CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Terbuthylazine

EC Number: 227-637-9

CAS Number: 5915-41-3

Index Number: Not yet assigned

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Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Terbuthylazine
EC number:	227-637-9
CAS number:	5915-41-3
Annex VI Index number:	Not yet assigned
Degree of purity:	> 96% w/w
Impurities:	Confidential. See Annex

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not applicable
Current proposal for consideration by RAC	Acute Tox. 4; H302 STOT RE 2; H373 Carc 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor: 10 Chronic M-factor: 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 STOT RE 2; H373 Carc 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410

	Acute M-factor: 10 Chronic M-factor: 10

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

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

2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox. 4; H302**	**None**	**None**	Not applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	**Carc 2; H351**	**None**	**None**	**Not applicable**
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	**STOT RE 2; H373**	**None**	**None**	Not applicable
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 Aquatic Chronic 1; H410**	**Acute M-factor: 10 Chronic M-factor: 10**	Not classified	Not applicable
5.1.	Hazardous to the ozone layer				

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

Labelling: Pictogram: GHS07, GHS08, GHS09

Signal word: Warning

Hazard statement codes:

H302; Harmful if swallowed

H373; May cause damage to organs through prolonged or repeated exposure

H351; Suspected of causing cancer

H410; Very toxic to aquatic life with long lasting effects)

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

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Terbuthylazine is an active substance in the scope of Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). There is no entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance.

At the time of submission the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Terbuthylazine is a broad spectrum herbicide belonging to the triazine group. It is effective against a wide range of annual and perennial broad leaved weeds. It also has activity on several annual grasses and contributes to the activity on grass weeds when used with mixture partners. In 2011, a positive opinion was given regarding the approval of the new active substance, (Reg 820/2011/EU). The UK were the Rapporteur Member State for the active substance. In accordance with Article 36(2) of the CLP Regulation, terbuthylazine should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints.

The EFSA conclusion proposed to classify terbuthylazine as Acute Tox 4; H302 due to the lowest observed oral LD50 (1000-1590 mg/kg). Refer to section 4.2 of this report for full details.

Due to an increased incidence of mammary adenocarcinomas in rats, classification with Carc 2; H351 was also proposed in the EFSA conclusion. Refer to section 4.10 of this report for full details.

A classification with Aquatic Acute 1; H400 and Aquatic Chronic; H410 was also proposed in the EFSA conclusion. Refer to section 5 of this report for full details.

In addition to the classification proposed in the EFSA conclusion, it is also proposed to classify terbuthylazine with STOT RE 2; H373 considering the effects observed following repeat dosing, (namely body weight loss, severe reductions in body weight and severe decreases in body weight gain). Refer to section 3.8 of this report for full details.

2.3 Current harmonised classification and labelling

Not applicable

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

Not listed

2.4 Current self-classification and labelling

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

Classification
Acute Tox 4; H302 - Harmful if swallowed
Aquatic Acute 1; H400 – Very toxic to aquatic life
Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects

Labelling
H302, H410
Signal word: Warning
Pictograms: GHS07, GHS09

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Terbuthylazine is a pesticidal active substance. In 2011, a positive opinion was given regarding the approval of the new active substance, (Reg 820/2011/EU). The UK were the Rapporteur Member State for the active substance. In accordance with Article 36(2) of the CLP Regulation, terbuthylazine should now be considered for harmonised classification and labelling. This proposal considers all human health and environmental endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

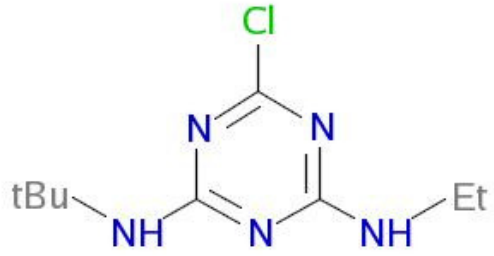
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	227-637-9
EC name:	Terbuthylazine
CAS number (EC inventory):	5915-41-3
CAS number:	5915-41-3
CAS name:	6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine
IUPAC name:	N-(tert-butyl)-6-chloro-N'-ethyl-1,3,5-triazine-2,4-diamine
CLP Annex VI Index number:	Not applicable
Molecular formula:	$C_9H_{16}ClN_5$
Molecular weight range:	229.7

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Terbuthylazine	≥ 98%		
Terbuthylazine	≥ 96 %		

Note that there are two producers of terbuthylazine

Current Annex VI entry: Not Applicable

Table 7a: Impurities (non-confidential information) – Manufacturer 1

Impurity	Typical concentration	Concentration range	Remarks
Simazine		≤ 3%	
Propazine		≤ 1%	

Table 7b: Impurities (non-confidential information) – Manufacturer 2

Impurity	Typical concentration	Concentration range	Remarks
Simazine		≤ 0.5%	
Atrazine		≤ 0.1%	

There are two manufacturers of terbuthylazine producing the technical material with a purity of ≥ 96 % and ≥ 98% respectively. There are a number of process impurities present in the terbuthylazine produced by both manufacturers and full information on the confidential impurities is provided in the IUCLID. Three of the impurities, propazine, simazine and atrazine have a harmonized classification on Annex VI of CLP (see below) and require further consideration when they are present in the final substance. Propazine and simazine are relevant impurities in the material produced by one manufacturer and can be present at levels of up to 1% and 3% respectively in the manufactured material, where they should be taken into consideration in the classification of the substance. Simazine and atrazine are relevant impurities in the technical material produced by the other manufacturer and can be present at levels of up to 0.5% and 0.1% respectively in the manufactured material. At these levels they would not individually contribute to the classification of the material.

The batches of terbuthylazine tested for the physical, human health and environmental hazards contained ≤ 1% of these impurities. There is no information to suggest that the effects observed in the studies with terbuthylazine can be attributed to the presence of these impurities alone. As such, it is proposed to classify terbuthylazine as outlined in this report based on the available data.

Current Annex VI entry:

Propazine

Carc 2; H351

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Simazine

Carc 2; H351

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Atrazine

STOT RE 2: H373

Skin Sens 1; H317

Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The batches of terbuthylazine tested were generally of higher purity than terbuthylazine as manufactured i.e., some of the tested batches did not contain all of the impurities found in the technical material. However, the available studies are considered appropriate to support the classification of terbuthylazine itself. The purity of the tested batches are specified in the relevant sections.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White crystalline powder	Das 1999b Flack 1995 a	Observation Purity 99.4% and 99.5%
Melting/freezing point	175.5 °C 175.7 °C	R Das 1999a Moller 2007	OECD 102 Purity 99.4% DSC Purity 99.6%
Boiling point	Decomposition observed at 224 °C before boiling point reached Decomposition observed at 230 °C before boiling point reached	R Das 2000a Moller 2007	OECD 103 Purity 99.4% DSC DSC Purity 99.6%
Relative density	1.2209	Flack 1994a	EEC Method A3 Purity 99.5%
Vapour pressure	9 x 10^{-5} Pa at 25 °C (extrapolated) 1.52 x 10^{-4} Pa at 22 °C	H Widmer 1999 Bacher 2004	EEC Method A4 Purity 99.4% EEC Method A4 Purity > 99%
Surface tension	71.8 mN/m at 20 °C 70.9mN/m	Martin 2000 Flack 1995a	OECD 115 Purity 96.5% EEC Method A5 Purity 96.8%
Water solubility	9 mg/l at pH 7.4 (25 °C) 6.64 mg/l at pH 7 (20°C)	Kettner 2000a Howes 1994	EEC Method A6 Purity 99.4% EEC Method A6 Purity 99.5%
Partition coefficient n-octanol/water	Log Pow = 3.4 (25 °C) Log Pow = 3.41 (20 °C)	Kettner 1999 Howes 1994	EEC method A8 Purity 99.4% EEC Method A8 Purity 99.5%
Flash point	Not relevant		
Flammability	Not considered flammable and experience in handling and use demonstrates the material will not ignite in contact with air or water	Angly 2000b Flack 1994a	EEC A10 Purity 96.8% and 96.5%

Explosive properties	Not explosive	Angly 2000d Flack 1994a	EEC A14 Purity 96.8% and 96.5%
Self-ignition temperature	No self ignition observed	Angly 2000c Flack 1994a	EEC A16 Purity 96.8% and 96.5%
Oxidising properties	Not oxidising	Angly 2000e Flack 1994a	EEC A17 Purity 96.8% and 96.5%
Granulometry	No data		
Dissociation constant	pKa = 1.95 (20 ^{o}C) pKa = 1.84 (20 ^{o}C)	Hormann 1999 Flack 1995e/Serri 2002	OECD 112 Purity 99.4% OECD 112 Purity 99.5%

2 MANUFACTURE AND USES

2.1 Manufacture

Terbuthylazine is manufactured both inside and outside of the EU.

2.2 Identified uses

Terbuthylazine is placed on the market in the EU as a herbicide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

Summary and discussion of physico-chemical properties

Refer to table 9.

Comparison with criteria

In a standard flammability study (EEC A10) terbuthylazine was found to be not flammable. Experience in handling and use indicates is not pyrophoric and does not react with water to liberate flammable gases. Further, it was also tested in a standard self ignition temperature study (EEC A16) and no spontaneous ignition was observed.

Terbuthylazine was tested in a standard explosivity study (EEC A14) where it was found to be not explosive under the influence of a flame and was not sensitive to impact or friction.

Terbuthylazine was tested in a standard study (EEC A17) and was not oxidising.

Conclusions on classification and labelling

Not classified

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary is based upon that in the Pesticide Draft Assessment Report (DAR) made for the review under Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC).

The toxicokinetics of terbuthylazine have been investigated in rats after single high and low dose administration and multiple dose administration.

Absorption

Terbuthylazine was found to be rapidly and extensively absorbed in the rat following oral administration of a single low dose (0.5 – 5 mg/kg bw/day: Tmax of 6-12 hours). A Tmax of 24-48 hours following administration of a single high dose (50-100 mg/kg bw/day) indicated delayed absorption. Tmax values of 8-12 hours following repeat administration are comparable with low dose administration.

Following single exposure (low and high dose), absorption was calculated to be 59-68 % in non-cannulated rats. In cannulated rats, oral absorption was estimated as 92 % in males and 79 % in females, although the value for females is likely to be an underestimate due to on-going excretion at study termination.

Distribution

Terbuthylazine was widely and evenly distributed in all tissues and organs investigated, with the exception of relatively high levels in the blood. Comparison of radioactivity levels in whole blood and plasma indicated an association with the cellular component and is consistent with significant and persistent binding of terbuthylazine (or metabolite) to erythrocytes. There was no evidence of bioaccumulation.

Metabolism

In rats, extensive metabolism of terbuthylazine was observed following oral administration, with no unchanged parent detected at low doses. At high doses, low levels were identified in the faeces (0.5-1.6 %). Metabolism proceeded via two major routes: 1) hydroxylation of the t-butyl moiety with further oxidation or conjugation or 2) oxidative cleavage of the amino-ethyl bond following further oxidation or conjugation. The major metabolites were identified as desethyl carboxylic acid (3U/M5) and a glucuronide conjugate of the desethyl analogue (5U/M3).

Excretion

Excretion via the urine (50-70 %) and faeces (31-40%) was observed. Excretion occurred primarily in the first 24 hours at low and repeated doses and between 24-48 h following a single high dose.

In bile-duct cannulated rats, urinary (13.6 %) and faecal excretion (1.7 %) were markedly reduced; however, levels of radioactivity remained high in the gastro-intestinal tract (28 %). Biliary excretion was extensive (45 %) consistent with enterohepatic circulation. Similar findings were observed in a second study.

