Annex VI Dossier

Proposal for Harmonised Classification and Labelling

Substance name: Thiacloprid
EC Number: Not yet allocated
CAS Number: 111988-49-9

Submitted by: UK REACH Competent Authority

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1 Identity of the substance and physical and chemical properties 8
1.1 Name and other identifiers of the substance 8
1.2 Composition of the substance 8
1.3 Physico-chemical properties 9
2 Manufacture and uses 10
2.1 Manufacture 10
2.2 Identified Use 10
2.3 Uses advised against 11
3 Classification and labelling 11
4 Environmental fate properties 12
4.1 Degredation 12
4.1.1 Stability 12
4.1.2 Biodegredation 12
4.1.2.1 Biodegredation estimation 12
4.1.2.2 Screening tests 12
4.1.2.3 Simulation tests 12
4.1.3 Summary and discusssion of persistenece 13
4.2 Environmental distribution 13
4.2.1 Adsorption/desoption 13
4.2.2 Volatilisation 13
4.2.3 Distribution modelling 14
4.3 Bioaccumulation 14
4.3.1 Aquatic bioaccumulation 14
4.3.1.1 Bioaccumulation estimation 14
4.3.1.2 Measured bioaccumulation data 14
4.3.2 Terrestrial bioaccumulation 14
4.3.3 Summary and discussion of bioaccumulation 14
4.4 Secondary poisoning 14
5 Human health hazard assessment 15
5.1 Toxicokinetics 15
5.2 Acute Toxicity 16
5.2.1 Acute toxicity: Oral 16
5.2.2 Acute toxicity: Inhalation 16
5.2.3 Acute toxicity: Dermal 17
5.2.4 Summary and discussion of acute toxicity 17
5.2.5 Summary and discussion of specific target organ toxicity – single exposure 17
5.3 Irritation 18
5.3.1 Skin 18
5.3.2 Eye 18
5.3.3 Respiratory tract 19
5.3.4 Summary and discussion of irritation 19
5.4 Corrosivity 20
5.5 Sensitisation 20
5.5.1 Skin 20
5.5.2 Respiratory system 20
5.5.3 Summary and discussion of sensitisation 20
5.6 Repeated dose toxicity 21
5.6.1 Repeated dose toxicity: Oral 21
5.6.1.1 Rat 21
5.6.1.2 Mouse 26

5.6.1.3 Dog....29
5.6.2 Repeated dose toxicity: Inhalation....31
5.6.2.1 Rat....31
5.6.3 Repeated dose toxicity: Dermal....32
5.6.3.1 Rat....32
5.6.4 Classification Rationale....33
5.7 Mutagenicity....33
5.7.1 *In vitro* data....33
5.7.2 *In vivo* data....34
5.7.3 Human data....34
5.7.4 Other relevent information....34
5.7.5 Summary and discussion of mutagenicity....34
5.8 Carcinogenicity....**Error! Bookmark not defined.**
5.8.1 Mechanistic studies....36
5.8.2 Summary and discussion of carcinogenicity....36
5.8.3 Classification rationale....38
5.9 Toxicity for reproduction....40
5.9.1 Effects on fertility....40
5.9.1.1 Additional mechanistic studies....46
5.9.1.2 Classification rationale....47
5.9.2 Developmental toxicity....49
5.9.2.1 Classification rationale....50
6 Human health hazard assessment of physico-chemical properties....52
6.1 Explosivity....52
6.2 Flammability....52
6.3 Oxidising potential....52
7 Environmental hazard assessment....53
7.1 Aquatic compartment (including sediment)....53
7.1.1 Toxicity test results....53
7.1.1.1 Fish....53
7.1.1.2 Aquatic invertebrates....53
7.1.1.3 Long-term toxicity to aquatic invertebrates....55
7.1.1.4 Algae and aquatic plants....55
7.1.1.5 Sediment organisms....55
7.1.1.6 Other aquatic organisms....55
7.1.2 Calculation of Predicted No Effect Concentration (PNEC)....55
7.2 Terrestrial compartment....55
7.3 Atmospheric compartment....55
7.4 Microbiological activity in sewage treatment systems....56
7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)....56
7.6 Conclusion on the environmental classification and labelling....56
8 Justification that action is required on a community-wide basis....57
9 References....58

Background to Proposal:

Thiacloprid is a chloronicotynyl 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 is currently waiting for listing in Annex I of the BPD Directive. Thiacloprid is not listed on Annex VI of Regulation (EC) 1272/2008.

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.

In accordance with Article 36(2) of EC Regulation 1272/2008, thiacloprid should now be considered for harmonised classification and labelling. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in Document IIA of the BPD assessment (attached as Annex I of this dossier).

Classification and labelling proposed in the BPD review of thiacloprid:

A proposal for classification and labelling of an active substance in accordance with Directive 67/548/EEC is required in the BPD dossier. The UK's initial proposal during this review was:

Class of danger	Xn: Harmful N: Dangerous for the environment
R-Phrases	R20/22: Harmful by inhalation and if swallowed. R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect R50/53: Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment

Since the initial biocides review, the classification for acute oral toxicity has been changed from harmful to toxic to take account of an extrapolated LD50 value obtained from an acute oral neurotoxicity study. It was also proposed that classification for dystocia seen in reproductive toxicity studies may be appropriate; however no proposal was put forward. Dystocia may be 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 in the offspring. It was noted in the biocides review that the current EU classification criteria do not explicitly cover dystocia and therefore, there was uncertainty about how to classify for this effect under the Dangerous Substance Directive. However, the criteria of the Globally Harmonized System for Classification and Labelling of Chemicals (GHS) (Annex 1, paragraph 3.7.2.1.1) seems to allow for the classification for effects on parturition. Consequently, the UK CA proposed a discussion on this novel issue at the Technical Committee for Classification and Labelling before making a firm proposal regarding the classification for this effect.

Classification and labelling proposed in the PPP review of thiacloprid:

A proposal for classification and labelling of an active substance in accordance with Directive 67/548/EEC is required in the PPP dossier. The UK's initial proposal during this review was:

Class of danger	Xn: Harmful N: Dangerous for the environment
R-Phrases	R20/22: Harmful by inhalation and if swallowed. R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect

R50/53: Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment

The proposed human health classification had the support of ECCO 109, the toxicology peer review meeting under Directive 91/414. Also, the proposed environment classification had the support of ECCO 110, the ecotoxicology peer review meeting.

Proposal for Harmonised Classification and Labelling

Substance Name:	Thiacloprid
EC Number:	A temporary EC number has been allocated (601-147-9).
CAS Number:	111988-49-9
Registration number:	None - Substance is in scope of REACH Article 15.
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:	The manufacturer has requested that impurities remain confidential. There are 9 process impurities; of these, the major impurity is present in a concentration range of ≥ 0.34% and ≤2%, with a typical concentration of 0.6%; the remainder are individually present at ≤ 0.2%. During the reviews under Directive 91/414/EEC and Directive 98/8/EC, none of the impurities were identified as contributing towards classification.

Proposed classification based on Directive 67/548/EEC:

Class of danger	T: Toxic N: Dangerous for the environment
R-Phrases	R25: Toxic if swallowed R20: Harmful by inhalation R40 (Carc. Cat 3): Limited evidence of a carcinogenic effect R62 (Repr Cat 3): Possible risk of impaired fertility R50/53: Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment

Proposed classification based on EC Regulation 1272/2008:

Signal Word	Danger
Classification	Acute Tox. 3 – Oral Acute Tox. 4 – Inhalation Carc. 2 Repr. 2f Aquatic Acute 1 Aquatic Chronic 1
H-Statements	H301: Toxic if swallowed H332: Harmful if inhaled H351: Suspected of causing cancer H361f: Suspected of damaging fertility H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects

Proposed labelling:

Directive 67/548/EEC:	T:R25 Xn:R20-40-62 N:R50/53 S2- S13-S23-S36/37-S46-S60-S61

CLP Regulation:

Pictogram: GHS06, GHS08, GHS09
Signal word: Danger
Hazard statement codes: H301, H332, H351, H361f, H400, H410
Precautionary statements : Not required as PS are not included in Annex VI.

Proposed specific concentration limits:

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.

Under GHS M factor 100 based on 0.001 <L(E)C_{50} ≤0.01 mg/l.

