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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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

Labelling: Indication of danger: T, N

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

S-phrases: 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

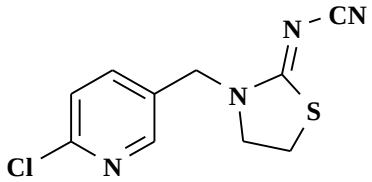
1 IDENTITY OF THE SUBSTANCE

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 that it does not contain	Mix, K.H. (1995)	

	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.

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 % No guideline stated	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)
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]	Not observed	No deaths and no clinical signs of toxicity at any dose.	CAR A6.9 (1998)

US-EPA guideline			
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**

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.

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	Average scores at 24, 48, 72 hours were Erythema: 1, 1, 0	No classification	CAR A.6.1.4 (1995c)

OECD 404	Oedema: 0, 0, 0		

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**

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.

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.

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	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

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

Non-GLP		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: 0, 3,.41, 27.9 or 115 mg/kg/day Purity: 96.6-97.5%	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. 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 14 days B6C3F1 mice: 5 males and 5 females per group OECD 407 GLP	0, 50, 200, 2000 or 10000 ppm Males: 0, 22, 84, 765 or 4143 mg/kg/day Females: 0, 30, 113, 1201 or 5450 mg/kg/day Purity 98.6%	There were no deaths, clinical signs of toxicity, or effects on body weight. 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	0, 100, 1000, or 10000 ppm	No deaths or clinical signs of toxicity observed. Decreased body weight gain and increased food consumption at 10,000 ppm in

B6C3F1 mice: 3 males and 3 females per group No guideline; non-GLP.	Males: 0, 30, 368 or 4141 mg/kg/day Females: 0, 64, 559 or 5785 mg/kg/day. Purity 98.6%	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**

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

4.9.6 Conclusions on classification and labelling

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

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* 2-year: 50 males and 50 females per group Interim sacrifice; 10 males & 10 females (1 yr). OECD 453 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. 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: 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	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.

[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.

GLP		[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 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 weeks old at start of study) to investigate hormone changes Oral / gavage 15 females/group	0 or 60 mg/kg in 0.5 % aqueous methylcellulose, administered once per day 4 days Sacrifice at either 2 hours or 8 hours after the last dose	Hormone levels were evaluated 2 and 8 hours after a 4-day exposure. 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 hormone levels and gene expression Oral / gavage 15 females/group	0 or 60 mg/kg in 0.5 % aqueous methylcellulose Single dose Sacrifice at 2, 8 or 24 hours after the dose	Hormone levels were evaluated 2, 8 and 24 hours after a single dose. There were no deaths, clinical signs or effects on body or 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.

[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.

4.10.2.2 Carcinogenicity: inhalation

No information available.

4.10.2.3 Carcinogenicity: dermal

No information available.

4.10.3 Human information

No information available.

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**

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	Oral, diet 0, 50, 300 or 600 ppm Calculated intake *circa*: 0, 3.7, 22 or 43 mg/kg/day (m+f)	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.

per dose group GLP	Purity: 96.7-97.5%	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	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;	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.

OECD 415 GLP	Females - 0, 2, 23 or 75 mg/kg/day. Purity not stated.	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 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]
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	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%	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]
Investigation of whether short-term exposure on gestation days 18-21 induces dystocia. Oral / gavage	0, 17.5, 35 or 60 mg/kg/day Purity: 96.7-97.5%	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.

<table>
<tr>
<td>Rat, Sprague-Dawley (Sasco)

30 pregnant females per dose (10 in the low-dose group)

GLP</td>
<td></td>
<td>Reproductive findings:
<table>
<tr><th></th><th colspan="4">Dose (mg/kg)</th></tr>
<tr><th></th><th>0</th><th>17.5</th><th>35</th><th>60</th></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>
<tr><td>No. of live births</td><td>253</td><td>102</td><td>192</td><td>81</td></tr>
<tr><td>Mean litter size</td><td>12</td><td>12</td><td>10</td><td>12.7</td></tr>
<tr><td>Mean No. of viable pups</td><td>12</td><td>11</td><td>9</td><td>7</td></tr>
<tr><td>No. of stillborn pups</td><td>4</td><td>5</td><td>28</td><td>34</td></tr>
<tr><td>No. cannibalised</td><td>0</td><td>2</td><td>11</td><td>13</td></tr>
<tr><td>Mean live birth index</td><td>99</td><td>94</td><td>83*</td><td>71*</td></tr>
</table>
*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]</td>
</tr>
<tr>
<td>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</td>
<td>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.</td>
<td>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]</td>
</tr>
<tr>
<td>One-generation phased exposure study, focus on steroid hormones</td>
<td>Oral / dietary
Ad libitum

0 or 800 ppm

54 or 61 mg/kg/day for</td>
<td>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,</td>
</tr>
</table>

<table>
<tr>
<td>Rat, Sprague Dawley (Sasco)

Non-guideline

GLP

10-16 females/group</td>
<td>males and females, respectively

Purity: 97%

Group 1: killed following a 9-week pre-mating 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>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 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</td>
</tr>
</table>

		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 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 video recording of parturition Rat, Sprague-Dawley (Sasco), 7 pregnant females Non-guideline	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 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)

		<table><tr><td></td><td>PND1</td><td>1.05 (0.21)</td><td>1.19[NS] (0.60)</td></tr><tr><td>Change between GD 20 & PND1***</td><td></td><td>0.16 (0.13)</td><td>0.03** (0.03)</td></tr></table> [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 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]
In vitro investigation of cytochrome P450 in liver microsomes. Non-GLP Non-guideline	Liver microsomes of male rats and dogs pre-treated with phenobarbital 0, 1000 μM Thiacloprid	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]
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	Oral / dietary *Ad libitum* 0 or 1000 ppm (mg/kg not known – estimated to be approx. 75 mg/kg/day) Purity: 97.2%	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]

gestation. Not GLP		

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 Developmental neurotoxicity study Rat (Sprague-Dawley) 25 females per dose group GLP	0, 50, 300 or 500 ppm	At 300 and 500 ppm, there were significant decreases in body weight and 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**

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; 10^{th} 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

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

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.