4.1.2 Human information

Non-available

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of terbuthylazine were investigated orally in single dose and repeat dose studies in rats. Following single and repeat administration, terbuthylazine was well absorbed and widely distributed. Terbuthylazine was extensively metabolised and excreted in the urine and faeces. Biliary excretion accounted for nearly all of the faecal excretion. There was no evidence of bioaccumulation.

4.2 Acute toxicity

Information on the acute toxicity of terbuthylazine is available from three oral studies in rats, one dermal study in rats and one in rabbits; and one inhalation study in rats.

Table 11: Summary table of relevant acute toxicity studies

Method	LD_{50}/LC_{50}	Remarks	Reference
Oral OECD 401 Tif: RAIf rat 5/sex/dose Vehicle: distilled water GS 13529 (99 % purity)	> 2000 mg/kg bw	One female died on day 2. Other effects (e.g. hunched posture, piloerection, dyspnea and reduced locomotor activity) indicative of general toxicity were observed. No gross lesions indicative of organ toxicity were observed at necropsy of surviving animals. Necropsy revealed a spotted thymus in the decedent female.	Hartmann (1989a)
Oral EPA (1985), MAFF (1985), FiFRA 81-1 Sprague Dawley rat 5/sex/dose Vehicle: distilled water TK 12669/1 (96.4 % purity)	Between 1000 and 1590 mg/kg bw	Deaths were observed at 1000 mg/kg bw (2M, 1F), 1590 mg/kg bw (3M, 3F) and 2510 mg/kg bw (2M, 4F). Reduced weight gain and piloerection was observed at all dose levels; diarrhoea and prostration were observed at 1590 and 2510 mg/kg bw. Gross necropsy of decedents revealed congestion of the lungs (all animals) and fluid in abdominal cavity (one animal). No treatment-related effects were observed in surviving animals	Mercier (1991a)
Oral OECD 401 Sprague Dawley rats 5/sex/group Vehicle: distilled water Lot no 29 (97 % purity)	> 6400 mg/kg bw	Mortality occurred at doses ≥ 4000 mg/kg bw Other effects (e.g. hunched posture and increase salivation) indicative of general toxicity were observed. No gross lesions indicative of organ toxicity were observed at necropsy	Gardner (1988)
Dermal OECD 402 Sprague-Dawley rats 5 sex/dose Vehicle: distilled water Semi-occlusive TK 12669/1 (96.4 % purity)	> 2000 mg/kg bw	No deaths or signs of toxicity were observed. Gross necropsy did not reveal any treatment-related findings.	Mercier (1991b)
Dermal Comparable with OECD 402 New Zealand White Rabbits 5/sex/dose Vehicle: distilled water Occlusive Lot 29 (97 % purity)	> 2000 mg/kg bw	No deaths and no signs of systemic toxicity were observed. Single incidences of adverse effects (pale medulla with dark cortico/medulla junction, pale kidneys with stippled appearance, pale raised nodules on surface of liver, pale mottled appearance of liver lobes) were observed at Gross necropsy	Kynoch, Parcell & Mullins (1989)

Inhalation (dust aerosol) OECD 403 Tif: Ralf rats 5/sex/dose GS13529 (99 % purity)	> 5.324 mg/L	No deaths were observed. Signs of toxicity (piloerection, hunched posture, dyspnea and reduced locomotor activity). Gross necropsy didn't reveal any treatment related findings.	Hartmann (1989b)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Via the oral route data are available from three studies in rats. The LD_{50}s from two studies were > 2000 mg/kg bw, whereas the LD_{50} value derived from the third was between 1000-1590 mg/kg bw. In accordance with the Guidance on the Application of the CLP Criteria, classification is, generally, based on the lowest LD_{50} value from the most sensitive species, unless a robust justification as to why this would not be appropriate can be provided. Comparison of the studies does not reveal any explanation for the difference; they were all to guideline, were of a similar purity (96.4-99 % purity), used Sprague-Dawley or Sprague-Dawley-derived rats (Tif: RaIf rat), and administered the substance in water. Consequently, it is proposed to base the classification on the lowest LD_{50} value.

4.2.1.2. Acute toxicity: inhalation

An inhalation LC_{50} of > 5.3 mg/l for 4 hours was derived from a study conducted with rats.

4.2.1.3. Acute toxicity: dermal

Dermal LD_{50} values of > 2000 mg/kg bw were derived from two studies conducted with rats and rabbit.

4.2.1.4. Acute toxicity: other routes

No data available

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

See Section 4.2.1

4.2.4 Comparison with criteria

Via the oral route, an LD_{50} of between 1000-1590 mg/kg bw meets the criteria for classification as Acute tox 4 ($300 < ATE \leq 2000$ mg/kg) under the CLP Regulation.

Via the dermal route, the LD_{50} was > 2000 mg/kg bw and no classification is required under CLP.

Via the inhalation route, classification is only required if the LC_{50} is ≤ 5 mg/l for dusts and mists under the CLP. Since the LC_{50} is > 5.3 mg/l, no classification is required under CLP.

4.2.5. Conclusions on classification and labelling

Acute Tox. 4; H302

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

4.3.1. Summary and discussion of Specific target organ toxicity – single exposure

Refer to table 11 for a summary of the effects observed following single exposure and section 4.4.3 for information on respiratory irritation.

All clinical signs were considered to be non-specific signs of general acute toxicity. A number of changes in various organs (spotty thymus, lung congestion, pale liver and kidney) were observed in decedents. However, these changes were not consistently observed intra- or inter studies. No effects were noted in surviving animals.

4.3.2 Comparison with criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure are classified in STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect.

Classification in STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

The signs apparent after single oral, dermal and inhalation exposure to terbuthylazine were indicative of non-specific, general acute toxicity. As there was no clear evidence of specific toxic effects on a target organ or tissue, no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) under CLP is proposed.

4.3.3 Conclusions on classification and labelling

Not classified, conclusive but not sufficient for classification.

4.4 Irritation

4.4.1 Skin irritation

Two skin irritation studies are available in rabbits.

Table 12: Summary table of relevant skin irritation studies

Method	Results: Average Scores	Remarks	Reference
OECD 404 New Zealand White Rabbits TK 12669/1 (96.4 % purity)	Scores at 24, 48 and 72 h Erythema: 0,0,0 Oedema: 0,0,0	Six animals tested Slight erythema (score of 1 in 2 animals) was observed after 1 h only	Mercier O (1990a)
OECD 404 New Zealand White Rabbits Lot 29 (purity 97 %)	Scores at 24, 48 and 72 h Erythema: 0,0,0 Oedema: 0,0,0	Six animals tested	Liggett MP (1988a)

4.4.1.1 Non-human information

The skin irritation potential of terbuthylazine has been investigated in two standard guideline studies in rabbits. The only sign of irritation was slight erythema observed in one study at the 1 h time point.

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

The skin irritation potential of terbuthylazine has been investigated in two standard guideline studies. The only sign of irritation was slight erythema observed in one study at the 1 h time point.

4.4.1.4 Comparison with criteria

Slight erythema was observed at 1 h in one study only. No other signs of irritation were observed;. As the relevant average scores for erythema and oedema were below the value of 2.3 (as specified in the CLP criteria) and the effects were not severe in any individual animals or persistent, no classification is required under CLP.

4.4.1.5. Conclusions on classification and labelling

Not classified , conclusive but not sufficient for classification

4.4.2 Eye irritation

Two eye irritation studies are available in rabbits.

Table 13: Summary table of relevant eye irritation studies

Method	Results: Average scores	Remarks	Reference
OECD 405 (1987) New Zealand White Rabbits TK 12659/1 (96.4 % purity)	Scores for 6 animals (averaged from observations at 24,48 and 72 h) Cornea: 0, 0, 0,0,0,0 Iris: 0.3,0, ,0.6, 0.3, 0.3, 0.6 Conjunctivae – redness: 1.3, 0.6, 0.3, 1.3, 0.3, 0.3 Conjunctiva – chemosis: 0.3, 0.3, 0, 0.3, 0, 0.3	Six animals tested Irritation still present in one animal at termination (72 hrs)	Mercier (1990b)
OECD 405 New Zealand White Rabbits Lot 29 (97 % purity)	Scores for 6 animals (averaged from observations at 24,48 and 72 h) Cornea: 0, 0, 0, 0, 0, 0 Iris: 0, 0, 0, 0, 0, 0 Conjunctivae – redness: 0, 0.3, 0.6, 0.3, 0.6, 0.3 Conjunctiva – chemosis: 0, 0, 0.3, 0, 0.3, 0	Six animals tested Irritation resolved by day 3	Liggett (1988b)

4.4.2.1 Non-human information

The eye irritation potential of terbuthylazine has been investigated in two standard guideline studies in rabbits. No effect on the cornea was noted. Mild effects were observed in the iris in one study. Effects on the conjunctivae were observed in both studies but were limited to erythema and mild oedema. Although the Mercier study was terminated at 72 hours, before the effects had fully resolved, the results of the Liggett study showed effects to be fully reversible within 3 days.

4.4.2.2 Human information

No data available

4.4.2.3 Summary and discussion of eye irritation

See section 4.4.2.1

4.4.2.4 Comparison with criteria

Since this study was conducted on six animals, the criteria within the CLP Regulation are not directly applicable. However, the "Guidance on the Application of the CLP Criteria" states that classification is required if the individual average (from observations at 24,48 and 72 hours) is greater than the cut off in 4 out of the 6 animals. No effects on the cornea were observed. The relevant average score for consideration of effects on the iris is ≥ 1 and for conjunctival redness and oedema the relevant value is ≥ 2. No individual animal average was greater than these values and therefore classification is not required under the CLP Regulation.

4.4.2.5 Conclusions on classification and labelling

Not classified ; conclusive but not sufficient for classification.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.2 Human information

No information available

4.4.3.3 Summary and discussion of respiratory tract irritation

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.4.4 Comparison with criteria

No signs of respiratory tract irritation were observed as outlined in either the CLP Regulation.

4.4.4.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.5 Corrosivity

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Refer to table 12			

4.5.1 Non-human information

Terbuthylazine is not irritating to skin (see section 4.4)

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

See section 4.5.1

4.5.4 Comparison with criteria

No signs of corrosivity were observed in an *in vivo* skin irritation study.

4.5.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification.

4.6 Sensitisation

4.6.1 Skin sensitisation

Three skin sensitisation studies are available in the guinea-pig.