Proposed notes: None

JUSTIFICATION

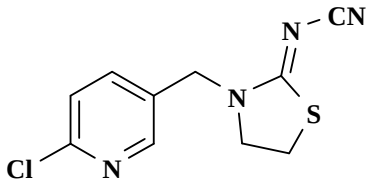
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE

Name: Thiacloprid
EC Number: A temporary EC number has been allocated (601-147-9).
CAS Number: 111988-49-9 [unstated stereochemistry]
IUPAC Name:
(Z)-N-{3-[(6-Chloro-3-pyridinyl)methyl]-1,3-thiazolan-2-yliden}cyanamide

1.2 COMPOSITION OF THE SUBSTANCE

Name: Thiacloprid
EC Number: A temporary EC number has been allocated (601-147-9).
CAS Number: 111988-49-9
IUPAC Name:
(Z)-N-{3-[(6-Chloro-3-pyridinyl)methyl]-1,3-thiazolan-2-yliden}cyanamide
Molecular formula: C10H9CIN4S
Structural formula:



Molecular Weight: 252.73
Typical Concentration (% w/w): 98.95% (no individual impurities present at > 2%)
Concentration range (% w/w): > 97% to ≤ 99.37%
Synonyms: YRC 2894

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	Yellow- brown solid	Reubke K.J (2001)
VII, 7.2	Melting/freezing point	4.2	136°C	92/69/EEC, A1 Krohn, J (1996)
VII, 7.3	Boiling point	4.3	The substance decomposed at 270 ^{o}C before boiling.	OECD 103 Krohn, J (1996)
VII, 7.4	Relative density	4.4 density	1.46 at 20 ºC	OECD 109 Krohn, J (1996)
VII, 7.5	Vapour pressure	4.6	8×10^{-10} Pa at 25 °C 3 x 10^{-10} Pa at 25 °C	OECD 104 Krohn, J (1996)
VII, 7.6	Surface tension	4.10	66 mN/m	OECD 115 Krohn, J (1996)
VII, 7.7	Water solubility	4.8	184 mg/L at pH 7 and 20 ^{o}C	OECD 105 Krohn, J (1996)
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7	1.26 at pH 7 and 20 ºC	OECD 107 (Shake flask method) Krohn, J (1996)
			0.73 at pH 7	OECD 117 (HPLC Method) Gruener R (2001)
VII, 7.9	Flash point	4.11	Not applicable since thiacloprid is a solid.	
VII, 7.10	Flammability	4.13	Thiacloprid is not highly flammable, does not liberate gases in hazardous amounts and has no pyrophoric properties.	92/69/EEC, A10 Mix, K.H. (1995) 92/69/EEC, A12 Mix, K.H. (1995) 92/69/EEC, A13 Mix, K.H. (1995)

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.11	Explosive properties	4.14	Thiacloprid is not explosive	92/69/EEC, A14 Mix, K.H. (1995)
VII, 7.12	Self-ignition temperature		No self ignition occurred.	92/69/EEC, A16 Mix, K.H. (1995)
VII, 7.13	Oxidising properties	4.15	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.	Mix, K.H. (1995)
XI, 7.16	Dissociation constant	4.21	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 by Bayer CropScience based in Germany. Formulation plants are located in Dormagen (Germany) and in Marle (France). The quantity manufactured is in the range of >100-1000 t. In 2008 about 550 metric tonnes have been synthesised, out of which > 98% are formulated as insecticidal plant protection product. Very small quantities are formulated in wood preservatives and in ready-to-use products for the use on ornamentals in gardens. The major market for thiacloprid is Europe.

2.2 IDENTIFIED USE

Thiacloprid is primarily used in agriculture as plant protection product in form of foliar spray applications. As such, it is not made available to the public in general but restricted to farm use by qualified and professionally trained farmers, operators and workers. Thiacloprid has been registered in the EU for PPP-purposes under the Directive 91/414 and has been included in Annex I until Dec. 31st, 2014.

Thiacloprid is mainly applied against sucking insects and beetles in arable crops (primarily

oilseed rape) as well as sucking pests and some lepidopteran species in orchards (primarily apples). There are also uses against sucking insects in vegetables, rice, cotton and some tropical crops. Thiacloprid is also approved for use in some “minor uses” such as tea, herbs and spices. A very small proportion of thiacloprid is formulated in ready-to-use formulations for the control of sucking insects on ornamentals in the garden

Beside the agricultural use thiacloprid will be marketed as a new biocidal active substance for the use in wood preservatives (PT 8), according to the procedures of Directive 98/8/EC concerning the placing of biocidal products on the market. The intended uses of thiacloprid containing products are either as manufacturing concentrates to be used in primers or stains to then be applied to wood constructions, or for industrial use in the protection of wood or wood based construction products from wood destroying insects (beetles, borers, termites).

Biocidal products containing thiacloprid will be used by industrial, professional and non-professional users. The industrial wood uses (as a timber treatment) would be at industrial facilities (professional/industrial applications) with application such as by dip, brush and rolling, or by vacuum/pressure processes. Such uses might also include use as a glue-line treatment in the industrial manufacture of wood composites such as laminated veneer lumber, plywood, or in wood plastic composites.

Thiacloprid containing products are to be used after dilution to give the desired in-use final concentration determined by the application method. The final concentration in a solution to be used on timber is expected to be between 10 and 200 ppm (0.001 and 0.02%).

2.3 USES ADVISED AGAINST

There are no uses which are specifically advised against.

3 CLASSIFICATION AND LABELLING

The substance is not classified in Annex VI of Regulation EC/1272/2008. There are no self-classifications available.

4 ENVIRONMENTAL FATE PROPERTIES

Presented below is the key information pertinent to determining a classification position based on the UK's review of thiacloprid under the BPD (Annex I). 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 DEGREDATION

4.1.1 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 ^{o}C at environmentally relevant pH conditions.

On the basis of two aqueous photolysis studies, thiacloprid is not expected to undergo significant photodegradation the environment. 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 and not considered representative of EU conditions. The second study following ECETOC and UBA[1] 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). For the purpose of classification and labelling, these conditions are considered representative of EU environmental conditions and demonstrate that thiacloprid is not considered to undergo significant photodegradation in the environment.

The stability in air is not considered relevant for this type of dossier given that air is not considered an environmental compartment of concern for thiacloprid (see Section 4.2.2).

4.1.2 BIODEGREDATION

4.1.2.1 BIODEGREDATION ESTIMATION

As measured data is available an estimation is not relevant for this dossier.

4.1.2.2 SCREENING TESTS

Thiacloprid is considered not readily biodegradable on the basis of a valid 28 day study (Manometric Respirometry OECD 301F) where 0% degradation was observed (Reis, 2005).

4.1.2.3 SIMULATION TESTS

Following BBA and SETAC methods, aerobic water/sediment degradation of thiacloprid was assessed (Riegner, 1997) using pond water/sediment and lake water/sediment in flasks over 100 days in the dark at 20 ±1 ^{o}C. The water DT_{50} ranges calculated based on first-order kinetics were 2.9-6.3 days for pond water and 10.6-10.8 days for lake water. The DT_{50} ranges

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

were 20.3-27.9 days and 12.1 days respectively based on the whole system. These study results are based on primary degradation and not ultimate mineralisation. Although this study demonstrates aerobic degradation of thiacloprid, it does not demonstrate rapid degradation in terms of ultimate mineralisation. On this basis, thiacloprid is not considered to meet the criteria of >70% degradation in the aquatic environment within 28 days required for classification and labelling.

Following US EPA guidelines, anaerobic water/sediment degradation of thiacloprid was assessed using pond water over 360 days in the dark at 20 ^{o}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.

An aerobic water/sediment microcosm study conducted with outdoor artificial ponds for 98 days between 11-26 ^{o}C [2] is available (Heimbach, 1997a). The water DT_{50} was 31 days and the sediment DT_{50} was 62 days. Although this study also demonstrates aerobic degradation, Thiacloprid is not considered to meet the criteria of >70% degradation in the aquatic environment within 28 days.

4.1.3 SUMMARY AND DISCUSSSION OF PERSISTENECE

Thiacloprid achieved 0% degradation in a standard ready biodegradation study. Although there is evidence of aerobic degradation within the aquatic environment, thiacloprid is not considered to meet the criteria of >70% degradation in the aquatic environment within 28 days.

Based on these studies, thiacloprid is not considered to undergo rapid and ultimate degradation under environmental conditions and is considered not readily biodegradable for the purpose of classification and labelling.

4.2 ENVIRONMENTAL DISTRIBUTION

4.2.1 ADSORPTION/DESOPTION

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.

4.2.2 VOLATILISATION

Thiacloprid has a low extrapolated vapour pressure of 8 x 10^{-10} Pa at 25 °C and a low Henry's Law Constant (5 x 10-10 Pa × m3× mol-1 at 20 °C) based on measured data (Krohn, 1996). On this basis thiacloprid is considered unlikely to partition the air.

[2] The mean recorded temperature over the first 30 days was 12.6±1.4^{o}C.

4.2.3 DISTRIBUTION MODELLING

Not relevant to this type of dossier.

4.3 BIOACCUMULATION

4.3.1 AQUATIC BIOACCUMULATION

4.3.1.1 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. For example, a BCF_{fish} of 2.35 can be estimated based on the higher log K_{ow} measurement, following Equation 74 in the TGD (2003). This log K_{ow} value is within the domain of the QSAR (log K_{ow} 2-6).

Thiacloprid was observed to be extensively metabolised in metabolism studies using rats (Section 5.1). 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.

4.3.1.2 MEASURED BIOACCUMULATION DATA

No experimental data are available.

4.3.2 TERRESTRIAL BIOACCUMULATION

Not relevant for this type of dossier.

4.3.3 SUMMARY AND DISCUSSION OF BIOACCUMULATION

Based on the low measured log K_{ow} values (0.73 and 1.26), the estimated BCF_{fish} (2.35) and evidence of extensive metabolism in rats, thiacloprid is considered to have a low bioaccumulation potential

4.4 SECONDARY POISONING

Not relevant for this type of dossier.

5 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 the BPD (Annex I). 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 TOXICOKINETICS

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 (See Annex I). 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.

