998). Radiculoneuropathy is a degenerative lesion in the ventral roots of spinal nerves (mainly lumber segment) characterised by cholesterol clefts and demyelination and infiltration by foamy, lipid-laden macrophages. The study report indicates that these findings are known to occur spontaneously in old rats (termed spinal radiculoneuropathy or degenerative myelopathy) and may be exacerbated by xenobiotics.

Retinal atrophy and degeneration of the lens were seen in the eyes of control and treated female animals. These potentially serious lesions were only seen after two-year (near lifetime) exposure of rats: similar effects were not seen after one year in this study or in the 90-day study of Sheets (1997), in which the eyes and optic nerves were examined by histopathology. Historical control data for these findings were not available.

In both the liver and thyroid there were changes consistent with an adaptive response to treatment. Hepatic enzymes were induced at 50 ppm and above. Histopathological changes, likely to be a secondary consequence of enzyme induction, were seen in the livers of males from 50 ppm and in females from 500 ppm. In males at 50 ppm these changes included centrilobular hypertrophy, cytoplasmic changes in the hepatocytes (eosinophilic cytoplasm with basophilic strands), and eosinophilic/clear cell foci. Thyroid follicular epithelial hypertrophy was also seen in males at 50 ppm, which was most likely to have also been secondary to hepatic enzyme induction.

Subtle changes in the uterine tissue, in the form of glandular hyperplasia, at one year and ovarian cysts at the two-year sacrifice indicated a possible treatment-related imbalance of steroid sex hormone levels. The implications of these findings are discussed further in the section on carcinogenicity (4.10). The incidence of galatocele and lacteal cysts in the mammary glands of females was decreased at 25 ppm and 50 ppm. The toxicological significance of this isolated effect is unknown, although it was not further observed.

Mouse

Table 18: Summary table of relevant oral repeated dose toxicity studies (14- and 21-day, mouse)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Diet, ad libitum	0, 50, 200, 2000 or 10000 ppm	There were no deaths, clinical signs of toxicity, or effects on body weight.

14 days B6C3F1 mice: 5 males and 5 females per group OECD 407 GLP	Males: 0, 22, 84, 765 or 4143 mg/kg/day Females: 0, 30, 113, 1201 or 5450 mg/kg/day Purity 98.6%	Increased absolute liver weights (up to 32 %) at 2000 ppm. The liver was the only organ assessed for histopathology. Hypertrophy of centrilobular hepatocytes and cytoplasmic changes at 200 ppm and above, predominantly in males. Increased lipid content (not severe fatty change) in hepatocytes at 2000 ppm and above (m+f). Dose related induction of hepatic cytochrome P450 enzymes at 200 ppm and above. Decreased cholesterol (male + female), increased serum protein (male), decreased albumin and bilirubin (female) at 10,000 ppm. NOAEL = 22 mg/kg/day (50 ppm); LOAEL = 84 mg/kg/day (200 ppm). [CAR A6.3.1 (1997a)]
Diet, ad libitum 21 days B6C3F1 mice: 3 males and 3 females per group No guideline; non-GLP.	0, 100, 1000, or 10000 ppm Males: 0, 30, 368 or 4141 mg/kg/day Females: 0, 64, 559 or 5785 mg/kg/day. Purity 98.6%	No deaths or clinical signs of toxicity observed. Decreased body weight gain and increased food consumption at 10,000 ppm in males. Decreased food consumption in females at 1000 ppm but no significant effect on body weight. Macroscopic examination of liver and kidneys only. Enlarged livers in 2/3 males at 10,000 ppm. Increased liver weight (absolute: *circa* 10 %) at 1000 ppm. Liver enzyme activity was not determined. NOAEL = 30 mg/kg/day (100 ppm); LOAEL 368 mg/kg/day (1000 ppm). [CAR A6.3.1 (1994)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

Adaptive changes in the liver were seen after dietary exposures of mice to approximately 100 mg/kg/day thiacloprid or more for 14 to 21 days. Even at much higher doses (up to 5000 mg/kg/day) there was little, if any, evidence of serious damage to the liver or any other tissues.

Table 19: Summary table of relevant oral repeated dose toxicity studies (90-day, mouse)

Method	**Dose levels**	**Observations and remarks** **(effects of major toxicological significance)**
Diet, ad libitum 90 days B6C3F1 mice: 10 males and 10 females per group OECD 408 GLP	0, 50, 250, 1250 or 6250 ppm Males: 0, 20, 103, 542 or 2819 mg/kg/day Females: 0, 27, 139, 704 or 3351 mg/kg/day Purity: 98.6-98.7%	No treatment-related deaths or clinical signs of toxicity. Decreased mean body weight of 14 % (male) at 6250 ppm. Increased food intake (male + female) of 8-12 % at 1250 ppm and above. No effects on T3 or T4. Decreased cholesterol in females at 250 ppm and above and in males at 6250 ppm; up to 30 % decreased at 6250 ppm. Decreased bilirubin at 1250 ppm (male + female) of up to 40 %. Dose-related liver enzyme induction (CYP 450), increase of 7, 14, 66.9** and 107.7** % (male), and 1, 11, 59** and 87.4** % (female), at 50, 250, 1250 and 6250 ppm, respectively. Increased liver weights at 1250 ppm (up 8 %*) and at 6250 ppm (up to 40 %*). Hepatocellular hypertrophy at 1250 ppm (male) and 6250 ppm (male + female). Increased adrenal weights (female) of 25, 50 and 42 % at 250, 1250 and 6250 ppm, respectively. Dose-related increase in the severity of fatty vacuolation of the adrenal X-zone leading to hypertrophy at 50 ppm and above. Mean grade was 1.7, 2.5, 3.4, 4.5 and 4.8 at 0, 50, 250, 1250 and 6250 ppm, respectively.

		Decreased old corpora lutea and activation of ovarian interstitial glands at 1250 ppm and above. NOAEL = not established; LOAEL = 27 mg/kg/day (50 ppm) [CAR A6.4.1 (1995)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

Significant findings at approximately 25 mg/kg/day were limited to the adrenals, in which there were dose-related increases in severity of fatty vacuolation of the X-zone leading to hypertrophy. At higher doses, in females, this was accompanied by small increases in adrenal weight. The X-zone is located between the zona reticularis and the adrenal medulla; its function is unclear. The presence of the X-zone appears to be dependent on age (it has been reported to be a transient feature in young mice) and reproductive status and has been described in mice, voles, red squirrels, shrews, rabbits and cats. Histologically similar tissue has been reported in the foetal zone of the human adrenal gland. The relevance of these changes seen in the absence of other signs of toxicity is largely unknown, but there is little or no evidence to indicate that it is a serious lesion of relevance to classification.

Table 20: Summary table of relevant oral repeated dose toxicity studies (2-years, mouse)

Method	**Dose levels**	**Observations and remarks (effects of major toxicological significance)**
Diet, *ad libitum* 2 year (inc 1 year interim) B6C3F1 mice 1y: 10 males & 10 females per group 2y: 50 males and 50 females per group. OECD 451 GLP	0, 30, 1250 or 2500 ppm Males: 0, 5.7, 234.1 or 564.4 mg/kg/day Females: 0, 10.9, 475.3, 872.5 mg/kg/day Purity: 96.8-97.2 %	No effects on survival rates or body weight. Small (< 10.7 %) increase in absolute and relative liver weight in males & females, only statistically significant for relative weights at the top dose. Histopathological changes in the liver, incidences at 0, 30, 1250 and 2500 ppm: hepatocyte hypertrophy (male: 0/50, 0/50, 46/50, 49/50; female: 0/50, 0/50, 2/50, 3/50), degeneration (male only: 1/50, 0/50, 5/50, 16/50), fatty change (male: 3/50, 4/50, 15/50, 21/50; female: 2/50, 3/50, 3/50, 7/50), and necrosis (male: 5/50, 3/50, 6/50, 31/50; female: 15/50, 17/50, 17/50, 25/50). Induction of hepatic enzymes was not assessed. Adrenal X-zone vacuolation in females: increased incidence and severity with increasing dose (mean grade at 1250 ppm 2.0 in comparison to 1.1 in controls; incidence of 67 %, 75 %, 96 % and 100 % at 0, 30, 1250 and 2500 ppm). Increased incidence (not statistically significant) of eosinophilic, luteinised cells in the ovaries, incidences at 0, 30, 1250 and 2500 ppm: 3/50, 0/50, 5/50 and 8/50. No effects on the uterus or thyroid. No assessment of T3/T4 or TSH concentrations. No histological findings in the neurological system. NOAEL = 5.7 mg/kg/day (30 ppm); LOAEL = 457. 3 mg/kg/day (1250 ppm) [CAR A6.7 (1998)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

Thiacloprid administered in the range of 5 to 10 mg/kg/day for two years induced no

toxicologically significant effects in mice. The liver was the main target organ, with increased weight, hypertrophy, fatty change and enzyme induction seen from 230-470 mg/kg/day, and severe microscopic lesions (hepatocellular degeneration and necrosis) at approximately 500 mg/kg/day.

A dose-related increase in vacuolation of the adrenal X-zone, and associated hypertrophy, was seen in this study from 230-470 mg/kg/day thiacloprid. As previously discussed, the toxicological significance of these effects is unclear. There were no effects on the adrenal glands at doses below the guidance cut-off values for classification. Also from this dose, there was an increase in the incidence of eosinophilic, luteinised cells in the ovaries, which may have been linked to the tumour findings in this tissue (see Section 5.8).

Dog

Table 21: Summary table of relevant oral repeated dose toxicity studies (dogs)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Diet, *ad libitum* 70 days Beagle dogs: 2 males and 2 females per group Similar to OECD 409 Non-GLP	0, 100, 300 or 1000 ppm (increased gradually from day 19 to 2500 ppm at day 38) On average, calculated intake was: 0, 3.3, 9.6 or 80 mg/kg/day + Satellite group exposed to 2500 ppm (65.7 mg/kg/day) for 4 weeks. Purity: 98.6%	No deaths, clinical signs of toxicity or treatment-related effects on reflex responses, pulse rates or body temperatures. The only effects of note were seen at the top dose; these included: Decreased food consumption and body weight gain in females (satellite group). Increased absolute prostate weights (up to 69 %) in both the main and satellite groups. Hepatocyte cytoplasmic changes in both the main and satellite groups. Slight increased liver enzyme activity. Decreased T4 and increased T3 and thyroxin-binding capacity (TBC) in females (satellite group). NOAEL = 9.6 mg/kg/day (300 ppm); LOAEL = 80 mg/kg/day (1000/2500 ppm) [CAR A6.4.1 (1998a)]
Diet, *ad libitum* 105-106 days Beagle dogs, 4 males and 4 females per group OECD 409 GLP	0, 250, 1000 or 2000 ppm Males: 0, 8.5, 34.9 or 68 mg/kg/day Females: 0, 8.9, 34.7 or 65.3 mg/kg/day. Purity: 96.8-97.2%	No deaths, clinical signs of toxicity or effects on body weight or food consumption, pulse rate or reflex reactions. Combined (male + female) liver weights were 16, 20 and 17 % above control at 250, 1000 and 2000 ppm, respectively. However, control liver weights (326 g) were below the historical control range (334-438 g). Liver xenobiotic metabolising enzymes were induced at 1000 ppm and above. T4 was decreased at 1000 ppm and above. Mean absolute prostate weight increased by 148 and 180 % at 1000 and 2000 ppm, respectively. In this tissue, slight to moderate hypertrophy of the glandular epithelium in all dogs at 1000 ppm and above. Increased incidence of spermatocytic degeneration in the testes (2/4 dogs) and/or epididymides (4/4 dogs, compared with 1 control) at 2000 ppm. The interstitial testicular cells also appeared to be slightly more prominent in 3 dogs at this dose. Such findings are reported to show a wide variation in severity and incidence in young dogs. Uterine weight was increased by 32, 26 and 71 % at 250, 100 and 2000 ppm, respectively. NOAEL = 8.5 mg/kg/day (250 ppm); LOAEL = 34.8 mg/kg/day (1000 ppm) [CAR A6.4.1 (1998)]
Diet, *ad libitum* 1 year Beagle dog,	0, 40, 100, 250 or 1000 ppm for 52 weeks or	No deaths, clinical signs of toxicity, effects on body weight, pulse rate, heart rate or body temperature. At 1000 ppm, hepatocellular cytoplasmic changes (pale perinuclear cytoplasm) were seen in males at week 26 but not at week 52. No hepatic enzyme induction observed. No other treatment-related changes were noted during the

4 males and 4 females per group for 52 weeks; or 3 males per group for 24 weeks OECD 452 GLP	0, 100 or 1000 ppm for 26 weeks Males: 0, 1.42, 3.60, 8.88 or 34.42 mg/kg/day Females: 0, 1.39, 3.27, 8.30 or 33.80 mg/kg/day. Purity: 96.8-97.1%	histopathology investigations (which included the neurological system). At week 52, there was an increase in group mean absolute prostate weight of 76% at 1000 ppm. Smaller increases were seen at 40 and 250 ppm (but not 100 ppm) at 52 weeks. NOAEL = 8.7 mg/kg/day (250 ppm); LOAEL = 34 .8 mg/kg/day (1000 ppm) [CAR A6.5 (1998b)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

In the 70-day dietary study, Beagle dogs showed signs consistent with adaptive hepatic enzyme induction, including increased enzyme activity, hepatocyte cytoplasmic changes and some changes to thyroid hormone parameters at approximately 80 mg/kg/day thiacloprid. Increases in prostate weights were also seen at this dose. Although the increase observed was relatively high (69% above controls), there was no evidence of organ dysfunction to support classification.

In the 106-day study, there was an induction of hepatic enzymes at approximately 35 mg/kg/day and above, associated with increased liver weight, which was most likely an adaptive response to increased metabolic need, owing to treatment. There were again significant increases in prostate weights, observed at approximately 35 and 65 mg/kg/day, together with slight hypertrophy of the prostate glandular epithelium at 65 mg/kg/day. This is not considered to represent dysfunction of the prostate. Uterine weights were increased at all three dose levels, but there was no evidence of organ dysfunction.

In the longer-term study, there were only minimal changes in the liver at the highest dose level (approximately 35 mg/kg/day). Prostate weights were increased most significantly at 35 mg/kg/day, and less so at lower doses. However, there was no evidence of glandular epithelium hypertrophy.

4.7.1.2 Repeated dose toxicity: inhalation

Repeated dose inhalation studies have been conducted in the rat.

Table 22: Summary table of inhalation repeated dose toxicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Inhalation, nose-only Wistar rats, 10 males and 10 females 6 h/day 5 days; + 2 week recovery period Similar to OECD403 & 412 GLP (but no QA)	0, 0.00197, 0.019 or 0.205 mg/l Aerosol MMAD = 2.9 - 3.3 µm Purity: 97.2%	No deaths occurred. At 0.205 mg/l, signs of general toxicity included un-groomed pelt, piloerection, reduced motility, tremor, laboured breathing pattern and emaciation. There was also evidence of slight respiratory tract irritation. Body weight was decreased on day 4 and 7. Also at 0.205 mg/l, increased mean absolute and relative liver weight and decreased mean absolute thymus weight (by up to 60%) that recovered by end of the study. Hepatic CYP P450 similarly induced. Dark spleens were noted at 0.019 mg/l and above in females after the treatment period, but not at terminal sacrifice. NOAEC = 0.019 mg/l ; LOAEC = 0.205 mg/l [CAR A6.3.3 (1995)]
Inhalation, nose-only Wistar rats, 10 males and 10 females 6 h/day 5 d/week for 28 days Similar to OECD 403 & 412 GLP (but no QA)	0, 0.002, 0.018 or 0.143 mg/l Aerosol MMAD = 2.9 µm Purity: 97.2%	No deaths occurred. At 0.143 mg/l, signs of general toxicity included decreased motility, tremor, laboured breathing pattern, piloerection, un-groomed hair-coat, atony, crepitation, salivation, decreased (slight) body weights and hypothermia. At 0.143 mg/l, decreased absolute and relative liver weights (up to 17 and 12% in males and females, respectively), slight hepatocellular hypertrophy and liver enzyme induction. Increased thyroid weight in males and females; slight hypertrophy of the follicular epithelium in 2 males. At 0.018 mg/l, slight hepatocellular and thyroid follicular epithelial cell hypertrophy. NOAEC = 0.018 mg/l LOAEC = 0.143 mg/l [CAR A6.3.3 (1998)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

Relatively minor changes in the liver and thyroid were reported after repeated exposure to 0.143 mg/l thiacloprid for 28 days. Although this exposure level is below the cut-off for classification as harmful (0. 6 mg/l, table 3.9.2.2 of the CLP guidance), none of the findings are judged to provide evidence of serious damage

The “dark” spleens observed in female rats at 0.019 and 0.205 mg/l after exposure in the 5-day study were resolved after recovery. The toxicological significance of these findings is unclear, but they are not judged to be of serious concern given that they resolved after cessation of exposure and were not reported in other repeated dose studies.

4.7.1.3 Repeated dose toxicity: dermal

A repeated dose dermal study has been conducted in the rat.

Table 23: Summary table of the dermal repeated dose toxicity study

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Dermal, 28 days Wistar rats, 5 males and 5 females 6 h/day, 5 d/week for the first 3 weeks, and 7 d/week for the final week. Followed by a 2-week recovery period. OECD 410 GLP	0, 100, 300 or 1000 mg/kg/day Purity: 97.2%	No deaths, clinical signs of toxicity, or effects on body weights observed. No treatment-related local skin effects. Increased absolute liver weights (up to 14 %) at 1000 mg/kg/day. Hepatic centrilobular hypertrophy associated with more homogeneously structured cytoplasm in males at 300 mg/kg/day and above and in females at 1000 mg/kg/day. These effects persisted in 2/5 males treated with 1000 mg/kg/day during the recovery period. Thyroid follicular cell hypertrophy at 1000 mg/kg (male + female), reversible in females but persisted in 1/5 males at the end of recovery. NOAEL = 100 mg/kg/day; LOAEL = 300 mg/kg/day. [CAR A6.3.2 (1997b)]

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed by the Technical Meeting of the Biocide Competent Authorities.

In the only available dermal study, which involved the repeated administration of thiacloprid over a 28-day period, there was evidence of an adaptive response in the liver from 300 mg/kg/day; this comprised hepatic centrilobular hypertrophy associated with more homogeneously structured cytoplasm in males only. No effects were seen at 100 mg/kg/day.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

No further relevant information.

4.7.1.7 Summary and discussion of repeated dose toxicity

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. 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/d. Additionally, more serious histopathological changes (degeneration, fatty change, necrosis) were reported in a two-year mouse study, but only from 234 mg/kg/d. The thyroid effects similarly 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.

In a rat 90-day neurotoxicity study, no neurotoxic, motor or locomotor effects were recorded up to the maximum tested dose of 115 mg/kg/d. However, prolonged oral administration of thiacloprid to female rats for two years resulted in degenerative myelopathy from 33.5 mg/kg/d, retinal atrophy from 3.3 mg/kg/d and degeneration of the lens from 33.5 mg/kg/d. No adverse histological findings occurred in the neurological system of mice at doses up to 875 mg/kg/d during a two-year study.

Increases in the weights of the prostate (associated with hypertrophy of the glandular epithelium) and uterus in dogs and of the adrenal glands in mice were reported, but since there was no evidence of organ dysfunction, these organ weight changes do not justify classification.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Under Directive 67/548/EEC, classification as R48 is reserved for substances that cause serious damage to health, generally at or below the guidance value of 50 mg/kg/d (for a classification of harmful) obtained in an oral 90-day study in rats. For 90-day inhalation and dermal studies, the guidance values are 0.25 mg/l/6 hr and 100 mg/kg/d, respectively. In several repeated-dose studies of durations from five days to two years, the only serious effects recorded at doses below this guidance value were neurotoxicological findings in a two-year oral rat study. In this study, a statistically significantly increased incidence of retinal atrophy occurred from 3.3 mg/kg/d, and degenerative myelopathy and degeneration of the lens occurred from 33.5 mg/kg/d in females. When the oral guidance value is adjusted from a 90-day study to one of 24-months' duration, a value of 6.25 mg/kg/d is obtained, which is clearly below the dose at which degenerative myelopathy and degeneration of the lens were reported. Additional considerations are that these effects only occurred after chronic exposure; some of the findings associated with the degenerative myelopathy are known to occur in aged rats and can be exacerbated by xenobiotics; and the degeneration of the lens was present in many control animals. Therefore, these two effects will not be considered further in deciding upon a classification for repeated-dose toxicity.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

A classification of R48 is indicated when serious effects that meet the following descriptions occur at or below the guidance values.

a) Substance-related deaths

There were no deaths.

b) Major functional changes in the central or peripheral nervous systems and/or other organ systems

Effects on the nervous systems include those on sight, hearing and the sense of smell. Retinal atrophy was reported in a two year study in rats from 3.3 mg/kg/d; however, there was also a high incidence in the control animals (15/50). A chronic exposure seemed to be necessary to induce this effect, since it was not seen after 90 days or one year of administration. No neurological effects occurred in a two-year mouse study in which thiacloprid was administered at doses up to 873 mg/kg/d. On balance, this finding does not provide a sufficient basis to justify the classification of thiacloprid for repeated dose toxicity.

c) Any consistent changes in clinical biochemistry, haematology or urinalysis parameters that indicate severe organ dysfunction

Although there were changes in some clinical chemistry parameters (liver enzymes and thyroid hormones) below the guidance value, these were indicative of adaptive changes. There was no evidence of severe organ dysfunction.

d) Severe organ damage noted in microscopic examination following autopsy

No effects indicative of severe organ damage (necrosis, fibrosis, granuloma formation in vital organs with regenerative capacity; severe morphological changes that are potentially reversible but are clear evidence of marked organ dysfunction; evidence of appreciable cell death in vital organs incapable of regeneration) were reported below the guidance value.

Additionally, there were no generalised changes that involved several organ systems or severe changes in the general health status of the animals.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Directive 67/548/EEC:	**No classification**

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Under CLP, STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day oral study. For 90-day inhalation (vapour) and dermal exposures, the guidance values are 1 mg/l/6 hr and 200 mg/kg/d, respectively. 'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

In several repeated-dose studies of durations from five days to two years, the only serious effects recorded at doses below this guidance value were neurotoxicological findings in a two-year oral

rat study. In this study, statistically significantly increased retinal atrophy occurred from 3.3 mg/kg/d, and degenerative myelopathy and degeneration of the lens occurred from 33.5 mg/kg/d in females. When the oral guidance value is adjusted from a 90-day study to one of 24-months' duration, a value of 12.5 mg/kg/d is obtained, which is below the dose at which degenerative myelopathy and degeneration of the lens were reported. Additional considerations are that these effects only occurred after chronic exposure; some of the findings associated with the degenerative myelopathy are known to occur in aged rats and can be exacerbated by xenobiotics; and the degeneration of the lens was present in many control animals. Therefore, these two effects will not be considered further in deciding upon a classification for repeated-dose toxicity.

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

A classification of STOT-RE is indicated when toxic effects that may include the following descriptions occur at or below the guidance values.

a) Morbidity or death resulting from repeated or long-term exposure

There were no treatment-related deaths or cases of moribund animals.

b) Significant functional changes in the central or peripheral nervous systems or other organ systems

This includes effects on special senses (sight, hearing and the sense of smell). Retinal atrophy was reported in a two year study in rats from 3.3 mg/kg/d; however, there was also a high incidence in the control animals (15/50). A chronic exposure seemed to be necessary to induce this effect, since it was not seen after 90 days or one year of administration. No neurological effects occurred in a two-year mouse study in which thiacloprid was administered at doses up to 873 mg/kg/d. On balance, this finding does not provide a sufficient basis to justify the classification of thiacloprid for repeated dose toxicity.

c) Any consistent and significant adverse changes in clinical biochemistry, haematology or urinalysis parameters

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 constitute a normal biochemical or physiological response and do not indicate classification.

d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination

There were no such effects at doses below the guidance values.

e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity

There were no such effects.