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406 – maximisation study Guinea-pig/ Pirbright White 20 animals GS 13529 technical (96.4 % purity)	Negative 1/19 test animals (1 animal died) 0/10 controls	Induction: Intradermal: 5% in peanut oil ± FCA or FCA alone Skin responses not reported Topical: 30 % in Vaseline Skin responses not reported Challenge: 10 % in Vaseline assessed at 24 and 48 hrs	Hagemann (1991)
OECD 406 (1981)– maximisation study Guinea-pig/ Dunkin Hartley 20 animals TK 12669/1 (96.4 % purity)	Negative 0/20 test animals 0/10 controls	Induction: Intradermal: 0.00085 % terbuthylazine ± FCA or FCA alone Skin responses not reported Topical: 68 % in water Skin irritation previously induced with 10 % SDS in paraffin Challenge: 68 % in water assessed at 24 and 48 hrs Positive control behaved as expected	Mercier (1991c)
OECD 406 – maximisation study Guinea-pig/ Dunkin Hartley Lot 29 (97 % purity)	Negative **25 %** / **Control** / **Test** **24 hr** 2/10 2/20 **48 hr** - 1/20 **72 hr** - 1/20 **50 %** **24 hr** 5/10 10/20 **48 hr** - 4/20 **72 hr** - 7/20	Induction: Intradermal: 5 % w/w in liquid paraffin ± FCA or FCA alone Skin responses not reported Topical: 50 % w/w in liquid paraffin Skin responses not reported Challenge: 25 % or 50 % w/w in liquid paraffin assessed at 24, 48 and 72 hr	Kynoch & Parcell (1988)

4.6.1.1 Non-human information

Skin sensitisation potential has been investigated in three standard guinea pig maximisation studies. Clear negative responses were observed in two studies employing challenge concentrations of either 10 % or 68 % terbuthylazine; although it should be noted the induction concentration in the latter study was very low raising concerns as to the quality of this study. Both studies only assessed sensitisation potential at 24 and 48 hours. In the third study, the proportion and severity of findings (grade 1 erythema) were similar in the control and test animals at 24 hours; however, whereas responses resolved in the control group, findings persisted or increased in severity in four animals in the 50 % group and one animal in the 25 % group at 48 and 72 hours, suggesting a sensitisation response. In addition, slight erythema (restricted to a small area of the challenge site) was observed in three additional animals in the 50 % group and 1 animal in the 25 % group at 72 hours. Findings in these additional animals are considered anomalous and not indicative of a sensitisation response as no reactions were observed in any of these animals at 48 hours and only one of these animals from the 50 % group had a slight reaction at 24 hours. Therefore, the number of animals considered to be clearly exhibiting a sensitisation response was 4/20.

4.6.1.2 Human information

No data available

4.6.1.3 Summary and discussion of skin sensitisation

The skin sensitisation potential has been investigated in three standard maximisation studies. No positive responses were observed in two studies. In a third study, the proportion of animals considered to be clearly exhibiting a sensitisation response was 4/20 animals.

4.6.1.4 Comparison with criteria

The sensitisation response was < 30 % in all guinea-pig maximisation studies. Therefore, no classification is required under the CLP Regulation.

4.6.1.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.6.2 Respiratory sensitisation

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data available			

4.6.2.1 Non-human information

No data available

4.6.2.2 Human information

No data available

4.6.2.3 Summary and discussion of respiratory sensitisation

Not applicable

4.6.2.4. Comparison with criteria

Not applicable

4.6.2.5 Conclusions on classification and labelling

Not classified - data lacking

4.7 Repeated dose toxicity

Repeated dose toxicity has been investigated extensively via the oral route in the rat (one 28-day, one 60-day, two 90-day and three chronic repeated dose studies (see section 4.10)), mouse (one 14-day, one 28-day and three chronic repeated dose studies (see section 4.10)), dog (one 1-year repeated dose study) and rabbit (two 28-day studies). Studies are also available via the dermal route in the rat (one 28-day study) and rabbit (two 28-day studies).

4.7.1 Animal information

4.7.1.1 Repeated dose toxicity: oral

Table 17a: Summary table of relevant oral repeated dose toxicity studies

Method	Results	Reference
28-day study Non-guideline Oral, diet Rat Sprague-Dawley 5/sex/dose 0, 400, 2000 and 10000 ppm corresponding to 0, 35, 150 or 359 mg/kg bw/day in males and 0, 39, 126 and 329 mg/kg bw/day in females Batch 29 (97 % purity)	**10000 ppm** - Sacrificed on day 9 Week 1: Weight loss in both sexes, 77/80 % ↓ food consumption (males/females) in week 1, ↓ adipose + small thymus in all animals **2000 ppm -** Sacrificed on day 9 Week 1: Weight loss in both sexes, 46/58 % ↓ food consumption (males/females) in Wk 1, ↓ adipose + small thymus in all animals **400 ppm** Week 4: 27/18 % ↓ bodyweight (males/females), 55/50 % ↓ bodyweight gain (week 4), 25/24 % ↓ food consumption (males/females) in weeks 1-4 LOAEL – 35/39 mg/kg bw/day based on bodyweight effects	Hopkins (1988)
60-day study Non-guideline Oral, diet Rat Wistar 10/sex/dose 0, 20, 50, 125 and 450 ppm corresponding to 0, 1.8, 4.4, 10.7, 38.1 mg/kg bw/day in males and 0, 2.0, 5.0, 11.4 and 39 mg/kg bw/day in females Batch 453/990/96 (96.6 % purity)	**450 ppm** 22/17 % ↓ week 8 bodyweight (males/females), 37/43 % ↓ bodyweight gain over weeks 0-8 (males/females), 27/23 % ↓ (males/females) food consumption over study *Organs;* 20 % ↑ relative adrenal weight (males), 10/11 % ↓ absolute kidney weight (males/females), 14 % ↑ relative kidney weight (males), 10 % ↓ absolute liver weight (females), 11 % ↑ relative liver weight (males), 30 % ↑ relative testes weight, 30/17 % ↓ absolute/relative ovary weight **125 ppm** 9 % ↓ week 8 bodyweight (females), 24 % ↓ bodyweight gain over weeks 0-8 (females), 17 % ↓ food consumption weeks in females over study **50 ppm** 6 % ↓ week 8 bodyweight (females), 14 % ↓ bodyweight gain over weeks 0-8 (females), ↓ food consumption weeks 3-4 **20 ppm** No adverse effects observed NOAEL: 10.7 mg/kg bw/day for males based on bodyweight effects at 38.1 mg/kg bw/day; 2 mg/kg bw/day for females based on reduced bodyweight gain at 5 mg/kg bw/day	Ramesh (1999)
90-day study (with 2-week recovery) OECD 408 Oral, diet Rat Tif:Ralf 10/sex/dose	**300 ppm** *Bodyweight and food consumption:* 24/ 22 % ↓ week 13 bodyweight(males/females), 31/32 % ↓ bodyweight gain over weeks 0-13 (males/females), 13-22/17-19 % (NS) ↓ food consumption (males/females) *Haematology, clinical chemistry and urology:* 5 % ↓ haemoglobin and 6 % ↓ haemocrit (females), 60/50 % ↑ neutrophils (males/females), 26/12 % ↓ glucose (males/females), 14/19 % ↑ urea (males/females), 16 % ↑ Creatinine (females), 20 % ↑ phosphate (females), 50 % ↑ ALP (other minor changes in	Bachmann (1995)

0, 6, 30, 100 or 300 ppm equivalent to 0, 0.407, 2.08, 7.11 or 22.3 mg/kg bw/day in males and 0, 0.405, 2.13, 7.18 or 22.8 mg/kg bw/day in females Batch: SG 6925 (96.4 % purity)	parameters are considered to be normal variation), 41 % ↓ volume, ↑ specific gravity and slight acidification in males **100 ppm** *Bodyweight and food consumption:*16/11 % ↓ week 13 bodyweight(males/females), 22/16 % ↓ bodyweight gain (males/females), 8.4-17/7-16 % (NS) ↓ food consumption (males/females) *Haematology, clinical chemistry and urinalysis:* 72 % ↑ neutrophils, 25 % ↑ phosphate (females), 27/14 % ↓ glucose (males/females), 27/31 % ↑ urea (males/females), 12 % ↑ creatinine (females), ↑ specific gravity and slight acidification in males **30 ppm** *Bodyweight and food consumption:* 8 % ↓ bodyweight gain (females), 10 % ↓ food consumption in females (week 3) *Haematology, clinical chemistry and urinalysis:* 74 % ↑ neutrophils, 14 % ↓ glucose (females), 13 % ↑ urea (males) **6 ppm** *Haematology, clinical chemistry and urinalysis:* 49 % ↑ neutrophils**,** 12 % ↑ urea (males) A NOAEL of 2 mg/kg bw/day in males and females based on bodyweight effects, changes in haematological parameters and clinical chemistry at ≥ 7 mg/kg bw/day	
90-day study EPA guideline Oral, diet Rat Charles River 10/sex/dose 0, 50,100, 200 or 400 ppm equivalent to 0, 4, 8, 14 or 30 mg/kg bw/day in males and 0, 4, 9, 18, 34 mg/kg bw/day in females Batch 29 (97 % purity)	**400 ppm** *Bodyweight and food consumption:* 25/14 % ↓ week 13 bodyweight (males/females), 35/26 % ↓ bodyweight gain over weeks 0-13 (males/females), 23/13 % ↓ food consumption (males/females) *Haematology and clinical chemistry*: 34 % ↓ white blood cells (neutrophils, lymphocytes, eosinophils) in males; 12 % ↓ glucose (males), 30/12 % ↑ BUN (males/females) *Organ weights:* 13/30 % ↑ absolute/relative liver weight (females) **200 ppm** *Bodyweight and food consumption:* 18 % ↓ week 13 bodyweight (males), 23/16 % ↓ bodyweight gain over weeks 0-13 (males/females), 19 % ↓ food consumption (males) *Organs:* 7/17 % ↑ absolute/relative liver weight (females). ↑ males with sinusoidal dilation/congestion of the liver (8 v 3 in controls) **100 ppm** *Bodyweight and food consumption:* 12 % ↓ week 13 bodyweight (males), 19 % ↓ bodyweight gain over weeks 0-13 (males), 9 % ↓ food consumption (males) **50 ppm** No adverse effects observed NOAEL of 4 mg/kg bw/day for both sexes based on the effects on bodyweight and food consumption and increased liver weight (females) at ≥ 8 mg/kg bw/day	Kirk (1990)
14-day study, oral, diet	**2000 ppm** *Bodyweight and food consumption:* 14/10 % ↓ bodyweight by end of study (males/females), 63/74 % ↓ bodyweight gain (day 0-14) (males/females), non-	Krishnappa (1999)

Mice Swiss 5/sex/dose OECD 407 0, 25,100, 400, 1600 or 2000 ppm equivalent to 0, 5, 20.1, 75.4, 320 or 390 mg/kg bw/day in males and 0, 5.4, 20.1, 88.7, 304 and 390 mg/kg bw/day in females Batch 453/990/96 (96.6 % purity)	statistically significant 20/30 % ↓ food consumption (males/females) **1600 ppm** *Bodyweight and food consumption:* 8 % ↓ terminal bodyweight (males), 40 % ↓ bodyweight gain over days 0-14 (males), non-statistically significant 39 % ↓ food consumption (males/females) **400, 100 or 25 ppm** No adverse effects observed A NOAEL of 75 mg/kg bw/day in males and 89 mg/kg bw/day in females was derived based on the bodyweight effects at ≥ 304 mg/kg bw/day	
28-day study (2 week recovery), oral, diet Mice Swiss Albino OECD 407 6/sex/dose 0, 200, 600, 1500 ppm equivalent to 0, 41, 120, 315 mg/kg bw/day in males and 0, 43, 131 and 324 mg/kg bw/day in females Batch 453/990/96 (96.6 % purity)	**1500 ppm** *Mortality, bodyweight and food consumption:* One female died (not considered treatment related). 13-15/9-14 % ↓ bodyweight (males/females weeks 1-3, but not 4), 13 % ↓ food consumption in males week 4 only and 10-15 % ↓ females throughout study *Haematology*: 12 % ↓ red blood cells (females); 17 % ↓ haemoglobin (females), 15 % ↓ haemocrit (females) during the treatment period. 11 % ↓ red blood cells (males); 11 % ↓ in haemoglobin (males), 10 % ↓ haemocrit (males) at the end of the recovery period only. *Clinical chemistry*: 30/40 % ↑ BUN (males/females), 17 % ↑ cholesterol (males) *Organs:* 17 % ↑ liver weight (females), hepatocyte necrosis 5/6 females v 3/6 in controls. Kidney lymphocyte infiltration 3/6 females v 0/6 in controls; 115 % ↑ Spleen weight (female) **600 ppm** Slightly ↓ food consumption in females *Clinical Chemistry:* 32 % ↑ BUN (females) *Organs:* , 10 % ↑ liver weight (female), 92 % ↑ spleen weight (female) **200 ppm** *Organs:* 69 % ↑ spleen weight (female) A NOAEL of 120 mg/kg bw/day in males was determined based on bodyweight effects and food consumption at 315 mg/kg bw/day. A LOAEL of 43 mg/kg bw/day was determined for females based on increased spleen weight at this dose level.	Suresh (1996)
52 week oral, diet Beagle dogs 4/sex/dose 0, 10, 50 or	**250/500 ppm** *Bodyweight and food consumption:* Due to palatability issues, top dose animals were initially dosed 250 ppm. This was increased to 500 ppm on day 22. Weight loss in both sexes resulted in cessation of treatment during weeks 7-11. Treatment with 250 ppm resumed in week 12, with 500 ppm on week 13.	Cope (1992)