5.2 ACUTE TOXICITY

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

5.2.1 ACUTE TOXICITY: ORAL

Table 5.1 Summary of acute oral toxicity studies

Species/Dose	LD_{50} (mg/kg)	Observations and remarks
Rat (5/sex/dose) 62.5 – 1000 mg/kg[1] Purity 97.3 % OECD 401	836 (males) 444 (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, tachypnea, dyspnoea and laboured breathing. [Krötlinger, 1996a]
Rat (5/sex/dose) 100- 5000 mg/kg[1] Purity 98.3 % No guideline stated	621 (males) 396 (females)	Clinical signs were seen at all dose levels and included decreased motility and reactivity, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnea, dyspnea, laboured breathing, diarrhoea. [Krötlinger, 1995a]
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 (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. [Sheets and Gilmore, 1997]
Rat Acute neurotoxicity study (12/sex/dose) 0, 3.1, 11 mg/kg[2] US-EPA guideline	Not observed.	No deaths and no clinical signs of toxicity at any dose. [Sheets, 1998]

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

5.2.2 ACUTE TOXICITY: INHALATION

Table 5.2 Summary of acute inhalation toxicity studies

Species/Dose	LC_{50} (mg/l)	Observations and remarks
Rat (5/sex/dose) 0, 0.08, 0.48, 1.5	> 2.5 (male) 1.2 (female)	No deaths occurred in males. Clinical signs of systemic toxicity observed up to day 6 post exposure included concentration-dependent bradypnea, dyspnoea, laboured breathing, rales, prostration, mydriasis, chromodacryorrhea, tremor, reduced motility, apathy, un-groomed hair,

or 2.5 mg/l for 4 hours, aerosol (MMAD <10µm) Purity 97.2 % OECD 403		hypothermia and piloerection. [Pauluhn, 1996]

5.2.3 ACUTE TOXICITY: DERMAL

Table 5.3 Summary of acute dermal toxicity studies

Species/Dose	LD_{50} (mg/kg)	Observations and remarks
Rat (5/sex/dose) 2000 mg/kg Purity 97.3 % OECD 402	> 2000	There were no deaths and no clinical signs of toxicity or local skin reactions. [Krötlinger, 1996b]

5.2.4 SUMMARY AND DISCUSSION OF ACUTE TOXICITY

The oral LD_{50} can be identified as 177-444 mg/kg. The lowest value lies within the range (20-200 mg/kg) for classification as T;R25 under Directive 67/548/EEC.

The oral LD_{50} also lies within the range (50-300 mg/kg) for classification as Acute Oral Tox. 3 (H301: Toxic is swallowed) under Regulation (EC) 1272/2008.

The inhalation LC_{50} of 1.2 mg/l lies within the range (1-5 mg/l/4h) for classification as Xn;R20 under Directive 67/548/EEC.

The inhalation LC_{50} also lies within the range (1-5 mg/l) for classification as Acute Inhalation Tox. 4 (H332: Harmful if inhaled) under Regulation (EC) 1272/2008.

The dermal LD_{50} lies above the classification cut-off of 2000 mg/kg under both Directive 67/548/EEC and Regulation (EC) 1272/2008 therefore no classification is proposed.

Directive 67/548/EEC: propose T;R25 Xn;R20

CLP Regulation: propose Acute Tox. 3 (H301), Acute Tox. 4 (H332)

5.2.5 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. There are no

human data to provide information on this end point. No classification as STOT-SE under Regulation (EC) 1272/2008 is proposed.

5.3 IRRITATION

5.3.1 SKIN

The skin irritation potential of thiacloprid (purity 97.3%) has been tested in a standard combined skin and eye irritation study in 3 male New Zealand White rabbits (Krötlinger, 1995c). Very slight erythema of the skin occurred in all three rabbits tested but all skin reactions had resolved by 72-hours post-application.

Table 5.4 Summary of skin irritation study

Species/ No per group	Average score 24, 48, 72 h		Reversibility (Y/N)	Result
	Erythema	Oedema		
Rabbit, New Zealand White, 3 males OECD 404	1, 1, 0	0, 0, 0	Yes	No classification [Krötlinger, 1995c]

5.3.2 EYE

The eye irritation potential of thiacloprid (purity 97.3%) has been tested in a combined skin and eye irritation study (Krötlinger, 1995c). 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.

Table 5.5 Summary of eye irritation study

Species/ No./group	Average score 24, 48, 72 h				Reversibility (Y/N)	Result
	Cornea	Iris	Conjunctiva			
			Redness	Chemosis		
Rabbit, New Zealand White, 3 males OECD 405	0, 0, 0	0, 0, 0	0.6, 0, 0	0.6, 0, 0	Yes	No classification [Krötlinger, 1995c]

5.3.3 RESPIRATORY TRACT

No information from humans is available. The respiratory tract irritation potential of thiacloprid has not been directly investigated in animals. In an acute toxicity study via the inhalation route clinical signs seen after exposure to 0.48 mg/l (4 h) thiacloprid included bradypnea, dyspnoea, laboured breathing, 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; Pauluhn, 1995; 1998), 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' (Pauluhn, 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 (Pauluhn, 1998). On balance, there is limited evidence from animals that thiacloprid has the potential to cause respiratory system irritation and therefore no classification is proposed.

5.3.4 SUMMARY AND DISCUSSION OF IRRITATION

Thiacloprid caused only slight (Score 1), reversible erythema and swelling of the skin, which is below the response required (mean score of 2 or more) for classification as a skin irritant under both Directive 67/548/EEC and Regulation (EC) 1272/2008.

Thiacloprid caused only mild, transient eye irritation characterised by conjunctival redness and swelling where the average score did 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 either Directive 67/548/EEC. Nor does this 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 Regulation (EC) 1272/2008.

There is no evidence that thiacloprid causes respiratory tract irritation.

In conclusion, no classification for skin, eye, or respiratory irritation is proposed.

Directive 67/548/EEC:	**no classification proposed**
CLP Regulation:	**no classification proposed**

5.4 CORROSIVITY

Thiacloprid did not lead to full thickness, or irreversible damage to the skin (Section 5.3) and therefore does not meet the criteria for classification as corrosive.

Directive 67/548/EEC :	**no classification proposed**
CLP Regulation:	**no classification proposed**

5.5 SENSITISATION

5.5.1 SKIN

Table 5.6 Summary of skin sensitisation study

Species	Number of animals sensitised/Total number of animals	Result
OECD 406 Guinea pig	1/10 thiacloprid 0/10 vehicle only controls Formulated in 2 % Cremophor	Negative [Stropp, 1996]

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% (Stropp, 1996). Skin reactions (grade 1) occurred in 1/10 animals and was 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 are available in which 2-mercaptobenzothiazole produced the expected responses.

5.5.2 RESPIRATORY SYSTEM

There is insufficient information to determine whether or not thiacloprid can cause occupational asthma.

5.5.3 SUMMARY AND DISCUSSION OF SENSITISATION

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%[3] of animals tested for classification under both Directive 67/548/EEC and the CLP Regulation.

There is no evidence to suggest that thiacloprid is a respiratory sensitiser. No classification is proposed.

Directive 67/548/EEC:	**no classification proposed**
CLP Regulation:	**no classification proposed**

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

5.6 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.

5.6.1 REPEATED DOSE TOXICITY: ORAL

5.6.1.1 RAT

There are 5 studies available: two of 14-day duration, two 90-day studies, and a 2 year-study.

Table 5.7. 14-day studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Daily gavage 14 days Wistar rats: 3 males and 3 females per group Non-GLP	0, 5, 10, 20, 60 or 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 ASAT, ALAT, AP increased at 120 mg/kg/day (up to 65 % above controls). Increased relative liver weights of approximately 20 % and 40 % were seen in males and females at 60 and 120 mg/kg/day, correlating with a slight untypical structure of the hepatocellular cytoplasm. Hepatic enzymes were induced at all doses. Increased cell proliferation in perivenular region of liver in 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 [Krötlinger, 1995a]
Diet, *ad lib*. 14 days Wistar rats: 5 males and 5 females per 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	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

	mg/kg/day mg/kg/d equivalents calculated from actual food intake Purity: 98.6%	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) [Krötlinger, 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 of Krötlinger (1995a; 1996c) both demonstrate that rats show an adaptive response to repeated dosing with thiacloprid.

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

In the second study, with addition of thiacloprid to the diet, there was a focus on the liver and the thyroid. At approximately 50 mg/kg/day and 190 mg/kg/day, there were again modest increases in liver weight and this was 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 5.8. 90-day studies

Dose schedule	Dose levels	Observations and remarks (effects of major toxicological significance)
Diet, ad libitum 90 days (+35 days recovery) Wistar rats, 10 males and 10 females per 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. Increased thyroid weight by 66 % in males at 1600 ppm; 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) [Krötlinger and Geiß, 1997]
Diet, ad libitum Fischer rats; 12 males and 12 females per 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). [Sheets, 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.

According to the classification criteria in Annex VI of Directive 67/548/EEC, substances inducing serious damage in 90-day studies at dose levels of 50 mg/kg/day or less should be classified as harmful.

In the dietary study of Krötlinger and Geiß (1997), rat body weight was significantly decreased and absolute liver weight was increased at approx 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 thyroxin (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 dietary study of Sheets (1997), focussing on neurotoxicity parameters, there were no significant body weight changes persisting throughout the 90-day treatment period. Small, transient decreases at approx 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.

There was no evidence of serious damage in either of these studies to justify classification of thiacloprid as harmful.

Table 5.9. 2-year study (non-tumour findings)

Dose schedule	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 mortality. 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 (eosinophillic cytoplasm with basophilic strands) and eosinophillic/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 (inc. 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 increased in males and females at 1000 ppm. 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. NOAEL = 1.2 mg/kg/day (25 ppm) ; LOAEL = 2.5 mg/kg/day (50 ppm). [Bomhard *et al.*, 1998]

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 2-year study were seen in females from 500 ppm (*circa* 33.5 mg/kg/day): 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 and are termed spinal radiculoneuropathy or degenerative myelopathy and may be exacerbated by xenobiotics. Also, the dose (*circa* 33.5 mg/kg/day for 2 years) at which these effects were seen is judged to be well-above the cut-off for classification. A simple extrapolation of the 90-day (3 month) cut off value of 50 mg/kg/day to 2-years (24 months) would give a cut-off value of 6.25 mg/kg/day.