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

There were no such effects.

g) Evidence of appreciable cell death (including cell degeneration and reduced cell numbers) in vital organs incapable of regeneration

There were no such effects.

Additionally, there were no generalised changes that involved several organ systems or significant/severe changes in the general health status of the animals.

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

CLP Regulation:	**No classification**

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.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

The genotoxic potential of thiacloprid has been investigated in several *in vitro* studies and an *in vivo* micronucleus test.

Table 24: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Concentrations tested	Result		Reference
		+S9	-S9	
IN VITRO				
Bacterial reverse mutation (Ames) *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100	Limit test Purity: 97.2%	Negative	Negative	CAR A6.6.1 (1995a)
Bacterial reverse mutation (Ames) *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 E. coli WP2/urvA	Limit test Purity: 96.8%	Negative	Negative	CAR A6.6.1 (1995)
In vitro mammalian chromosome aberration Chinese hamster V79 cells	0.075, 0.3, 0.75 mg/ml Purity: 96.8 – 97.2% 4 hour exposure	Negative	Negative	CAR A6.6.1 (1995c)
In vitro HPRT gene mutation Chinese hamster V79 cells	0.015, 0.031, 0.063, 0.12, 0.25, 0.5 mg/ml Purity: 97.2%	Negative	Negative	CAR A6.6.1 (1996b)
In vitro unscheduled DNA synthesis Sprague-Dawley rat hepatocytes	0.075, 0.15, 0.3, 0.35, 0.4, 0.45, 0.5 mg/ml	Negative		CAR A6.6.1 (1996a)
IN VIVO				
Mammalian erythrocyte micronucleus Mouse, NMRI, male and female, 5/sex/dose	0 or 60 mg/kg i.p. Purity: 96.8 – 97.2%	Negative		CAR A6.6.1 (1995b)

4.9.2 Human information

No human data are available.

4.9.3 Other relevant information

There are no other relevant information available.

4.9.4 Summary and discussion of mutagenicity

Thiacloprid was negative in several *in vitro* assays and in an *in vivo* micronucleus assay.

4.9.5 Comparison with criteria

Thiacloprid did not meet the criteria for classification as a germ cell mutagen

RAC evaluation of germ cell mutagenicity

Summa

CLP Regulation: No classification

Directive 67/548/EEC: No classification

In a batt milar to, internatio mosome aberratior on 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.

4.9.6 Conclusions on classification and labelling

4.10 Carcinogenicity

The potential carcinogenicity of thiacloprid has been evaluated in standard studies in rats and mice after two years of dietary exposure. Tumours occurred in the thyroid and uterus of rats and the ovaries of mice, as summarised in the following table. The non-neoplastic observations from these studies are summarised in Section 4.7.

Table 25: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat, Wistar Diet, *ad libitum*	0, 25, 50, 500 or 1000 ppm	No effects on survival rates. At two years, survival of males was 39, 38, 36, 40, 41 and of females was 22, 28, 36, 31, 33 at 0, 25, 50, 500 and 1000 ppm. In females only, decreased body weight at 500 and 1000 ppm:

2-year: 50 males and 50 females per group Interim sacrifice; 10 males & 10 females (1 yr). OECD 453 GLP	Males: 0, 1.2, 2.5, 25.2 or 51.7 mg/kg/day Females: 0, 1.6, 3.3, 33.5 or 69.1 mg/kg/day Purity 96.8 – 97.2 %	maximal difference from controls of 15 % between weeks 55 – 77 and remained above 10 % until termination (500 ppm); max. 21 % in week 69/71 (1000 ppm). In females, there were increased uterine tumours at 500 and 1000 ppm. Tumour incidences (out of 50) at 0, 25, 50, 500 and 1000 ppm were: malignant adenocarcinoma (6, 3, 3, 14, 18), benign adenoma (0, 0, 1, 1, 2), malignant adenosquamous carcinoma (0, 0, 0, 1, 2). Although the study authors described these findings as not statistically significant[2], the mean incidence of uterine adenocarcinoma at 500 (28 %) and 1000 ppm (36 %) was well above the mean historical control value and outside the range for this laboratory [mean 6.6 %; range 0 – 24 %]. The historical control incidence for adenosquamous carcinoma is not known. Female rats also showed a very slight increase in the incidence of thyroid follicular cell adenoma (0/50, 1/50, 1/50, 1/50 & 2/48), which was just outside the historical control range (0-2%, mean 0.8%). In male rats, there was an increase in the incidence of thyroid follicular cell adenoma (0/50, 0/50, 1/50, 5/50 and 8/49), which was statistically significant at the highest two doses. The mean historical control incidence for this tumour type was 1.6 % (range 0 – 5 %). One follicular cell adenoma was observed in a male at 1000 ppm at the 1-year interim sacrifice. There were no other significant tumour findings. [CAR A6.5/6.7 (1998)]
Mouse, B6C3F1 Diet, *ad libitum* 2 year (inc. 1 year interim sacrifice) 1 year: 10 males & 10 females per group 2 years: 50 males and 50 females per group. OECD 451 GLP	0, 30, 1250 or 2500 ppm Males: 0, 5.7, 234.1 or 564.4 mg/kg/day Females: 0, 10.9, 475.3, 872.5 mg/kg/day Purity 96.8 – 97.2 %	No treatment effects on survival rates or body weight. In females, there was an increase in benign ovarian luteomas: 0/47, 1/48, 5/49 (10.2 %) and 5/47 (10.6 %). A single malignant luteoma was seen in one mouse at 2500 ppm. None of these findings was statistically significant, but the values at the top two doses were above historical control values for this mouse strain. Historical control data (i) laboratory performing the test: luteomas occurred in 6/29 studies at incidences of 2, 2, 2, 2, 4 and 6.25 %. Mean incidence = 0.64 %. Historical control data (ii) National Toxicology Programme: luteomas occurred in 3/927 animals examined (0.3 %). There was no evidence of thyroid or uterine tumours in mice. [CAR A6.7 (1998)]

4.10.1 Mode of action studies

There has been some effort by Industry to establish the mode of action behind the tumours seen

[2] Statistical evaluation in the study report of neoplastic lesions was undertaken in a two-step process. The lesions were first assessed by a trend test, which for the uterine adenocarcinomas was highly significant (P = 0.0005). Because of the significant result, the results were then further analysed by a pair-wise comparison of control and dose groups, which for the control/high-dose group analysis resulted in a P value of 0.054. The study authors therefore reported these findings as not being of statistical significance. However, it should be borne in mind that the incidence in the control group was relatively high (12%, being double that of the two lowest dose groups), which would have affected this P value.

in the thyroid and uterus (summarised in the following table). These studies are further discussed in section 4.10.2.1.

Table 26: Summary of carcinogenicity mode of action studies

Method	Dose level	Result
In vitro study on the inhibition of thyroid peroxidase from hog thyroid extracts.	483 or 870 µM	Thiacloprid had no direct inhibitory action on thyroid peroxidase catalysis of guaiacol oxidation or the formation of iodine from iodide. Car A6.10 [1994]
In vivo study of aromatase activity Rat, Wistar Oral, dietary 15/sex/group at 0, 100 and 1000 ppm. 10/sex/group at 200 and 500 ppm.	0, 100, 200, 500 or 1000 ppm. Calculated intake: 0, 6.6, 20.4, 47.5 or 60.4 mg/kg/day. 4 weeks	A dose-related increase was observed in hepatic aromatase: statistically significant at 200, 500 and 1000 ppm (1.8, 2.1 and 2.4-fold increase, respectively). There was no induction of ovarian aromatase. No serum hormone levels were measured. CAR A6.10 [1998a]
In vivo study, including observations of aromatase activity and changes in plasma hormone levels Mouse, B6C3F1 Oral / dietary 30 females/group	0, 30, 250 or 2500 ppm. Calculated intake: 0, 6, 18, 139 and 1101 mg/kg/day. 13 weeks	No deaths or body weight effects. Increased liver weight at 2500 ppm (26% increase above controls). Hepatic aromatase was induced significantly at dose levels >250 ppm (11.9, 14.5, 19.6 and 56.2 pmol/g/min at 0, 30, 250 and 2500 ppm, respectively). Slight decrease in serum oestradiol at 250 ppm (8 % decrease) and 2500 ppm (19 % decrease). Increase in serum progesterone levels at 2500 ppm (29 %). CAR A6.10 [1998b]
In vivo uterotrophic assay in the immature rat Rat, Wistar (19 days old at the start of the study) 7 females/group S.C. injection	0 or 70 mg/kg/d in arachis oil 3 days, then samples collected 24 hours after last dose	There were no deaths, but the body weights of thiacloprid-treated animals were reduced by up to 20% compared with the controls. Thiacloprid had no effect on uterine weights, histopathological findings, mitoses (stromal and epithelial cells of the endometrium) or cell proliferation (endometrial stromal and luminal epithelial cells). In contrast, the positive control chemicals 17 β-oestradiol (direct agonism of the oestrogen receptor) and androstenedione (indirect mechanism of action: conversion to oestrogens catalysed by aromatase activity) caused increases in the uterine weight, endometrial hyperplasia, mitotic index and proliferative index. [2007]
In vivo study on young female Wistar rats (11	0 or 60 mg/kg in 0.5 % aqueous	Hormone levels were evaluated 2 and 8 hours after a 4-day exposure.

weeks old at start of study) to investigate hormone changes Oral / gavage 15 females/group	methylcellulose, administered once per day 4 days Sacrifice at either 2 hours or 8 hours after the last dose	There were no mortalities, but thiacloprid resulted in a mean body weight reduction of 7 % to 8 % by day 3. Statistically significant changes in absolute organ weights in treated animals were: liver: reductions of 15% and 17% at 2 h and 8 h (no microscopic examination); adrenal glands: increases of 36 % and 21 % at 2 h and 8 h; ovary: reductions of 12 % and 15 % at 2 h and 8 h. The changes in adrenal and ovary weights were not associated with any morphological changes. Uterine weight was unaffected. There were no changes to the oestrus cycle, as judged by vaginal epithelial cell structure. Statistically significant increases in plasma progesterone were measured at 2 h (70 %) and 8 h (54 %). No oestradiol was detected in any of the samples, possibly because of a technical problem. Results for testosterone were inconclusive; however, it was more readily detected in the plasma of treated animals (17/30) than in the controls (1/30). Plasma FSH was unaffected by thiacloprid. [2009a]
In vivo study in female Wistar rats (11 weeks old at start of study) to investigate changes in hormone levels and gene expression Oral / gavage 15 females/group	0 or 60 mg/kg/d in 0.5 % aqueous methylcellulose 4 days Sacrifice at 24 hours after the last dose	Hormone levels were evaluated 24 hours after a 4-day exposure. Two females died during the course of the study, one on day 4 and one on the day of sacrifice. Mean body weight was reduced by 10 % on day 4 in the treatment group. Statistically significant changes in relative organ weights in treated animals were: liver, increase of 22 %; adrenals, increase of 63 %; ovary and uterus, reduction in each of 17 %. There were no changes to the oestrus cycle, as judged by vaginal epithelial cell structure. There was a statistically significant increase (74 %) in the plasma progesterone concentration, together with slight increases in plasma oestradiol (28 %) and FSH (14 %) concentrations that were not statistically significant. Results for testosterone were inconclusive; however, it was more readily detected in the plasma of treated animals than in the controls. Analysis of ovary, liver and adrenal tissue samples by quantitative polymerase chain reaction showed a tendency towards the up-regulation of genes associated with the hormone biosynthesis pathway in these organs. [2009b]
In vivo study in female Wistar rats (11 weeks old at start of study) to investigate changes in	0 or 60 mg/kg in 0.5 % aqueous methylcellulose	Hormone levels were evaluated 2, 8 and 24 hours after a single dose. There were no deaths, clinical signs or effects on body or

hormone levels and gene expression Oral / gavage 15 females/group	Single dose Sacrifice at 2, 8 or 24 hours after the dose	organ weights. Plasma progesterone was significantly increased 8 (57 %) and 24 (81 %) hours after treatment. Results for testosterone were inconclusive; however, it was more readily detected in the plasma of treated animals than in the controls. There were no significant changes in plasma oestradiol concentrations. Analysis of 24-hour liver and ovary tissue samples by quantitative polymerase chain reaction showed an up-regulation of some genes in the liver responsible for the metabolism and catabolism of the steroid sex hormones; there were no statistically significant changes in gene expression in the ovary. [2009c]
In vivo study on female Wistar rats (7 weeks old at start of study) Oral / dietary 15 females/group	100, 1000 and 1600 ppm Calculated intake: 8.0, 75.2, 107.7 mg/kg/day 28 days	Cytochrome P450 isoenzyme activity in the liver, aromatase enzyme activity in the ovaries, plasma sex steroid hormone concentrations and the expression of a number of genes associated with steroid synthesis and metabolism were determined after 28 days of exposure. There were no deaths, but all animals of the 1600 ppm group had a wasted appearance. Body weight gain over the duration of the study was significantly reduced at 1000 and 1600 ppm, associated with reduced food consumption. Relative and absolute liver weights were increased at 1000 and 1600 ppm (associated with enlargement and dark colouration). There was no dose response in the ovary weights. There were no changes to the oestrus cycle, as judged by vaginal smears and microscopic examination. Plasma progesterone concentrations were marginally (not statistically significant) increased in all the treatment groups. The results for testosterone were inconclusive. Plasma oestradiol concentrations were significantly increased at 1000 ppm (by 65 %) and 1600 ppm (by 60 %). The plasma FSH level was marginally but not significantly increased at 1600 ppm. Total P450 content and PROD and BROD activities in the liver were elevated at 1000 and 1600 ppm. Aromatase activity in ovarian tissue was marginally but non-significantly inhibited at 1000 and 1600 ppm. Thiacloprid treatment had no effect on hepatic aromatase activity. In ovarian tissues, the only noticeable change in the expression of steroidogenic genes was an increase in ovarian *Cyp17a1* in all treatment groups. One ovarian gene associated with metabolism (*Akr1c18*) was also up-regulated in all the treatment groups. In the liver, there were no statistically significant increases in the genes associated with steroidogenesis. Some hepatic genes associated with metabolism were up-regulated by thiacloprid, whilst another was down-regulated. *Cyp19* (the gene encoding for aromatase) could not be detected

		in any sample. [2009d]
In vivo study on aged female Wistar rats (72 weeks old at start of study) Oral / dietary 25 females/group	0 or 1000 ppm Calculated intake: 0 and 31.5 mg/kg/day 28 days	For humane reasons, one animal of the treatment group was sacrificed on day 21. The mean body weight was reduced by between 5 % (day 8) and 13 % (day 29); cumulative body weight loss between days 1 and 29 was statistically significant. Histopathological evaluations of the uterus and vagina revealed changes that were consistent with effects on the oestrus cycle: a reduced incidence of repetitive pseuodpregnancy (52 % at 0 ppm, 27 % at 1000 ppm) and an increased incidence of 'ambiguous phase of the oestrus cycle' (non-correspondence of the oestrus cycle stage in the vagina and uterus; 8 % at 0 ppm, 27 % at 1000 ppm). Treatment-related differences in vaginal mucification were also recorded: reduced incidence (64 % incidence at 0 ppm, 32 % at 1000 ppm) and reduced severity (minimal to moderate at 0 ppm, minimal to slight at 1000 ppm). A 19 % increase in plasma oestradiol in animals that were in repetitive pseudopregnancy plus ambiguous oestrus cycle was considered by the study author to be treatment-related. Because of large inter-animal variability, the effects on progesterone levels were difficult to interpret. [2009e]
In vitro study to investigate thiacloprid's steroidogenic effect on H295R adrenal cells	50 µM, 100 µM, 500 µM, 1 mM Cells exposed for 24 or 48 hours	The most marked effect of thiacloprid was a consistent and concentration-related inhibition of testosterone secretion at 24 and 48 hours. An increase in progesterone secretion at 24 hours was also recorded; this change had disappeared at 48 hours. The effects on oestradiol secretion were difficult to interpret because of the low and variable control values. [2010a]
In vitro study to investigate the effects of thiacloprid on progesterone and oestradiol secretion by Wistar rat preantral follicles	50 µM, 100 µM, 500 µM Cells exposed for 24 or 48 hours	Preantral follicles were isolated from young (7 weeks old) female rats. Treatment with 500 µM thiacloprid produced a clear and consistent increase in progesterone at both 24 and 48 hours. This concentration also resulted in a consistent increase in oestradiol secretion at both time points. [2010b]

4.10.2 Non-human information

4.10.2.1 Carcinogenicity: oral

The available evidence indicates that thiacloprid treatment results in increased frequencies of malignant uterine tumours and benign thyroid tumours in rats and of mostly benign ovarian

luteomas in mice.

i) *Rat uterine tumours*

The study showed that repeated treatment with thiacloprid (approximately 33.5 and 69 mg/kg bw/day, via the diet) induces malignant tumours in the rat uterus.

Although there is no clear understanding of the mechanism(s) leading to increased uterine tumours in exposed Wistar rats, thiacloprid is presumed to be non-genotoxic in this tissue, given the profile of results seen in the tests conducted to assess mutagenicity (see section 4.9).

An initially hypothesised mode of action involved thiacloprid-mediated induction of hepatic aromatase, resulting in elevated plasma oestradiol concentrations (1998a and b; see Table 26). Following prolonged agonism of oestrogen responsive tissues such as the uterus, this could lead to increased tumour formation. In support of this, the 1998 study (see Table 28, section 4.11.1.) observed increased serum oestradiol concentrations in pregnant and non-pregnant rats receiving repeated doses of about 60 mg/kg bw/day thiacloprid via the diet. However, hepatic aromatase activity was not detected in control or treated animals in later studies. Subsequently, it was realised that the assay to measure aromatase activity used in the earlier studies was non-specific and was likely to have been measuring generally increased liver enzyme activity, as indicated by a dose-related increase in total P450 content and in BROD and PROD activity (2009d; table 28); these increases in enzyme activity were consistent with the increases in the expression of genes associated with metabolism in the liver samples of treated females. In contrast, direct measurement of hepatic aromatase enzyme activity by determination of oestradiol production indicated an absence of this enzyme in all control and treated liver microsome samples investigated. Furthermore, *cyp19*, the gene coding for aromatase, could not be detected in any liver samples. It is therefore concluded that a thiacloprid-induced increase in hepatic aromatase activity is not responsible for the uterine tumours in rats.

Consequently, further investigations have sought to elucidate a mode of action for the induction of these tumours. After short-term exposure (4 to 28 days) in young female rats (7 to 11 weeks old at the start of the study), there were no morphological changes to the uterus and the oestrus cycle was unaffected by thiacloprid administration. Additionally, in a short-term immature rat uterotrophic assay, thiacloprid did not cause changes in the uterine weight or morphology, indicating that it did not have an oestrogenic effect on the uterus either directly or indirectly (via aromatase induction). A consistent finding in the studies on young adult rats that ranged between a single dose and 28 days' duration was increased serum progesterone. The effect of thiacloprid on serum oestradiol differed with the study and the duration, with the level being unaffected after a single dose but increased after 28 days. Plasma FSH was largely unaffected. Ovarian function, as indicated by increased progesterone and oestradiol secretion by preantral follicles (2010b; table 26), was affected by exposure to thiacloprid for 24 or 48 hours.

In contrast to the findings in young rats, there were treatment-related effects on the oestrus cycle in aged rats (72 weeks old at the start of the study). Additionally, the incidence and severity of vaginal mucification was reduced in rats that had received thiacloprid (2009e; table 26). Oestrogen stimulates vaginal cornification, whilst progesterone stimulates mucification of the vaginal epithelium. In those animals that were in pseudopregnancy plus ambiguous oestrus cycle, oestradiol was significantly increased, whilst progesterone appeared to be decreased, although there was a lot of inter-animal variability. This was consistent with the changes in vaginal mucification observed in these aged, treated females being indicative of an increase of

the oestradiol/progesterone (E/P) ratio. Prolonged exposure to thiacloprid induced the development of uterine glandular hyperplasia (at one year) and ovarian cysts (at two years) in rats (1998; amended 2007, see Table 17). Both of these morphological findings were consistent with a treatment-related imbalance of steroid sex hormone levels; ovarian cyst development has been associated with hormone changes, and the proliferative characteristics of oestradiol and progesterone on the glandular epithelium and the uterine stroma are well documented.

Based on the above observations, Industry has concluded that the primary target of thiacloprid is the ovarian follicle, leading to changes in progesterone and oestradiol secretion that eventually result in uterine changes (as evidenced in the glandular hyperplasia, ovarian cysts and oestrus cycle changes) in aged females as the alterations in ovarian hormone secretion associated with the ageing process are exacerbated by thiacloprid. It is proposed that, ultimately, these then lead to uterine tumours. In view of the evidence, this hypothesis would seem to be plausible.

ii) *Rat thyroid tumours*

In the 1998 study, thiacloprid induced relatively small numbers of benign follicular cell adenomas in male Wistar rats at 25 (incidence of 10%) and 52 mg/kg/d (incidence of 16 %, compared with none in the controls). At these doses, in the same study, there were changes in the liver and thyroid consistent with an adaptive response to treatment.

There are a number of possible mechanisms by which non-genotoxic chemicals may induce thyroid tumours in rodents, acting via a disturbance of the thyroid-pituitary axis.

One such mechanism whereby the thyroid-pituitary axis may be disturbed is liver enzyme induction; this can lead to increased conjugation and excretion of thyroid hormones, which in turn leads to increased thyroid stimulating hormone (TSH) secretion from the pituitary and then compensatory hyperplasia in the thyroid, possibly ultimately leading to the formation of tumours. Histopathological and direct evidence from a number of studies (section 4.7.1.) indicated that thiacloprid induced liver enzymes in rats; these enzymes included cytochrome P-450, UDP-glucuronyl transferase, PROD and BROD. The induction of UDP-glucuronyl transferase, in particular, has been associated with thyroid tumours in rodents, since it is responsible for the metabolism of T4, which is compensated for by an increased production of TSH by the pituitary. The 1998 study observed that plasma TSH was consistently increased in male and female rats at approximately 50 mg/kg/d thiacloprid, with some increase of TSH in male rats also being measured at 25 mg/kg/d; tumours occurred at both of these doses. There were no clear effects seen on T3 or T4 levels in this study, but transient (as would be expected in the levels of hormones that are controlled by feedback mechanisms) changes in T3/T4 were reported from 2 mg/kg/d thiacloprid in a 90-day study (1997; table 16). Support for this mechanism of action was provided by an increase in functional thyroid activity, as indicated in several rat studies by increased thyroid weight (dose-related from 2 mg/kg/d, statistically significant at 123 mg/kg/d, 90-day study), mitotic rate (from 50 mg/kg/d, 14-day study) and follicular cell hypertrophy and hyperplasia (from 2.5 mg/kg/d, two-year study) (section 4.7.1.). It has been accepted previously by the Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reproductive toxicity (1-2 September 1999) that humans are considerably less sensitive than rodents (especially rats) to the formation of thyroid tumours via prolonged perturbation of thyroid hormone homeostasis induced by non-genotoxic substances.