250/500 ppm equivalent to 0, 0.4, 1.8 or 8.8 mg/kg bw/day in males and 0, 0.4, 1.6 or 8.3 mg/kg bw/day Batch: GS 13529 (96.4 % purity)	Bodyweight loss was again recorded and animals were terminated on week 16. 20/14 % ↓ (male/female) in food consumption over study period **50 ppm** *Bodyweight and food consumption:* 36/48% ↓ in male/female bodyweight gain over the study. 13/17 % ↓ in male/female in food consumption over 0-52 weeks. **10 ppm** No adverse effects noted A NOAEL of 0.4 mg/kg bw/day is derived based on bodyweight effects.	
28-day oral toxicity study, Oral, gavage New Zealand White Rabbits 5/sex/group (+ high dose recovery group) 0, 5, 50 or 500 mg/kg bw/day for three days then reduced to 0, 5, 20 or 100 mg/kg bw/day for 25 days. Recovery group 500/100 mg/kg bw/day Vehicle: aqueous 0.1 % polysorbate 80 and 0.5 % carboxymethylcellulose GS 13529 (99.8 % purity)	**500/100 mg/kg bw/day** *Mortality and clinical signs:* Six males and five females died during the study period (all but one by day 6). Marked signs of sedation, dyspnea, ruffled fur, curved/ventral body position, diarrhoea and tremor. Surviving animals: 1 male and 3 females from the main group and 4 males and 2 females from the recovery group *Bodyweight and food consumption:* 36/41 % ↓ bodyweight week 4 (males/females), 40-90 % ↓ food consumption *Haematology (week 4) :* 25/13 % ↓ Red blood cells (males/females), 25/13 % ↓ haemocrit (males/females), 24/17 % ↓ haemoglobin (males/females), 50/21 % ↓ white blood cells (males/females), *Clinical chemistry (week 4):* 28/30 % ↓ plasma phosphate, 37 % ↓ urea (females) *Organs:* ↓ All absolute organ weights in surviving animals. 70 % ↓ relative testes to brain weight Thymus: 79/94 % ↓ relative thymus to brain weight (surviving male/females), haemorrhage (1/10 male), mottled (2/10 males and 2/10 females), small (1/10 males and 3/10 females), atrophy (5/10 females), Spleen: 22/41 % ↓ relative spleen to brain weight (males/females), haemosiderosis (1/10 males, 4/10 females), atrophy (2/10 males), Lymph node: atrophy (2/10 females) **50/20 mg/kg bw/day** *Clinical signs:* Moderate signs of sedation, dyspnea, ruffled fur, curved/ventral body position, diarrhoea and tremor. *Bodyweight and food consumption:*: 17/9 % ↓ bodyweight by end of study (males/females), 30-45 % ↓ food consumption (males) *Clinical chemistry:* 26 % ↓ urea (females) *Organs:* 32 % ↓ relative testes to brain weight Thymus: 37/16 % ↓ relative thymus to brain weight (male/females), mottled (1 female) Spleen: 38/13 % ↓ relative spleen to brain weight (males/females), haemosiderosis (3 male, 4 females)	Seifert (1984a)

	5 mg/kg bw/day *Clinical signs:* moderate signs of sedation, dyspnea, ruffled fur, curved/ventral body position, diarrhoea and tremor. *Bodyweight*: 9 % ↓ bodyweight (females) *Organs:* 16 % ↓ relative thymus to brain weight (male), 30 % ↓ relative spleen to brain weight (males) and haemosiderosis (2 females) **0 mg/kg bw/day** Spleen haemosiderosis (2 females) A LOAEL of 5 mg/kg bw/day was derived for this study based on clinical signs and bodyweight effects observed at the lowest dose level.	
28-day toxicity Study, oral, gavage New Zealand White rabbits 5/sex/group 0, 0.05, 0.5 or 5 mg/kg bw/d Vehicle: 3% aqueous corn starch FL 860558 (97 % purity)	**5 mg/kg bw/day** *Bodyweight and food consumption:*17 % ↓ (NS) weight gain (males), 9 % ↓ food consumption (males) *Haematology:* 4 % ↑ MCHC **0.5 and 0.05 mg/kg bw/day** No effects observed A NOAEL of 5 mg/kg bw/day based on absence of adverse effects at top dose.	Schiavo, Hazelette & Green (1987a)

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting. ↓= decrease compared to control. ↑/↓ = increased/decreased compared to control.

Rat

Seven studies in rat are available: one 28-day, one 60-day, two 90-day studies and three chronic studies. Apart from the 28-day study, the top dose level in all studies was below the relevant cut-off dose level for classification. The route of administration was via the diet for all studies.

In the 28-day study, top (350 mg/kg bw/day) and mid (150 mg/kg bw/day) dose animals lost weight during the first week, this led to the sacrifice of all animals on day 9. Terminal bodyweight in the low dose was significantly lower than the controls. Food consumption was also reduced in all dose groups. No other adverse effects were observed.

In the 60-day study, compared to controls, bodyweight gain was reduced from 4 mg/kg bw/day in females, with bodyweight adversely affected (> 10 %) at the top dose (~ 38 mg/kg bw/day) in both sexes. Food consumption was also reduced throughout the dosing period at the top dose and intermittently at > 4 mg/kg bw/day. Increases in relative organ weights (adrenal, kidney, liver and testes) were observed at the top dose. Since absolute weights were, in general, slightly lower than control at this dose level, and there were no corresponding histopathological effects, the increase in relative weight is likely to be secondary to bodyweight effects and not relevant for classification.

In the first 90-day study (Bachmann, 1995), female bodyweight was lower than controls from 30 ppm with the reductions in bodyweight and bodyweight gain being considered adverse (> 10 %) in both sexes from 7 mg/kg bw/day. Increased weight gain was observed in top dose males during the recovery phase. Food consumption was also decreased, particularly at the beginning of the study. Very minor decreases in haematological parameters (haemoglobin and haemocrit), and changes in clinical chemistry (↑ phosphate, ↓ glucose, ↑ urea and ↑ creatinine) and urinalysis (↓volume and slight acidification) parameters were also observed at > 7 mg/kg bw/day. Haematological and clinical chemistry effects were at least partly reversible by the end of the recovery phase. Absolute organ weights tended to be lower in both sexes at the top two doses, whereas relative weights were higher. In the absence of any gross or microscopic pathology findings in these organs, these are considered to be secondary to the reduction in bodyweight.

In the second 90-day study (Kirk, 1990), compared to controls, male bodyweight and bodyweight gain was adversely reduced (> 10 %) at > 8 mg/kg bw/day. Female bodyweight was significantly lower than controls (14 %) at 34 mg/kg bw/day and bodyweight gain was affected at 18 mg/kg bw/day (16 % ↓). Food consumption was also lower in males from 8 mg/kg bw/day and in females at the top dose (34 mg/kg bw/day). There were some changes in clinical chemistry parameters (↓ white blood cells, ↓ glucose, ↑ BUN) observed at the top dose (30/34 mg/kg bw/day in males/females). Dose-related decreases in the absolute weight of a number of organs at 4 mg/kg bw/day were observed. In the absence of any other effects these are likely to be secondary to the decrease in bodyweight. A dose-related increase in absolute/relative liver weight was observed in females in the mid (7/17 %) and top (13/30 %) dose. This increase was not accompanied by any histopathological changes.

In the three chronic rat studies available, the lead effect was a significant reduction in bodyweight observed from low doses (> 1 mg/kg bw/day). Other effects observed included a dose related increase in the incidence of liver biliary cysts in females and Leydig cell hyperplasia from 1 mg/kg bw/day in one study (Gfeller, 1983a) and endometrial and cervix epithelial hyperplasia and cholesterol clefts in the sciatic nerve of females in another at 53 mg/kg bw/day (Ramesh, 2001).

Mouse

Five studies, a 14-day study, a 28-day study and three chronic studies have been conducted on mice. All studies were administered via the diet.

In a 14-day study, a significant reduction in body weight (and food consumption) was observed in both sexes at ≥ 320 mg/kg bw/day compared to controls.

In a 28-day study, compared to the controls, bodyweight was adversely reduced (> 10 %) in both sexes at the top dose (> 314 mg/kg bw/day) during the treatment period (an increase in weight gain was observed in both sexes in the top dose during the recovery period). Decreases in haematological parameters (red blood cells, haemoglobin and haemocrit) were also observed in females at the end of the treatment period, and in males at the end of the recovery period only. Female liver weight was adversely increased (10 %) at ≥ 130 mg/kg bw/day and was accompanied by a slight increase in hepatocyte necrosis at the top dose. In females, the increase in spleen weight was dose related, but reversible. Due to the magnitude of the increase and the association with altered haematological parameters, this effect was considered to be toxicologically significant.

In all the three chronic studies, reduction in bodyweight was the lead effect. In two of the three studies, effects on bodyweight were observed at doses < 20 mg/kg bw/day (Kumar

(2000), Frankhauser (1999)), whereas in the other study effects were only noted at the top dose (79 mg/kg bw/day; Gfeller, 1982). Other effects observed included a dose-related increase in pituitary weight in females in one study (Gfeller, 1982) and vacuolar changes in the optic nerve, degenerative changes in the testes and spermatic granuloma at the top dose only (99/120 mg/kg bw/day in males/females) of another (Kumar, 2000).

Dog

A one-year repeated dose study is available in the dog. In this study, terbuthylazine was administered in the diet.

In the one-year study, body weight loss lead to the sacrifice of all top dose animals (> 8.3 mg/kg bw/day) on week 16. Bodyweight gain as compared to the control was also significantly reduced in both sexes at the intermediate dose level as was food consumption. No other adverse effects were observed.

Rabbit

Two 28-day studies are available in the rabbit. The route of administration was via gavage.