Retinal atrophy and lens degeneration were seen in the eyes of control and treated female animals. These potentially serious lesions were only seen after 2-year (near lifetime) exposure of rats: similar effects were not seen after 1 year in this study or in the 90-day study of Sheets (1997), in which the eyes and optic nerves were examined by histopathology. It is questionable whether these findings justify classification. On balance, given that these degenerative changes were seen in many control animals, it appears that these findings do not provide a sufficient basis to justify classification of thiacloprid as harmful. Historical control data for these findings were not readily 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 – this is most likely to have also been secondary to hepatic enzyme induction.

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 is not further observed, nor does there appear to be a functional consequence in reproductive toxicity studies at doses relevant for classification.

5.6.1.2 MOUSE

Table 5.10. 14- and 21-day studies

Dose Schedule	Dose levels	Observations and remarks (effects of 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). [Krötlinger, 1997a]
Diet, ad libitum 21 days B6C3F1 mice: 3 males and 3 females per group No guideline; non-GLP.	0, 100, 1000, or 10000 ppm Males: 0, 30, 368 or 4141 mg/kg/day Females: 0, 64, 559 or 5785 mg/kg/day. Purity 98.6%	No deaths or clinical signs of toxicity observed. Decreased body weight gain and increased food consumption at 10,000 ppm in males. Decreased food consumption in females at 1000 ppm but no significant effect on body weight. Macroscopic examination of liver and kidneys only. Enlarged livers in 2/3 males at 10,000 ppm. Increased liver weight (absolute: *circa* 10 %) at 1000 ppm. Liver enzyme activity was not determined. NOAEL = 30 mg/kg/day (100 ppm); LOAEL 368 mg/kg/day (1000 ppm). [Wirnitzer, 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 14-21 days (Krötlinger, 1997a; Wirnitzer, 1994). Even at much higher doses (up to 5000 mg/kg/day) there is little, if any, evidence of serious damage to the liver or any other tissues. These studies do not support the classification of thiacloprid for repeated-dose toxicity.

Table 5.11. 90-day study

Dose Schedule	Dose levels	Observations and remarks (effects of toxicological significance)
Diet, ad libitum 90 days	0, 50, 250, 1250 or 6250 ppm	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.

B6C3F1 mice: 10 males and 10 females per group OECD 408 GLP	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 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 increased 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) [Wirnitzer and Rühl-Fehlert, 1995]

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.*

Only the lowest dose in this study was below the cut-off value of 50 mg/kg/day for classification of the chemical as harmful (Wirnitzer and Rühl-Fehlert, 1995).

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 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. Without further evidence to indicate that this is a serious, relevant lesion, the induction of fatty vacuolation of the X-zone in mice only is not judged to justify classification of thiacloprid as harmful.

Table 5.12. 2 year study (non-tumour findings)

Dose Schedule	Dose levels	Observations and remarks (effects of toxicological significance)
Diet, *ad libitum* 2 year (inc 1 year interim) B6C3F1 mice 1y: 10 males	0, 30, 1250 or 2500 ppm Males: 0, 5.7, 234.1 or 564.4 mg/kg/day Females: 0, 10.9, 475.3,	No effects on mortality 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,

& 10 females per group 2y: 50 males and 50 females per group. OECD 451 GLP	872.5 mg/kg/day Purity: 96.8-97.2 %	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 eosinophillic, 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) [Wirnitzer, and Geiss, 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 dosing in the range 5 to 10 mg/kg/day for 2 years induced no toxicologically significant effects in mice (Wirnitzer and Geiss, 1998). 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. At this high dose, no classification is justified.

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, although there appeared to be no clear evidence of an effect on adrenal function. 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). Given the high doses at which these effects were seen, they do not justify classification of thiacloprid as harmful.

5.6.1.3 DOG

Table 5.13 Dog studies

Dose Schedule	Dose levels	Observations and remarks (effects of 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) [Wetzig, and Geiß, 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 to 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 thought 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) [Wetzig and Rinke, 1998]

Diet, *ad libitum* 1 year Beagle dog, 4 males and 4 females per group for 52 weeks; or 3 males per group for 24 weeks OECD 452 GLP	0, 40, 100, 250 or 1000 ppm for 52 weeks or 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%	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 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) [Wetzig and Geiß, 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 of Wetzig and Geiß, (1998a), 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 (Wetzig and Rinke, 1998), 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 due 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 (Wetzig and Geiß, 1998b), 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.

None of the findings in dogs appear to indicate sufficiently serious tissue/organ damage to justify classification for repeated dose toxicity.

5.6.2 REPEATED DOSE TOXICITY: INHALATION

5.6.2.1 RAT

Table 5.14 Inhalation exposure studies in the rat

Exposure Schedule	Exposure levels	Observations and remarks (effects of toxicological significance)
Inhalation, nose-only Wistar rats, 10 males and 10 females 6 h/day 5 days; + 2 week recovery Similar to OECD403 & 412 GLP (but no QA)	0, 1.97, 19 or 205 mg/m^3 Aerosol MMAD = 2.9 - 3.3 µm Purity: 97.2%	No deaths occurred. At 205 mg/m^3, 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 205 mg/m^3, 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 19 mg/m^3 and above in females after the treatment period, but not at terminal sacrifice. NOAEC = 19 mg/m^3 ; LOAEC = 205 mg/m^3 [Pauluhn, 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, 2, 18 or 143 mg/m^3 Aerosol MMAD = 2.9 µm Purity: 97.2%	No deaths occurred. At 143 mg/ m^3, 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 143 mg/ m^3, 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 18 mg/m^3: slight hepatocellular and thyroid follicular epithelial cell hypertrophy. NOAEC = 18 mg/m^3 LOAEC = 143 mg/m^3 [Pauluhn, 1998]

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

None of the findings in these 2 studies are judged to provide evidence of serious damage following repeated inhalational exposure of rats to thiacloprid. There is evidence from the 28-day study of relatively minor changes in the liver and thyroid after repeated exposure to 143 mg/m^3 thiacloprid (Pauluhn, 1998). Although this exposure level is below the cut-off for classification as harmful (750 mg/m^3), the effects are not of sufficient concern to justify classification. More significant changes are likely to be induced at higher exposure levels, but

no classification is proposed given the absence of data.

The "dark" spleens observed in female rats at 19 and 205 mg/m^3 after exposure in the 5-day study were resolved after recovery (Pauluhn, 1995). The toxicological significance of these findings is unclear, but they are not judged to be of serious concern given that they resolve after cessation of exposure and have not been reported in other repeat dose studies.

5.6.3 REPEATED DOSE TOXICITY: DERMAL

5.6.3.1 RAT

Table 5.15 Dermal exposure studies in the rat

Dose/ Schedule	Dose levels	Observations and remarks (effects of 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. 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 homogenously 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. [Krötlinger, 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 study (Krötlinger, 1997b), involving repeated dosing over a 28-day period, there was evidence at 300 mg/kg/day and above of an adaptive response in the liver. This was similar to that seen after oral and inhalational dosing. At 300 mg/kg/day, which is the cut-off level for classification as harmful for this type of study, the only significant finding was in males: hepatic centrilobular hypertrophy associated with more homogeneously structured cytoplasm in males. No effects were seen at 100 mg/kg/day. In the absence of any serious damage following repeated dosing at 300 mg/kg/day or less, no classification is justified.

5.6.4 CLASSIFICATION RATIONALE

As discussed in detail, at relevant exposure levels thiacloprid does not appear to cause serious damage (clear functional disturbance or morphological change which has toxicological significance) following repeated or prolonged exposure by oral, inhalation or dermal routes. Consequently, no classification for repeated dose toxicity under Directive 67/548/EEC or STOT-Repeat under the CLP Regulation is proposed.

Directive 67/548/EEC:	**no classification proposed**
CLP Regulation:	**no classification proposed**

5.7 MUTAGENICITY

5.7.1 *IN VITRO* DATA

The *in vitro* genotoxic potential of thiacloprid has been well investigated in a number of valid, standard tests. Thiacloprid tested negative in two bacterial gene mutation tests and a mammalian cell gene mutation test at the HPRT locus and in an unscheduled DNA synthesis test. Thiacloprid also tested negative in a chromosomal aberration test using Chinese hamster V79 cells.

Table 5.16 Summary of *in vitro* mutagenicity studies.

Method	Organism/Strain	Concentrations tested	Result		Reference
			+S9	-S9	
Bacterial reverse mutation test	*Salmonella typhimurium* TA1535, TA1537, TA98 and TA100	Limit test Purity: 97.2%	Negative	Negative	Herbold, (1995a)
Bacterial reverse mutation test	*Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 *E. Coli* WP2/urvA	Limit test Purity: 96.8%	Negative	Negative	Otha, (1995)
In vitro mammalian chromosome aberration test	Chinese hamster V79 cells	0.075, 0.3 or 0.75 mg/ml Purity: 96.8-97.2% 4 h exposure	Negative	Negative	Herbold, (1995c)
In vitro HPRT gene mutation test	Chinese hamster V79 cells	0.015, 0.031, 0.063, 0.12, 0.25 or 0.5 mg/ml Purity: 97.2%	Negative	Negative	Brendler-Schwaab, (1996b)
In vitro unscheduled	Sprague Dawley rat hepatocytes	0.075, 0.15, 0.3, 0.35, 0.4 ,0.45 or	Negative		Brendler-Schwaab,

DNA synthesis		0.5 mg/ml Purity: 96.7-97.2%		(1996a)

5.7.2 *IN VIVO* DATA

The *in vivo* genotoxic potential of thiacloprid has been investigated in a standard micronucleus test. No statistically significant increases in incidence of micronuclei were observed at a dose that led to significant signs of toxicity and mortality.