Another mechanism involves the inhibition of thyroid peroxidase (TPO). TPO is an enzyme found mainly in the thyroid that plays a critical role in the formation of T3 and T4. Inhibition of

this enzyme could disturb the thyroid-pituitary axis and impact on the synthesis of thyroid hormones which may result in thyroid tumours. An *in vitro* study investigated the possibility that thiacloprid or its metabolites could exert a direct effect on TPO (1994; see Table 26). Interactions of 435 and 870 μM thiacloprid with TPO-catalysed reactions were evaluated with a partially purified fraction of hog thyroid glands as an enzyme source. TPO-catalysed guaiacol oxidation and iodine formation were used as measures for peroxidase activity. Plasma extracts from rats treated with 2000 ppm thiacloprid for 14 days were also screened for an inhibitory effect on TPO-catalysed iodine formation. The results show that thiacloprid did not inhibit either TPO-catalysed guaiacol oxidation or iodine formation from iodide. The plasma extracts also had no inhibitory effect on the TPO-catalysed iodine formation. Therefore, it was concluded that thiacloprid and its metabolites had no direct inhibitory effect on TPO.

iii) *Mouse ovarian tumours*

There was a dose-related increase in benign ovarian luteomas in female B6C3F1 mice treated orally with thiacloprid for two years (1988; table 25) (incidences of 0%, 2.1%, 10.2%, 10.6% at 0, 10.9, 475.3 and 872.5 mg/kg/d). A single malignant tumour, in the high-dose group, was observed. Ovarian luteomas are seen only rarely in control mice; however, there is some evidence that in instances when they do occur, they tend to be clustered within studies. Historical control data in the RITA[3] database from studies conducted in CD-1 mice indicate that, for 13 studies of two-years' duration, the historical control range was 0-10% (mean 1.7%). Of these studies, six of them were without any incidences of ovarian luteoma. In the remainder, the mean was 3.5%, with 1, 2 or 5 animals being affected in each study.

As discussed above, the primary target of thiacloprid is presumed to be the ovarian follicle, leading to hormonal perturbation. As well as the changes in hormone levels induced in rats, thiacloprid administration also affected hormone levels in mice, with a 13-week administration resulting in increased serum progesterone and slightly decreased serum oestradiol (1998b; table 26). It is therefore possible that the ovarian tumours in mice could be a consequence of direct ovarian follicle toxicity or of prolonged changes in circulating hormone levels.

4.10.2.2 Carcinogenicity: inhalation

No information available.

4.10.2.3 Carcinogenicity: dermal

No information available.

4.10.3 Human information

No information available.

[3] Registry of Industrial Toxicology Animal Data. This is a database of historical control data from animal carcinogenicity and chronic studies collected from European and American companies and maintained by the Fraunhofer Institute of Toxicology and Experimental Medicine.

4.10.4 Other relevant information

Thiacloprid was negative in a series of *in vitro* and *in vivo* assays to detect its genotoxic potential (section 4.9).

4.10.5 Summary and discussion of carcinogenicity

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, and mainly benign ovarian tumours in mice.

The mechanistic studies conducted to supplement the carcinogenicity studies indicated that thiacloprid interfered with sex hormone biosynthesis, resulting in changes in circulating plasma sex hormones *in vivo* and changes in sex hormone secretion by adrenal cells and preantral follicles *in vitro*.

In the case of the rat uterine tumours, the long-term perturbation of steroid sex hormones, as a result of thiacloprid's targeting of ovarian follicles, was implicated in the tumour induction. In particular, oestradiol levels were elevated over time in a temporal relationship that suggested a shift to oestrogen dominance as the duration of exposure increased; after a single dose, plasma oestradiol was unaffected whilst progesterone was increased (57 to 81%) and continued to be so when measured 24 hours after the last of four daily doses (74%); whereas after 28 days of exposure, oestradiol was increased by up to 65% whilst any increase in progesterone was only marginal. Based on information available from public sources, Industry has reported that circulating oestradiol levels in rats are generally lower than those in women and has therefore postulated that rats are more sensitive to small changes in circulating oestradiol levels than women. However, there is no evidence to support this claim, and so the uterine tumours are regarded as being of relevance to humans. This secondary mechanism of action, through prolonged disturbance of the sex hormones, may be expected to be a threshold effect.

Direct evidence for thiacloprid having an effect on ovarian function was provided by a preantral follicle study, together with the increased incidence of ovarian cysts in the rat two-year study and the occurrence of ovarian luteomas in mice. These tumours are considered to be relevant to humans.

The thyroid tumours in rats may also have been a consequence of prolonged hormone perturbation on the thyroid-pituitary axis. To enable a conclusion to be made that disturbance of the thyroid-pituitary axis was responsible for the thyroid tumours, information on the following aspects is needed: evidence for a (histo)pathological sequence of events characteristic of prolonged thyroid stimulation; evidence for sustained alterations in circulating hormones; and information or experimental evidence on the mode of action (van Raaij, 2001). Taking each point in turn, thiacloprid had the following effects at or below the doses at which tumour induction occurred. Histopathological observations in repeated-dose rat studies included increased thyroid weight, mitotic rate and follicular cell hypertrophy. Changes in TSH were recorded in the two-year rat study, and mainly temporary alterations in T3/T4 were reported in a 90-day study. Evidence of the mode of action was provided by thiacloprid's induction of liver enzymes in a similar pattern to strong inducers of P450 (induction of cytochrome P450, PROD, BROD and UDP-glucuronyl transferase); such substances are thought to induce thyroid tumours through a perturbation of the thyroid-pituitary axis as a consequence of liver enzyme induction. Liver enzyme induction in rats leads to an enhanced metabolism and excretion of thyroid

hormones and a consequent prolonged thyroid stimulation by increased production of TSH. Humans are less sensitive to this mechanism of action because of the reservoir of thyroid hormone that is bound to thyroxine binding globulin. It therefore seems reasonable to conclude that the thyroid tumours observed in rats were of low relevance to humans.

Taking into account the above considerations, the classification for carcinogenicity will be based mainly on the uterine tumours in rats and the ovarian tumours in mice.

4.10.6 Comparison with criteria

The tumour findings in rats and mice treated orally with thiacloprid indicate that this substance has a carcinogenic potential, and therefore that classification for carcinogenicity is justified.

In accordance with the CLP criteria, classification in category 1A for carcinogenicity is not justified given that there is no evidence of thiacloprid having caused cancer in humans. It is therefore necessary to decide whether to classify thiacloprid in Category 1B or Category 2.

Since there was a combination of benign and malignant neoplasms in two species, a simple case for classification as Category 1B could be made. However, there are several additional factors that should be considered in making the decision.

Increased incidences of malignant uterine tumours in rats were only observed alongside relatively severe toxicity, indicating that the maximum tolerated dose had been achieved or exceeded. In these animals, body weights were reduced by a maximum of 15 – 20 % and histopathological changes were noted which included degenerative myelopathy in the nervous system characterised by radiculoneuropathy, sciatic nerve degeneration and subsequent skeletal muscle atrophy (see Table 17). In the eyes, retinal atrophy and lens degeneration were noted. Likewise, the ovarian tumours in mice occurred at doses that were associated with histopathological changes in the liver, including necrosis. It is generally accepted that tumours that occur only at excessive doses, associated with severe toxicity, have a more doubtful potential for carcinogenicity in humans. If such a confounding effect of excessive toxicity is suspected, a classification in Category 2 rather than 1B is supported.

Another consideration is the tumour type and background incidence. Apart from a single malignant ovarian tumour, the luteomas observed in female mice were benign and the increased incidence was not statistically significant, although it was slightly above the historical control range from the same laboratory. Additional historical control data suggests that incidences of this tumour type are often clustered within studies. In terms of the rat uterine tumours observed, historical control data from Wistar rats indicates that uterine tumours tend to be malignant rather than benign (Bomhard & Rinke, 1994).

The absence of evidence for a genotoxic mechanism of action lessens the level of concern for humans. The most likely mechanism of action, at least for the uterine tumours, was a secondary one through the prolonged perturbation of sex hormones; this mechanism is associated with a threshold effect, which is further evidence that the lower classification is the most appropriate. An additional factor is that the tumours observed were species specific: mice did not exhibit uterine tumours, and ovarian tumours did not occur in rats.

Taking into account the overall tumour profile in rats and mice, classification as a Category 2 carcinogen according to the CLP criteria is judged to be most appropriate. There are no grounds

to draw attention to a particular route of exposure on the label.

The equivalent classification in accordance with Directive 67/548/EEC is Category 3.

4.10.7 Conclusions on classification and labelling

CLP Regulation:	**propose Carc 2; H351**
Directive 67/548/EEC:	**propose Carc. Cat 3; R40**

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.

Additional key elements

During public consultation, industry provided additional data from the rat 2-year study on thiacloprid on incidences of mammary gland tumours, mammary gland secretion (lacteal cysts, galactoceles), pituitary adenomas, ovaries cysts and chronic progressive nephropathy (CPN) in females. According to industry, the observed pattern of response was similar to the response following bromocriptine (dopamine agonist) treatment and/or restricted diet, both associated with hypoprolactinemia.

Additional data from the rat 2-year study on thiacloprid.

Dose (ppm)	0	25	50	500	1000	0	25	50	500	1000
Mammary gland	Males					Females				
N° examined	50	50	50	50	50	50	50	50	50	50
Fibroadenoma		1				7	7	4	6	3
Adenocarcinoma						2	1	1		2
Adenoma						1		1	1	1
Lacteal cysts	1	3	4	1	1	18	15	10	12	6

Galactoceles	0	0	0	0	0	3	3	4	2	0

Dose (ppm)	0	25	50	500	1000	0	25	50	500	1000
Pituitary gland	Males					Females				
N° examined	50	50	50	50	50	50	50	50	50	50
Pars distalis adenoma	7	12	11	7	14	19	17	18	18	14
Pars intermedia adenoma		1		2						
Pars distalis carcinoma								1		

Dose (ppm)	0	25	50	500	1000
Chronic Progressive Nephropathy (CPN)	Females				
N° examined	50	50	50	50	50
Grade 1	20	19	17	19	9
Grade 2	8	9	15	3	2
Grade 3	3	4	2	1	1
Grade 4	1	0	1	2	0
Grade 5	1	0	0	0	0
Total	33	32	35	25	12

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.

4.11 Toxicity for reproduction

The reproductive toxicity of thiacloprid has been investigated in several fertility, development and reproductive function studies.

4.11.1 Effects on fertility

Table 27: Summary table of relevant reproductive toxicity studies - fertility

Method Species	Exposure conditions, & doses	Observations and remarks
Two-generation (similar to OECD 416) Rat, Sprague-Dawley (Sasco) 30 males and 30 females per dose group GLP	Oral, diet 0, 50, 300 or 600 ppm Calculated intake *circa*: 0, 3.7, 22 or 43 mg/kg/day (m+f) Purity: 96.7-97.5%	There was no evidence of an effect on mating, fertility or implantation. However, in P0 pregnant females there was dystocia (described as 'difficulty delivering') leading to death in 0, 0, 4 and 3 dams at 0, 50, 300 and 600 ppm (gestation days 23-24). Parturition started (i.e. some, but not all, pups delivered) in 3 dams with dystocia, but not in the remaining 4 (all pups found dead *in utero*). Several gross observations including pallor, wet/stained perineal areas, red vaginal discharge were generally associated with dystocia. 'Pinpoint red foci' in the liver were also noted in one of the 600 ppm females with dystocia. There was no dystocia in F1 females. At 600 ppm, the number of stillbirths was increased (live-birth index decreased) in F1 and F2 generations: F1 5.7 % at 600 ppm, 0.6% controls; F2 5% at 600 ppm, 2.9 % in controls. The viability index was reduced at day 4 in F1 (82.8 %^{NS} at 600 ppm, 97.4 % in controls) mainly owing to cannibalization of pups; in the F2, the viability index was 91.6 %^{NS} at 600 ppm and 93.9 % in controls. Pup weights at birth were unaffected by exposure to thiacloprid but by day 21 they were reduced by 15 %* at 600 ppm compared with controls. These effects were probably non-specific effects secondary to maternal

		toxicity. Decreased body weights at 600 ppm in P0 (parental) + F1 (males and females). There was no evidence that body weight decreases were especially pronounced in dams suffering from dystocia. Significant toxicity was seen in the liver and thyroid at 600 and 300 ppm, as follows: Statistically significant increased absolute liver weight: 600 ppm – P0 males (29%), P0 females (19.7%), F1 females (20.9%); 300 ppm – P0 males (17%), F1 females (18.8%). Minimal to moderate hepatocellular necrosis occurred in each of the mid-and high-dose females that died or were sacrificed because of dystocia. Necrosis was distributed in a scattered, patchy manner through the parenchyma and appeared to be an acute response with minimal inflammatory infiltrate of neutrophils. Necrosis was not observed in dams that delivered successfully. Increased incidence of hepatocyte hypertrophy: P0 males (0/30, 0/30, 10/29 and 28/30), P0 females (0/30, 0/30, 10/30 and 26/30), F1 males (0/30, 0/29, 18/30, 27/30), F1 females (0/30, 0/30, 16/30 and 29/30). Increased thyroid weights: 600 ppm - P0 males (25 %), P0 females (21%); P0 females 300 ppm (14 %). No change in F1 males or females. Thyroid follicular cell hypertrophy: P0 males (5/30, 4/30, 7/29, 20/30), P0 females (0/30, 0/30, 5/30, 17/30), F1 males (6/30, 7/29, 13/30, 19/30), females (4/30, 4/30, 18/30 and 25/30). [CAR A6.8.2 (1997)]
One-generation, range-finding (similar to OECD 415) Rat, Crl:CD BR 7 males and 7 females per dose group GLP	Oral, diet 0, 100, 400 or 1600 ppm for entire study period, from 28 days before mating Daily mg/kg intake was not calculated in the study report Purity 98.6%	There was no evidence of an effect on mating, fertility or implantation. Decreased maternal body weight gain seen throughout study period in top dose animals. During the pre-mating phase body weight gain was 48 % lower than controls in animals treated with 1600 ppm. Hepatocyte hypertrophy and cytoplasmic changes: P0 females (0/7, 1/7 and 7/7 at 0, 400 and 1600 ppm); P0 males (0/7, 7/7 at 0 and 1600 ppm); F1 females (0/7, 7/7) and F1 males (0/7, 6/7). Thyroid follicular cell hypertrophy: P0 males (0/7, 1/7 and 4/7 at 0, 400 and 1600 ppm), P0 females (0/7, 6/7), F1 males (0/7, 2/7) and F0 females (0/7, 1/7). Increased F1 pup deaths at 1600 ppm only (16 compared with 3 in controls between PND 0 and 4). This contributed to a decrease in pup viability index on day 4 at 1600 ppm only (mean 83.9 %^{NS} compared with 96.4 in controls). Decreased pup weights from day 4 after birth (weight gain from birth to termination was 28 %* less than controls in the 1600 ppm group). These findings are considered as a secondary non-specific consequence of maternal toxicity. [CAR A6.8.2 (1995)]

^{NS} = Not significant
* = $P \leq 0.01$

4.11.1.1 Non-human information

Thiacloprid showed no effects on mating performance or fertility in either of these studies.

In a one-generation range-finding study, there were no treatment-related effects on reproductive performance. The number of pup deaths (day 0-4) was significantly increased at 1600 ppm, resulting in a slightly lower viability index for this group. However, dystocia was not reported in this study. It should be noted that the small group sizes would have reduced the likelihood of dystocia being observed in this study, and it was conducted with a different strain of rat from the

other studies. Hepatocyte hypertrophy, vacuolisation and a 'ground glass' appearance of the hepatocyte cytoplasm were noted at 400 ppm and above. Thyroid follicular cell hypertrophy was also observed from 400 ppm.

In a two-generation study, effects in the liver and thyroid of parental animals were noted in animals treated with 300 and 600 ppm. The effects seen in dams (increased incidences of hepatocyte hypertrophy and thyroid follicular cell hypertrophy) were consistent with hepatic enzyme induction, as was observed in the repeated dose studies. Parental body weights were also reduced at 600 ppm, particularly in females. In the offspring, decreased F1 and F2 pup viability and pup weight gain occurred at 600 ppm and were likely to represent secondary non-specific consequences of maternal toxicity.

Dystocia (described as 'difficulty in delivering') occurred in 4/30 animals at 300 ppm and in 3/30 at 600 ppm in P0 females. Several gross observations seen, including pallor, wet/stained perineal areas and red vaginal discharge, were signs of maternal distress as a consequence of dystocia. Pinpoint red foci in the liver were also noted in one of the 600 ppm females with dystocia. Slight to moderate necrosis of the liver was seen only in dams with dystocia. Dystocia was not seen in F1 females, although there was an increase in stillbirths and a slight reduction in pup viability in this generation.

A series of studies have further investigated the induction of dystocia in rats treated with thiacloprid. In addition, an *in vitro* and an *in vivo* study have been conducted in order to help elucidate the mode of action by which thiacloprid might increase the levels of certain steroid hormones. These studies are summarised in the following table.

Table 28: Summary of dystocia mode of action studies

Method **Species**	**Exposure conditions & doses**	**Observations and remarks**
One-generation fertility study Rat, Sprague-Dawley (Sasco) 15 males per dose + 30 females per dose OECD 415 GLP	Oral / dietary *ad libitum* 0, 25, 300 or 1000 ppm Entire study period (starting 10 weeks before mating). Males - 0, 2, 20 or 69 mg/kg/day; Females - 0, 2, 23 or 75 mg/kg/day. Purity not stated.	There was no evidence of an effect on mating, fertility or implantation. No histopathological examinations were made. The only significant findings were at 1000 ppm (LOAEL): Clinical signs of toxicity included paleness, laboured breathing and cold to touch. Mean body weights of females were significantly lower during the last three weeks of the pre-mating phase (5.8 %), during gestation (4.9-10.4 %) and during lactation days 0-4 (10-13 %). Mean body weight gain was significantly reduced (16.6 %) during gestation; no clear treatment-related effects on food consumption were noted. In females, there was an increase of 22 % in group mean liver weight and of 17 % in thyroid weight. Most significantly, there were deaths on GD 23 and 24 from dystocia in 2/28 females that delivered several pups followed by a long period of time with no further deliveries. Additionally, one [1/28] female died on gestation day 24 without any signs of initiation of labour (incidence of dystocia 0/30 in controls and lower dose groups). Clinical signs in these animals were consistent with difficult labour. There were no gross pathological findings in the organs (including liver) of these animals. There was a very slight reduction in the number of live-born pups in the top dose group, but pups born in this group had a statistically significant

<table>
<tr><td></td><td></td><td>lower mean weight (13 % lower than controls) and, on day 4 of lactation, a reduced viability index (76 %, compared with 98 % in controls) and body weight were noted.

CAR A6.10 [1998a]</td></tr>
<tr><td>One-generation fertility study
+
physiological assessments of uterus and cervix

Rat, Sprague-Dawley (Sasco)

30 males per group +
30 females per group (multiple female groups per dose)

GLP</td><td>Oral / dietary

0 or 1000 ppm

Entire study period (starting 10 weeks before mating)

Approximately 75 mg/kg/day (based on Eigenberg, 1998a)

Purity: 96.7-97%</td><td>There was no evidence of an effect on mating, fertility or implantation. Body weight of treated dams was statistically significantly reduced compared with controls on GD 16 (-7%) and 22 (-9%).

One dam in the dosed group (1/30) died from dystocia on day 22 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; one of these was not pregnant, and another was suspected to have pregnancy toxaemia (not related to thiacloprid administration).

When data was combined from all subgroups of the study, there was a statistically significant decrease in the overall number of foetuses per litter (treated 10.2 compared with 12.3 in controls). This was influenced by the findings in the sub-groups for pathological investigations (10/group), in which the mean number of foetuses per dam was 7.7 (range 0-14) in the treated animals compared with 12.9 (range 11-15) in the controls; one of those treated (the dam that died of suspected pregnancy toxaemia) had 10 implantation sites but no foetuses (100% resorption) and six others had fewer than 10 pups. However, in another sub-group the number of foetuses per dam was only very slightly reduced at GD 16 (10.1 compared with 11.7 in the controls), with no difference at GD 22 (10.6 compared with 10.7).

No treatment-related effect on uterine electrophysiology, cervical extensibility, cervical collagen content or uterine alpha adrenergic receptor concentration. Microscopy did not reveal any effects on the uterus or cervix.

Non-statistically significant, although reproducible, decrease in uterine contractility at gestation day 22.

CAR A6.10 [1998b]</td></tr>
<tr><td>Investigation of whether short-term exposure on gestation days 18-21 induces dystocia.

Oral / gavage

Rat, Sprague-Dawley (Sasco)

30 pregnant females per dose (10 in the low-dose group)</td><td>0, 17.5, 35 or 60 mg/kg/day

Purity: 96.7-97.5%</td><td>There was no dystocia but there were deaths of 1/27, 0/9, 7/29, 8/25 dams between GD 20 and 24 at 0, 17, 35 and 60 mg/kg/d.

The mean body weights of the 35 and 60 mg/kg bw/day groups were significantly lower than the control group during the dosing period and there was a marked decrease in weight gain in all dose groups (negative weight gain at 35 and 60 mg/kg/d). Significant reductions in food intake were seen on gestation days 18-21 at all dose levels. Clinical signs of toxicity in the dams from 35 mg/kg included hypoactivity, chromorhinorrhoea and clear vaginal discharge.

Reproductive findings:
<table>
<tr><td></td><td colspan="4">Dose (mg/kg)</td></tr>
<tr><td></td><td>0</td><td>17.5</td><td>35</td><td>60</td></tr>
<tr><td>No. of animals</td><td>27</td><td>9</td><td>29</td><td>25</td></tr>
<tr><td>No. pregnant</td><td>21</td><td>9</td><td>29</td><td>16</td></tr>
<tr><td>No. of litters</td><td>21</td><td>9</td><td>22</td><td>11</td></tr>
<tr><td>Total No. of pups</td><td>257</td><td>109</td><td>231</td><td>128</td></tr>
</table></td></tr>
</table>

GLP		*No. of live births* 253 / 102 / 192 / 81 *Mean litter size* 12 / 12 / 10 / 12.7 *Mean No. of viable pups* 12 / 11 / 9 / 7 *No. of stillborn pups* 4 / 5 / 28 / 34 *No. cannibalised* 0 / 2 / 11 / 13 *Mean live birth index* 99 / 94 / 83* / 71* *statistically significant The reproductive findings in this study appear to have been related to maternal toxicity (poor condition of the dams) rather than to a direct toxic effect on the pups themselves. CAR A6.10 [1998c]
Investigation of whether short-term exposure on gestation days 18-21 induces dystocia. Oral gavage Rat, Sprague-Dawley (Sasco) 10 pregnant females in the control group, 36 in the treated group GLP	0 mg/kg/d, or 100 mg/kg/d on GD 18 & 19 then 50 mg/kg/d on GD 20 Some control & treated animals were maintained under conditions of an alternative light cycle (14 hours of light, 10 hours of dark) on GD 8 to 22; the remainder were maintained under the standard 12 hour light cycle.	Severe toxicity at 100 mg/kg/d resulted in the dose being lowered to 50 mg/kg/d on GD 20. The intention was to administer a dose on GD 21, but as the treated animals began to deliver early on GD 21, the last dose was given on GD 20. The following clinical signs were observed: nasal stain, no stool, reduced stool, hypoactivity, tremors, laboured breathing, cold to touch. Clinical signs of toxicity to the 100 mg/kg/d dose occurred the day after dosing was initiated. Mean body weights of the treated groups were about 13% lower than those of controls on GD 20. 7/10 control and 26/36 of the treated rats were pregnant. 'Numerous' animals in the treated group had already delivered or were in labour on the morning of GD 21. Of the treated animals, one was found dead on GD22 after having delivered; one began labour but was found dead on GD 22; and two were sacrificed during delivery on GD 22 and GD 21. One of the latter animals had been in labour for 22 hours and so was classified as having dystocia, but this was considered to be the result of necrosis of a uterine horn together with general toxicity of thiacloprid rather than a direct action on the birth process. The alternative light cycle did not have an effect on the time of delivery. CAR A6.10 [1998d]
One-generation phased exposure study, focus on steroid hormones Rat, Sprague Dawley (Sasco) Non-guideline GLP	Oral / dietary *Ad libitum* 0 or 800 ppm 54 or 61 mg/kg/day for males and females, respectively Purity: 97% *Group 1*: killed following a 9-week pre-mating	There was no evidence of an effect on mating, fertility or implantation. Dystocia was seen in 2/12 group 3 dams. Significant reductions in mean body weight gain were noted in dosed animals during the pre-mating and gestation period. They also had increased liver weights and hepatocytomegaly. Group mean absolute liver weights were increased by 21 %; < 16 %; < 10 % (groups 1, 2 and 3, respectively). At termination, there was increased hepatic aromatase, cytochrome P450, n-demethylase and o-demethylase activity in treated rats. No changes were seen in serum concentrations of FSH, T4, T3, TSH, oxytocin or prolactin. Circulating cholesterol was statistically significantly increased in cycling rats that received thiacloprid, but not on GD 18 or LD 2. Cervical and uterine prostaglandin E_2 and F_2alpha content were unaffected

<table>
<tr>
<td>10-16 females/group</td>
<td>phase

Group 2: killed following a 9-week pre-mating phase
+
mating/pregnancy phase + gestation, then sacrificed day 18 or 21 of gestation.