In the first study (Seifert, 1984a), terbuthylazine was administered via oral gavage and adverse effects were observed from 5 mg/kg bw/day. At this dose level, effects consisted of moderate clinical signs (sedation, dyspnea, ruffled fur, curved/ventral position, diarrhoea and tremors), as well as dose-related reductions in organ weights (thymus and spleen). At the next dose level (50 mg/kg bw/day), additional effects included reduced male bodyweight (>10 %) and food consumption as compared to the control, an increased incidence of spleen haemosiderosis and reduced relative testes weight. At the highest dose (500 mg/kg bw/day), high mortality (six males and five females) was observed, resulting in the dose being reduced to 100 mg/kg bw/day from day 3. At this dose, bodyweight was lower in both sexes compared to the control (> 30 %). Marked decreases in haematological parameters (red blood cells, haemoglobin and haemocrit) and splenic haemosiderosis and atrophy were also observed in surviving animals (1 male and 3 females). At this dose level, absolute organ weights were lower in both sexes, but reliable interpretation was hampered by effects on bodyweight and low animal number. Dose-related reductions in relative thymus and testes weight were observed in males and spleen weight (relative to brain weight) was lower in top dose females. In decedents, mottled lungs and thymus, gastric haemorrhage and fluid in the thoracic cavity was observed.

In the second study (Schiavo et al (1986)), terbuthylazine was administered via oral gavage and effects were limited to lower weight gain in top dose males (5 mg/kg bw/day) as compared to the controls and a marginal decrease in food consumption. The increase in MCHC in top dose males is not thought to be of toxicological significance in the absence of effects on other haematological parameters.

4.7.1.2 Repeated dose toxicity: inhalation

No data available

4.7.1.3 Repeated dose toxicity: dermal

Table 17b: Summary table of relevant dermal repeated dose toxicity studies

Method	Results	Reference
28-day dermal toxicity study Tif: Ralf rats 5/sex/group 0, 1, 10, 100 or 1000 mg/kg bw/day SG 8201 (96.8 % purity)	**1000 mg/kg bw/day** *Bodyweight and food consumption:* 12-15% ↓ bodyweight over week 2-4 (males), 12-22 % ↓ food consumption over week 1-4 (males), 18 % ↓ food consumption week 1 only (females) *Clinical chemistry:* 38/ 25 % ↑ ALT/ALP (males), *Organs:* 30 % ↓ thymus weight (males), minimal splenic extramedullary heamatopoiesis in 4 males (1 control) **100 mg/kg bw/day** *Bodyweight and food consumption:* 11-12 % ↓ bodyweight between week 2-4 (males), 11-16 % ↓ food consumption week 1-4 (males), 22 % ↓ food consumption week 1 only (females) *Organs:* 30 % ↓ thymus weight (males) **10 and 1 mg/kg bw/day** No adverse effects observed A NOAEL of 10 mg/kg bw/day based on effects on bodyweight and lower thymus weight in males at 100 mg/kg bw/day	Marty (1992)
28-day dermal toxicity study New Zealand White Rabbits 5/sex/dose 0, 5, 50 or 500 mg/kg bw/day Batch EN 16727 (99.8 % purity)	**500 mg/kg bw/day** *Bodyweight and food consumption:* Weight loss observed at various time points. Terminal bodyweight was 16/13 % ↓ controls (males/females), 86/73 % ↓ bodyweight gain week 0-4 (males/females), 15-70/8-34 % ↓ food consumption over study period (males/females) *Clinical signs:* slight dermal irritation, diarrhoea, sedation, curved or ventral body position, ruffled fur, ataxia and tremors *Organs:* 29/23 % ↓ thymus weight (males/females), thymic atrophy (1 male), 10/36 % ↓ gonads (males/females), 22 % ↓ kidney weight (females) **50 mg/kg bw/day** *Clinical signs:* slight dermal irritation, sedation, curved or ventral body position, ruffled fur, ataxia and tremors *Organs:* 14 % ↓ thymus weight (males) **5 mg/kg bw/day** *Clinical signs:* slight dermal irritation, sedation, curved or ventral body position, ruffled fur and tremors A LOAEL of 5 mg/kg bw/day was derived based in signs of toxicity	Seifert (1984b)
28-day dermal	**500 mg/kg bw/day** *Mortality and clinical signs:* One female died. Prior to death this female	Schiavo (1987b)

toxicity study New Zealand White Rabbits 5/sex/dose 0, 0.05, 0.5 or 500 mg/kg bw/day Batch FL 860558 (97 %)	showed clinical signs of toxicity (few faeces, muscle wasting, lethargy, hypoactivity, hypothermia and cachexia). Effects in other animals consisted of slight dermal irritation in 8/10 animals, occasional observations of few faeces and soft faeces and perineal staining in 1 female *Bodyweight and food consumption:* Initial weight loss in both sexes during week 1, weight gain observed weeks 2-4. Overall, minimal weight gain over weeks 0-4. 76-11 % and 89-18 % ↓ food consumption in males/females *Organs:* 18/34 % ↓ thymus weight (not statistically significant) **0.5 mg/kg bw/day** *Bodyweight and food consumption:* 18 % ↓ food consumption week 1 (females) **0.05 mg/kg bw/day** No toxicologically significant effects A NOAEL of 0.5 mg/kg bw/day was determined based on mortality, food consumption and bodyweight effects at the top dose level	

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting. ↓= decrease compared to control. ↑ = increased compared to control.

There is a 28-day rat study and two 28-day rabbit studies available.

Rat

In the available 28-day study in rats, adverse effects in males started to occur from 100 mg/kg bw/day. There were no toxicological adverse effects observed in females. At 100 mg/kg bw/day effects consisted of reductions in male bodyweight as compared to the control (> 10 %), food consumption (more severe at the beginning of the study) and thymus weight. In high dose males (1000 mg/kg bw/day), additional effects included clinical chemistry changes (↑ alkaline phosphatase, alkaline transferase) and minimal splenic extramedullary haematopoiesis.

Rabbit

In the first of the available studies (Seifert, 1984b), slight dermal irritation and clinical signs (sedation, curved or ventral body position, ruffled fur and tremors) were observed at all dose levels (≥ 5 mg/kg bw/day), ataxia (mid and high) and diarrhoea (high dose only) were also observed at the higher dose levels. Reduced thymus weight was observed in the mid (50 mg/kg bw/day) and high dose levels. At the highest dose level (500 mg/kg bw/day), additional effects consisted of reduced bodyweight compared to the control (> 10 %), reduced food consumption (more apparent at the beginning of the study), and reduced organ weights (thymus, gonads and kidney).

In the second study (Schiavo, 1987b), apart from a reduction in food consumption in mid-dose females during week one, adverse effects were only observed at the highest dose (500 mg/kg bw/day). At this dose level, effects included the death of one female (day 29), signs of dermal irritation, lower thymus weight (not statistically significant), weight loss and a marked decreased in food consumption, particularly at the beginning of the study.

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

Not applicable

4.7.1.7 Summary and discussion of repeated dose toxicity

Oral

The oral repeated-dose toxicity of terbuthylazine has been investigated in one 28-day, one 60-day, two 90-day and three chronic studies (see section 4.10) in the rat; one 14-day, one 28-day and three chronic studies available in the mouse (see section 4.10), a one-year study in the dog and two 28-day studies in the rabbit.

Mortality

In a 28-day rabbit study (Seifert, 1984a), 11 top dose animals died. All but one of these deaths occurred by day six. In this study, the high dose group were initially administered 500 mg/kg bw/day before the dose level was reduced on day 3 to 100 mg/kg bw/day for the remainder of the study. These deaths are likely to be due to the initial dosage (500 mg/kg bw/day).

In a 28-day rat study (Hopkins, 1988), all rats dosed > 129 mg/kg bw/day were terminated on day 9 due to 'extreme unpalatability of the diet' and bodyweight loss. Similarly, a 52-week study in dogs (Cope, 1992) was also terminated on week 16 due to bodyweight loss.

No deaths were observed in any other study or with any other species. However, it should be noted that apart from the studies in mice (Krishnappa (1999) and Suresh (1998)), this highest dose levels were below 50 mg/kg bw/day.

Bodyweight

Effects on bodyweight were observed in all studies (see table 17c below) as were effects on food consumption. In the 28-day studies, the rabbit was the most sensitive species, with reduced bodyweight gain observed (9 % and 17 %) in both the Seifert (1984a) and Schiavo *et al* (1987a) studies at 5 mg/kg bw/day (the top dose in the latter study). In a 28-day (Hopkins, 1988) and 60-day study (Ramesh, 1999) in rats, bodyweight gain was significantly lower than controls at 35 and 4 mg/kg bw/day, respectively. In the former study, bodyweight loss was observed at higher doses (> 126 mg/kg bw/day) and animals were sacrificed on day 9 due to extreme unpalatability of the diet. Mice were the least sensitive species, with effects on bodyweight only observed at doses > 300 mg/kg bw/day.

In the available 90-day studies in rats, significant reductions in bodyweight (> 10 %) and bodyweight gain were observed at doses > 7 mg/kg bw/day ((Bachmann (1995) and Kirk (1990)). These decreases were accompanied by reductions in food consumption. In a 52

week dietary study in dogs, a reduction in bodyweight gain (2 mg/kg bw/day) was also observed and was accompanied by a reduction in food consumption.

Similarly, in the chronic studies conducted in mice and rats effects on bodyweight was the lead effect.

Table 17c: summary of the bodyweight effects at doses relevant to classification from repeat dose oral toxicity studies

Study Duration	**Species route of administration**	**BW effects observed at doses relevant for classification**	
		300 mg/kg bw/day	**30 mg/kg bw/day**
14-day	Mice (diet)	304 mg/kg bw/day 8 % ↓ BW (M), 40 % ↓ BW gain (M)	No adverse effects
28-day	Rat (diet)	329 and 126 mg/kg bw/day Sacrificed on day 9. Weight loss in both sexes	35 mg/kg bw/day 27/18 % ↓ BW & 55/50 % ↓ BW gain in M/F
	Mice (diet)	315 mg/kg bw/day 13-15/9-14 % ↓ BW (M/F weeks 1-3)	43 mg/kg bw/day No bw effects
	Rabbit (gavage) Doses reduced day 3	500/100 mg/kg bw/day Deaths. 36/41 % ↓ BW (M/F)	50/20 mg/kg/bw/day 17/9 % ↓ BW (M/F)
	Rabbit (gavage)	Top dose 5 mg/kg bw/day	
Cut-off doses for classification		**100 mg/kg bw/day**	**10 mg/kg bw/day**
60-day	Rat (diet)	38 mg/kg bw/day 22/17 % ↓ BW & 37/43 % ↓ BW gain (M/F)	11 mg/kg bw/day 9 % ↓ BW & 24 % ↓ BW gain (F)
90-day	Rat (diet)	22 mg/kg bw/day 24/ 22 % ↓ BW & 31/32 % ↓ BW gain (M/F)	7 mg/kg bw/day 16/11 % ↓ BW (M/F) & 22/16 % ↓ BW gain (M/F)
90-day	Rat (diet)	30 mg/kg bw/day 25/14 % ↓ BW & 35/26 % ↓ BW gain (M/F)	8 mg/kg bw/day 12 % ↓ BW & 19 % ↓ BW gain (M)
52 weeks	Dog (diet)	Top dose 8.8 mg/kg bw/day	8.8 mg/kg bw/day study terminated weeks 7-11 (BW loss due to palatability issues)

Legend: BW- Bodyweight, M – male, F- female, (NS) – non-statistically significant

Haematology

In mice (Suresh, 1996) and rabbits (Seifert, 1984a), blood cell parameters (↓ red blood cells, ↓ haemoglobin,↓ haemocrit) were affected in the top dose group in 28-day studies (315 mg/kg bw/day and 500/100 mg/kg bw/day, respectively). These effects were accompanied by increased spleen weight in the mouse; slightly increased splenic haemosiderosis and clinical signs indicative of anaemia (sedation and dyspnea) in the rabbit. In mice, females were affected during the dosing period, whereas effects in males were only observed at the end of the recovery period (effects had mostly recovered in females by this time). In the rabbit, effects were observed at 100 mg/kg bw/day; however, interpretation is hampered by the high mortality rate at this dose.