Table 5.17 Summary of *in vivo* data

Method	Strain	Concentrations tested	Result	Reference
Mammalian erythrocyte micronucleus test	Mouse, NMRI male + female 5/sex/dose	0 or 60 mg/kg i.p. Purity: 96.8-97.2%	Negative	Herbold, (1995b)

5.7.3 HUMAN DATA

No human data are available.

5.7.4 OTHER RELEVENT INFORMATION

No further relevant information available.

5.7.5 SUMMARY AND DISCUSSION OF MUTAGENICITY

Data indicate that thiacloprid is not mutagenic *in vitro* or *in vivo* and does not meet the criteria for classification as a mutagen.

Directive 67/548/EEC:	**no classification proposed**
CLP Regulation:	**no classification proposed**

5.8 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 in these studies are summarised in Section 5.6.

Table 5.18 Summary of carcinogenicity studies

Species/ Dose schedule	Dose levels	Carcinogenicity observations and remarks
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 %	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, the mean incidence of uterine adenocarcinoma at 500 and 1000 ppm was well above the mean historical control value for this laboratory [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), but this was within 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), and this was statistically significant at the highest 2 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. [Bomhard *et al*., 1998]
Mouse, B6C3F1 Diet, *ad libitum* 2 year (inc. 1 year interim sacrifice) 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 %	In females, there was an increase in benign ovarian luteomas: 0/47, 1/48, 5/49 and 5/47. A single malignant luteoma was seen in the mice at 2500 ppm. None of these findings were statistically significant, but the values at the top two dose levels appear to have been 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. [Wirnitzer, and Geiss, 1998]

5.8.1 MECHANISTIC STUDIES

There has been some effort by industry to establish the mode of action behind tumours seen in the thyroid and uterus (summarised in the following table). These studies are further discussed in Section 5.8.2.

5.19 Summary of carcinogenicity mechanistic studies

Method	Dose level	Result
In vitro study on the inhibition of thyroid peroxidase from hog thyroid extracts.	483 or 870 μM Purity: 98.6%	Thiacloprid had no direct inhibitory action on thyroid peroxidase catalysis of guaiacol oxidation or the formation of iodine from iodide. [Freyberger, 1994]
In vivo study of aromatase activity Rat, Wistar Oral, dietary 15/sex/dose at 0, 100 and 1000 ppm. 10/sex/dose 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. [Andrews *et al*., 1998a]
In vivo study, including observations of aromatase activity and changes in plasma hormone levels Mouse, B6C3F1 Oral / dietary 30 females per dose	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 %). [Andrews *et al*., 1998b]

5.8.2 SUMMARY AND DISCUSSION OF CARCINOGENICITY

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*

Bomhard *et al.,* (1998) have shown that repeated treatment with thiacloprid (approximately 30 and 60 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

5.7).

A hypothesised mode of action involves thiacloprid-mediated induction of hepatic aromatase resulting in elevated plasma oestradiol concentrations (Andrews *et al*., 1998a and b; see Table 5.19). Following prolonged agonism of oestrogen responsive tissues such as the uterus, this could lead to increased tumour formation. In support of this, Christenson (1998; see Table 5.21) 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, this doesn't explain why increased tumours where only seen in the uterus, and not in other oestrogen-responsive tissues.

ii) *Rat thyroid tumours*

In the study by Bomhard *et al*., (1998), thiacloprid induced relatively small numbers of benign follicular cell adenomas in Wistar rats. The increased incidence of these tumours was seen at dietary exposures equivalent to approximately 25 or 50 mg/kg bw/day thiacloprid. At these doses, in the same study, there were changes in the liver and thyroid consistent with an adaptive response to treatment. Body weight was significantly increased in females but not in males.

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 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. 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 this mechanism.

An i*n vitro* study investigated the possibility that thiacloprid or its metabolites could exert a direct effect on TPO (Freyberger, 1994; See Table 5.19). Interactions of 435 and 870 µM thiacloprid with TPO-catalysed reactions were evaluated using 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 have no direct inhibitory effect on TPO.

There is only limited information available to inform about other possible mechanisms. For example, it is known that liver enzyme induction can lead to increased conjugation and excretion of thyroid hormones which in turn leads to compensatory hyperplasia in the thyroid and ultimately may lead to the formation of tumours. Humans appear to be less sensitive to the effects of enzyme-inducing compounds on thyroid hormone metabolism. Bomhard *et al.,* (1998) observed that plasma TSH was increased in male and female rats at approx 50 mg/kg bw/day thiacloprid. This may have been related to the inducing effect that thiacloprid has on liver metabolism. Increased TSH might lead to over-stimulation of the thyroid gland and

subsequently to thyroid tumours. However, there were no clear effects seen on T3 or T4 levels in this study and increased TSH was not seen at the lower dose of 25 mg/kg/day at which an increased frequency of tumours was also seen. Consequently, the mode of increased thyroid tumour formation in rats treated with thiacloprid is not fully elucidated and should therefore be considered as relevant to humans.

iii) *Mouse ovarian tumours*

Small increases in benign ovarian luteomas were seen in female B6C3F1 mice treated with approx. 475 and 875 mg/kg bw/day thiacloprid in the diet for 2 years (Wirnitzer and Geiss, 1988). A single malignant tumour was seen in the higher dose group. Ovarian luteomas are seen only rarely in control mice.

It is possible that the increased incidence in these tumours may have been a consequence of an over-stimulation of the ovaries following hormonal perturbation (e.g. perturbation of prolactin release by oestrogens) by repeated exposure to thiacloprid. This substance has an inducing effect on hepatic aromatase, which could increase oestradiol levels. However, this has not been established as the mode of action and some of the available data do not fit very well. For example, contrary to what might have been expected, Andrews (1998b), who treated B6C3F1 female mice with approx 1100 mg/kg bw/day thiacloprid in the diet for 13 weeks, observed slightly increased plasma progesterone and slightly reduced plasma oestradiol levels, concomitant with an increase in hepatic aromatase.

The mechanism by which thiacloprid treatment resulted in increased ovarian tumours remains unknown and should therefore be considered relevant to humans.

5.8.3 CLASSIFICATION RATIONALE

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

In accordance with the criteria in Directive 67/548/EEC, classification in category 1 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 2 or category 3.

Since increased tumours have been seen in 2 species, a simple argument for category 2 classification can be made. However, on consideration of all the available data, there are a number of factors that indicate classification in category 3 would be more appropriate. Most significantly, there is the lack of genotoxicity seen with thiacloprid in short-term tests *in vitro* and *in vivo*. Also, there is the lack of consistency between the tumour findings in rats and mice suggesting that they could be species-specific. This is particularly relevant to the rat thyroid tumours.

Malignant tumours are generally considered to be of most concern for classification. The criteria indicate that when seen at very high dose levels exceeding the “maximal tolerated dose”, the significance of such tumours in laboratory studies is reduced and category 3 may be the more appropriate classification. With thiacloprid, malignant tumours were only seen in

the uteri of rats 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 5.9). In the eyes, retinal atrophy and lens degeneration were noted.

In view of these observations, the available evidence is considered to best match the criteria for classification as a category 3 carcinogen.

Similarly, according to Regulation EC/1272/2008, classification as a category 2 carcinogen is judged to be appropriate. There are no grounds to draw attention to a particular route of exposure on the label.

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

5.9 TOXICITY FOR REPRODUCTION

5.9.1 EFFECTS ON FERTILITY

The effects of thiacloprid on fertility were initially studied in a one-generation range finding study and a standard two-generation study in rats.

Table 5.20 Summary of fertility studies

Method Species	Exposure conditions, & doses	Observations and remarks
2-Generation (similar to OECD 416) Rat, Sprague-Dawley 30 males and 30 females per dose group GLP	Oral, diet 0, 50, 300 or 600 ppm Calculated intake *circa*: 0, 3.7, 22 or 43 mg/kg/day (m+f) Purity: 96.7-97.5%	There was no evidence of an effect on mating, fertility or implantation. However, in P0 pregnant females there was dystocia (described as 'difficulty delivering') leading to death in 0, 0, 4 and 3 dams at 0, 50, 300 and 600 ppm (gestation days 23-24). Parturition started (i.e. some, but not all, pups delivered) in 3 dams with dystocia, but not in the remaining 4 (all pups found dead *in utero*). Several gross observations including pallor, wet/stained perineal areas, red vaginal discharge were generally associated with dystocia. 'Pinpoint red foci' in the liver were also noted in one of the 600 ppm females with dystocia. There was no dystocia in F1 females. At 600 ppm: decreased live birth index in F1 and F2 generations of approximately 8%^{NS} below controls; increased incidence^{NS} of still-born pups: F1 5.7 % at 600 ppm, 0.6% controls; F2 5% at 600 ppm, 2.9 % in controls; reduced viability index at day 4: F1 82.8 %^{NS} at 600 ppm, 97.4 % in controls; F2 91.6 %^{NS} at 600 ppm, 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 secondary non-specific effects to maternal toxicity. It is not clear from the study report whether these findings only occurred in pups from dams with dystocia. 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 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 due to 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).