Group 3:
killed on lactation day 2 following a 9-week pre-mating phase, mating, gestation and parturition.</td>
<td>by thiacloprid administration, and there was no change in uterine oestrogen or progesterone receptor concentrations. Likewise, there was no alteration in GSH in the liver or the uterus. Uterine weight did not change in any group.

There was increased serum oestradiol compared with controls at the same time-points: group means increased by 40 %, 26 % and 151 % in groups 1, 2 and 3, respectively. In the group 3 animals that had dystocia, oestradiol levels were 37.5 and 103.3 pg/ml, compared with a group mean of 48.7 pg/ml (range 23.2 to 103.3) and a control mean of 19.4 pg/ml (range 8.6 to 35.6).

Aromatase activity in the ovary increased significantly in both controls and treated animals during gestation. Group mean values were similar in controls and treated animals at termination in both groups 1 and 2. At lactation day 2 (group 3), the treated animals showed a similar group mean aromatase activity to that seen at gestation day 18 (group 2), whereas the control value had decreased significantly.

There was a slight increase in the mean progesterone concentration in treated animals compared with the controls at GD 18 and lactation day 2 (statistically significant at this time point). Progesterone concentrations were lower on lactation day 2 than on gestation day 18 in both the control and treated animal groups. However, individual animal data varied considerably, as shown in the following table (circulating serum progesterone levels, ng/mL):

<table>
<tr><th colspan="2">Group 2 (gestation day 18)</th><th colspan="2">Group 3 (lactation day 2)</th></tr>
<tr><th>Control</th><th>800 ppm</th><th>Control</th><th>800 ppm</th></tr>
<tr><td>61.32</td><td>40.11</td><td>32.29</td><td>29.80</td></tr>
<tr><td>66.22</td><td>102.08</td><td>8.83</td><td>18.52</td></tr>
<tr><td>85.44</td><td>72.79</td><td>14.81</td><td>28.81</td></tr>
<tr><td>86.20</td><td>101.56</td><td>18.28</td><td>24.42</td></tr>
<tr><td>62.02</td><td>96.92</td><td>18.36</td><td>17.87</td></tr>
<tr><td>65.94</td><td>68.92</td><td>16.86</td><td>[24.22]*a</td></tr>
<tr><td>43.63</td><td>67.68</td><td>13.10</td><td>[16.49]*b</td></tr>
<tr><td>84.36</td><td>87.56</td><td>12.15</td><td>32.65</td></tr>
<tr><td>84.80</td><td>79.79</td><td>13.87</td><td>29.76</td></tr>
<tr><td>88.16</td><td>81.92</td><td>11.67</td><td>22.49</td></tr>
<tr><td>91.40</td><td>176.37</td><td>12.88</td><td>27.10</td></tr>
<tr><td>66.07</td><td>-</td><td>28.25</td><td>17.96</td></tr>
<tr><td>59.90</td><td>-</td><td>16.86</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td>17.12</td><td>-</td></tr>
<tr><td>Mean: 72.73</td><td>Mean: 88.70</td><td>Mean: 16.81</td><td>Mean: 25.71**</td></tr>
</table>

* Dams sacrificed because of dystocia. The first dam (a) delivered several pups on GD 23, but did not complete parturition by GD 24; 4 pups remained in the uterus, 2 of which were viable and 2 of which were dead (one in an advanced state of autolysis). The second dam (b) showed slight indications that parturition had been initiated on GD 22 but died on GD 23 without successfully delivering any pups, with one pup lodged in the birth canal. Additional dead pups found in utero were reported to be ‘very large’. Other dams in the group had delivered successfully.
** Excluding the two dams with dystocia.

Circulating corticosterone and luteinizing hormone were increased compared with the controls in all three of the treated groups (statistically significant for corticosterone in all groups; statistically significant for</td>
</tr>
</table>

		luteinizing hormone in cycling rats and at lactation day 2). In the two animals with dystocia, the levels of these two hormones were not increased compared with the animals that did not have dystocia. CAR 6.10 [1998; and 1998b]
First feasibility assay for the video recording of parturition Rat, Sprague-Dawley (Sasco), 10 pregnant females per group Non-guideline	All animals were untreated. Group 1: controls Group 2: blood samples for hormone analysis were collected from the retro-orbital venous plexus on GD 17 and on lactation day 2 Between GD 21 and 22, parturition was recorded with a camera recorder	The technique and detailed procedure of video recording were tested and optimised. A possible influence of video recording and blood sampling on the process of parturition was investigated. Parturitional length was defined as the time that passed between the moment the first pup appeared at the vaginal orifice and the moment the last pup was expelled. One female of group 2 was not pregnant. Another animal in group 2 was killed for humane reasons (severe damage to the eye during blood collection) on GD 22, before parturition had begun. <u>*Dystocia*</u> Blood-sampling did not affect the start date of parturition. Dystocia-like symptoms were recorded in 3/18 animals (two from the control group and one from the blood-sampling group). 1). In the first control animal, parturition onset was on GD 22, with the time to expulsion of the first pup being 31 minutes. At necropsy (GD 23), most pups were alive, but 4 placentae were found in the cervix, together with the presence of liquid in the abdominal cavity (incomplete parturition). 2). In the second control animal, parturition began on GD 22 at 12:31 am, and at least 7 pups were delivered by 2:20 pm. On GD 24, the 4 remaining pups in the cage were dead. At necropsy (GD 25), 6 placentae were noted in the uterine horns (incomplete parturition). 3). In the female from the blood sampling group, parturition began on GD 22 at 2:08 pm and was still ongoing at 5:33 pm. On GD 23, the 10 pups in the cage were cold to the touch and had no milk in the stomach; therefore, the dam and litter were sent to early necropsy. Two dead pups and five placentae were noted in the uterus (incomplete parturition). The progesterone level was 172 379 pg/ml on GD 17 and 41 620 pg/ml after parturition. The oestradiol level was 8.5 pg/ml on GD 17 and below the level of detection after parturition. A further female from the blood sampling group had an eye damaged during blood collection on GD 17. Vaginal discharge was noted on GD 21 but parturition began only on GD 23. On this day, piloerection was noted and the female was killed for humane reasons. Four dead pups and 7 placentae were noted in the uterus. The progesterone level was 142 449 pg/ml on GD 17 and 27 646 pg/ml after parturition. The oestradiol level was 9.6 pg/ml on GD 17 and 4.2 pg/ml at necropsy. <u>*Hormone levels*</u> In group 2 animals, progesterone levels ranged between 109 433 and 172 380 pg/ml on GD 17 and 18 853 and 41 620 pg/ml after parturition. Oestradiol levels ranged between 5.8 and 9.8 pg/ml on GD 17 and, for most animals, were below the detection level after parturition. [2011a]
Second feasibility assay for the	All animals were untreated	The aim of the study was to optimise the study design to determine if it would be possible to clearly determine the end of parturition and to avoid, as much as possible, stress of the animals. To this end: bedding material

video recording of parturition Rat, Sprague-Dawley (Sasco), 7 pregnant females Non-guideline		was removed from the cages during parturition; blood samples for hormone measurement were not collected; technicians were less present in the room during parturition; and activity and noise in the room were minimised. One female (8097) was disturbed by the absence of bedding material and scratched the bedding material in an attempt to prepare a nest; she delivered 11 pups within a normal timeframe (1:55 hours). Of the 7 females, one had total resorptions and did not deliver. Five delivered on GD 22 and one on GD 23. The duration of parturition ranged between 1:02 and 3:10 hours. At necropsy, three days after the delivery of 3 pups, one female (8089) had a dead foetus in the uterine horn, still attached to the placenta. Additionally, one placenta was still present in the uteri of dams 8071 and 8087, at least two days after parturition. [2011b]
Special one-generation dietary reproduction study Rat, Sprague-Dawley (Sasco) 43 females, 25 males per group Non-guideline	0 or 800 ppm, corresponding to: 0, 50.5 mg/kg in males 0, 60.9 mg/kg in females (pre-mating phase) 0, 54 mg/kg in females (gestation phase) Administered for at least 10 weeks prior to and during mating and throughout pregnancy.	Main animals: on GD 21, 24 presumed pregnant females per group were placed under video camera recorders to determine the duration of parturition (defined as the time between the first appearance of a pup at the vaginal orifice and the moment the last pup was expelled). Females were sacrificed and necropsied on the day following the completion of parturition (PND 1) or as soon as practical after GD 25 if no parturition was observed. Prior to necropsy, a blood sample was taken from the abdominal aorta. Thiacloprid, progesterone and oestradiol concentrations of pregnant females were measured. Satellite animals: a blood sample was collected from the retro-orbital venous plexus from 4-6 pregnant females/group on GD 20, 21 or 22 for determination of thiacloprid, progesterone and oestradiol concentrations. Females sampled on GD 20 were placed under video camera recorders on GD 21; following parturition, blood samples were collected from the retro-orbital venous plexus. Females sampled on GD 21 or 22 were necropsied after the sample had been taken. There were no treatment-related clinical signs. Non-treatment-related signs attributed to stress were hyper-reactivity to external stimuli, aggression and/or resistance to handling. During the pre-mating phase, the mean food consumption was slightly lower than that of controls, with mean body weight gain being occasionally lower. During gestation, the mean body weight gain and food consumption of treated animals were slightly lower than in the controls. *Dystocia* Dystocia was recorded in 3/26 animals that received thiacloprid but in none of the untreated controls. 1). A female of the main group showed clinical signs indicative of pain and a difficult parturition (piloerection, reddish soiled anogenital region, reduced motor activity) and was killed during parturition on GD 23. At necropsy there was a marked uterus prolapse and three live pups in the uterine horn. The progesterone concentration was 71 687 pg/ml (455% above the mean value of 15 748 pg/ml measured in the satellite group on GD 22). The oestradiol level was below the limit of detection of 16.4 pg/ml (the mean value of 22.0 pg/ml was recorded in the satellite control group on GD 22). The concentration of thiacloprid was 5.8 mg/l. 2). A female of the main group was found dead on GD 24 after delivery of 12 pups within 266 minutes; the mean duration of parturition

in the treated group was 105.6 ± 42.9 minutes. One dead pup was found in the uterus. Because the animal was found dead, no blood sample was collected.

3). A female of the satellite group (blood sampling on GD 20 and terminal sacrifice) had a parturition duration of 210 minutes (GD 23) for 14 pups (compared with a mean duration of 128 ± 55 minutes in the treated satellite group) and showed clinical signs after parturition (piloerection, general pallor, soiled anogenital and abdominal regions). Hormonal values on GD 20 and at terminal sacrifice were within the normal range of values. Thiacloprid concentrations were 25.9 and 5.76 mg/l on GD 20 and at terminal sacrifice, respectively.

Onset and duration of parturition
In the animals in the main group, treatment had no effect on the onset of parturition or its duration. In those satellite animals from which a blood sample was collected on GD 20, the mean duration of parturition was prolonged compared with the main-group animals, in both control and treated groups, with the effect being more marked in the controls than the treated animals. The onset of parturition was slightly delayed in treated satellite animals (GD 22.6) compared with the control satellite group (GD 21.8).

Hormone and thiacloprid levels
The hormone and thiacloprid levels measured are reported in the tables below.

Mean hormone levels measured during gestation (GD) and at terminal sacrifice on PND 1 (SD).

Hormone	GD	Untreated	Thiacloprid 800 ppm
PROGESTERONE (pg/ml)	20	90 219.8 (17 336.8)	108 176.8^{NS} (16042.1)
	21	26 687.5 (4 053.0)	26 334.0^{NS} (13 786.0)
	22	15 747.8 (6 464.5)	14 268.0^{NS} (3 087.5)
	PND1	15 753.4 (2 827.6)	16 975.2^{NS} (6 265.6)
Change between GD 20 & PND1		-74 466.4 (16 951.2)	-91 201.6^{NS} (14 008.2)
OESTRADIOL (pg/ml)	20	27.0 (8.1)	20.2^{NS} (3.2)
	21	21.0 (6)	39.5* (12.4)
	22	22.0 (5.1)	28.8^{NS} **(**14.7)
	PND1	16.0 (0.0)	17.4^{NS} (2.2)
Change between GD 20 & PND1		-11.0 (8.1)	-2.8** (3.1)
RATIO E/P (oestradiol / progesterone x 1000)	20	0.31 (0.11)	0.19** (0.02)
	21	0.78 (0.15)	1.88^{NS} (0.97)
	22	1.54 (0.62)	1.93^{NS} (0.70)
	PND1	1.05 (0.21)	1.19^{NS} (0.60)
Change between GD 20 & PND1***		0.16 (0.13)	0.03** (0.03)

^{NS} = not significantly different from the untreated group. * = Significantly ($p \leq 0.05$) higher than the untreated group. ** = Significantly ($p \leq 0.01$) lower than the untreated group.
*** = Calculated as (oestradiol value measured at PND1 – oestradiol

<table>
<tr>
<td></td>
<td></td>
<td>value measured on GD20) / (progesterone value measured on PND1 – progesterone value measured on GD 20) x 1000.

Mean (SD) plasma concentrations of thiacloprid (mg/l)
<table>
<tr><th>Main animals</th><th colspan="4">Satellite animals</th></tr>
<tr><th>TS</th><th>GD 20</th><th>GD 21</th><th>GD 22</th><th>TS</th></tr>
<tr><td>16.9 (5.6)</td><td>22.1 (3.4)</td><td>16.1 (1.5)</td><td>14.8 (3.6)</td><td>9.8 (3.0)</td></tr>
<tr><td>n = 22</td><td>n = 5</td><td>n = 4</td><td>n = 4</td><td>n = 5</td></tr>
</table>

Gross and histopathology findings
Mean terminal body weights were lower and absolute and relative liver and thyroid weights were higher than controls. Histopathology revealed a minimal to moderate diffuse centrilobular hepatocellular hypertrophy and a minimal to moderate diffuse follicular cell hyperplasia/hypertrophy in 25/26 and 20/26 females (including those affected by dystocia), respectively, compared with 0/25 and 2/25 cases in controls.

[2011c]</td>
</tr>
<tr>
<td>In vitro investigation of cytochrome P450 in liver microsomes.

Non-GLP

Non-guideline</td>
<td>Liver microsomes of male rats and dogs pre-treated with phenobarbital

0, 1000 µM Thiacloprid</td>
<td>Thiacloprid was found to be a very weak inhibitor of 7-ethoxycoumarin-deethylase (ECOD) in rat and dog microsomal preparations.

Thiacloprid was shown to induce enzymes that metabolise the steroid testosterone to androstenedione.

There was no inhibition of the main hydroxylation and oxidation reactions of testosterone. Effects on oestradiol/progesterone were not studied.

[1998a]</td>
</tr>
<tr>
<td>Plasma levels of thiacloprid

Rat, Sprague-Dawley (Sasco)

8 pregnant, 12 non-pregnant exposed rats; 5 pregnant, 5 pregnant controls

rats treated during pre-mating and up to 21 days of gestation.

Not GLP</td>
<td>Oral / dietary
Ad libitum

0 or 1000 ppm

(mg/kg not known – estimated to be approx. 75 mg/kg/day)

Purity: 97.2%</td>
<td>No treatment-related deaths or clinical signs of toxicity or body weight changes were reported. Mean gestation length and frequency of stillborn pups were similar in control and treated groups.

In non-pregnant rats a constant group mean concentration of approx. 60 nmol unmetabolised thiacloprid/ml plasma was seen. In pregnant rats, levels tended to increase from a group mean of 60 to approx. 80 nmol/ml by the end of gestation. The plasma levels of thiacloprid increased during gestation and reached a peak at the end of the gestation period.

[1998]</td>
</tr>
</table>

Dystocia was a consistent finding in the one-generation studies described in Table 28. Although this effect was not reported in a short-duration study in which thiacloprid was administered on gestation days 18-21, there was a dose-related increase in the incidence of stillbirths (1998c;

table 28). Increased incidences of stillbirths following thiacloprid administration were also found in one-generation studies from the same laboratory (1997; table 27 and 1998a; table 28); they seem to have been secondary to the poor condition of the mothers rather than direct toxicity to the pups. In another short-duration study (1998d, table 28), thiacloprid administration was associated with adverse effects on parturition (early onset), deaths of dams (either spontaneous or sacrificed for humane reasons) at the time of parturition and dystocia; however, the high dose used was considered to have resulted in excessive general toxicity rather than a direct effect on the birth process.

Several of the studies in Table 28 incorporated elements to attempt to elucidate the reason(s) for thiacloprid's induction of dystocia. The 1998b study made physiological assessments of the uterus and cervix of pregnant rats that had been treated with 0 or 1000 ppm (approximately 75 mg/kg bw/day) thiacloprid. Apart from a small decrease in uterine contractility on gestation day 22, functional and morphological investigations did not reveal any compound-related effects on the cervix or uterus. Cervical and uterine prostaglandin E_2 and F_2alpha (which mediate myometrial contractility) contents were unaffected by thiacloprid administration up to 800 ppm (approximately 61 mg/kg/d) (1998; 1998b; table 28).

Consistent with the findings in the repeated dose toxicity studies, the liver was a target organ and liver enzyme induction was apparent. The cytochrome P450 enzyme aromatase (CYP19) was one of the liver enzymes that appeared to be induced by thiacloprid. However, as discussed in the section on carcinogenicity (4.10.), the assay to measure aromatase activity used in these earlier studies was non-specific; later studies included in section 4.10, in which a more specific assay was used, did not detect increased hepatic aromatase activity after thiacloprid administration. Despite this, the finding of increased ovarian aromatase activity in both controls and treated animals during gestation (1998 and1998b; table 28) was consistent with Industry's demonstration that ovarian *cyp19* gene expression and aromatase activity increase in pregnancy in the rat (data not shown). At lactation day 2, the ovarian aromatase activity remained elevated in the treated animals (whereas it had fallen in the controls).

Attempts were made to measure steroidal hormones at different time-points before, during and after gestation. In the study (1998; table 28), serum oestradiol was raised during pre-mating, gestation and on lactation day 2 in treated compared with control groups, but particularly so on lactation day 2 (151 % increase compared with controls); this was consistent with the sustained levels of ovarian aromatase activity recorded at this time-point compared with the controls. Of the two animals that suffered dystocia, one had a serum oestradiol level that was increased by 212 % compared with the group mean, whereas the level in the other was below the group mean. In the same study, corticosterone and luteinizing hormone levels were raised in the treated animals at all time points. No changes were detected in FSH, T4, T3, TSH, oxytocin or prolactin levels. Between gestation day (GD) 18 and lactation day 2, progesterone concentrations fell in both the control and treated animal groups, although the levels were slightly higher in the latter than the former at both time points. However, progesterone levels in the animals that exhibited dystocia were not raised at lactation day 2 compared with the animals that delivered normally. Despite the increased circulating oestradiol and progesterone with thiacloprid administration, there was not a corresponding increase in uterine oestrogen and progesterone receptor concentrations.

In an attempt to further establish a causal relationship between effects on hormone levels (progesterone and oestradiol) and dystocia, video recording of female rats during parturition together with blood sampling on GD 20, 21 and 22 was performed (2011c; table 28). Dystocia

occurred in 3/26 animals that received thiacloprid, two of the main group (blood not collected around the time of parturition) and one of the satellite group (blood collected from the retro-orbital venous plexus on GD 20 and at terminal sacrifice). In the case of the latter, the hormone levels of this animal were within the normal range but the stress of the blood sampling might have been a contributory factor to the induction of dystocia. In contrast, the progesterone concentration at GD 23 of one of the main-group animals was 455% higher than the mean value measured in the satellite control group on GD 22; oestradiol was below the limit of detection. Hormones were not measured in the third affected animal.

Overall, the main hormonal changes noted in all groups (treated and untreated) were a decrease in progesterone between GD 20 and GD 22 and an increase in the oestradiol/progesterone ratio between GD 20 and GD 22 (five-fold in the controls, ten-fold in the treated animals). Whilst oestradiol decreased in the controls between GD 20 and the terminal sacrifice, it increased in the treated groups between GD 20 and GD 22. For both the oestradiol concentration and the oestradiol/progesterone ratio, the change between terminal sacrifice and GD 20 was statistically significantly less in the thiacloprid-treated animals than in the controls. Notwithstanding, an association between the hormonal changes and dystocia was apparent only for the individual with the high progesterone level at the time of parturition (i.e., the normal fall in progesterone that initiates parturition in rats was absent). The hepatocellular hypertrophy in the liver and follicular cell hyperplasia / hypertrophy in the thyroid gland reported in most of the treated animals were consistent with thiacloprid's induction of liver enzymes observed in repeated dose toxicity studies (section 4.7); there was not an association between the severity of these effects and the occurrence of dystocia.

Although an *in vitro* study of cytochrome P450 in liver microsomes (Schmidt, 1998a) did not reveal an inhibiting effect of thiacloprid on enzymes involved in steroid degradation, it did show that thiacloprid treatment of microsomes can stimulate the metabolism of testosterone to androstenedione. However, the relevance of these observations to pregnant female rats exposed to thiacloprid is not established, and consequently if thiacloprid-mediated changes in oestradiol metabolism might be involved in the processes leading to dystocia in some of these animals. One *in vivo* study that monitored the circulating plasma levels of unchanged thiacloprid in female rats treated during pregnancy showed that the plasma levels continued to increase during gestation and reached a peak at the end of the gestation period (1998; table 28), although this finding was not replicated in a later study (2011c; table 28).

Unusually for the studies reported herein, dystocia was reported in untreated animals in two feasibility studies (2011a,b; table 28). Since the studies involved intensive investigations and presence of technicians in the room, and, in some cases, blood sampling, the occurrence of dystocia was attributed to non-specific causes of parturition disorders induced by stress, to which the study authors concluded the strain of rat used (Sprague-Dawley (Sasco)) was particularly susceptible.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

The studies of developmental toxicity in rats and rabbits are summarised in the following table.