Apart from very minor haematological changes observed in one 90-day rat study (Bachmann, 1995) no effects were observed in the rat or dog; however, in these species it should be noted that either lower doses were employed or the study was terminated early.

Dermal

The dermal repeated-dose toxicity has been investigated in a 28-day rat study and two 28-day rabbit studies.

Mortality

One rabbit receiving 500 mg/kg bw/day was found dead on day 29 (Schiavo, 1987b). Prior to death this female showed clinical signs of toxicity (few faeces, muscle wasting, lethargy, hyperactivity, hypothermia and cachexia).

Bodyweight

In the 28-day study in rats (Marty, 1992), bodyweight (and food consumption) was decreased (10-15 %) at both the mid (100 mg/kg bw/day) and top dose (1000 mg/kg bw/day). In rabbits, effects on bodyweight were only observed at the top dose levels in both studies (500 mg/kg bw/day) (Seifert, 1984b and Schiavo, 1987b).

4.7.1.8 Comparison with criteria of repeated dose toxicity findings relevant for classification

Oral

Mortality

Deaths were observed in one 28-day rabbit study. Since the deaths occurred at the beginning of the study (all but one occurred within the first 6 days), they are likely to be due to the initial dosage (500 mg/kg bw/day) and therefore occurred above the cut-off for classification (300 mg/kg bw/day).

A 28-day rat study and 52-day dog study were terminated due to effects on bodyweight/food consumption. The significance of these effects is discussed below.

Bodyweight

A reduction in bodyweight/bodyweight gain was observed below the relevant cut-off for classification in all species (apart from mice) and in all study durations. Effects on bodyweight were the main effect, and were severe enough to ensure dose levels in the majority of studies were below the cut-off level for classification. In one rat study (dosed > 129 mg/kg bw/day) and one dog study (dosed 9 mg/kg bw/day) bodyweight effects were so severe they led to early termination of those dose groups. At these dose levels, food consumption was also significantly reduced (46-80 % in the rat study).

In two 90-day rat studies, significant reductions in bodyweight (> 20 %) and bodyweight gain (> 30 %) were observed at the top dose (22 mg/kg bw/day in Bachmann (1995) and 34 mg/kg bw/day in Kirk (1990)); below the cut-off for classification (100 mg/kg bw/day). In these studies, food consumption was also reduced between 13-23 %. It is possible unpalatability of the diet caused the reduction in bodyweight. However, this is unlikely to be the sole cause since reduced bodyweight and food consumption were also observed following administration via oral gavage and dermal (occlusive) administration of terbuthylazine to

rabbits. As the effects on bodyweight were marked, dictated the dose levels employed in the studies and cannot be attributed solely to 'palatability' issues, they are considered sufficiently severe to warrant classification. In some studies, bodyweight was significantly reduced at dose levels relevant for classification in STOT RE category 1 (particularly rabbit); however, severity of these effects was marginal and varied between sex (and may also have been influenced by palatability in the dog). Therefore, classification as STOT RE Category 2 is considered more appropriate.

Haematology

Significant effects on haematological parameters were observed in the 28-day study in mice and one 28-day study in rabbit. Minor effects were observed in one 90-day study in rats at the top dose (22 mg/kg bw/day); failure to observe any haematological effects in other studies may be due to the low dose levels employed.

In mice, haematological effects were only observed at the top dose (315 mg/kg bw/day), suggesting terbuthylazine may cause anaemia. As this dose level is close to the cut-off for classification, these effects are considered relevant for classification. At this dose level, a significant decrease in red blood cell number (12 %), haemoglobin levels (17 %) and haemocrit (10 %) was observed in female mice. This was accompanied by increased spleen weight, but no other effects indicative of anaemia (e.g., haemosiderin and reticulocytisis). No effects were observed in males during the treatment period; however, red blood cell parameters were reduced at the end of the recovery period (to a lesser extent than in females). Although the effects on blood cell parameters are considered adverse, in the absence of other signs of anaemia, the extent of these effects are not considered of sufficient severity to justify classification.

In the rabbit study, at the top dose (100 mg/kg bw/day), a reduction in haematological parameters (red blood cells, haemoglobin and haemocrit), slightly increased heamosiderosis in the spleen and clinical signs consistent with anaemia (dyspnea and sedation) were observed. The extent of the effects on blood cell parameters in females was between 13 – 17 %, whereas in males the effects were more severe (~ 25 %). Although adverse, the extent of the effects observed in females are not of sufficient severity to warrant classification; a conclusion on the significance of the effects in males is not possible due to the low animal number in this dose group (one male) due to deaths early on in the study.

Dermal

Mortality and bodyweight

No deaths occurred below the dermal cut-off for classification (200 mg/kg bw/day). Reduced bodyweight were observed below the cut-off for classification under the CLP of 200 mg/kg bw/day. Although adverse, the extent is not considered severe enough to support classification.

4.7.1.9 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to CLP

STOT RE 2; H373

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18a: Summary table of relevant *in vitro* mutagenicity studies

Method	Results	Remarks	Reference
Ames (OECD 471 (1983)) *S. typhimurium* TA98, TA100, TA1535 and TA 1537 *E.coli* WP2 uvrA (pKM101) Five concentrations between 312.5 – 5000 µg/plate SG 8201 (96.8 % purity)	- S9: Negative + S9: Negative	Positive controls included	Hertner (1995a)
Ames (OECD 471) *S. typhimurium* TA1535, TA 1537, TA1358, TA98 and TA 100 Five concentrations between 500-8000 µg/plate Batch 29 (97 % purity)	- S9: Negative + S9: Negative	Positive controls included	Forster (1998)
Ames (OECD 471) *S. typhimurium* TA1535, TA 1537, TA 98, and TA 100 *E.Coli* WP2 urvA Five concentrations between 50 – 5000 ug/plate Batch 088495038 (98% purity)	- S9: Negative + S9: Negative	Positive controls included	Bowles (2009)
Mammalian cell gene mutation (HGPRT) (OECD 476 (1983)) Chinese Hamster Cells V79 Four concentrations between 14.07 - 380 ug/plate SG 8201 (96.8 % purity)	- S9: Negative + S9: Negative	The level of cytotoxicity was less than recommended by the guideline in both experiments with S9 and one without Positive controls included	Hertner (1995b)
Mammalian cell gene mutation (HGPRT) (OECD 476) Chinese Hamster Cells V79 Six concentrations between 50 - 800 ug/plate Batch 29 (97 % purity)	- S9: Positive + S9: Negative	In the absence of S9 there was a slight increase in mutation frequency at ≥ 600 ug/ml, observed in the presence of cytotoxicity (20-30 % survival compared to the control) Less than the recommended cytotoxicity was observed in the presence of S9 Positive controls included	Seeberg (1988a)

In vitro cytogenetics (OECD 473 (1983)) Chinese Hamster cells 23.75 – 380 ug/plate SG 8201 (96.8 % purity)	- S9: Negative + S9: Negative	Positive controls included	Hertner (1995c)
In vitro cytogenetics (OECD 473) Chinese Hamster Ovary cells 17.2 – 371 ug/ml Batch not indicated (97 % purity)	- S9: Negative + S9: Negative	Although no increase in chromosome aberrations there was increased incidence of endoreduplication and polyploidy at the top dose level with metabolic activation Positive controls were included	Mosesso (1988a)
Unscheduled DNA Synthesis US EPA TSCA test guidelines HELA S3 cells 8 – 800 ug/ml Batch 29 (97 % purity)	- S9: Negative + S9: Negative	Study not acceptable 3 instead of 6 replicates used The positive control did not demonstrate the sensitivity of the assay in the presence of S9	Seeburg (1988b)

Table 18b: Summary table of relevant *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
Bone Marrow Micronucleus, OECD 474 (1983), Oral, gavage TIF: MAGF Mice 8/sex/group 1st experiment: 0 &5000 mg/kg bw 2nd experiment: 0, 1250, 2500 and 5000 mg/kg bw Vehicle: aqueous carboxymethylcellulose Batch SG 6925	Negative	Two experiments conducted. In the repeat experiment one death at the top and mid dose was observed. Positive controls responded as expected	Hertner (1989)
Bone Marrow micronucleus, oral, gavage OECD 474 Swiss mice (5/sex/group) 0, 2500, 5000 mg/kg bw Vehicle: corn oil Batch 29	Negative	Clinical signs: piloerection, ungroomed appearance, decreased activity, hunched posture, (semi-) closed eyes, muscular tremor, blanching, tachipnoea, yellow spot/yellowing urigenital region, ataxia, urinary incontinence P/N ratio increased in treated and positive control groups. Positive controls responded as expected	Mosesso (1988b)

4.9.1 Non-human information

4.9.1.1 In vitro data

The genotoxicity of terbuthylazine was investigated in three Ames tests, two mammalian cell gene mutation assays (HGPRT), two chromosome aberration assays and an unscheduled DNA synthesis assay. Positive controls were included in all assays and behaved as expected in all except the *in vitro* UDS assay; as a result, this assay was considered unacceptable and excluded from the assessment of terbuthylazine's mutagenic potential.

A positive response was observed in the absence of metabolic activation in one HGPRT study (Seeburg, 1988a). These responses were only observed at doses with 20-30 % survival and may be a result of the high cytotoxicity at this dose level. No evidence of mutagenicity was observed in another HGPRT assay (conducted at lower dose levels) or in the Ames tests, further reducing concern for these findings. In addition, although the results of the two *in vitro* cytogenetics studies were negative for clastogenicity, signs of endoreduplication and polyploidy were observed in one study at the top dose in the presence of metabolic activation. Similar results were not observed in the *in vivo* micronucleus studies (see below).

4.9.1.2 In vivo data

Two studies have evaluated the potential for terbuthylazine to induce cytogenetic damage in the bone marrow of mice. No evidence of micronucleus formation was found in either study. In both studies, the test substance was judged to have reached the target organ.

Overall, the results of these studies provide reassurance that terbuthylazine has no *in vivo* mutagenic potential.

4.9.2 Human information

No information available

4.9.3 Other relevant information

No information available

4.9.4 Summary and discussion of mutagenicity

Data indicate terbuthylazine is not mutagenic *in vitro* or *in vivo*.

4.9.5 Comparison with criteria

Data indicate terbuthylazine is not mutagenic *in vitro* or *in vivo* and does not require classification.

4.9.6 Conclusions on classification and labelling

No classification for mutagenicity is required.

Not classified; conclusive but not sufficient for classification

4.10 Carcinogenicity

There are three carcinogenicity studies available in the rat and three carcinogenicity studies available in the mouse.