		[Eigenberg and Hamilton, 1997]
1-Generation, range-finding (similar to OECD 415) Rat, Sprague-Dawley 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 to 3 in controls). 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. [Porter *et al*., 1995]

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

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 (Porter, 1995). 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. 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 (Eigenberg, 1997). Effects seen in dams were increased incidences of hepatocyte hypertrophy and follicular cell hypertrophy in the thyroid, and are consistent to those indicative of enzyme induction outlined previously in repeat dose studies. Parental body weights were also reduced at 600 ppm particularly in females. No effect was seen on male or female mating performance or on fertility. 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 delivering') occurred in 4/30 animals at 300 ppm and in 3/30 at 600 ppm in P0 females. This effect was not seen in F1 females although it is possible that dystocia may have contributed to reduced pup-viability seen in this generation. Several gross observations seen including pallor, wet/stained perineal areas, 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. The occurrence of dystocia after thiacloprid treatment has been confirmed in further studies in rats (see Table 5.21).

In a series of studies, Eigenberg (1998 a, b and c) and Christensen (1998)/Schmidt (1998a)

further investigated the induction of dystocia in rats treated with thiacloprid. These studies are summarised in the following table:

Table 5.21 Summary of dystocia studies

Method Species	Exposure conditions, & doses	Observations and remarks
One-generation fertility study Rat , Sprague-Dawley 15 males per dose + 30 females per dose OECD 415 GLP	Oral / dietary *ad libitum* 0, 25, 300 or 1000 ppm Entire study period (starting 10 weeks before mating). Males - 0, 2, 20 or 69 mg/kg/day; Females - 0, 2, 23 or 75 mg/kg/day. Purity not stated.	There was no evidence of an effect on mating, fertility or implantation. No histopathological examinations were made. The only significant findings were at 1000 ppm (LOAEL): Clinical signs of toxicity included paleness, laboured breathing and cold to touch. Mean body weights of females were significantly lower during the last three weeks of the pre-mating phase (5.8%), during gestation (4.9-10.4%) and during lactation days 0-4 (10-13%). Mean body weight gain was significantly reduced (16.6%) during gestation; no clear treatment-related effects on food consumption were noted. In females, there was an increase of 22% in group mean liver weight and of 17% in thyroid weight. Most significantly, there were deaths due to dystocia: 2/28 females, in addition to one [1/28] female that died on gestation day 24 without any signs of initiation of labour (incidence of dystocia 0/30 in controls and lower dose groups). 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. [Eigenberg, 1998a]
One-generation fertility study + physiological assessments of uterus and cervix Rat, Sprague-Dawley 30 males per dose + 30 females per dose (multiple-dosing groups)	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. One dam (1/30) died from dystocia on day 22 with 3 pups born and 12 *in utero*. Statistically significant decrease in overall number of foetuses per litter (treated 10.2 compared to 12.3 in controls, combined data from all subgroups of the study). No treatment related effect on uterine electrophysiology, cervical extensibility, cervical collagen content of uterine α_1-receptors. Microscopy did not reveal any effects on the uterus or cervix. Non-statistically significant, although reproducible decrease in uterine contractility at gestation day 22. [Eigenberg, 1998b]

<table>
<tr><td>GLP</td><td></td><td></td></tr>
<tr><td>Investigation of whether short-term exposure on gestation days 18-21 induces dystocia.

Oral / gavage

Rat, Sprague-Dawley

30 pregnant females per dose

GLP</td><td>0, 17, 35 or 60 mg/kg/day

Purity: 96.7-97.5%</td><td>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. Significant reductions in food intake were seen on gestation days 18-21 at all dose levels.

Reproductive findings:
<table><tr><th></th><th colspan="4">Dose (mg/kg)</th></tr><tr><th></th><th>0</th><th>17</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 findings in this study appear to have been related to problems associated with the birth of the pups, rather than to a toxic effect on the pups themselves.

[Eigenberg, 1998c]</td></tr>
<tr><td>One generation phased exposure study, focus on steroid hormones

Rat, Sprague Dawley

Non-guideline

GLP

10-16 females/group</td><td>Oral / dietary
Ad libitum

0 or 800 ppm

54 or 61 mg/kg/day for males and females, respectively

Purity: 97%

Group 1: killed following a 9 weeks’ pre-mating phase

Group 2: killed following a 9 weeks’ pre-mating phase</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. Increased liver weight and hepatocytomegaly. Group mean absolute liver weights were increased by 21 %; < 16 %; < 10 % (groups 1,2 and 3, respectively).

At termination, there was increased hepatic aromatase, cytochrome P450, n-demethylase and o-demethylase activity in treated rats. No changes seen in serum concentrations of FSH, T4, T3, TSH, oxytocin or prolactin.

There was increased serum oestradiol compared to controls: group means increased by 40 %, 26 % and 151 % in groups 1, 2 and 3, respectively. No change in uterine oestrogen or progesterone receptor concentrations. No change in uterine weight in any group.

Aromatase activity in the ovary increased significantly in both controls and treated animals during gestation. Group mean values</td></tr>
</table>

	+ mating/pregnancy phase + gestation, then sacrificed day 18 or 21 of gestation. Group 3: Killed following post-delivery (lactation).	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 no significant difference between progesterone concentrations in controls and treated animals. 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): **Group 2 (gestation day 18)** **Group 3 (lactation day 2)** **Control** **800 ppm** **Control** **800 ppm** 61.32 40.11 32.29 29.80 66.22 102.08 8.83 18.52 85.44 72.79 14.81 28.81 86.20 101.56 18.28 24.42 62.02 96.92 18.36 17.87 65.94 68.92 16.86 [24.22]*a 43.63 67.68 13.10 [16.49]*b 84.36 87.56 12.15 32.65 84.80 79.79 13.87 29.76 88.16 81.92 11.67 22.49 91.40 176.37 12.88 27.10 66.07 - 28.25 17.96 59.90 - 16.86 - - - 17.12 - Mean: 72.73 Mean: 88.70 Mean: 16.81 Mean: 25.71** * Dams sacrificed because of dystocia. The first dam (a) delivered several pups, but did not complete parturition. The second dam (b) died without successfully delivering any pups, with one pup lodged in the birth canal. Other dams in the group had delivered successfully. ** Excluding the two dams with dystocia [Christenson, 1998; and Schmidt, 1998b]

In the study reported by Eigenberg (1998a), the effects of dystocia and increased stillbirths were again observed. The mean body weights of females treated with 1000 ppm (approximately 75 mg/kg bw/day) thiacloprid were significantly reduced; mean body weight gain was significantly reduced during gestation. No clear treatment-related effects on food consumption were noted. There were significant increases in mean relative liver weight at 300 and 1000 ppm.

At 1000 ppm, the 2 dams that died were considered to be suffering with dystocia. Several pups were delivered followed by a long period of time with no further deliveries. One dam delivered 1 pup on gestation day 22 but delivered no further pups, and was found dead on gestation day 23. The second dam delivered 10 pups on gestation day 23 and was found dead on gestation day 24. A third dam considered to have dystocia was found dead on gestation day 24 without ever having been observed in labour. Clinical signs in these animals were consistent with difficult labour. There were no gross pathological findings in the organs (including liver) of these animals. Histopathology was not assessed. There was no evidence of dystocia in female rats treated with the lower doses of thiacloprid.

In a second one-generation study, Eigenberg (1998b) 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. One of the exposed dams (1/30) died whilst giving birth, the likely cause for this was dystocia. Treated rats had lower body weights than control animals and there was a significant decrease in the number of foetuses per litter in treated dams used for the pathological investigations. However, 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.

A study is available that investigated whether short term treatment on gestation days 18-21 with thiacloprid could induce dystocia (Eigenberg, 1998c). Pregnant Sprague-Dawley rats were initially gavaged with 0, 35 and 60 mg/kg thiacloprid on gestation days 18-21. Because of severe toxicity and deaths in the 35 and 60 mg/kg dose groups, an additional dose level of 17.5 mg/kg was introduced into the study. Clinical signs of toxicity were seen at dose levels >35 mg/kg and included hypoactivity, chromorhinorrhoea and clear vaginal discharge. Marked toxicity was observed at all dose levels and included death, clinical signs, body weight changes and reduced food intake at 35 mg/kg and above. Dystocia was not observed but there was a dose-related increase in the incidence of stillbirths at 35 mg/kg. One-generation studies from the same laboratory (Eigenberg, 1998 a;b) have provided evidence to suggest that this was related to a maternal effect of thiacloprid on parturition itself or generally related to severe maternal toxicity.

A further one-generation study also investigated the increased incidence of dystocia and stillbirths after treatment with thiacloprid, along with possible effects on circulating hormone levels (Christenson, 1998; and Schmidt, 1998b). Groups of rats were administered diet containing 0 or 800 ppm (approximately 60 mg/kg/day) thiacloprid for 10 weeks before mating and during pregnancy. Among the rats scheduled for sacrifice after delivery, one female was found dead and two pregnant females were sacrificed due to prolonged or incomplete parturition (dystocia). One of these females successfully delivered several pups but did not complete the delivery of all pups. The second female showed some indications that parturition had been initiated but did not deliver. One pup was found lodged in the birth canal of this dam. In both cases, the animals were given at least 24 hours to complete the process. Both rats were killed on days 23-24 of gestation (normally gestation is 22 days long).

Liver weight was increased in the treated animals at necropsy. Microscopy revealed centrilobular hepatocytomegaly and proliferation of the smooth endoplasmic reticulum in the liver. Tissues from the two animals with dystocia were however not examined microscopically. The hepatic enzyme activities, including cytochrome P450 and aromatase, in treated animals were elevated at all time points.