Table 29: Summary of developmental studies

Method Species	Exposure conditions & doses	Observations and remarks
Oral, gavage Developmental toxicity (OECD 414); GLP Rat (Wistar) 28-35 females per dose group Purity: 97 to 97.3%	0, 2, 10 or 50 mg/kg/day Days 6-19 *post coitum*	No maternal deaths occurred. At 50 mg/kg, there was a statistically significant decreased food consumption (reduced by 64 %) and weight loss from day 6-9. Body weight gain from days 6-19 was 45 % lower than controls. Body weight at day 20 was 12 % lower than controls. No significant toxicity at 10 mg/kg/day. At 50 mg/kg: total resorption in 1 female, increased post implantation loss as late resorptions (20% compared with 7% in controls), decreased mean litter size (9.3 foetuses/female compared with 11.5), decreased foetal weight (15% decrease) and increased skeletal retardation. These are all considered to be secondary, non-specific effects that were a consequence of maternal toxicity. No significant findings at 10 mg/kg/day. Also at 50 mg/kg: increased incidence of forelimb malformations characterised by bone dysplasia (8/270 (3 %) in 6/20 litters compared with 1/321 (0.3 %) in controls). This lies within the historical control range for the laboratory (0-3.45 %) and is concluded to represent a chance finding. The dysplasia was described as comprising thickened, shortened, bent, kinked or constricted limb bones. [1997, amended by 2000]
Oral, gavage Developmental toxicity (OECD 414); GLP Rabbit, Himalayan 24 females per dose group Purity 97.3%	0, 2, 10 or 45 mg/kg/day Days 6-28 *post coitum*	No maternal deaths occurred. At 45 mg/kg, there was a statistically significant decreased corrected terminal maternal body weight (6 %). Decreased weight gain at 45 mg/kg days 6-11 (-113 g compared with -17 g in controls) and 6-28 (+5.4 g compared with +154 g in controls). Decreased food consumption was evident between days 6-11 (76% lower than controls) and days 24-29 (20% lower). At 10 mg/kg: maternal body weight gain was reduced between days 6-11 (-113 g compared with -17 g in controls) as was food consumption (28% lower than controls). There were no significant findings at 2 mg/kg/day. At 45 mg/kg, there were 2 abortions and 3 total litter resorptions among the 24 dams; and increased incidence in skeletal retardations (reduced or delayed ossification) and marginally increased incidence of supernumerary 13th ribs with or without supernumerary lumbar vertebrae. There was also a statistically significant decrease in foetal weight (21% lower than controls for males and females combined) and an increased incidence of arthrogryposis (4.4% compared with 2% in controls). The historical background rate for this lesion was 0.05-6%. All these findings are concluded to have been secondary non-specific effects as a consequence of maternal toxicity. A change in the sex ratio of litters in the high-dose group was considered to have been a chance finding. The number of male foetuses (% litter mean) was decreased (42 % compared with 76% of controls). At 10 mg/kg: a small (6%) decrease in foetal weight (males + females combined). [CAR A6.8.1 (1996)]
Oral, diet	0, 50, 300 or	At 300 and 500 ppm, there were significant decreases in body weight and

Developmental neurotoxicity study Rat (Sprague-Dawley) 25 females per dose group GLP	500 ppm	food consumption of dams and pups. There was also a delay in sexual maturation of pups at these two dose levels. There were no signs of developmental neurotoxicity. [2001]

4.11.2.1 Non-human information

In the 1997 study, signs of maternal toxicity were seen in rats at 50 mg/kg/d and included reduced body weight and food consumption. Maternal body weight loss was particularly evident on gestational days 6 to 9. During this period animals treated with 50 mg/kg/d lost 17.7 g in body weight compared with the controls, which gained 7.6 g. During pregnancy, the main findings were total resorption in one high-dose dam, an increased post-implantation loss, a decrease in foetal weight and an increased incidence of skeletal retardations. These effects are considered to have been secondary non-specific consequences of the maternal toxicity induced by thiacloprid.

The 1996 study exposed pregnant rabbits to thiacloprid by gavage on days 6-28 post-coitum. Maternal toxicity was evident at 10 and 45 mg/kg/d. Signs of maternal toxicity were decreased body weight and food consumption. At 45 mg/kg/d, three total litter resorptions, an increased incidence of skeletal retardations (reduced or delayed ossification), marginally increased incidence of supernumerary 13^{th} ribs with or without supernumerary lumbar vertebrae, along with a 20 % reduction in foetal weight were observed. These effects were considered to be secondary non-specific consequences of maternal toxicity. There was also an increased incidence of arthrogryposis, but this was within the historical control range for the laboratory and therefore not regarded as a significant finding. A slight alteration of the sex ratio (decreased males) is also considered to have been a chance finding.

In a study designed specifically to investigate developmental neurotoxicity, there was a decrease in pup weight that is regarded as a secondary non-specific consequence of maternal toxicity (2001). A delay in the sexual maturation of pups was explained by the low pup weight.

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

Historical control data for dystocia in different strains of rat and obtained from different sources were provided by Industry. Dystocia data from rats treated with xenobiotics were also provided, to assess if general toxicity caused by the administration of substances other than thiacloprid is associated with dystocia. This information is presented in the table below, together with the incidences observed in the studies reported within this report.

Table 30: Incidences of dystocia in control and xenobiotic-exposed rats in one- and two-generation studies

Incidence of dystocia	Controls	Dose groups without toxicity	High-dose groups with toxicity
Wistar, historical data, 1989 to 2008, Bayer Toxicology, Wuppertal, Germany			
Animals:	6 / 1822 = 0.33 %	10 / 3638 = 0.27 %	6 / 1674 = 0.36 %
Studies:	6 / 56 = 10.7 %	10 / 56 = 17.9 %	6 / 48 = 12.5 %
General toxicity observed:		In high-dose groups, included decreased food intake and body weight, piloerection, bloody noses, changes in clinical chemistry, increased liver weight and poor general condition.	
Sprague-Dawley (Sasco), historical data 1988 to 1997, Bayer Toxicology, Stilwell, USA			
Animals:	11 / 908 = 1.2 %	20 / 2591 = 0.77 %	6 / 635 = 0.94 %
Studies:	8 / 26 = 30.8 %	9 / 25 = 36 %	6 / 18 = 33.3 %
General toxicity observed:		In high-dose groups, included decreased body weights/body weight gains and ovary weights, chronic pneumonia, increased liver, kidney and lung weights, decreased litter size, cannibalization of pups, hypoactivity, tremors.	
Sprague-Dawley (Sasco), thiacloprid studies, 1995-1998, Bayer Toxicology, Stilwell, USA			
Animals:	0 / 165 = 0 %	0 / 120 = 0 %	13 / 192 = 6.7 %
Studies:	0 / 4 = 0 %	0 / 2 = 0 %	4 / 4 = 100 %
General toxicity observed:		Decreased body weights, increased liver and thyroid weights, hypoactivity, pallor, laboured breathing, hepatocytomegaly.	
Sprague-Dawley (Sasco), thiacloprid studies, 2011, Bayer SAS, France*			
	Control	No thiacloprid but blood sampled	Thiacloprid
Animals:	5 / 41 = 12 %	1 / 24 = 4.2 %	3 / 39 = 7.7 %
Studies:	2 / 3 = 66.7 %	1 / 2 = 50 %	1 / 1 = 100 %
General toxicity observed:	Signs of stress: hyper-reactivity, aggression.	Signs of stress not recorded.	Decreased body weights, liver and thyroid cell hyperplasia / hypertrophy.
Crl:CD BR, thiacloprid study, 1995, Miles Inc., Elkhart, USA (Porter, 1995)			
Animals:	0 / 7 = 0%	0 / 14 = 0%	0 / 7 = 0% (1600 ppm)
General toxicity observed:		In top-dose group, decreased maternal body weight gain, hepatocyte hypertrophy, thyroid follicular cell hypertrophy.	

*Two of the included studies were feasibility studies in which thiacloprid was not administered; the cases of dystocia in control animals occurred in these studies.

The data in this table indicate that general toxicity associated with the administration of substances does not increase the incidence of dystocia compared with untreated controls; this is

in contrast to the findings with thiacloprid conducted at Stilwell, in which dystocia was reported in all studies and only in the high-dose groups. The data also indicate that the findings of the feasibility studies are not directly comparable with those of the other studies, since the extra stress associated with the procedures was likely to be a complicating factor.

4.11.4 Summary and discussion of reproductive toxicity

4.11.4.1 Fertility

Thiacloprid administration to Sprague-Dawley (Sasco) rats resulted in problems with parturition that had serious toxicological consequences. The onset of parturition was delayed or absent, and signs of difficulties with delivery included prolonged labour, pallor, wet/stained perineal areas, red vaginal discharge, reduced motor activity and the death of some dams. In some cases, parturition was incomplete, as indicated by pups lodged in the birth canal, live or dead pups *in utero* and undelivered placentae. These indications of dystocia were a consistent finding in the fertility studies in which thiacloprid was administered to Sprague-Dawley rats from 10 weeks prior to mating until the end of pregnancy and usually occurred at doses of about 60 mg/kg/d and above. An effect on reproductive toxicity, specifically on parturition, was evident even after a short exposure: thiacloprid administration from GD 18 to 20 was associated with early onset of parturition, although excessive systemic toxicity was a confounding factor in this study. Dystocia is a rare spontaneous event in rats, as demonstrated in historical control animal incidences of 0.33 % in Wistar and 1.2 % in Sprague-Dawley rats.

Levels of the sex steroids progesterone and oestrogen are tightly controlled in rats before and during parturition to, firstly, maintain the pregnancy and then, secondly, to induce parturition. Progesterone blocks myometrial contractions and reduces the sensitivity of the smooth muscle cells towards oxytocin. In contrast, oestradiol enhances the sensitivity of the smooth muscle cells towards oxytocin and stimulates spontaneous, rhythmic contractions of the myometrium. Therefore, for a successful pregnancy and delivery, a certain ratio of progesterone and oestradiol is required. In the rat, the corpus luteum is responsible for the maintenance of progesterone and oestradiol levels throughout pregnancy. In the early and middle stages of pregnancy, progesterone levels remain fairly constant. Likewise, the levels of oestradiol are constant in early and mid pregnancy. At term, increasing prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations result in the death of the corpus luteum, with a consequent rapid decrease in progesterone levels without a change in the number of uterine progesterone receptors. Hence, in the rat, a marked decrease of the serum progesterone concentration at term is a prerequisite for the initiation of parturition. Simultaneously, serum oestradiol levels increase between GD 19 and delivery, together with increases in uterine oestradiol receptors and oxytocin (between GD 16 and 19). Overall, the oestrogen/progesterone (E/P) ratio increases five-fold between GD 19 and the onset of labour (Fang *et al.,* 1996).

The effects of substances on the timing of onset and the duration of parturition and associated hormone levels in rats are difficult to study, in particular as the tightly-controlled mechanism of parturition is easily disturbed by, for example, the stress of blood-sampling. Nevertheless, Industry has invested considerable effort into trying to establish an explanation for the dystocia induced by thiacloprid; the explanations offered are discussed in depth in Annex I. Thiacloprid has been shown in a number of carcinogenicity mechanistic (section 4.10.1.) and reproductive toxicity studies to interfere with sex hormone biosynthesis and result in changes in the absolute levels and ratios of sex hormones. Based on the pattern of findings that has emerged, the

following sequence of events seems the most plausible.

Thiacloprid, via liver-enzyme induction, results in more circulating cholesterol being available for steroidogenesis up to the synthesis of androstenedione and testosterone in the theca interna cells of the ovaries. This steroidogenesis is assisted by the increased expression of the ovarian *Cyp17a1* gene, which encodes for 17α-hydroxylase; one of this enzyme's roles is to convert 17α-hydroxyprogesterone to androstenedione (a precursor of oestradiol). The increased levels of androgen precursors together with an increased ovarian aromatase activity then lead to increased oestradiol production by the ovarian granulosa cells and ultimately an alteration of the normal E/P ratio. There was an indication from one study that the pups were over-grown, which might then have led to difficulties in their delivery.

The pertinent data in relation to each of these key events is summarised below.

- ***Liver enzyme induction:*** Histopathological and direct evidence from a number of studies (section 4.7.1.) indicated that thiacloprid induced liver enzymes in rats, mice and dogs. The liver can represent an unregulated source of steroidal hormone (i.e., high-dose chemical induction of P450-dependent enzymes involved in synthesis and metabolism, such as CYP19 aromatase). The 1998 study (table 28) proposed that an effect of thiacloprid on the liver was affecting the animals' ability to regulate steroid homeostasis via increased cholesterol, since cholesterol is a precursor in the process of steroidal hormone synthesis. Thiacloprid was also able to induce enzymes that metabolise the steroid testosterone to androstenedione in an *in vitro* assay with rat and dog liver microsomes (1998a; table 28). Dose-related liver effects and liver enzyme induction were observed in the repeated dose toxicity studies in rats (section 4.7.1.), and in the reproductive toxicity studies they corresponded with the doses and times at which dystocia occurred. Although levels of testosterone were not obtained, it was more easily detected in plasma samples from animals that received 60 mg/kg/d thiacloprid (2009a,b, section 4.10.1, table 26).
- ***Increased androgen precursors:*** The gene that encodes for ovarian 17α-hydroxylase, *Cyp17a1,* showed increased expression in a carcinogenicity mechanistic study (2009d, section 4.10.1, table 26). *Cyp17a1* converts 17α-hydroxyprogesterone to androstenedione. Androstenedione was not measured in any study, so it is not possible to confirm this proposed key event.
- ***Increased ovarian aromatase activity:*** In rats, aromatase in the granulosa cells of the ovaries catalyses the conversion of androgens to oestrogens (the most potent of which is oestradiol). Ovarian aromatase activity increases in pregnant rats and results in the increased oestradiol levels that occur in late pregnancy (1998; table 28). The increased ovarian aromatase activity that was recorded in thiacloprid-treated rats on lactation day 2 at a dose at which dystocia was observed, compared with a fall in the untreated controls, was consistent with the raised serum oestradiol in these rats at this time point (1998; table 28). An effect via aromatase was also consistent with thiacloprid not having a direct oestrogenic effect in an immature rat uterotrophic assay (2007, see section 4.10; table 26), and with the primary target of thiacloprid in rat and mouse carcinogenicity studies being identified as the ovarian follicle.
- ***Increased oestradiol production:*** In a normal rat pregnancy, the circulating oestradiol level gradually increases between GD 19 and 21 and then rapidly falls so that it is withdrawn by GD 22 (Inoué, 1981). Thiacloprid administration resulted in raised serum oestradiol levels compared with controls during pre-mating, gestation and on lactation day 2, with the latter being particularly pronounced (2.5 × the control values); there was not a corresponding

increase in uterine oestrogen receptor concentrations (1998; table 28). Of the two animals in this study in which dystocia occurred, the serum oestradiol level of one was increased by 212 % compared with the group mean, whereas the level of the other was below the group mean. In the 2011c study (table 28), oestradiol was below the limit of detection at GD 23 in one animal with dystocia, was within the normal range for controls at GD 20 and terminal sacrifice in a second, and was not measured in a third. Overall, whilst the oestradiol levels fell in the control groups between GD 20 and the terminal sacrifice, they increased by 1.3 to 1.9 × the control values in the thiacloprid groups between GD 20 and 22, with a very slight (not statistically significant) decrease by terminal sacrifice. The change in the oestradiol level between GD 20 and terminal sacrifice was less in the thiacloprid-treated animals than in the controls.

- ***Alteration of the normal E/P ratio:*** A normal time of onset and duration of parturition is initiated in rats when a relatively small amount of progesterone is combined with a gradual increase in and then rapid withdrawal of oestradiol, with both hormones completely withdrawn by GD 21 and 22, respectively (Inoué, 1981). Overall, the oestrogen/progesterone (E/P) ratio increases five-fold between GD 19 and the onset of labour (Fang *et al.,* 1996). This magnitude of E/P ratio increase in untreated controls was confirmed (2011c; table 28), whereas, in contrast, the E/P ratio increased 10-fold between GD 20 and 22 when thiacloprid (approximately 60 mg/kg/d) was administered. At terminal sacrifice (after the onset of parturition), the increase in the E/P ratio of the thiacloprid-treated animals from GD 20 was still double that of the controls. An increase in the E/P ratio was also observed in aged rats treated with thiacloprid for 28 days (2009e; section 4.10.1; table 26). In the study by Inoué (1981), a normal progesterone profile combined with a larger and longer (until GD 23) infusion of oestradiol (1.5 × the normal total amount) resulted in difficulties in parturition: the timing of parturition varied, its duration was much prolonged and undelivered foetuses were found *in utero*. This pattern of effects mirrored those observed after thiacloprid administration and is consistent with the increased mean oestradiol levels in treated groups.
- ***Over-growth of pups:*** Some of the undelivered pups in one study (1998;1998b table 28) were reported to be 'very large', which might have resulted in the difficult deliveries. A delay in the onset of labour has been reported to be responsible for difficult deliveries when the normal E/P ratio is disrupted (i.e., the pups grow bigger than normal) (Inoué, 1981); however, in the dam in the 1998 study (table 28), the onset of parturition was not delayed (there were signs that it had begun on GD 22), although it was prolonged (the dam died on GD 23). Information on the weights or size of pups obtained from the dams with dystocia in other studies was not available to support this hypothesised key event.

Because the maintenance of pregnancy and onset of parturition is tightly controlled in rats and is dependent on levels of progesterone and oestrogens, a disturbance of this hormonal regulation is a biologically plausible explanation for thiacloprid's mode of action. 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 should be acknowledged that hormone levels are variable throughout the day and so 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, this proposed mode of action should be regarded as speculative.

There are some important species differences between rats and humans in pregnancy maintenance and the control of parturition onset. For example, in rats, it is the corpus luteum that is responsible for the synthesis of progesterone throughout pregnancy; in contrast, in humans,

progesterone synthesis is switched from the corpus luteum to, primarily, the placenta after the first few weeks of pregnancy. There is also a species difference in the site of oestrogen production: in pregnant and non-pregnant rats, the ovaries are the source, whereas in pregnant humans oestrogens are produced mainly by the placenta. In humans, but not rats, the foetus and placenta interact in the formation of steroid hormones. The most striking difference in the hormonal control of parturition between rats and humans is the requirement for a rapid fall in circulating progesterone levels to trigger the onset of parturition in the former, which is absent in the latter. Overall, it has been suggested that parturition in humans is not as precisely regulated as it is in rodents but is, rather, a multifactorial process (Mitchell and Taggart, 2009). Moreover, the hormonal modifications that occur in humans during gestation appear to be much greater and more diverse than those that have been determined in all other mammalian species studied, indicating that the human reproductive processes are more evolutionarily advanced (Casey & McDonald, 1997). Petraglia *et al.* (2010) concluded that the control of pregnancy and parturition is highly species specific, and that in humans there is not a simple chain of events as there are in many other species. In their view, the evidence indicates that there are multiple paracrine/autocrine events, foetal hormonal changes and overlapping maternal/foetal control mechanisms that trigger parturition in humans. As a result, the decrease or absence of a single component can be compensated by changes in other pathways. A mode of action that involves disturbance of the normal progesterone and oestradiol levels during late gestation and parturition may therefore be of reduced concern for adverse effects on human parturition.

Other possible explanations for the dystocia are discussed in Annex I but were found not to be supported by the data presented in these studies.

4.11.4.2 Developmental toxicity

In three studies to investigate developmental toxicity in rats and rabbits, increased post-implantation loss, total litter resorptions, decreases in foetal weight, increased incidences of skeletal variations and retardations, and a delay in sexual maturation were observed and were associated with exposure to thiacloprid. However, all these effects occurred only together with maternal toxicity (indicated by reduced body weight and food consumption) and so do not provide evidence of a specific effect on development.

4.11.5 Comparison with criteria

4.11.5.1 Fertility

Thiacloprid consistently induced dystocia in rats. Since, in accordance with the CLP criteria, adverse effects on parturition are included under the heading ‘adverse effects on sexual function and fertility’, classification under this end-point should be considered.

Category 1A is for known human reproductive toxicants and, in the absence of human data, is clearly not an appropriate classification for thiacloprid.

Category 1B is for presumed human reproductive toxicants where there is clear evidence of an adverse effect that is not a secondary non-specific consequence of other toxic effects. The adverse effects on parturition caused by thiacloprid occurred in several studies at moderate doses and had serious toxicological consequences for the dams and the pups. The liver-enzyme induction observed after thiacloprid administration might have resulted in unregulated

steroidogenesis, but since this was coupled with ovarian enzyme induction, particularly of aromatase, the proposed resultant distortion in the E/P ratio in pregnant rats and the profound effect on parturition that this had should not be dismissed as non-specific, general maternal toxicity.

Notwithstanding, there are factors that could lead to a classification in Category 2 rather than 1B. Adverse effects on parturition were only recorded at doses of thiacloprid that were maternally toxic in other 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 non-maternally toxic doses. Another consideration is that the mechanistic information did not clearly demonstrate relevance of the proposed mode of action to humans. Rather, it is more likely to lessen the level of concern for humans: parturition in humans does not seem to be as tightly regulated as it is in rodents and it has been proposed that there is redundancy in the control of human parturition, such that if one pathway is disrupted, others can compensate. In further support of the lower classification, the incidence of adverse effects on parturition was rather low: of the studies conducted at Stilwell, the overall mean animal incidence of dystocia in high-dose groups with toxicity was 6.7 % (Table 22).

Overall, therefore, classification as a suspected human reproductive toxicant in Category 2 (H361f) for adverse effects on sexual function and fertility (CLP) is considered to be the most appropriate.

The reproductive toxicity classification criteria in Directive 67/548/EEC do not provide clearly for the classification of substances that cause adverse effects on parturition. However, dystocia is considered to be a manifestation of reproductive toxicity taken in its widest sense, as it indicates an adverse effect on parturition that can potentially result in adverse effects to the offspring and dams. To ensure a harmonisation of classification and labelling, it is proposed to classify thiacloprid in category 3 for reproductive toxicity under Directive 67/548/EEC (R62).

4.11.5.2 Developmental toxicity

In the available studies, there are no indications of thiacloprid having induced a direct developmental effect (e.g. a teratogenic effect), and there are no clear signs of developmental toxicity in a wider sense in the absence of maternal toxicity. On this basis, classification in category 1B or 2 of CLP (category 2 or category 3 according to the Directive 67/548 criteria) would be inappropriate. The effects in pups that are seen (increased post-implantation loss, pup mortality, decreased body weight, delayed maturation of pups) are judged as likely to be secondary non-specific consequences of generalised maternal toxicity. On this basis, it is judged that no classification is required for developmental toxicity.

4.11.6 Conclusions on classification and labelling

CLP Regulation	**Propose Repr 2; H361f**
Directive 67/548/EEC:	**Propose Repr. Cat 3; R62**

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.

Additional key elements

Some additional information was provided by Industry.

In the dose range-finding study (1995), the doses of 100, 400 and 1600 ppm were estimated to be approximately 7.6, 31.1 and 117 mg/kg bw/d, respectively. The increase in stillbirths in this range-finding study was 3.1% at the top dose vs 5.6% in controls.

In the DAR, provided as an annex to the CLH report, details on maternal bodyweights and on reproductive findings are available for each fertility and developmental study, which enabled a comparison of the findings in pups with maternal toxicity at different time points. For instance, in the range-finding study, a decrease in pup weights occurred from post-natal day 4 to 35 while maternal mean body weights were normal in this time range at the same dose (decreased maternal body weight gain and mean body weight as compared to controls were observed only during pre-mating and gestation periods), and similar findings were observed in the two-generation study.

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 ± 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.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No information available.

4.12.1.2 Immunotoxicity

No information available.

4.12.1.3 Specific investigations: other studies

No information available.