Table 19: Summary table of relevant carcinogenicity studies

Method	Results Remarks	Reference
> 2-year study, pre-guideline, Oral, Diet Rat TIF (RAIf) Sprague-Dawley-derived Dosed for 24 months, terminated day 848 (f) and day 779 (m) when survival < 20 % 80/sex/dose: Final group: 50/sex/group Interim sacrifice (12 months): 10/sex/group Interim sacrifice (24 months): 20/sex/group 0, 30, 150, 750 ppm equivalent to 1, 7 and 42 mg/kg bw/day in males and 1, 8 and 53 mg/kg bw/day in females GS13529 (98 % purity)	***Non-neoplastic findings*** 750 ppm *Bodyweight and food consumption:* ↓ bodyweight in males (18-43 %) and females (17-42 %) over the study period. 17-51 %/44-61 % ↓ bodyweight gain in males/females over the study period. food consumption in males (19-35 % week 1 – 54, but not 110) and females (21 % on week 1 and 10 % on week 54) *Clinical Chemistry:* 14-18 % ↓ white blood cells (wk 17 and 26) in males, ↓ glucose 15-19 % (wk 17 and 26) in males and females early on, ↓ BUN 14-62 % in males and 47-93 % in females up to week 78, 43-57 % ↑ ALP on weeks 17 and 26 in females *Organs:* 20/14 % ↓ liver weight (males/females), Liver biliary cysts (2/14 males/females), 23/31 % ↓ kidney weight (males/females) Alveolar foam cells in lung (46/35 males/females), Thyroid cell hyperplasia (1/4 male/female), Leydig cell hyperplasia (21 males) 150 ppm *Bodyweight and food consumption:* ↓ bodyweight in males (22-25 % on weeks 28 and 54, but not at 110 weeks) and females (18-26 % from week 12 onwards). 22-28 %/30-35 % ↓ bodyweight gain in males/females over the study period. ↓ food consumption in males (10-16 % week 1 – 54, but not week 110) and females (18 % on week 1 and 10 % on week 54) *Clinical Chemistry:* 16-18 % ↓ white blood cells (week 17 and 26) in males, 26-43 % ↓ BUN (females up to week 78) *Organs:* Liver biliary cysts (3/10 males/females), Lung alveolar foam cells (23/14, males/females), thyroid cell hyperplasia (1/1 male/female), Leydig cell hyperplasia (5 males) 30 ppm *Bodyweight and food consumption:* ↓ bodyweight in males (8-9 % on weeks 28 and 54, but not at 110 weeks) and females (8-10% from week 12-54 only), 10-16 %↓ bodyweight gain in females over the study period, ↓ food consumption (10 % week 1 in both sexes and 9/5% in males/females on week 54) *Organs:* Liver biliary cysts (9 females), lung alveolar foam cells (24/12 males/females), thyroid cell hyperplasia (2/2 male/female), Leydig cell hyperplasia (3 males) 0 ppm Liver biliary cysts (1/4 males/females), lung alveolar foam cells (24/6 males/females), thyroid cell hyperplasia (1 male), leydig cell hyperplasia (6 males)	Gfeller (1983a)

	Neoplastic findings **Neoplastic findings** **Male** / **Female** Dose: 0 / 30 / 150 / 750 / 0 / 30 / 150 / 750 Group size (interim and terminal): 79 / 79 / 80 / 80 / 80 / 80 / 80 / 80 **Mammary gland** Carcinoma: 2 / 1 / 2 / 0 / 4 / 9 / 3 / 14* Laboratory historical control: Range 4/80 – 17/80 Fibroadenoma: 2 / 0 / 1 / 0 / 16 / 17 / 9 / 8 Laboratory historical control: Range 19/80-37/80 Adenoma: 0 / 1 / 0 / 0 / 3 / 4 / 2 / 1 Laboratory historical control: Range 0/80 – 4/80 Carcinosarcoma: 0 / 0 / 0 / 0 / 0 / 0 / 1 / 1 Laboratory historical control: no data Total: 4 / 2 / 3 / 0 / 23 / 30 / 15 / 23 **Leydig cell tumours**: 3 / 4 / 2 / 10 Laboratory historical control: 0-7.5 % * One of these carcinomas was detected in the one year interim kill A NOAEL could not be derived based on effects on bodyweight and food consumption at 30 ppm (1.2 and 1.3 mg/kg bw/day in males and females, respectively)	
Chronic study, oral, diet Rat TIF(RAIf) Sprague-Dawley derived pre-guideline Dosed for 24 months, terminated week 116 (males) and week 121 (females) when survival < 20 % Final sacrifice: 50/sex/group. Interim sacrifice (12 months): 10/sex/group	30 ppm ↓ bodyweight in males/females (generally < 10 %), 16 % ↓ liver weight in females (interim sacrifice only) 6 ppm No significant adverse effects A NOAEL of 6 ppm (equivalent to 0.35 and 0.36 mg/kg bw/day in males and females, respectively) was derived.	Gfeller (1983b)

<table>
<tr>
<td>Additionally, 10/sex/group included for haematological investigations and 10/sex/group for clinical chemistry investigations.

0, 6 or 30 ppm equivalent to 0, 0.35 and 1.59 mg/kg bw/day in males and 0-0.36 and 1.6 mg/kg bw/day in females

GS13529
98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two year, chronic study, oral, diet
Rat

Wistar

OECD 453

Final sacrifice: 50/sex/group.

Interim sacrifice (12 months): 20/sex/group

0, 10, 40, 120 ppm equivalent to 0, 0.4, 1.7 and 5.5 mg/kg bw/day in males and 0, 0.6, 2.4 and 7.6 mg/kg bw/day in females

453/990/96
Purity 96.6%</td>
<td>120 ppm
15/18 % ↓ bodyweight in males/females, 18/26 % ↓ bodyweight gain at termination. 6 % ↓ food consumption (females)

19 % ↑ testes weight, 17/19 % ↑ relative liver weight (males/females), ↑ spleen heamosiderosis (27 males compared to 17 in control), cholesterol clefts in sciatic nerve (8 females compared to 0 in controls), endometrial hyperplasia (14 females compared to 7 in controls), cervix epithelial hyperplasia (5 compared to 1 in controls)

40 ppm
11 % ↑ relative liver weight (females), endometrial hyperplasia (12 females compared to 7 in controls), ↑ spleen heamosiderosis (25 males compared to 17 in control),

10 ppm
No significant findings

<table>
<tr><th colspan="5">Neoplastic findings</th></tr>
<tr><td>Dose</td><td>0</td><td>10</td><td>40</td><td>120</td></tr>
<tr><td>Group size</td><td>50</td><td>49</td><td>49</td><td>49</td></tr>
<tr><td colspan="5">Mammary gland</td></tr>
<tr><td>Adenocarcinoma</td><td>1 (2%)</td><td>4 (8%)</td><td>4 (8%)</td><td>8 (16%)</td></tr>
<tr><td colspan="5">Laboratory historical control: mean 4.9 % range: 0 -12 %</td></tr>
<tr><td>Fibroadenoma</td><td>6 (12%)</td><td>7 (14%)</td><td>10 (20%)</td><td>6 (12%)</td></tr>
<tr><td colspan="5">Laboratory historical control: mean 8.9 % Range 0-18 %</td></tr>
<tr><td>Adenoma</td><td>1 (2%)</td><td>3 (6%)</td><td>1 (2%)</td><td>1 (2%)</td></tr>
<tr><td colspan="5">Laboratory historical control: mean 0.9 % Range 0-2 %</td></tr>
</table>
</td>
<td>Ramesh, (2001)</td>
</tr>
</table>

	A NOAEL of 10 ppm (equivalent to 0.4 and 0.6 mg/kg bw/day in males and females) was derived	
Two year chronic study, oral, diet Pre-guideline Mouse Tif:MAGf 50/sex/group. 0, 30, 150, 750 ppm equivalent to 0, 3, 16 and 76 mg/kg bw/day in males and 0, 3, 15 and 79 mg/kg bw/day in females Batch 590305 Purity 98%	750 ppm 14-21 % ↓ bodyweight in females (weeks – 28-76) 538 % ↑ lymphocytes (females), 88 % ↓ lymphocytes (males) and 87 % ↓ white blood cells (males), 69 % ↓ BUN (males) 28 % ↑ adrenal weight (females), 52 % ↓ ovary weights, 75 % ↑ pituitary weight (females) 150 ppm 344 % ↑ lymphocytes in females, 50 % ↑ pituitary weight (females) 30 ppm 38 % ↑ pituitary weight (females) * No neoplastic findings A NOAEL of 30 ppm (equivalent to 2.97 mg/kg bw/day) for females based on increase pituitary weight at 150 ppm. A NOAEL of 150 ppm (equivalent to 15.8 mg/kg bw/day) for males was derived based on bodyweight effects, food consumption and white blood cells	Gfeller (1982)
Eighteen month, oral, diet OECD 451(1981) Mouse Tig:MAGF 50/sex/group, plus 10/sex/group for haematological parameter investigation 0, 10, 50, 500 or 1000 ppm equivalent to 0, 1, 6, 58 and 126 mg/kg bw/day in males and 0, 1, 5, 56 and 121 mg/kg bw/day in females Batch SG 8201 Purity 96.8%	1000 ppm 12/22 % ↓ bodyweight (males/females), Slight ↓ food consumption (males/females), 28/13.6 % ↓ liver weight (males/females), 9 % ↓ in haemocrit (males) 500 ppm 6/18 % ↓ bodyweight (males/females), 22/7.5 % ↓ liver weight (males/females) 50 and 10 ppm No significant adverse effects observed No neoplastic findings observed A NOAEL of 50 ppm was note for both sexes (equivalent to 5.61 and 5.24 mg/kg bw/day in males and females, respectively) was derived	Frankhauser (1999)
Eighteen months,	750 ppm	

oral, diet OECD 451 Mouse Swiss Albino 50/sex/group Histopathology was only carried out on high dose animals 0, 100, 250 and 750 ppm equivalent to 0, 15, 37 and 99 mg/kg bw/day in males and 0, 16, 40 and 118 mg/kg bw/day in females Batch 453/990/96 Purity 96.6%	↓ bodyweight in females throughout study period (5-13 %). 23 % ↓ bodyweight gain in males up to 9 months. 27 % ↓ bodyweight gain in females. Emaciation (5 males/ 2 females), vacuolar changes in optic nerve (8 males and 4 females compared to 4 males and 0 females in the control) Cystic uterus glands in 5 females compared to 0 in control, degenerative changes in the testes of 21 males compared to 11 controls. Spermatic granuloma observed in 3 males compared to 0 in controls 250 ppm 12-16 % ↓ bodyweight gain in females between 9 and 15 months, emaciation (2 males) 100 ppm Emaciation (2 males) No evidence of carcinogenicity observed A NOAEL of 100 ppm (equivalent to 14.6 and 15.5 mg/kg bw/day) in males and females was derived	

* Not statistically significant

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

<u>Rat</u>

As shown in table 19, in the three available rat studies, increased incidences of tumour findings were seen in the mammary gland and Leydig cells. A detailed analysis and discussion of these tumour findings is presented below.

Mammary Gland tumours

Description of the results

An increased incidence in mammary gland carcinoma was observed in the low and high dose (but not the mid dose) of a study conducted in Sprague-Dawley derived rats (TIF(RaIf)) (14 in the top dose compared to 4 in the control) (Gfeller (1983a)) and in the top dose of a study conducted in Wistar rats (8 in the top dose compared to 1 in the control) (Ramesh (2001)). In the Ramesh study, it should be noted that the tumour incidence in the low and mid dose was also higher than in the controls.

Whilst the incidence of mammary gland tumours was increased, there are some additional considerations that need to be outlined before a conclusion on classification can be reached.

Effect of study design on results

The Ramesh study was a guideline study. The Gfeller (1983a) study on the other hand was a non-standard study. In this study, animals were dosed for 2 years and then the study continued until survival was 20 % in one group (interim groups at one and two years were also included). In this case, the study was terminated in females after 848 days. This is of significance as a higher number of treated animals survived to termination than controls and therefore, had the tumours been age-related this may have confounded the results of the study. However, as shown in table a below, most tumours were observed during the first two years of the study and therefore the increased study length does not appear to have had any significant impact on tumour development.