A number of hormones were monitored in treated and control rats during and after pregnancy; gestation day 18 or 21 and lactation day 2, respectively. These data do not provide a clear explanation for the effects seen in treated animals and therefore do not impact significantly on how thiacloprid should be classified. Briefly, the picture observed can be described as follows. Oestradiol plasma concentrations were significantly increased at the end of the pre-mating period and on lactation day 2. Corticosterone levels were significantly raised at all time points. No changes were detected in FSH, T4, T3, TSH, oxytocin or prolactin levels. Uterine oestrogen and progesterone receptor concentrations were not affected by treatment, but progesterone concentrations varied amongst treated animals. No

aromatase induction was detected in the ovaries at the end of the pre-mating phase or during gestation. Ovarian aromatase activity, however, was increased at 800 ppm on day 2 of lactation. In particular, levels were especially high in animals that had dystocia. It is unclear how these hormone related parameters related to those during parturition.

5.9.1.1 ADDITIONAL MECHANISTIC STUDIES

There has been some effort by industry to understand the mode of action for the dystocia seen in thiacloprid-treated rats, with a focus on whether increased serum progesterone levels in pregnant rats might be a key event (see Annex 2). In the rat, a marked decrease of serum progesterone concentrations at term is a prerequisite for the initiation of parturition. An increase in progesterone concentrations could therefore lead to prolonged gestation and dystocia. In humans, progesterone concentrations remain high during parturition; down-regulation of progesterone receptor activity is thought to be a key event in the initiation of parturition. Such a species difference in physiology could mean that increases of progesterone concentrations after exposure to thiacloprid may not lead to dystocia in pregnant women.

However, as noted in the previous section, the available evidence is not sufficient to enable a clear conclusion to be reached on this subject. For example, if a decrease in serum progesterone concentration is required to initiate parturition in the rat, sustained serum progesterone levels would actually inhibit its onset. However, although parturition failed to complete, it was initiated (as shown by the delivery of some pups) in some of the rats with dystocia. Also, dystocia was not seen in dams that had the highest pre-and post parturition progesterone concentrations (Christenson, 1998; Schmidt 1998b). This suggests that the mode of action by which thiacloprid causes dystocia involves more than a simple increase in progesterone concentrations.

In addition, there is one *in vitro* and one *in vivo* study that have been conducted in order to help elucidate the mechanism by which thiacloprid might increase the levels of steroid hormones such as oestradiol and progesterone, and subsequently help explain how thiacloprid might induce dystocia.

Table 5.22 Summary of mechanistic studies

Method	Test system and concentration range	Observations and remarks
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. No inhibition of the main hydroxylation and oxidation reactions of testosterone. Effects on oestradiol/progesterone were not studied. [Schmidt, 1998a]
Plasma levels of thiacloprid	Oral / dietary *Ad libitum*	No treatment-related deaths or clinical signs of toxicity or body weight changes were reported. Mean gestation length

Rat , Sprague Dawley 8 pregnant, 12 non-pregnant exposed rats; 5 pregnant, 5 pregnant controls rats treated during pre-mating and up to 21 days of gestation. Not GLP	0 or 1000 ppm (mg/kg not known – estimated to be approx. 75 mg/kg/day) Purity: 97.2% rats treated up to 21 days (during gestation for pregnant animals)	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 un-metabolised 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. [Andrews and Schmidt, 1998]

Although the *in vitro* study did not reveal any inhibiting effect on enzymes involved in steroid degradation, it did show that thiacloprid treatment of microsomes can stimulate the metabolism of testosterone to androstenedione (Schmidt, 1998a). However, further investigation would be needed to establish whether any of these observations are relevant to pregnant female rats exposed to thiacloprid, and consequently whether thiacloprid-mediated changes in oestradiol metabolism might be involved in the processes leading to dystocia in some of these animals.

In addition to studies on hormone levels, an *in vitro* study monitored the circulating plasma levels of unchanged thiacloprid in female rats treated during pregnancy, compared to that in non-pregnant rats (Andrews and Schmidt, 1998). This study investigates potential differences in the pharmacokinetics between pregnant and non-pregnant rats and potential susceptibility to toxicity. However, this study does not provide useful information on the mode of action that leads to dystocia.

Consequently, it is concluded that the mechanism(s) by which thiacloprid caused dystocia in rats has not been established.

5.9.1.2 CLASSIFICATION RATIONALE

There is no evidence from any of the available studies that thiacloprid has an adverse effect on fertility: i.e. it has not been shown to have an adverse effect on mating performance or any of the standard parameters used in the assessment of fertility in 1- and 2-generation studies.

Dystocia has been seen in rats treated with 22 mg/kg bw/day thiacloprid. This adverse effect has been seen most commonly in rats treated before mating through to the end of gestation.

Although it is not known how treatment with thiacloprid leads to dystocia in rats, some plausible suggestions are available. Given that thiacloprid may modify the metabolism of steroid hormones, at least in the liver, it has been suggested that a rat-specific mechanism involving perturbation of progesterone levels at the time of parturition may be involved. Equally, signs of maternal toxicity have been seen at doses causing dystocia, most significantly decreased body weights and minimal to moderate liver necrosis. The effects seen are not judged to represent “severe maternal toxicity”, but they may still have had a role in the disruption of the physiology of pregnant rats contributing to the processes leading to dystocia.

The reproductive toxicity classification criteria in Directive 67/548/EEC do not provide clearly for the classification of substances that cause dystocia.

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. This is reflected in the criteria of Regulation EC/1272/2008, which make provision for classification for effects on parturition under 'sexual functioning and fertility'.

Considering the criteria in Regulation (EC) 1272/2008, classification in either category 1 or category 2 for reproductive toxicity is possible. Category 1A is inappropriate because the adverse effect of dystocia has not been reported in thiacloprid- exposed humans. As there is some uncertainty over the extent to which maternal toxicity has contributed to dystocia and, although not proven, there is a plausible mechanism (i.e. perturbation of hormonal mechanisms controlling parturition) that would limit the concern for human health, classification into category 1B also seems inappropriate. Given this uncertainty, it is therefore proposed to classify thiacloprid in category 2, labelling with phrase H361f.

Since it is important to ensure a harmonisation of classification and labelling, for example to avoid confusion over different labels and safety data sheets, it is also proposed to classify thiacloprid in category 3 for reproductive toxicity under Directive 67/548/EEC, with label R62. Category 3 under these criteria is considered equivalent to category 2 under Regulation (EC) 1272/2008.

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

5.9.2 DEVELOPMENTAL TOXICITY

There are studies of developmental toxicity in rats and rabbits, as summarised in the following table.

Table 5.23 Summary of developmental studies

Method Species	Exposure conditions	Doses	Observations and remarks
Developmental toxicity (OECD 414); GLP Rat (Wistar) 28-35 females per dose group	Oral, gavage Days 6-19 *post coitum*	0, 2, 10 or 50 mg/kg/day Purity: 97 to 97.3%	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: decreased implantations (79; against 93 in controls), increased post implantation loss (21; 7), increased late resorptions (20; 7), decreased mean litter size (9.3 foetuses; 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 deviations characterised by bone dysplasia (8/270, 3 %; 1/321, 0.3 %). This lies within the historical control range for the laboratory (0-3.45 %) and is concluded to represent a chance finding. [Stahl, 1997, amended by Boman and Klaus, 2000]
Developmental toxicity (OECD 414); GLP Rabbit, Himalayan 24 females per dose group	Oral, gavage Days 6-28 *post coitum*	0, 2, 10 or 45 mg/kg/day Purity 97.3%	No maternal deaths occurred. At 45 mg/kg, there was statistically significant decreased terminal maternal body weight (6 %). Decreased weight gain at 45 mg/kg days 6-11 (controls -17 g, top dose -113 g) and 6-28 (controls +154 g, top dose +5.4 g). 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 (control -17 g, 10 mg/kg -113 g) 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; increased incidence in skeletal retardations (reduced or delayed ossification) and marginally increased incidence of supernumerary 13th ribs with or without supernumerary lumbar vertebra. 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%; 2% in controls). This historical background rate for this lesion was 0.05-6%. All these findings are concluded to have been secondary non-specific effects, a consequence of

			maternal toxicity. A change in the sex ratio of litters at the top dose considered to have been a chance finding. The number of male foetuses (% litter mean) was decreased (controls 76% male, and 42 % at the top dose). At 10 mg/kg: a small (6%) decrease in foetal weight (males + females combined). [Holzum, 1996]
Developmental neurotoxicity study Rat (Sprague-Dawley) 25 females per dose group GLP	Oral, diet	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. [Hoberman, 2001]

In the study by Stahl (1997), signs of maternal toxicity were seen in rats at 50 mg/kg and included reduced body weight and food consumption. Maternal body weight loss was particularly evident on gestational days 6 – 9. During this period animals treated with 50 mg/kg lost 17.7 g in body weight compared to controls that gained 7.6 g. During pregnancy the main findings were an increased post-implantation loss, a decrease in foetal weight and an increased incidence of skeletal variations. These effects are considered to have been secondary non-specific consequences of the maternal toxicity induced by thiacloprid. The NOAEL for both maternal and foetal toxicity in this study was 10 mg/kg.

Holzum (1996) exposed pregnant rabbits to thiacloprid by gavage on days 6-28 post-coitum. Maternal toxicity was evident at 10 and 45 mg/kg. Signs of maternal toxicity were decreased body weight and food consumption. At 45 mg/kg, 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 vertebra, 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. The NOAEL for both maternal and foetal toxicity in this study was 2 mg/kg.

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 (Hoberman, 2001). A delay in the sexual maturation of pups can readily be accounted for by the low pup weight.