4.12.1.4 Human information

No information available.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Presented below is the information to determining a classification based on the UK's review of thiacloprid under the Biocidal Products Directive (98/8/EC). The test substance used in all of the following studies has been judged to be equivalent to the thiacloprid covered by this proposal.

5.1 Degradation

Table 31: Summary of relevant information on degradation

Method	Results	Remarks	Reference
US EPA, Subdivision N, 161-1	Hydrolysis DT_{50} at 25°C: pH 5, 7, 9 > 1 year	Stable to hydrolysis	Brumhard, 1998a CAR A7.1.1.1.1
ECETOC and UBA	Photolysis DT_{50} 0-5cm pure water >1000 days	Stable to photolysis	Hellpointner, 1995a CAR A7.1.1.1.2
OECD 301F	0% biodegradation	Not readily biodegradable	Reis, 2005 CAR A7.1.1.2.1
BBA Part IV, 5-4 and SETAC	Whole system DT_{50} at 20°C: 10.7 – 20.3 days	Aerobic system Primary degradation Day 100 CO_2 = 4%	Riegner, 1997 CAR A7.1.2.2.2
US EPA, Subdivision N, 162-3	Whole system DT_{50} at 20°C: 1041 days	Anaerobic system	Fritz, 199 8 CAR A7.1.2.2.2
BBA Part IV, 4-1 US EPA, Subdivision N, 162-1	Mean soil DT50 at 20°C: 2.33 days	Mean of results for four soils	Fritz & Bornatsch (1998) CAR A7.2.1

5.1.1 Stability

The results of a hydrolysis study following US EPA guidelines showed thiacloprid is hydrolytically stable under acidic (pH 5), neutral (pH 7) and alkaline (pH 9) conditions (Brumhard, 1998a). The DT_{50} 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 DT_{50} of 79.7 days (Henneböle and Bornatsch, 1998). This value

is based on 324 solar summer days in Arizona, USA. The second study following ECETOC (Determination of Quantum Yield) and UBA[4] (Phototransformation of chemicals in water) methods resulted in a DT_{50} of >1000 days for all seasons based on; pure water; 0-5 cm depth; clear sky; 10th degree longitude; and, 30^{o}, 40^{o}, 50^{o} and 60^{o} latitude (Hellpointner, 1995a).

On the basis of the two aqueous photolysis studies, thiacloprid is not expected to undergo significant photodegradation in the environment.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

The ready biodegradability of thiacloprid was investigated in a Manometric Respirometry Test (OECD guideline 301F) over a period of 28 days (Reis, 2005). Inoculum prepared using activated sludge from a domestic wastewater treatment was exposed to an initial test concentration of 102 mg a.s./l (i.e. below the water solubility of thiacloprid, 184 mg/l). Zero per cent biodegradation of thiacloprid was observed by the end of the study and so it is not readily biodegradable. Thiacloprid was shown not to inhibit the activated sludge micro-organisms (with 103% degradation, within 14 days, recorded in the toxic control with the reference substance aniline).

5.1.2.3 Simulation tests

Water-sediment degradation tests

Aerobic water/sediment degradation of thiacloprid in pond and lake systems was assessed following BBA and SETAC methods in a GLP study (Riegner, 1997).

Samples 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, the Netherlands; pH 8.3) and associated sand sediment (pH 8.4; 0.39% organic carbon). Radiolabelled thiacloprid at approximately 0.120 mg a.s./l, was added in acetonitrile to 18 flasks for each system. All flasks were then incubated at 20 ± 1°C in the dark for up to 100 days under aerobic conditions.

Applied radioactivity (AR) in the supernatant water attributed to parent substance decreased rapidly and values of <2% AR were detected after 35 days of incubation (thiacloprid was not detectable after 100 days). Thiacloprid partitioned between water and sediment (Table 32) and 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 (figure 1) were predominantly found in the aqueous phase in Lienden samples, but were more equally distributed between water and sediment in the Hönniger samples. At study termination, M02 accounted for 8 and 50% AR in Hönniger and Lienden water samples, respectively, and 36 and 6.4% AR in sediment samples for the two systems, respectively.

[4] European Centre for Ecotoxicology and Toxicology of Chemicals and Das Umweltbundsesamt

Non-extractable residues increased after 100 days to 22% AR for the Hönniger system and 17% AR for the Lienden system, respectively, whilst carbon dioxide increased to 4% AR at the end of the study for both systems. No organic volatiles other than carbon dioxide were detected.

Primary degradation rates were determined for both the water compartment and the whole system. The water DT_{50} ranges assuming first-order kinetics were 2.9-6.3 days for pond water and 10.6-10.8 days for lake water. The whole system DT_{50} 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. This ratio lies between the requirements of an OECD 308 aquatic simulation study (3:1-4:1) and an OECD 309 surface water simulation study (suspended solid content 0.01 – 1 g/l). The test is closer to a sediment simulation study, but is also relevant to the classification criteria for degradation in the aquatic environment.

Table 32: Concentrations of thiacloprid and its significant metabolites in water and sediment samples

	Hönniger				Lienden			
	Water		Sediment		Water		Sediment	
	Max (%AR)	Peak (day)	Max (%AR)	Peak (day)	Max (%AR)	Peak (day)	Max (%AR)	Peak (day)
Thiacloprid	N/A	N/A	50.6	3	N/A	N/A	10.2	1 & 3
M02	16.6	35	36.5	62	61.9	35	7.2	35 & 62
M30	5.3	100	1.2	100	9.5	100	0.3	100
Unknown metabolite	1.0	100	0.8	62	2.3	62	0.3	35

N/A – not applicable

Table 33: First-order dissipation rates for thiacloprid

	First-order kinetics by linear regression			First-order kinetics by curve fitting		
	DT_{50} (days)	DT_{90} (days)	r^2	DT_{50} (days)	DT_{90} (days)	r^2
Hönniger pond						
whole system	27.9	92.6	0.97	20.3	67.4	0.99
water	6.3	21.0	0.97	2.9	9.7	0.96
Lienden Lake						
whole system	10.7	35.4	0.99	12.1	40.1	1.00
water	10.6	35.2	0.98	10.8	35.7	1.00

An aerobic water-sediment microcosm study is available in which a formulation was applied to the water surface (Heimbach, 1997a), but the study design and results are not relevant for the evaluation of the degradability of the active substance so it is not summarised here.

Anaerobic sediment study

Following US EPA guidelines, the anaerobic water/sediment degradation of thiacloprid was assessed using pond water over 360 days in the dark at 20°C (Fritz, 1998). The whole system

DT_{50} was 1041 days based on one valid test concentration. On this basis thiacloprid is considered anaerobically stable.

Soil degradation
The route and rate of degradation of thiacloprid (> 98%) was investigated according to the methods BBA (Part IV, 4-1) and US-EPA (Pesticide Assessment Guidelines Subdivision N, Series 162-1) using soil 'Howe' (Indiana, US, top 15 cm) and rate of degradation alone 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) (Fritz and Bornatsch, 1998).

Samples of [^{14}C]-thiacloprid were prepared to a concentration of 0.371 mg a.s./kg dry soil. Treated soil samples (100 g dry weight equivalent) were incubated aerobically at 20 ± 1 °C in the dark for up to 100 days (BBA 2.1, BBA 2.2, Höfchen) or 365 days (Howe). Additionally, 1 kg of the Howe soil was treated at a 20-fold application rate (the exact concentration was not stated) and incubated under the same conditions to enable structure elucidation of the metabolites.

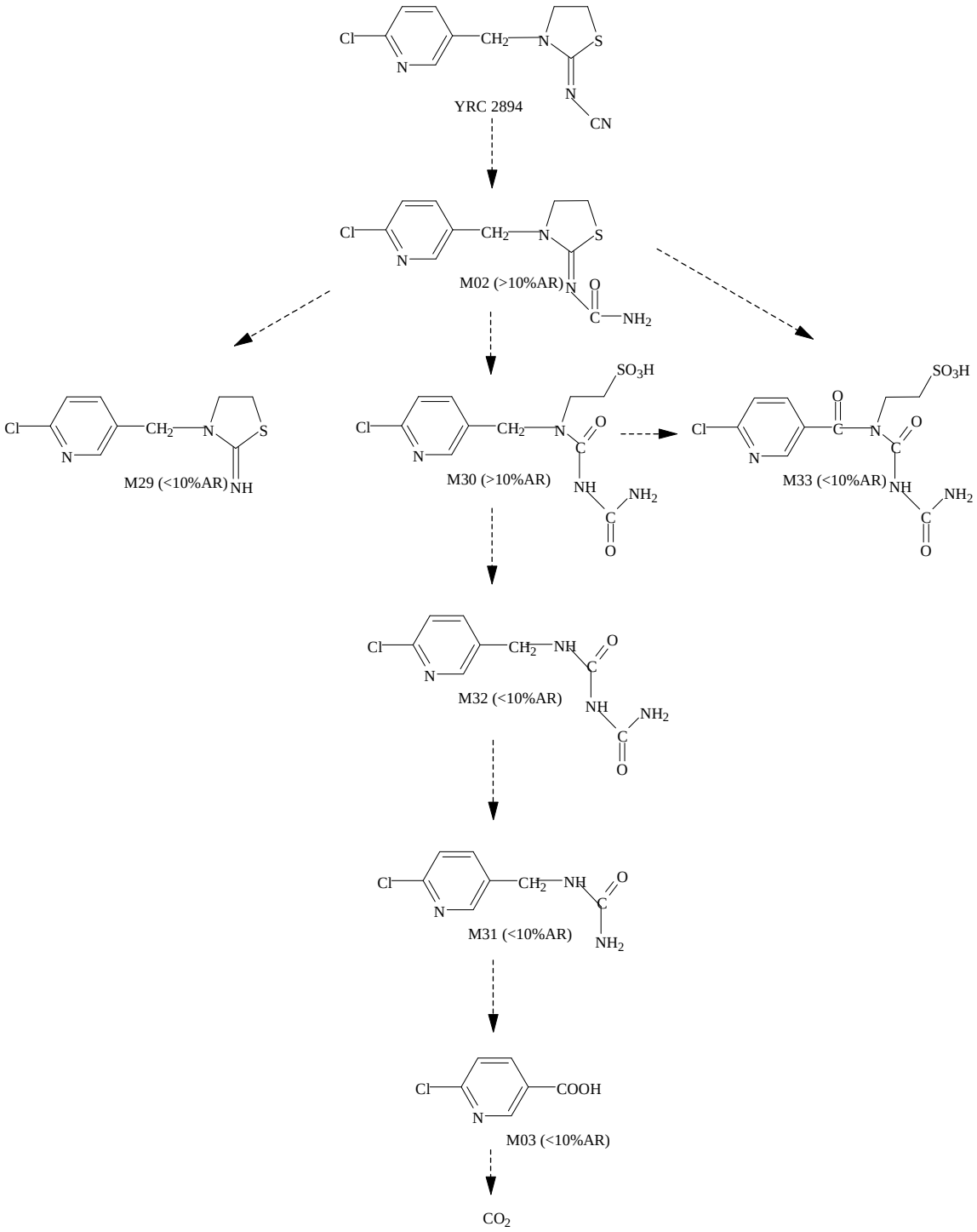
The mean value of the experimental disappearance time (DT_{50}) for thiacloprid was estimated to be 2.33 days (at 20 ± 1°C) for all soils based on first order kinetics. Bound residues accounted for 21.7 - 29.9% AR at the end of the study. Two metabolites (M02 and M30) occurred above 10% of the AR, with the amide derivative of thiacloprid (M02) shown to be the most abundant metabolite in all three soils investigated (see Figure 1). Mineralisation of thiacloprid, based on measured $^{14}CO_2$, was between 6.5 and 34% by the end of the test (100 days).

Table 34: Aerobic degradation of thiacloprid in soil

Soil type & characteristics	**Degradation rates (d)**		**% AR-distribution and products**			
			Max/Peak (DAT)	**End of the test (100 d)**		
	DT_{50}	**DT_{90}**	**Metabolites**	**CO_2**	**Bound residues**	**Metabolites and Thiacloprid conc.**
BBA 2.1: sand, pH 5.9, 0.57% OC	2.4	11.7	M02= 59.9 (8 d) M30 = 19.7 (60 d)	9.6	21.8	M02 = 20.7 M30 = 17.6 Thiacl. = 1.4
BBA 2.2: loamy sand, pH 6.3, 2.48% OC	1.5	11.8	M02= 72.3 (8 d) M30 = 5.2 (100 d)	14.7	22.7	M02 = 38.9 M30 = 5.2 Thiacl. = 1.3
Höfchen: silt loam, pH 6.0 2.4% OC	0.7	3.8	M02= 73.8 (3 d) M30 = 4.5 (14 d)	33.6	29.9	M02 = 16.9 M30 = 5.32 Thiacl. = 0.6
Howe: sandy loam pH 6.7, 1.12% OC	4.7	26.9	M02= 66.4 (30 d) M30 = 8.4 (120 d)	6.5	21.7	M02 = 47 M30 = 8.5 Thiacl. = 2.0 Further metabolites at highest dose

Thiacl. = thiacloprid

Figure 1: Degradation pathway in soil



5.1.3 Summary and discussion of degradation

Thiacloprid was abiotically stable in hydrolysis and photolysis studies.

Thiacloprid is not readily biodegradable as 0% biodegradation was observed in an OECD 301F study.

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 308 Test Guideline. 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 (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).

Thiacloprid is stable in an anaerobic water-sediment simulation study (DT_{50} = 1041 days), although this is not relevant for classification purposes.

An aerobic soil simulation study indicated rapid primary degradation of thiacloprid, but not rapid ultimate degradation.

Overall, thiacloprid is not rapidly degradable for the purposes of classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Following US EPA guidelines, adsorption and desorption constants were determined for thiacloprid using various soils (Henneböle, 1994). Six soils, ranging from sand to silty clay were used. The K_{oc} adsorption constant range was 393-870. The geometric mean K_{oc} adsorption constant was 595.8 and the geometric mean K_{oc} desorption constant was 718.7.

5.2.2 Volatilisation

Thiacloprid has a low extrapolated vapour pressure of 8×10^{-10} Pa at 25 °C and a low Henry's Law Constant (5×10^{-10} $Pa.m^3.mol^{-1}$ at 20°C) based on measured data (Krohn, 1996). On this basis thiacloprid is considered unlikely to partition from water to air.

5.2.3 Distribution modelling

Not relevant to this type of dossier.

5.3 Bioaccumulation

Table 35: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD Guideline 117	Log K_{ow} = 0.73	-	Gruener, 2001
OECD Guideline 107	Log K_{ow} at 20°C = 1.26	-	Krohn, 1996

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Thiacloprid has measured log K_{ow} values of 0.73 (OECD 117) (Gruener, 2001) and 1.26 (OECD 107) (Krohn, 1996). Such low values indicate a low bioaccumulation potential.

Thiacloprid was observed to be extensively metabolised in metabolism studies using rats (Section 4.1). Although a slower rate of metabolism could be expected in fish, an aquatic bioaccumulation study has not been conducted, and it is assumed that thiacloprid is unlikely to bioaccumulate in fish.

5.3.1.2 Measured bioaccumulation data

No experimental data are available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the low measured log K_{ow} values (0.73 and 1.26) and evidence of extensive metabolism in rats, thiacloprid is considered to have a low bioaccumulation potential in aquatic organisms.

5.4 Aquatic toxicity

Table 36: Summary of relevant aquatic toxicity information on technical thiacloprid

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Thiacloprid (97.3%)	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	30.5 mg/l	Static Measured	*CAR A7.4.1.1 1995a*
Thiacloprid (97.3%)	*Lepomis macrochirus*	OECD 203	96-h LC_{50}	25.2 mg/l	Static Measured	*CAR A7.4.1.1 1995a*
Thiacloprid (97.3%)	*Oncorhynchus mykiss*	OECD 210	97-d NOEC growth	0.244 mg/l	Flow-through Measured	*CAR A7.43.2, 1997*
Thiacloprid (97.3%)	*Daphnia magna*	OECD 202	48-h EC_{50}	> 85.1 mg/l	Static Measured	*Heimbach, 1995a CAR 7.4.1.2*
Thiacloprid (99.2%)	*Asellus aquaticus*	OECD 202	48-h EC_{50}	0.0758 mg/l	Static Nominal	*Manson, 2002a CAR 7.4.1.2*
Thiacloprid (99.2%)	*Gammarus pulex*	OECD 202	48-h EC_{50}	0.027 mg/l	Static Nominal	*Manson, 2002c CAR 7.4.1.2*
Thiacloprid (99.2%)	*Ecdyonurus sp.*	OECD 202	48-h EC_{50}	0.0077 mg/l	Static Nominal	*Manson, 2002d CAR 7.4.1.2*
Thiacloprid (97.2%)	*Hyalella azteca*	US EPA FIFRA 72-2	96-h EC_{50}	0.0407 mg/l	Static Measured	*Bowers, 1996 CAR 7.4.1.2*
Thiacloprid (97.4%)	*Daphnia magna*	OECD 202	21-d NOEC parent length	0.58 mg/l	Semi-static Measured	*Heimbach, 1996a CAR 7.4.3.4*
Thiacloprid (97.5%)	*Chironomus riparius*	BBA / OECD 219	28-d NOEC	0.0005 mg/l	Static Measured	*Heimbach, 1996b CAR 7.43.5.1*
Thiacloprid (96.8%)	*Scenedesmus subspicatus*	OECD 201	72-h E_rC_{50} 72-h NOE_rC	96.7 mg/l 32 mg/l	Static Nominal	*Anderson, 1995b CAR 7.4.1.3*
Thiacloprid (96.8%)	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC_{50}	>100 mg/l	Static Nominal	*Anderson, 1995a CAR 7.4.1.3*
Thiacloprid (96.8%)	*Lemna gibba*	US EPA	15-d EC_{50} frond numnber 15-d NOEC	>95.4 mg/l 46.8 mg/l	Measured	*Dorgerloh, 1996 CAR 7.4.3.5.2*

Studies on the acute toxicity of the thiacloprid metabolites/degradation products, M02 and M30 to aquatic life are fully evaluated and determined to be reliable in the biocides CAR and pesticides DAR for thiacloprid. For completeness therefore, these are summarised in the

following tables:

Table 37 Summary of the acute toxicity of the metabolite M02 to aquatic life.

Species	Test type and duration	Actual conc.n (as % of nominal)	LC/EC50 in mg/l (95% CL)	NOEC in mg/l	Test guideline[1]	Reference
a) Fish						
Oncorhynchus mykiss	static 96 h (limit test) purity of test substance 97.4%	98.8-99.6	>79.4[2]	79.4[2]	US EPA FIFRA 72-1	*CAR 7.4.1.1 1998*
Lepomis macrochirus	static 96 h (limit test) purity of test substance 97.4%	95.4-99.8	>78.6[2]	<78.6[2,3]	US EPA FIFRA 72-1	*CAR 7.4.1.1 1997a*
b) Invertebrates						
Hyalella azteca	static 96 hour purity of test substance 97.4%	81-96	96h EC50 >47.6[2]	5.55[2,4]	US EPA FIFRA 72-2	*Bowers 1997. Bayer Report No. 107719 CAR 7.4.1.2*
c) Algae						
Pseudo -kirchneriella subcapitata (*Selenastrum capricornutum*) (green alga)	static 96 h purity of test substance 97.1%	94.8-103.4	72h:ErC50 >100	NOErC 100	OECD 201, EC Method C3	*Dorgerloh 1998. Report No. DOM 98055 CAR 7.4.1.3*

[1] All tests conducted in accordance with guideline and to GLP.
[2] Based on mean measured concentrations, otherwise based on nominal.
[3] There were 2 mortalities out of 30 fish (6.7% mortality) at this concentration.
[4] Based on effects on behaviour (i.e. sublethal parameters) at ≥12.0 mg/l. No mortality (immobility) was observed at any test level (highest test concentration: 47.6 mg/l).

Table 38 Summary of the acute toxicity of the metabolite M30 to aquatic life.

Species	Test type and duration	Actual conc.n (as % of nominal)	LC/EC_{50} in mg/l (95% CL)	NOEC mg/l	Test guideline[1]	Reference
a) Fish						
Oncorhynchus mykiss	static 96 hour (limit test) purity of test substance 95.7% as sodium salt	99.3	>90.1[2]	90.1[2]	OECD 203	*CAR 7.4.1.1 1995c*
b) Invertebrates						
Daphnia magna	static 48 hour. purity of test substance 95.7% as sodium salt	96-101	>100[2]	100[2]	OECD 202, US EPA FIFRA 72-2	*Heimbach 1995. Report No. HBF/ Dm 152 CAR 7.4.1.2*
c) Algae						
Scenedesmus subspicatus	static 72 hour (limit test) purity of test substance 95.7%	97.6	ErC50 >100[2]	NOErC 100[2]	OECD 201, EC Method C3	*Anderson 1996. GLP Study No. E 323 0980-5 CAR 7.4.1.3*

[1] All tests conducted in accordance with guideline and to GLP.
[2] Nominal concentration in terms of free thiacloprid sulfonic acid.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Two static 96-hour acute toxicity studies are available (OECD 203) using *Oncorhynchus mykiss* (rainbow trout) and *Lepomis macrochirus* (bluegill sunfish). Measured concentrations were ≥ 93% of nominal and results were based on measured concentrations. The 96-h LC_{50} for *Oncorhynchus mykiss* was 30.5 mg/l. The 96-h LC_{50} for *Lepomis macrochirus* was 25.2 mg/l.

Additional acute studies performed with thiacloprid degradants (M02 and M30) were also conducted (further details are available in Document IIA to the BPD assessment and the pesticide DAR). The endpoints are summarised in the above tables. These degradants were less toxic than thiacloprid and would not, in themselves, warrant an environmental classification based on these acute data. As thiacloprid is considered not rapidly biodegradable, these results are not considered further.

5.4.1.2 Long-term toxicity to fish

The toxicity of thiacloprid in the early life stage of *O. mykiss* (rainbow trout) was investigated. The 97-day flow-through test was carried out according to OECD guideline 210. There were no deviations from the guideline and validity criteria were fulfilled. Measured concentrations were used although recovery rates were always above 80%. The mean measured concentrations were 0.122, 0.244, 0.483, 0.918, 1.91 and 3.91 mg/l.

Egg viability was assessed 13 days after fertilisation by taking 4 groups of 50 eggs; mean viability was 80% (range 76 - 90%). Fry survival was assessed on study day 69 (post hatch day

34) and was comparable with the control up to 0.483 mg/l. At concentrations above this there were significant differences from the control. This effect was no longer apparent at the end of the study, due to mortality in the controls in the same range. At day 97, fry survival was not significantly different between the treatments and the controls, being between 95 and 100%.

Egg hatchability was analysed at day 38 and was in the range 87 - 97% after correction for embryo viability. There were no significant differences between any of the treatments and the pooled controls. There was also no effect of any of the treatments on the time to hatch. Swim up of newly hatched fry occurred on study day 51. There was only a significant difference from the controls at 3.91 mg/l.

There was a significant difference in fry growth on study day 69 as length was significantly reduced ($p = 0.05$) at concentrations above 0.122 mg/l. By study day 97 there was a significant effect ($p = 0.05$) on fry growth at 1.91 and 3.91 mg/l, with fry dry weight was also significantly reduced at day 97 at concentrations above 0.122 mg/l. Observations were made for morphological and behavioural effects, but there were no dose related effects except a dark coloration and quiescence at 3.91 mg/l.