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.

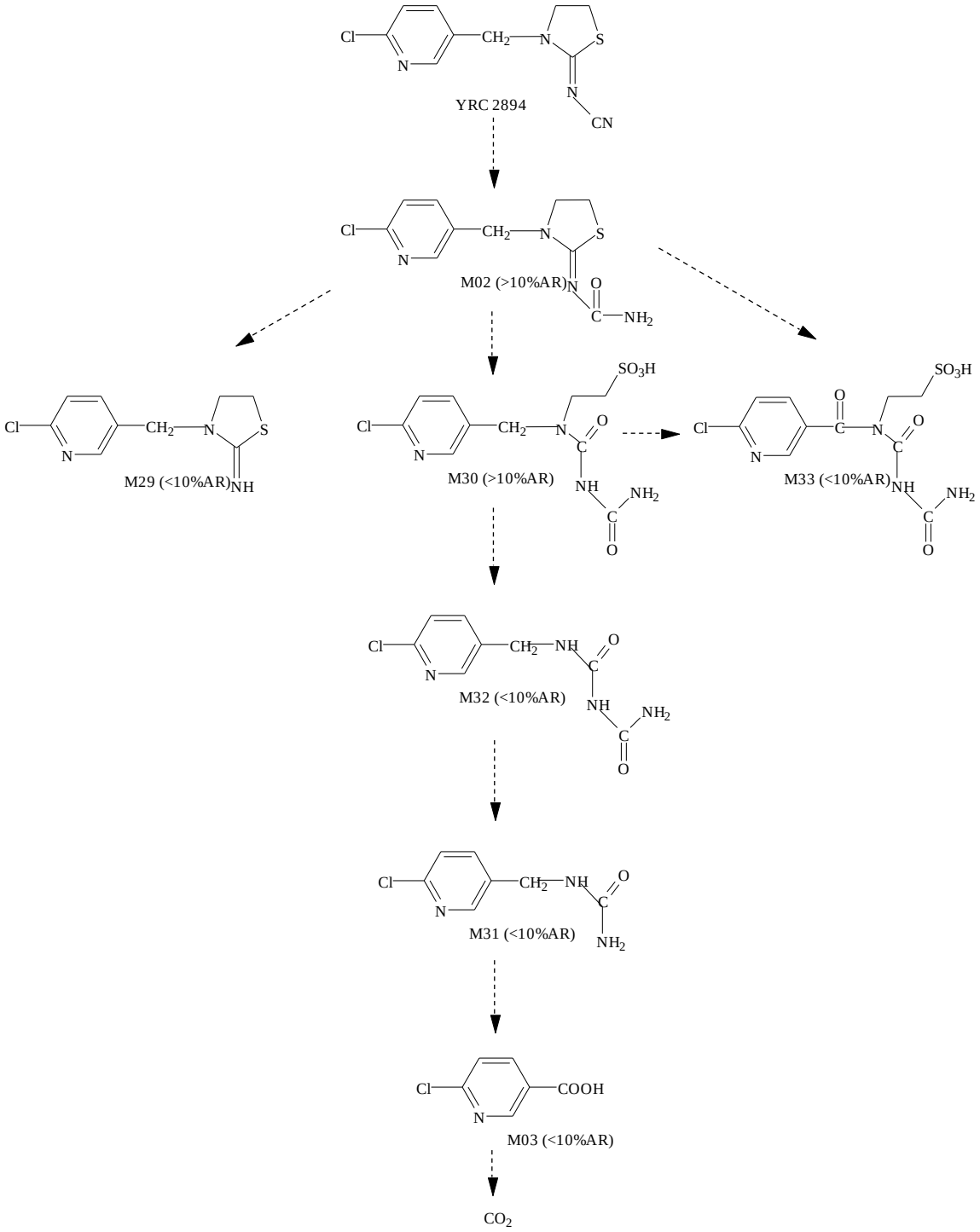
The mean value of the experimental disappearance time (DT_{50}) for thiacloprid was estimated to be 2.33 days (at 20 ± 1°C) for all soils based on first order kinetics. Bound residues accounted for 21.7 - 29.9% AR at the end of the study. Two metabolites (M02 and M30) occurred above 10% of the AR, with 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^{o}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 ≤ 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 ≥ 0.25%	0.025% ≤ Cn <0.25%	0.0025% ≤ Cn <0.025%

Where Cn is the concentration of thiacloprid in the preparation.

6 OTHER INFORMATION

7 REFERENCES

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	1995b	YRC 2894 techn.: acute toxicity (96 hours) to bluegill (*Lepomis macrochirus*) in a static test. Date: 1995-08-20
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	1998b	An experimental study to investigate the cause of dystocia and stillbirths in rats treated with technical grade YRC 2894. Date: 1998-09-02
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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-hydroxy-progesterone 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.