5.9.2.1 CLASSIFICATION RATIONALE

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 signs of developmental toxicity in a wider sense in the absence of maternal toxicity. On this basis, classification in category 1 or category 2 (Directive 67/548) would be inappropriate. The effects in pups that are seen (increased pup mortality, decreased body weight, delayed maturation of pups) can all be accounted for by secondary non-specific consequences of generalised maternal toxicity. On this basis, it is judged that no classification, rather than classification in category 3, is appropriate.

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

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 EXPLOSIVITY

In a standard study (Mix, 1995) thiacloprid was found not to exhibit any explosive properties.

No classification for explosivity is proposed.

6.2 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.

6.3 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.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Presented below is the key information pertinent to determining a classification position based on the UK's review of thiacloprid under the BPD (Annex I). The test substance used in all of the following studies has been judged to be equivalent to the thiacloprid covered by this proposal.

7.1 AQUATIC COMPARTMENT (INCLUDING SEDIMENT)

7.1.1 TOXICITY TEST RESULTS

7.1.1.1 FISH

Short-term toxicity to fish
Two static 96-hour acute toxicity studies are available (OECD 20) using *Oncorhynchus mykiss* (rainbow trout) (Dorgerloh, 1995a) and *Lepomis macrochirus* (bluegill sunfish) (Dorgerloh, 1995b). 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 studies performed with thiacloprid degradants were also conducted (further details are available in Annex I). The degradants were less toxic than thiacloprid, but as thiacloprid is considered stable in acute aquatic studies, they are not relevant to this type of dossier.

Long-term toxicity to fish
In a 97-day long-term toxicity study following OECD 210 and flow-through conditions, measured concentrations were ≥ 80% of nominal and results based on measured concentrations (Dorgerloh, 1997). The following endpoints and NOECs were recorded; time to hatch 3.91 mg/l; hatchability 3.91 mg/l; fry survival 3.91 mg/l; growth 0.244 mg/l; and, morphological and behavioural effects 1.91 mg/l. The lowest NOEC is 0.244 mg/l for growth based on length and weight.

7.1.1.2 AQUATIC INVERTEBRATES

Short-term toxicity to aquatic invertebrates
Four static 48-hour acute invertebrate toxicity studies following OECD 202 (modified where appropriate) and four different species; *Daphnia magna* water flea (Heimbach, 1995a), *Asellus aquaticus* freshwater hog louse (Manson, 2002a), *Gammarus pulex* freshwater shrimp (Manson, 2002c), and *Ecydonurus sp*. (mayfly larvae) (Manson, 2002d) are available. An additional static 96-hour acute toxicity to *Hyalella azteca* (freshwater amphipod) (Bowers, 1996) following US EPA guidelines is available.

All studies include immobilisation as an endpoint. The studies using *Gammarus pulex, Asellus aquaticus* and *Ecydonurus sp*. also included mortality as an endpoint. In all cases measured concentrations were >80% of nominal and unless specified, results are based on nominal data.

The test guidelines using *Gammarus pulex* and *Ecydonurus sp.* were modified for test organism – i.e. the temperature was adjusted to reflect the preferred range for the specific species. All guideline validity criteria were met and the studies are considered valid.

The measured 48-h EC_{50} for *Daphnia magna* was > 85.1 mg/l with a NOEC of 9.10 mg/l. 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 48-h EC_{50} for *Ecydonurus sp.* larvae was 0.0077 mg/l (mortality and immobilisation) with a NOEC of 0.004 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.

A range-finding study (OECD 202) with *Sericostoma personatum* (caddis fly) larvae is also available (Manson, 2002b). The 48-hour static study used nominal concentrations of 0.001, 0.01, 0.1 and 1.0 mg/l, and involved three replicates (*i.e.* three larvae) for the control and lowest test concentration, and four replicates (*i.e.* four larvae) for the remaining test concentrations. Variability in the dose-response relationship was observed with 75 per cent immobilisation at 1.0 mg/l, zero immobilisation at 0.1 mg/l, 25 per cent immobilisation at 0.01 mg/l and 33 per cent immobilisation at 0.001 mg/l. A follow-up definitive study was not conducted, although the range finding study indicates the 48-h EC_{50} lies between 0.1 and 1.0 mg/l. As the study does not include a sufficient number of replicates or include analytical support, it is not suitable for classification and labelling purposes. However, it can be considered as supporting data.

The lowest observed acute EC_{50} is 0.0077 mg/l for *Ecydonurus sp*. larvae based on mortality and immobilisation. The study was performed according to GLP, and followed the OECD 202 test guideline, and all validation criteria were met (including control mortality). *Ecydonurus sp* are considered a representative aquatic species for the invertebrate trophic level and they appear to be four orders of magnitude more sensitive than *Daphnia magna* (three other Crustacean species are also significantly more sensitive than *Daphnia*).

Two additional acute studies were performed with one major and one minor degradant, conducted with *Hyalella azteca* and *Daphnia magna* respectively. Further details are available in Annex I. The major degradant was less toxic than thiacloprid for the same species whilst no EC_{50} could be calculated for *D. magna* as no toxicity was observed. As thiacloprid is considered stable in acute aquatic studies, these results are not relevant to this type of dossier.

Long-term toxicity to aquatic invertebrates
A semi-static 21-day long-term *Daphnia magna* toxicity study following OECD 211 is available (Heimbach, 1996a). Measured concentrations were ≥ 80 % of nominal and results are based on measured concentrations. No mortalities occurred in the parental *Daphnia* but there was a significant effect on number of offspring per parent, parental body length and dry weight. The following endpoints and NOECs were recorded; number of offspring per parent 3.3 mg/l; parental body length 0.58 mg/l; and parental dry weight 1.85 mg/l. The lowest NOEC is 0.58 mg/l based on body length of the parent.

7.1.1.3 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 OECD 201 guideline (1984). Measured concentrations were >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 NOE_rC of 32 mg/l.

For *Pseudokirchneriella subcapitata*, the study report 5-day (120-hour) E_rC_{50} for was >100 mg/l with a quoted 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 0-72 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* following US EPA guidelines is also available (Dorgerloh, 1996). Based on measured data and frond number, the EC_{50} was >95.4 mg/l with a NOEC of 46.8 mg/l.

Two additional studies performed with thiacloprid degradants were conducted (further details are available in Annex I). In both cases the EC_{50} values were >100 mg/l, but as thiacloprid is considered stable in acute aquatic studies, they are not relevant to this type of dossier.

7.1.1.4 SEDIMENT ORGANISMS

Not relevant for this type of dossier.

7.1.1.5 OTHER AQUATIC ORGANISMS

7.1.2 CALCULATION OF PREDICTED NO EFFECT CONCENTRATION (PNEC)

Not relevant for this type of dossier.

7.2 TERRESTRIAL COMPARTMENT

Not relevant for this type of dossier.

7.3 ATMOSPHERIC COMPARTMENT

Not relevant for this type of dossier.

7.4 MICROBIOLOGICAL ACTIVITY IN SEWAGE TREATMENT SYSTEMS

Not relevant for this type of dossier.

7.5 CALCULATION OF PREDICTED NO EFFECT CONCENTRATION FOR SECONDARY POISONING (PNEC_ORAL)

Not relevant for this type of dossier.

7.6 CONCLUSION ON THE ENVIRONMENTAL CLASSIFICATION AND LABELLING

Thiacloprid exhibited limited acute toxicity to algae / aquatic macrophytes, compared to other trophic levels, with the lowest 72-h E_rC_{50} of 96.7 mg/l. It 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* although significant acute toxicity was observed for other invertebrates. Acute EC_{50} values <1 mg/l were observed for *Asellus aquaticus* (freshwater hog louse), *Gammarus pulex* (freshwater shrimp), *Ecydonurus sp*. (mayfly larvae), and *Hyalella azteca* (freshwater amphipod).

The lowest observed acute result is a 48-h EC_{50} of 0.0077 mg/l for *Ecydonurus sp*. larvae based on mortality and immobilisation. The study was performed according to GLP and standard test guidelines and all validation criteria were met. *Ecydonurus sp* are considered a representative aquatic species for the invertebrate trophic level and appear to be significantly more sensitive than *Daphnia magna* and other Crustaceans. In summary, the study is acceptable for the purpose of classification under Directive 67/548/EEC and Regulation (EC) 1272/2008 since a suitable test method and representative aquatic species were used.

Thiacloprid degradants were found to be less acutely toxic than thiacloprid.

There is some experimental evidence to suggest that thiacloprid may undergo aerobic degradation in the aquatic environment. However, thiacloprid achieved 0 % degradation in a standard ready biodegradation study and for the purpose of classification and labelling under Directive 67/548/EEC, is considered to be not readily biodegradable. Under the Regulation (EC) 1272/2008, thiacloprid is not considered to undergo rapid degradation in the environment.

Based on the low measured log K_{ow} values (0.73 and 1.26) and the estimated BCF_{fish} (2.35), thiacloprid is considered to have a low bioaccumulation potential.

Following Directive 67/548/EEC, thiacloprid should be classified Dangerous for the Environment with the following risk phrases:
N Dangerous for the Environment
R50 Very toxic to aquatic organisms
R53 May cause long term effects in the environment

Given the very low acute toxicity to invertebrates, 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.

Based on the Regulation (EC) 1272/2008, thiacloprid should be classified:
Aquatic Acute 1, Aquatic Chronic 1
Labelling: H400 ‘Toxic to aquatic life’ and H410 ‘Very toxic to aquatic life with long lasting effects’
Signal word ‘Warning’ and environmental warning label.
M factor 100 based on $0.001 < L(E)C_{50} \leq 0.01$ mg/l should apply.

8 JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Thiacloprid is a chloronicotynyl 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 and was included into Annex I of the Directive in 2004.

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.

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