The NOEC for time to hatch, hatching success and fry survival was 3.91 mg/l. The NOEC for morphological and behavioural effects was 1.91 mg/l. The most sensitive endpoint was for growth (length and weight), where the 97-d NOEC was 0.244 mg/l.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Four static 48-hour acute invertebrate toxicity studies following OECD 202 (modified where appropriate) and four different species are available. These are for: *Daphnia magna* (water flea), *Asellus aquaticus* (freshwater hog louse), *Gammarus pulex* (freshwater shrimp), and *Ecdyonurus sp*. (mayfly larvae). An additional static 96-hour acute toxicity study with *Hyalella azteca* (a freshwater amphipod) following US EPA guidelines is also available.

The most sensitive invertebrate was found to be *Ecdyonurus sp*. (mayfly larvae) based on mortality and immobilisation (Manson, 2002d). These organisms were collected from the River Nidd, Knaresborough, North Yorkshire, UK on the 13th June 2002. The study used mayflies from the *Ecdyonurus* genus (Family *Ecdyonuridae*) although the exact species were not determined and therefore the test organisms are referred to as *Ecdyonurus* sp. in this report. They were tested over 48 hours in a GLP study following OECD 202. The temperature was adjusted to 13.5°C to be in the preferred range for the organism. The study was performed using static conditions and used one mayfly per vessel, with ten replicates per concentration. Nominal concentrations were 0.004, 0.009, 0.019, 0.041 and 0.09 mg/l, and a control. The 24 and 28 hour EC50 (mortality and immobilisation) and LC50 (mortality) values were calculated using probit analysis after $\log_{10}$ transformation of the nominal exposure concentrations. Analysis indicated that measured concentrations were 75, 84, 90, 92 and 93% of nominal. While one of these is slightly below 80%, it does not affect the order of magnitude for the EC_{50}, and so the value is used as reported in the study. As the mean measured concentration was 87% of nominals, the endpoints are quoted in terms of nominal concentrations. The 48-h EC_{50} was 0.0077 mg/l, and the NOEC was 0.004 mg/l. Further information is included in the following table:

Table 37: Immobilisation of mayfly larvae *Ecdyonurus sp*. during the definitive test over a 48 hour period

Nominal conc.n (mg/L)	Number affected								
	3 hours			24 hours			48 hours		
	a	b	c	a	b	c	a	b	c
Control	10	0	0	10	0	0	10	0	0
0.004	10	0	0	10	0	0	10	0	0
0.009	10	0	0	8	2	0	2	6	2
0.019	9	1	0	1	7	2	0	3	7
0.041	9	1	0	0	6	4	0	3	7
0.09	5	4	1	1	2	7	0	3	7

(*a = number of mobile mayflies, b = number of immobile mayflies, c = number of dead mayflies*)

The remaining studies used immobilisation as the endpoint, with the *Gammarus pulex* and *Asellus aquaticus* tests also measuring mortality (Manson, 2002c and Manson, 2002a). In all cases measured concentrations were above 80% of nominal and unless specified, results are based on nominal data. All guideline validity criteria were met and the studies are considered valid. The test using *Gammarus pulex* was also modified by adjusting the temperature to reflect the preferred range for that species. The measured 48-h EC_{50} for *Daphnia magna* was > 85.1 mg/l with a NOEC of 9.10 mg/l (Heimbach, 1995a). The 48-h EC_{50} for *Asellus aquaticus* was 0.0758 mg/l (mortality and immobilisation) with a NOEC of 0.041 mg/l. The 48-h EC_{50} for *Gammarus pulex* was 0.027 mg/l (mortality and immobilisation) with a NOEC of 0.009 mg/l. The 96-h EC_{50} for *Hyalella azteca* was 0.0245 mg/l (immobilisation and surface floaters) with a NOEC of 0.011 mg/l (Bowers, 1996).

A 48-hour static range-finding study with *Sericostoma personatum* (caddis fly) larvae is also available (Manson, 2002b). This was based on OECD 202, and used a limited number of animals per test concentration (nominal 0.001, 0.01, 0.1 and 1.0 mg/l). Although 33% immobilisation was seen at the lowest test concentration, the dose-response relationship was unclear, and a follow-up definitive study was not conducted. This study is not fully valid, but suggests that acute toxicity for this species occurs at a similar concentration as for the other invertebrates, so it is mentioned here as supporting data.

Two additional acute studies were performed with one major (M02) and one minor degradant (M30), conducted with *Hyalella azteca* and *Daphnia magna* respectively. Further details are available in Document IIA to the BPD assessment and the pesticide DAR). The endpoints are summarised in the above tables. These degradants were less toxic than thiacloprid and would not, in themselves, warrant an environmental classification based on these acute data. As thiacloprid is considered not rapidly biodegradable, these results are not considered further.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A 21-day reproduction study was performed on technical thiacloprid (97.4% pure) by Heimbach (1996a) according to OECD guideline 202 and US-EPA guideline 72-4 using *Daphnia magna* (first instar <24 hours) under static renewal conditions. There were 10 replicates (1 daphnid/vessel) for each concentration and the control to monitor reproduction and growth. There were also three vessels (5 daphnia/vessel) to monitor survival. The daphnia were transferred to freshly prepared test media three times per week. 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 mean measured concentrations were 0.11, 0.33, 0.58, 1.05, 1.85, 3.3, 5.8 and 10 mg/l. The determination of the test substance in the test medium showed measured concentrations were above 80% of nominal, results were therefore based on mean measured concentrations. The physical-chemical parameters remained within the requirements of the protocols throughout the test period. The validity criteria were also considered fulfilled.

No mortalities occurred in the parental *Daphnia* at any dose (0.10 - 10 mg/l), with the exception of one at 0.58 mg/l. There was a significant effect (p = 0.05) on the sum of offspring per parent at 5.8 and 10.0 mg a.s./l. The numbers were 74.2% and 36.6% respectively relative to the control. There was also a significant effect on the number of offspring per parent and the day of first reproduction at 5.8 and 10.0 mg a.s./l. The values relative to the control were 76.6% and 40.7 % respectively. There was a significant effect (p = 0.05) on body length of parents at all the concentrations from 1.05 to 10.0 mg a.s./l. The lengths for the control and for the treatments 1.05, 1.85, 3.30, 5.80 and 10.0 mg a.s./l were as follows; 4.54 mm,4.33 mm, 4.02 mm, 3.80 mm, 3.60 mm and 3.03 mm. There was also a significant effect on dry weight at 3.3, 5.80 and 10.0 mg a.s./l. The dry weights were 0.45, 0.38 and 0.29 mg respectively, compared with the control at 0.65 mg. The NOECs for the parameters measured in the test are summarised in the following table:

Table 40: Summary of NOECs for Daphnia magna in a 21-day static renewal study

Test organism	*Daphnia magna*			
Findings/ Results Parameters	Sum of offspring/ parent	Number of offspring/parent and first reproduction day	Body length of parent animals	Dry weight of parent animals
Highest tested conc. without toxic effect (NOEC) mg a.s./l	3.3	3.3	0.58	1.85

Overall, the lowest 21-d NOEC is a mean measured 0.58 mg/l based on the body length of parent animals.

5.4.3 Algae and aquatic plants

Two static algal growth inhibition studies are available using *Scenedesmus subspicatus* and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (Anderson 1995b and 1995a). The studies follow the OECD 201 guideline (1984). Measured concentrations were above 80% of nominal and results were based on nominal concentrations. For *Scenedesmus subspicatus*, the calculated 72-h E_rC_{50} was 96.7 mg/l with a 72-h NOE_rC of 32 mg/l. For *Pseudokirchneriella subcapitata*, the study report indicates a 5-day (120-hour) E_rC_{50} >100 mg/l with a quoted 120-h NOE_rC of 18 mg/l. While the study length is longer than 72 hours, cell concentrations had increased by a factor of 16 between 0 and 72 hours. At 72 hours, 46.6% growth rate inhibition was observed for the highest exposure concentration of 100 mg/l. This indicates that the 72-h E_rC50 for *Pseudokirchneriella subcapitata* is also >100 mg/l based on nominal concentrations.

A 15-day study of toxicity to *Lemna gibba* (duckweed) following US EPA guidelines is also available (Dorgerloh, 1996). Based on measured concentration data and frond number, the 15-d EC_{50} was >95.4 mg/l with a 15-d NOEC of 46.8 mg/l.

Two additional studies performed with thiacloprid degradants (M02 and M30) were conducted (further details are available in Document IIA to the BPD assessment and in the pesticide DAR). The endpoints are also summarised in tables above. In both cases the EC_{50} values were >100 mg/l and these degradants would not, in themselves, warrant an environmental classification. As thiacloprid is also considered not rapidly degradable, these results are not considered further.

5.4.4 Other aquatic organisms (including sediment)

A 28-day study using *Chironomus riparius* was conducted using thiacloprid with a purity of 97.5% (Heimbach, 1996b). The study was undertaken in accordance with GLP and using a BBA method similar to OECD 219. There were no deviations from the protocol. Each test container was filled with a 2 cm layer of artificial sediment and 20 cm reconstituted overlying water (inc. nutrient solution). The test sediment contained 69% sand, 10% peat, 20% kaolin and 1% calcium carbonate. There were five replicates each containing five first instar larvae per test concentration, plus controls. Thiacloprid was introduced beneath the water surface and gently mixed to give initial nominal test 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.

During the study, number, sex and time of emergence of emerged midges were determined daily. The emergence rate of male and female midges was pooled for the statistical analysis, as this effect was not treatment related.

The measured test concentrations of three dose levels analysed (nominal 0.00032, 0.0018 and 0.010 mg a.s /l) were 83 to 113% of nominal (on average 98.3%) after one hour. Therefore, the results were based on nominal initial concentrations. However, the concentration of active substance in the water phase did decline over the course of the study, with mean measured values of 64.5% (day 7) and 15.7% (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. Recorded temperatures, pH values and oxygen levels were within guideline limits and were similar between the different treatments and the control.

The study showed that no adult midges emerged at test concentrations higher than 0.0018 mg/l. At 0.0018 mg/l, the day of first emergence was delayed until day 16 compared with the control (and lower concentrations), which was shown to be on day 14. No dose-response relationship was evident at concentrations ≤ 0.0018 mg/l (those with successful emergence) and the response above 0.0018 mg/l was so steep that a statistical calculation of this parameter was not possible. 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 (CAR), the UK Competent Authority recalculated the NOEC 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 test concentration 0.001 mg/l, using time 0 (nominal) and predicted concentrations on days 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. The test method meant that there is some uncertainty in the form of exposure for the organisms. The rapid dissipation of the substance to sediment observed in the aquatic simulation study (section 5.1.2.3) suggests organism exposure in the OECD 219 study may have been via sediment

contact and ingestion as well as through the water phase and pore water. However, in the CAR and DAR, the chironomid result was considered suitable for the pelagic risk assessment.

Two additional midge studies were conducted with thiacloprid degradants M02 and M30 using the same methodology as the a.s. study (further details are available in Document IIA to the BPD assessment and in the pesticide DAR). In both cases no effects were observed at the single limit concentrations tested (28-day EC_{50}s and NOECs for M02 were >0.0826 mg/l and 0.0826 mg/l, and for M30 they were >74.8 mg/l and 74.8 mg/l respectively) These were 'corrected' endpoints from the biocide CAR results, taking into account the measured concentrations in the overlying water over 28 days. As thiacloprid is considered not rapidly degradable, these results are not considered further.

The effect of thiacloprid on aquatic microorganisms was carried out in a single test performed according to ISO 8192, which generally corresponded to the OECD guideline 209 (Muller, 1995). The nominal concentrations ranged from 1000 to 10,000 mg/l, which exceed the water solubility. All validity criteria of the test method were met. The results based on nominal concentrations were EC_{50} = 6330 mg/l and EC_{10} = 1340 mg/l. It was concluded that thiacloprid shows a very low inhibitory effect on activated sludge.

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

Thiacloprid achieved 0% degradation in a standard ready biodegradation study. Based on the water solubility and aquatic micro-organism tests, biodegradation was not limited by solubility or micro-organism toxicity. Thiacloprid exhibits primary aerobic degradation in the aquatic environment. However, less than 70% degradation is expected within 28 days (i.e. $t_{1/2}$ was >16 days). On this basis, thiacloprid is not considered to undergo rapid and ultimate degradation, and not considered to be rapidly degradable for the purposes of classification.

Based on the low measured log K_{ow} values (0.73 and 1.26) thiacloprid is considered to have a low bioaccumulation potential in aquatic organisms.

Thiacloprid is acutely toxic to fish with a lowest 96-h LC_{50} of 25.2 mg/l. No acute toxicity was observed with *Daphnia magna* (48-h EC_{50} > 85.1 mg/l) but significant acute toxicity was seen for other invertebrates: acute EC_{50} values below 1 mg/l were observed for *Asellus aquaticus* (freshwater hog louse), *Gammarus pulex* (freshwater shrimp), *Ecdyonurus sp*. (mayfly larvae) and *Hyalella azteca* (freshwater amphipod). It is less acutely toxic to plants, the lowest 72-h E_rC_{50} for algae being 96.7 mg/l and a 15-d EC_{50} of >95.4 mg/l for aquatic macrophytes.

The lowest observed acute result is a 48-h EC_{50} of 0.0077 mg/l for *Ecdyonurus sp*. larvae based on mortality and immobilisation. This value indicates significantly higher sensitivity than for *Daphnia magna*, and around an order of magnitude greater sensitivity than the other invertebrates. Thiacloprid is an insecticide, so it is appropriate to use the results for aquatic insects even though they might not be a 'standard' test organism. Otherwise, the classification would not reflect the hazard towards organisms that may have particular sensitivity to the substance. This result is therefore considered acceptable for classification purposes.

Chronic aquatic toxicity data for fish and *Daphnia* are of a similar order of magnitude, the most sensitive result being a 97-d NOEC of 0.244 mg/l for fish; algal and aquatic macrophyte NOECs are above 1 mg/l. No chronic data are available for the most acutely sensitive species *Ecdyonurus sp*. A 28-d study using another insect species, *Chironomus riparius*, is available,

with a NOEC of 0.0005 mg/l. There is some uncertainty about whether the organisms were exposed mainly via sediment rather than water, so whilst the result has been used for risk assessment purposes in the CAR, it is considered as supporting information for classification purposes.

Regulation EC 1272/2008
Based on acute aquatic toxicity data with $L(E)C_{50}$ values below 1 mg/l, classification with Aquatic Acute 1 is applicable. The EC_{50} for *Ecdyonurus sp*. of 0.0077 mg/l means that an acute M-factor of 100 is applicable (since $0.001 < L(E)C_{50} \leq 0.01$ mg/l).

A full set of chronic data for the three trophic levels is available, although the most acutely sensitive species is not represented. Based on the most sensitive standard test organism data (97-d NOEC of 0.244 mg/l for fish), and lack of rapid degradability, the substance would be classified as Aquatic Chronic 2. However, based on the surrogate approach using the *Ecdyonurus sp*. 48-h EC_{50} result and lack of rapid degradation, classification with Aquatic Chronic 1 is appropriate with an M-factor of 100 (since $0.001 < L(E)C_{50} \leq 0.01$ mg/l). The same classification and M-factor are also suggested by the chironomid result ($0.0001 < NOEC \leq 0.001$ mg/l), although as there is some uncertainty about exposure routes in the test, it is not the key data for chronic classification. The same classification is also indicated by the acute toxicity data for other aquatic invertebrates (only the M-factor would be different).

Directive 67/548/EEC
As thiacloprid is not rapidly degradable and exhibits acute aquatic toxicity $L(E)C_{50}$ values below 1 mg/l, it should be classified N: Dangerous for the Environment with the following risk phrases: R50 Very toxic to aquatic organisms; and R53 May cause long term effects in the environment.

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

Based on Regulation (EC) 1272/2008, thiacloprid should be classified:
Aquatic Acute 1, Aquatic Chronic 1
Labelling: H410 'Very toxic to aquatic life with long lasting effects'
Signal word 'Warning' and environmental warning label.
Acute and Chronic M factors of 100 based on $0.001<L(E)C_{50}\leq 0.01$ mg/l should apply.

Following Directive 67/548/EEC, thiacloprid should be classified Dangerous for the Environment with the following risk and safety phrases:
N Dangerous for the Environment
R50 Very toxic to aquatic organisms
R53 May cause long term effects in the environment
S60 This material and its container must be disposed of as hazardous waste
S61 Avoid release to the environment. Refer to special instructions/Safety Data Sheet

In accordance with Directive 67/548/EEC the following Special Concentration Limits should apply:

Classification of the preparation		
N, R50-53	N, R51-53	R52-53
$Cn \geq 0.25\%$	$0.025\% \leq Cn < 0.25\%$	$0.0025\% \leq Cn < 0.025\%$

Where Cn is the concentration of thiacloprid in the preparation.

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.

Additional key elements

There was no information on the degradation of the two degradation products of thiacloprid in the CLH dossier. However, this information can be found from the Document IIA of the Biocide assessment report annexed to the CLH report. The information is used to assess if the degradation products would be classifiable.

In order to determine the degradation rate of the main soil metabolite, M02 (amide metabolite) in soil, data from the aerobic soil degradation study was analysed further. First-order kinetics was applied to the two individual steps of the degradation pathway (transformation of thiacloprid to M02, degradation of M01) with the simplifying assumption that degradation of thiacloprid followed first-order kinetics. Using peak concentration for M02 of 60-74% AR after 3-30 days in the 4 aerobic soils tested, a mean DT_{50} value of 69.5 days (ranging from 32 to 142 days) at 20°C was derived.

A study to investigate the degradation of the metabolite M30 was conducted according to BBA and SETAC Guidelines. Three biologically active pre-incubated soils 'Howe', 'BBA 2.1', and 'BBA 2.2' were used. Each soil was exposed to [^{14}C] M30 at a concentration of 0.194 mg/kg dry soil and incubated in the dark at 20 ± 1°C for up to 101 days. Three metabolites were observed. Two were identified as a sulfonic acid amide derivative (M34) and thiacloprid diamide (M32). The DT_{50} value for the metabolite M30 was calculated by linear regression (1st order kinetics). The mean DT_{50} for M30 was 37.67 days (16-79 days) at 20°C.

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} ≤ 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.

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8 ANNEXES

8.1 Annex I: aids to facilitate the discussion of reproductive toxicity

Thiacloprid administration resulted in problems with parturition in rats. The onset of parturition was delayed or absent, and signs of difficulties with delivery included prolonged labour, pallor, wet/stained perineal areas, red vaginal discharge, reduced motor activity and the death of some dams. In some cases, parturition was incomplete, as indicated by pups lodged in the birth canal, live or dead pups *in utero* and undelivered placentae. These indications of dystocia were a consistent finding in the fertility studies in which thiacloprid was administered to Sprague-Dawley rats from 10 weeks prior to mating until the end of pregnancy. An effect on reproductive toxicity, specifically on parturition, was evident even after a short exposure: thiacloprid administration from GD 18 to 20 was associated with early onset of parturition, although excessive systemic toxicity was a confounding factor in this study. Dystocia is a rare spontaneous event in rats, as demonstrated in historical control animal incidences of 0.33 % in Wistar and 1.2 % in Sprague-Dawley rats.

Industry has proffered some explanations for the occurrence of dystocia in the thiacloprid studies, which are discussed below.

Increased susceptibility of the Sasco strain of Sprague-Dawley rat to stress-induced dystocia

One proposed explanation for the dystocia is that it was a consequence of maternal stress either on its own or in combination with general toxicity from administration of an active substance. In particular, it has been suggested that the Sprague-Dawley (Sasco) rat has an increased susceptibility to disorders of parturition which may be induced by stress; Industry cites the occurrence of dystocia in control animals of the studies that employed video-recording and retro-orbital collection of blood samples (2011a,b; tabl e28) to support this argument. In all but one (range-finding) thiacloprid study, the Sprague-Dawley (Sasco) rat was used. Industry has claimed that this strain is more sensitive to parturition disorders than other strains, but has not produced any data to support this statement. However, an insight has been gained by the inclusion of data from studies conducted on other substances and their comparison with those studies reported herein.

Certainly, comparison of the incidences of dystocia in different strains (Table 22) indicates that the Sprague-Dawley (Sasco) strain appears to have a higher susceptibility to dystocia than Wistar rats (incidence of 1.2 % of control animals in non-thiacloprid studies, compared with an overall incidence of 0.33 % amongst control Wistar rats). (The data on Crl CD BR rats (which were derived from Sprague-Dawley rats several decades ago) are from a range-finding study (Porter *et al.*, 1995) with small group sizes (7 females per group), making the interpretation of this negative finding difficult.) The absence of dystocia in all the control (and low/mid-dose) groups of the studies in which thiacloprid was administered may have been a consequence of the smaller number of animals investigated, but in any case confirms that the spontaneous occurrence of this condition is low. In contrast, dystocia was reported in 2011a,b; table 28). However, the findings in these studies were not completely consistent with stress-induced dystocia; for example, in the first feasibility study, dystocia occurred in two animals without blood sampling compared with one animal from which blood was collected from the retro-orbital venous plexus, a procedure that one would predict would increase stress in the animals.

Also, in the second feasibility study, there was incomplete parturition in three females with no overt indications of stress, whereas a female that did exhibit obvious signs of stress delivered normally. Whilst it cannot be excluded that stress was involved in the dystocia in these two studies conducted in France, possibly owing to the collection of blood samples and/or the video-recording procedure (cage moving, presence of technicians in the room, additional noise, also transport of the animals from USA to France), the fact that there were no cases of dystocia in control, low or mid-dose groups in any of the thiacloprid studies conducted at Stilwell, USA, argues against stress alone being responsible for the effect in these latter studies.

Therefore, the possibility of the combined effects of stress and general toxicity resulting from the administration of an active substance are considered next. The historical data on Wistar and Sprague-Dawley (Sasco) rats show that the incidence of dystocia was largely unaffected by administration of the test substances, even when the animals received doses that resulted in toxicity. This was in striking contrast to the situation with thiacloprid, in which dystocia only occurred in animals that received doses that resulted in toxicity. When the high-dose groups were compared, the incidence of dystocia per animal, per generation and per study was markedly higher in the thiacloprid studies than in the studies on other substances (6.7 % of animals in the studies conducted at Stilwell, compared with 0.94 % of Sprague-Dawley (Sasco) rats in the non-thiacloprid studies). There is no reason to suppose that the animals in the thiacloprid studies suffered more non-specific stress than those in the other studies, as the general toxicity recorded (decreased body weight, increased liver weight, hypoactivity) appeared to be similar between the thiacloprid and non-thiacloprid studies.

In an attempt to further clarify the hypothesis that stress and general toxicity were responsible for the induction of dystocia in the thiacloprid studies, the clinical observations reported from individual affected animals are reported in the table below.

Table 41: Toxicity in female Sprague-Dawley (Sasco) rats after thiacloprid administration

Study & location	Incidences of dystocia	Toxicity in females with dystocia	Toxicity in females without dystocia
1997 Stilwell, USA Two-generation	0/30, 0/30, 4/30, 3/30 at 0, 3.7, 22, 43 mg/kg/d (P animals)	P - 22mg/kg/d: FV2104: lacrimation, moderate hepatocellular necrosis FV2116: no clinical signs during study, slight hepatocellular necrosis FV2122: no clinical signs during study, minimal hepatocellular necrosis FV2125: no clinical signs during study, minimal hepatocellular necrosis P – 43 mg/kg/d: FV3104: weak, pale during week 14, pin-point red foci in the liver, slight hepatocellular necrosis, fluid present in thorax and abdomen FV3117: no clinical signs during study, slight hepatocellular necrosis and minimal inflammation of the	P: Pallor, hypoactivity, alopecia, increased liver & thyroid weights, hepatocytomegaly (minimal / slight / moderate) ± inflammation of the liver (minimal), thyroid follicular cell hypertrophy F1: Reduced terminal body weights, increased liver & thyroid weights, hepatocytomegaly ± inflammation, hepatocellular cytoplasmic vacuolization, thyroid follicular cell hypertrophy, lacrimation, slight liver cell necrosis (one high-dose animal)

		liver FV3126: no clinical signs during study, moderate hepatocellular necrosis There were no cases of dystocia in the F1 animals.	
1998a Stilwell, USA One-generation	0/30, 0/30, 0/30, 3/28 at 0, 2, 23, 75 mg/kg/d	IZ3121 (died GD 23 during parturition): no remarkable clinical observations weeks 1 to 15. IZ 3127 (died GD 24 during parturition): laboured breathing, paleness, cold to touch during week 15. IZ3123 (died GD 24, parturition not started): no remarkable clinical observations weeks 1 to 15.	Mainly no remarkable observations, but occasionally lacrimation, paleness, vaginal discharge prior to week 15, hypoactivity, laboured breathing, cold to touch. No histopathology investigations.
1998b Stilwell, USA One-generation	0/30, 1/30 at 0, 75 mg/kg/d	JE1120: no clinical observations, body weight unaffected	Decreased body weight, three other deaths before GD 18 (1 not pregnant, another unrelated to thiacloprid administration), hypoactivity, cold to touch. Histopathology only on the cervix and uterus.
1998c Stilwell, USA	0/30, 0/30, 0/30, 0/30 at 0, 17, 35, 60 mg/kg/d GD 18-21	No dystocia but maternal deaths from GD 20.	At all doses, decrease in body weight gain and food consumption. At 35 & 60 mg/kg/d, death, hypoactivity, dose-related increase in stillbirths (proposed to be related to maternal toxicity / effects of thiacloprid on parturition).
1998d Stilwell, USA	0/7, 1/26 Other effects on parturition: early onset, absence of onset, deaths during or after delivery at 0 mg/kg/d, or 100 mg/kg/d on GD 18 & 19 then 50 mg/kg/d on GD 20	6 (labour didn't begin, found dead on GD 22): hypoactivity & reduced stool GD 19-20; laboured breathing GD 21. 32 (delivered, found dead on GD 22): hypoactivity & no stool GD 19-20; nasal stain GD 21. 38 (sacrificed on GD 22 after being in labour for 22 hours – dystocia): hypoactivity & no stool GD 19-20; necrosis of uterine horn 41 (sacrificed during delivery on GD 21): hypoactivity & no stool GD 19-20; tremors & cold to touch on GD 19.	Hypoactivity and no/reduced stool was a common finding in treated animals, also laboured breathing, tremors and nasal stains. The excessive toxicity on GD 19-20 led to the dose being reduced on GD 20.
1998 Stilwell, USA One-generation	0/15, 2/12 at 0, 61 mg/kg/d	KF1169: pallor, tissues normal, individual body weight not recorded KF1171: pallor, tissues normal, individual body weight not recorded (histopathology was not performed	Reduced terminal body weights, centrilobular hepatocytomegaly, increased liver weights.

		on these two animals)	
2011c One-generation	*Main group*: 0/24, 2/24 *Satellite group* (blood samples at GD 20 + TS, or GD 21 or GD 22): 0/16, 1/15 0, 800 ppm (corresponding to 0, 60.9 mg/kg/d in the pre-mating phase & 0, 54 mg/kg/d in the gestation phase)	*Main group:* UR2F0481: slightly lower body weight gain between GD 14 and 21 than other treated females. Liver hypertrophy was moderate; thyroid follicular cell hyperplasia / hypertrophy was minimal. UR2F0463: no clinical signs up to start of parturition. Liver hypertrophy was moderate; thyroid follicular cell hyperplasia/hypertrophy was normal *Satellite group:* UR2F0451 (blood sample taken on GD 20): no clinical signs up to start of parturition. Liver hypertrophy was slight; thyroid follicular cell hyperplasia / hypertrophy was slight.	Hyper-reactivity to external stimuli, aggression (attributed to stress). Mean body weight gain during gestation was reduced by 14 %. Microscopic liver hypertrophy ranged from minimal to moderate. Microscopic thyroid follicular cell hyperplasia / hypertrophy ranged from minimal to moderate.

On looking at this table, it is apparent from the 1997 study that dystocia occurred in conjunction with liver necrosis, since all dams with dystocia also showed a degree of hepatocellular necrosis; in contrast, no liver necrosis occurred in P dams that delivered normally. The repeated dose studies (section 4.7.) demonstrate that the liver is a target organ of thiacloprid, exhibiting enzyme induction, hypertrophy, degeneration, fatty change and necrosis. Liver hypertrophy (slight to moderate) was reported in those animals in the 2011 c study in which dystocia occurred, but it also occurred (graded minimal to moderate) in the majority of dosed animals that did not have dystocia (reported in 25/26 treated animals). Hepatocellular necrosis was not reported in this or any of the other reproductive studies, although histopathology of the liver was not always included in the investigations. An association betwee increased incidence and/or severity of liver toxicity and dystocia has therefore not been demonstrated.

In only one study report was dystocia presumed to be a non-specific consequence of maternal toxicity (1998d). In this study, thiacloprid was administered at a dose of 100 mg/kg/d on GD 18 and 19, then because of excessive toxicity the dose was reduced to 50 mg/kg/d on GD 20; the one case of dystocia recorded was considered by the authors to be associated with necrosis of a uterine horn and general maternal toxicity rather than a direct effect on the birth process. In all the other studies, lower doses were administered. Overall, the conclusion from the analyses shown in Tables 22 and 23 is that general maternal toxicity by itself does not lead to dystocia in the Sprague-Dawley (Sasco) or Wistar rat.

The cytochrome P450 enzyme aromatase (CYP19) is one of the liver enzymes induced by thiacloprid, resulting in enhanced levels of oestradiol. This leads to a discussion of the possibility that a specific effect of thiacloprid, either on its own or together with stress, was responsible for the dystocia.

Changes to normal steroid hormone levels with effects on parturition onset and uterine contractility

Levels of the sex steroids progesterone and oestrogen are tightly controlled before and during

parturition to, firstly, maintain the pregnancy, and then, secondly, to induce parturition. Progesterone blocks myometrial contractions and reduces the sensitivity of the smooth muscle cells towards oxytocin. In contrast, oestradiol enhances the sensitivity of the smooth muscle cells towards oxytocin and stimulates spontaneous, rhythmic contractions of the myometrium. For a successful pregnancy and delivery, therefore, a certain ratio of progesterone and oestradiol is required. Thiacloprid has been shown in a number of carcinogenicity mode of action (section 4.10.1.) and reproductive toxicity studies to interfere with sex hormone biosynthesis and result in changes in the absolute levels and ratios of sex hormones. Although Industry does not dispute this effect, it has argued that such consequences in rats are not relevant to parturition in humans, because of differences in how parturition is initiated between the two species.

In the rat (and also in mice and rabbits), the corpus luteum is responsible for the maintenance of progesterone and oestradiol levels throughout pregnancy. In the early and middle stages of pregnancy, progesterone levels remain fairly constant. Likewise, the levels of oestradiol are constant in early and mid pregnancy. At term, increasing prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations result in the death of the corpus luteum, with a consequent rapid decrease in progesterone levels without a change in the number of uterine progesterone receptors. Hence, in the rat, a marked decrease of the serum progesterone concentration at term is a prerequisite for the initiation of parturition. Simultaneously, serum oestradiol levels increase between GD 19 and delivery, together with increases in uterine oestradiol receptors and oxytocin (between GD 16 and 19). Overall, the oestrogen/progesterone (E/P) ratio increases five-fold between GD 19 and the onset of labour (Fang *et al.*, 1996). Administration of the oestrogen-receptor blocker tamoxifen results in lower oestradiol levels than those in controls after GD 21 and a much smaller increase in the E/P ratio (Fang *et al.*, 1996). In these animals, the onset of parturition was delayed by 24 hours. The occurrence of parturition, albeit delayed, despite the low oestradiol levels, indicated that the decline in progesterone was more important than the rise in serum oestradiol.

An investigation by Inoué (1981) into the effects of different E/P ratios on the onset and duration of parturition in hormone-infused, ovariectomised pregnant rats demonstrated how finely balanced this ratio must be for a successful and timely parturition in this species. This study showed that a predominance of progesterone at term (GD 22 to 23) resulted in a prolonged gestation until GD 24/25 and a difficult labour (owing to over-grown foetuses), or, more commonly, no onset of parturition. Conversely, high levels of progesterone administered until GD 23 in combination with higher and earlier oestradiol brought forward delivery of the pups (GD 22 to 24) but still with a difficult and prolonged labour. Withdrawal of both progesterone and oestradiol on GD 22 resulted in individual differences in the timing of delivery (GD 22 to 23). A relatively small amount of progesterone together with a gradual increase in and then rapid withdrawal of oestradiol, with both hormones completely withdrawn by GD 21 and 22, respectively, resulted in a delivery of normal duration on GD 22. In contrast, the same progesterone programme combined with higher and longer administration of oestradiol (until GD 23) resulted in a difficult parturition, with variation in the time of onset (GD 21 to 23) but a much prolonged duration. Progesterone by itself, withdrawn on GD 21, resulted in delivery on GD 21 or 22, whereas oestradiol by itself advanced the timing of parturition (GD 20 to 21.5).

Uterine contractility is affected by the sensitivity of the myometrium to oxytocin. In both humans and rats, myometrial sensitivity to oxytocin increases through late gestation, in parallel with an increase in myometrial oxytocin receptor concentrations. Oestrogen increases oxytocin binding in ovariectomised pregnant rats and progesterone inhibits this oestrogen-induced rise. The increase in oxytocin receptors is correlated with the concentration of oestrogen receptors.

Oxytocin stimulates the secretion of uterotonic prostaglandins from the pregnant rat endometrium and human decidual cells.

In rats, uterine PGE_2 increases progressively in late gestation, reaching a peak the evening before delivery (GD 21.5). In the few hours before parturition, there is also an abrupt, five-fold increase in uterine oxytocin receptors, which was almost completely inhibited (on GD 22) by administration of tamoxifen. Notwithstanding, a significant increase in oxytocin receptors, to control levels, did occur before the delayed parturition, even with continued tamoxifen treatment. Likewise, although tamoxifen resulted in PGE_2 concentrations that were significantly lower than controls on GD 21 and 21.5, peak PGE_2 tissue concentrations at the time of tamoxifen-delayed parturition were similar to those in the control animals at normal onset of parturition (Fang *et al*., 1996). Other studies have indicated that endometrial oxytocin mRNA is increased in both humans and rats by oestrogen treatment, although there has been speculation that locally-produced oestrogen may be more important than that in the serum. Oxytocin stimulates endometrial PGE_2 production, so the decrease in PGE_2 with tamoxifen administration may have been the result of an interrupted oestrogenic stimulus to oxytocin or oxytocin receptor synthesis. In rat endometrium, oxytocin receptor synthesis is increased by PGE_2, giving rise to the likelihood of a positive feedback mechanism to increase myometrial contractility. Progesterone appears to be the predominant regulator of this feedback loop: higher levels of progesterone prevent uterine contractions, whilst progesterone withdrawal enables the feedback loop to progressively increase contractions.

Fang *et al*. (1996) concluded that there are considerable similarities between rats and humans during pregnancy and parturition, and therefore argued that the rat model has relevance to humans. Lately, however, it has been suggested that the rat may not be the most appropriate model for many aspects of human pregnancy and parturition. As stated above, in rats, rabbits and mice it is the corpus luteum that is responsible for the synthesis of progesterone throughout pregnancy; in contrast, in humans, progesterone synthesis is switched from the corpus luteum to, primarily, the placenta after the first few weeks of pregnancy. There is also a species difference in the site of oestrogen production: in pregnant and non-pregnant rats, the ovaries are the source, whereas in pregnant humans oestrogens are produced mainly by the placenta. The rat placenta is capable of producing androstenedione via 17α-hydroxylase activity in the second half of pregnancy, but since it lacks aromatase, it is not able to convert androstenedione to oestradiol; the latter is instead synthesised in the ovaries. In contrast, human placental oestrogen formation is dependent on 17α-hydroxylase activity of the foetus to provide androgen precursors, which are then converted to oestrogens in the placenta via aromatase activity; therefore in humans, the foetus and placenta interact in the formation of steroid hormones.

The most striking difference in the hormonal control of parturition between rats/rabbits/mice and humans is the requirement for a rapid fall in circulating progesterone levels to trigger the onset of parturition in the former, which is absent in the latter. In fact, in humans progesterone levels remain high and unchanged, or even increase, in the time preceding and during parturition; it is only once the placenta has been expelled that progesterone levels fall. Because of this fundamental difference between rats and humans, Industry has argued that the dystocia seen when thiacloprid is administered to pregnant rats, which it has hypothesised is because the necessary rapid fall in plasma progesterone does not occur, is not relevant to humans.

To explain how parturition can occur in humans in the absence of a fall in circulating progesterone levels, several concepts have attempted to describe a ‘functional progesterone withdrawal’. In sheep, a sharp increase in the maternal plasma E/P ratio, as a consequence of a

shift in steroidogenesis towards oestrogen production at the expense of progesterone production, precedes the onset of spontaneous labour. This change is thought to stimulate local prostaglandin production, leading to the onset of labour. Although no significant, consistent changes in oestrogen and progesterone are observed in human maternal serum before parturition, both oestrogen and progesterone have been demonstrated to be synthesised in human chorio-decidual tissue, leading to the hypothesis that local synthetic mechanisms may increase the E/P ratio at the time of labour onset. The reported increase in the E/P ratio in amniotic fluid during human parturition by Romero *et al.* (1988) may reflect this. More recently, it has been shown that progesterone and oestrogen are produced prior to the onset of labour, but once labour has begun, the predominant products are inactive progesterone metabolites and the biologically-active oestradiol (Mitchell and Taggart, 2009). Another hypothesis (reviewed by Zakar and Hertelendy, 2007) is that endocrine and paracrine factors (oxytocin, $PGF_{2\alpha}$ and PGE_2) instigate a switch from progesterone receptor B (PR B) to the isoforms A and C, which are inhibitors of PR B. The production of more oestrogen receptor α is then stimulated. The positive feedback mechanism that steadily increases myometrial contractility via oxytocin receptors, oxytocin and endometrial PGE_2 is thus still predominantly regulated by progesterone, but the ratio of receptor isoforms rather than the amount of progesterone determines if the feedback mechanism is inhibited or promoted. In humans, the output of labour-promoting prostaglandins (PGE_2 and $PGF_{2\alpha}$) by the placenta and the amnion increases with advancing pregnancy.

Overall, it has been suggested that parturition in humans is not as precisely regulated as it is in rodents but is, rather, a multifactorial process (Mitchell and Taggart, 2009). Moreover, the hormonal modifications that occur in women during gestation appear to be much greater and more diverse than those that have been determined in all other mammalian species studied, indicating that the human reproductive processes are more evolutionarily advanced (Casey & McDonald, 1997). Petraglia *et al.* (2010) concluded that the control of pregnancy and parturition is highly species specific, and that in women there is not a simple chain of events as there are in many other species. In their view, the evidence indicates that there are multiple paracrine/autocrine events, foetal hormonal changes and overlapping maternal/foetal control mechanisms that trigger parturition in humans. As a result, the decrease or absence of a single component can be compensated by changes in other pathways. However, this also seems to be the case in some other species, at least to some extent, since Petraglia *et al*. (2010) note that specific gene-knockout mice are able to deliver normally (for example, when oxytocin is knocked-out) or with an altered timing but normal uterus emptying (when, for example, certain enzymes of prostaglandin synthesis are knocked-out).

In rats, thiacloprid resulted in several effects on parturition that had serious toxicological consequences: a delayed onset of parturition or absence of its onset, difficulties in labour, and incomplete parturition. Some of the undelivered pups in one study (1998; 1998b; table 28) were reported to be ‘very large’, which might have resulted in the difficult deliveries and might have been a consequence of a delay in the onset of labour (i.e., the pups grew bigger than normal); information on the weights or size of pups obtained from the dams with dystocia in other studies was not available to support this hypothesis. The experimental evidence to indicate how thiacloprid might have influenced parturition in rats is summarised below.

Progesterone withdrawal

As described by Inoué (1981), progesterone withdrawal by GD 21 is required for a normal onset and duration of parturition in rats. In the 1998 study (table 28), thiacloprid did not prevent a fall in the mean progesterone concentration between GD 18 and lactation day 2, although the levels were slightly raised compared with the control animals at both time points. However, individual

animal data varied considerably and the progesterone levels in the two animals that exhibited dystocia were not higher at lactation day 2 than those in the same treatment group that delivered normally. Thiacloprid did not alter the uterine progesterone receptor concentration. In a second study (2011c; table 28), dystocia was recorded in three females; of these, progesterone levels were increased by 455 % at GD 23 in one animal, were within the normal range in the second, and were not measured in the third. Overall, progesterone levels fell between GD 20 and terminal sacrifice in all the groups, with no difference between those that had received thiacloprid and those that had not.

The available evidence is therefore not very supportive of the hypothesis that thiacloprid prevents the pre-parturition fall in progesterone levels.

Serum oestradiol

In a normal rat pregnancy, the circulating oestradiol level gradually increases between GD 19 and 21 and then rapidly falls so that it is withdrawn by GD 22 (Inoué, 1981). Thiacloprid administration resulted in raised serum oestradiol levels compared with controls during pre-mating, gestation and on lactation day 2, with the latter being particularly pronounced; there was not a corresponding increase in uterine oestrogen receptor concentrations, however (1998; table 28). Of the two animals in this study in which dystocia occurred, the serum oestradiol level of one was increased by 212 % compared with the group mean, whereas the level of the other was below the group mean. In the 2011c study (table 28), oestradiol was below the limit of detection at GD 23 in one animal with dystocia, was within the normal range for controls at GD 20 and terminal sacrifice in a second, and was not measured in a third. Overall, whilst the oestradiol levels fell in the control groups between GD 20 and the terminal sacrifice, they increased in the thiacloprid groups between GD 20 and 22, with a very slight (not statistically significant) decrease by terminal sacrifice. The change in the oestradiol level between GD 20 and terminal sacrifice was less in the thiacloprid-treated animals than in the controls.

Therefore, although there was not always a positive association between increased oestradiol levels and the occurrence of dystocia in individual animals, thiacloprid administration did result in increased group mean oestradiol levels.

E/P ratio

A normal time of onset and duration of parturition is initiated in rats when a relatively small amount of progesterone is combined with a gradual increase in and then rapid withdrawal of oestradiol, with both hormones completely withdrawn by GD 21 and 22, respectively (Inoué, 1981). Overall, the oestrogen/progesterone (E/P) ratio increases five-fold between GD 19 and the onset of labour (Fang *et al.*, 1996). This magnitude of E/P ratio increase in untreated controls was confirmed in the 2011c study (table 28), whereas, in contrast, the E/P ratio increased 10-fold between GD 20 and 22 when thiacloprid was administered. At terminal sacrifice (after the onset of parturition), the increase in the E/P ratio of the thiacloprid-treated animals from GD 20 was still double that of the controls. An increase in the E/P ratio was also observed in aged rats treated with thiacloprid for 28 days (2009e; section 4.10.1; table 26).

In the study by Inoué (1981), a normal progesterone profile combined with a larger and longer (until GD 23) infusion of oestradiol resulted in difficulties in parturition: the timing of parturition varied, its duration was much prolonged and undelivered foetuses were found *in utero*. This pattern of effects mirrored those observed after thiacloprid administration and is consistent with the increased mean oestradiol levels in treated groups.

Ovarian aromatase activity

In rats, aromatase in the granulosa cells of the ovaries catalyses the conversion of androgens to oestrogens (the most potent of which is oestradiol). Ovarian aromatase activity increases in pregnant rats and results in the increased oestradiol levels that occur in late pregnancy. The increased ovarian aromatase activity that was recorded in thiacloprid-treated rats on lactation day 2, compared with a fall in the controls, was consistent with the raised serum oestradiol in these rats at this time point (1998; table 28). An effect via aromatase was also consistent with thiacloprid not having a direct oestrogenic effect in an immature rat uterotrophic assay (2007, see section 4.10; table 26), and with the primary target of thiacloprid in rat and mouse carcinogenicity studies being identified as the ovarian follicle.

In pregnant humans, the main source of oestrogens, and site of aromatase activity, is the placenta. The formation of androgen precursors of oestrogens is dependent on the foetus. It should be assumed that thiacloprid, with a molecular weight of 252.73 g/mol and an octanol/water partition coefficient of 1.26 at pH 7, is able to cross the human placenta.

Another ovarian enzyme whose activity might have been increased by thiacloprid administration was 17α-hydroxylase, since the gene that encodes for it, *Cyp17a1,* showed increased expression in a carcinogenicity mechanistic study (2009d, section 4.10.1; table 26). One of the roles of 17α-hydroxylase is to catalyse the conversion of 17α-hydroxyprogesterone to androstenedione.

Liver enzyme induction

Histopathological and direct evidence from a number of studies (section 4.7.1.) indicated that thiacloprid induced liver enzymes in rats, mice and dogs. The liver can represent an unregulated source of steroidal hormone (i.e., high-dose chemical induction of P450-dependent enzymes involved in synthesis and metabolism, such as CYP19 aromatase). The 1998 study author (table 28) proposed that an effect of thiacloprid on the liver was affecting the animals' ability to regulate steroid homeostasis via increased cholesterol, since cholesterol is a precursor in the process of steroidal hormone synthesis.

An *in vitro* assay with rat and dog liver microsomes indicated that thiacloprid was able to induce enzymes that metabolise the steroid testosterone to androstenedione. As already noted, androstenedione is a precursor of oestradiol.

Oxytocin

In the one study in which it was measured, thiacloprid administration did not affect oxytocin levels (1998; table 28). Thiacloprid administration resulted in decreased uterine contractility on GD 22, but there were no associated effects on uterine electrophysiology, cervical extensibility, cervical collagen content or uterine α_1-receptors.

Prostaglandins E_2 and $F_{2\alpha}$

Cervical and uterine prostaglandin E_2 and $F_{2\alpha}$ content were unaffected by thiacloprid administration at approximately 61 mg/kg/d (1998; table 28), a dose at which dystocia occurred. There is therefore no evidence that thiacloprid prevented the death of the corpus luteum via an inhibitory effect on prostaglandin $F_{2\alpha}$. Likewise, the normal increase in uterine PGE_2 during late gestation was not inhibited. This was consistent with oxytocin levels also not being affected, indicating that the positive feedback mechanism of endometrial prostaglandin E_2 / oxytocin to increase myometrial contractility was not disturbed.

Conclusion

The effects of substances on the timing of onset and the duration of parturition and associated hormone levels in rats are difficult to study, in particular as the tightly-controlled mechanism of parturition is easily disturbed by, for example, the stress of blood-sampling. Nevertheless, Industry has invested considerable effort into trying to establish an explanation for the dystocia induced by thiacloprid. Based on the pattern of findings that has emerged, the following sequence of events is proposed.

Thiacloprid, via liver-enzyme induction, results in more circulating cholesterol being available for steroidogenesis up to the synthesis of androstenedione and testosterone in the theca interna cells of the ovaries. This steroidogenesis is assisted by the increased expression of the ovarian *Cyp17a1* gene, which encodes for 17α-hydroxylase; one of this enzyme's roles is to convert 17α-hydroxyprogesterone to androstenedione. The increased levels of androgen precursors together with an increased ovarian aromatase activity then lead to increased oestradiol production by the ovarian granulosa cells and ultimately an alteration of the normal E/P ratio.