Table a) Survival and mammary gland carcinoma rates in Sprague Dawley rat study (Gfeller, 1983a)

	Number of animals				Number of mammary gland carcinomas			
Dose level [ppm]	0	30	150	750	0	30	150	750
Animals found dead or killed moribund day 0-736	30	21	24	31	1	2	3	9
Animals found dead or killed moribund 736-848	16	15	10	11	2	3	0	2
Scheduled sacrifice of year 1	10	10	10	9	0	0	0	0
Scheduled sacrifice of year 2	13	15	14	12	1	2	0	1
Scheduled terminal sacrifice	11	19	22	17	0	2	0	2
Total scheduled sacrifices					4	9	3	14
Total of all animals	80	80	80	80				

Relevance of historical control

Laboratory historical control data is available for both studies. Mammary gland tumours are a common spontaneous tumour in female Sprague-Dawley rats (NTP, 2005) and the incidences in the Gfeller (1983a) study are reported to be within the laboratory historical control range (top dose 14/80; historical control range: 4/80 – 17/80). The designs of these studies are similar to the Gfeller study and therefore will be of variable duration (terminal sacrifice determined by 20 % survival) making it difficult to conclusively state that the tumour incidence fell within the historical control range as the breakdown of tumour incidence over time for these studies is not known. However, since these tumours occur at a high spontaneous rate in Sprague-Dawley rats and the majority occurred within the standard 2-

year period, these tumours are probably not treatment related. The incidence of mammary gland tumours in Wistar rats was within the historical control range at the low and mid-dose groups and marginally above the laboratory historical control range at the top dose (16 %; historical control mean 4.9 % and range 0-12 %). On this basis, a treatment related effect can not be ruled out. Overall, a marginal carcinogenic effect of potential concern to humans was observed in Wistar rats.

Effect of toxicity on the results

According to the guidance supporting the CLP Regulation (page 307), the highest dose in a carcinogenicity study should induce minimal toxicity, such as characterised as a 10 % reduction in bodyweight gain (maximal tolerated dose). In the Ramesh (2001) study, Wistar rat bodyweight gain was reduced by > 10 % at the top dose, which was the only dose at which an increased incidence of mammary gland adenocarcinoma was observed. This indicates that, strictly, the increased tumour frequency only occurred above the maximum tolerated dose (MTD). However, in spite of this, there is no evidence to indicate the tumours were the result of excessive toxicity and, therefore, they cannot be dismissed on this basis.

Potential Modes of Action

Terbuthylazine is a chlorotriazine. Other members of this group include atrazine, which has been shown to cause mammary gland tumours in Sprague-Dawley (SD) rats.

A significant amount of mode of action work has been carried out with atrazine and the results of this work have been published in accordance with the IPCS framework (Meek et al (2003)). In this paper, it was postulated that atrazine affects the hypothalamic-pituitary-ovary axis. Atrazine acts by suppressing the secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus, which in turn suppresses the release of luteinising hormone (LH) from the pituitary, preventing ovulation. The failure to ovulate results in the persistent secretion of oestrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin. This leads to accelerated reproductive aging, which in SD female rats is characterised by persistent hyperestrogenemia and hyperprolactinemia with low levels of LH and follicle stimulating hormone (FSH).

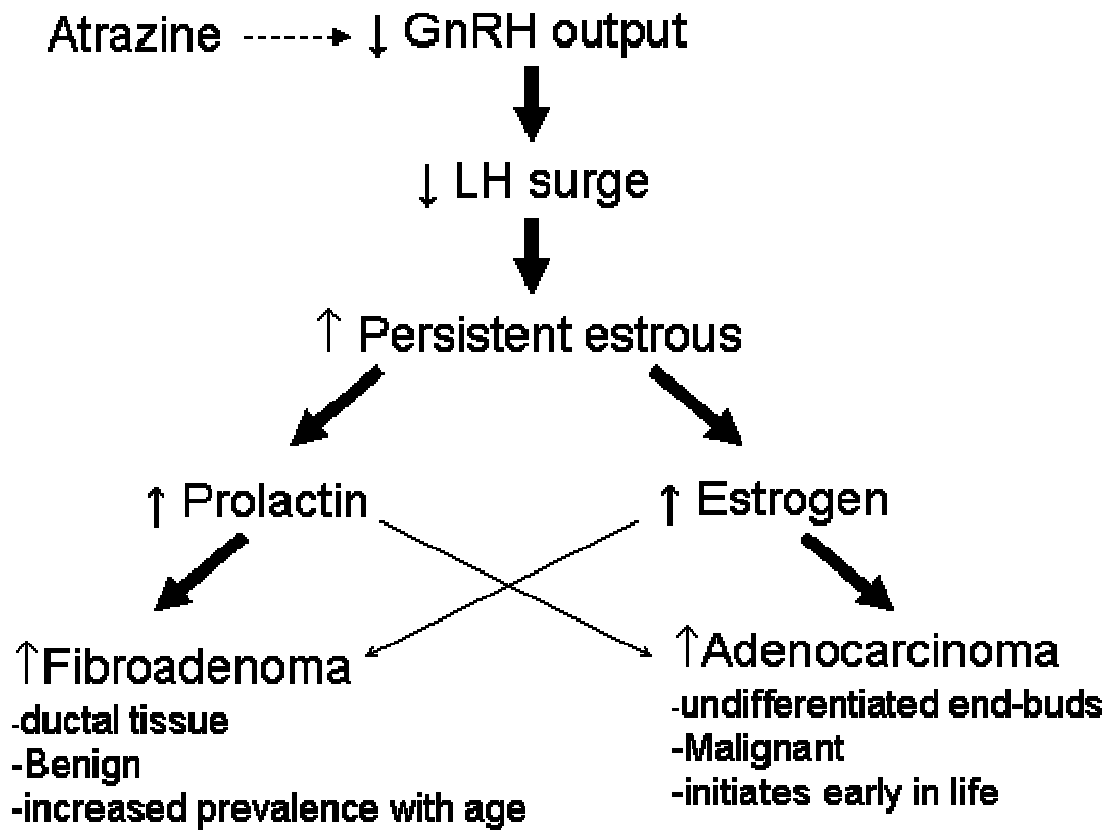


Figure 1. Key events associated with the earlier appearance and increased incidence of mammary tumours in atrazine-treated female SD rats. Figure produced from Simpkins et al (2011).

Contrastingly, in women, reproductive aging and menopause is characterised by exhaustion of the ovarian follicles resulting in low levels of oestrogen and prolactin and high levels of LH and FSH. The main differences in reproductive senescence between SD rat and women are outlined in table b (adapted from Simpkins et al, (2011)).

Table b. Differences in reproductive senescence between SD rats and women (adapted from Simkins et al (2011))

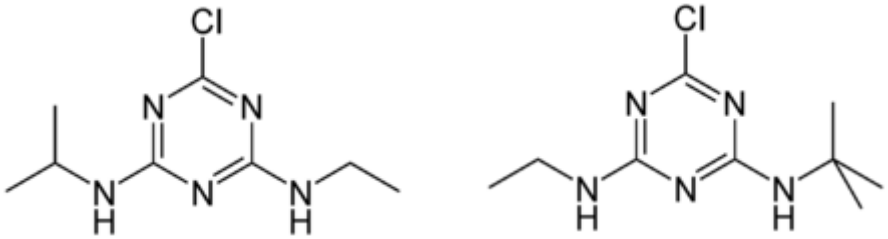
Parameter	*SD rat*	*Women*
Start of senescence (% of normal lifespan)	30-40%	60-70%
Principle cause of senescence	Hypothalamic failure to stimulate LH/FSH	Depletion of ovarian follicle content
LH surge capability	Lost	Maintained
Predominant cycle pattern	Persistent oestrus	Menopause
Oestrogen/progesterone ratio	Elevated/prolonged	Reduced
Prolactin secretion	Persistently elevated	Reduced

Spontaneous mammary tumour incidence (lifetime)	30-40%	8-10 %
Principal known factors that increase MT Risk	Prolactin, oestrogen, chemical mutagens	Oestrogen, nullparity, family history
Prolactin dependence	High	None

Based on the differences in reproductive physiology between SD rats and humans, the atrazine response in SD rats was not considered relevant to humans. The EU TC C&L group accepted these tumours were not relevant to humans.

Industry has proposed to read-across the information available for atrazine to terbuthylazine based on the clear structural similarities and toxicological profile of the two substances (see figure 2.). No mechanistic studies have been conducted with terbuthylazine to confirm it behaves similarly to atrazine; however, the clear structural similarity suggests that these tumours may occur by the same mode of action, providing further reassurance that the tumours in this strain of rat are not relevant for classification.

Figure 2. The structures of the chlorotriazines: atrazine and terbuthylazine



In addition, industry has proposed that the tumours observed in Wistar rats occurred via a similar mechanism as in Sprague-Dawley rats. Unlike for Sprague-Dawley rats, no carcinogenic studies are available for Wistar rats with atrazine and the mode of action argument has not so far been extended to this strain of rat. There is, however, one study available demonstrating that atrazine inhibits lutenising hormone release without altering pituitary sensitivity to GnHR in female Wistar rats, which is consistent with the mechanism of action in SD rats (Foradori et al (2009)). Furthermore, industry have argued that since Wistar rats share a similar ancestry (Sprague-Dawley rats being derived from Han Wistar rats) their reproductive ageing processes are very similar and under similar neuroendocrine control. Further information is provided in Annex I. This is in contrast to Fischer 344 rats, which do not share a common ancestry and are known not to be susceptible to disruption of the hypothalamic-pituitary-ovary axis in the same way. Although plausible, there is currently insufficient data to conclude that the proposed mode of action is applicable to Wistar rats, making it difficult to rule out the tumours on this basis.

Summary

In summary, mammary tumours were observed in two carcinogenicity studies, one in Sprague-Dawley rats and one in Wistar rats.

In Sprague-Dawley rats, mammary tumours have generally been found to occur at a high spontaneous rate. There was an increased incidence of mammary tumours at the top dose level in Sprague-Dawley rats. Although interpretation of the study is slightly confounded by the varied study durations (of both this study and the studies used to provide the historical control range), in this study the incidences fell within the historical control range. Furthermore, terbuthylazine is structurally very similar to another pesticide of the chlorotriazine class, atrazine. Atrazine also causes mammary tumours in Sprague-Dawley rats and, after detailed review, the relevance of these tumours to humans has been discounted previously within both the EU Pesticides and EU Classification and Labelling frameworks. Atrazine is not classified for carcinogenicity. On this basis, the mammary gland tumours in terbuthylazine-treated Sprague-Dawley rats are not considered relevant for classification.

The increased incidence of mammary gland adenocarcinoma in terbuthylazine-treated Wistar rats at the highest dose level tested was marginally above the historical control range. Although this was a lower dose than that which appeared to increase the incidence of mammary gland carcinoma in Sprague-Dawley rats, there was a group mean decreased body weight gain of 26% at study termination, indicating strictly that the MTD had been slightly exceeded. However, only limited data are available to establish whether chlorotriazines have a comparable effect on the hypothalamic-pituitary-ovary axis in Wistar rats as they have in Sprague-Dawley rats (Foradori *et al* (2009)). Therefore, there remains doubt about the human relevance of the increased mammary gland tumours seen in Wistar rats. On this basis, although of borderline significance, the increased tumour frequency observed in this strain of rat cannot be dismissed completely.

Leydig cell tumours

An increased incidence of Leydig cell tumours was observed in top dose Sprague-Dawley male rats. However, there was no such increase in the study conducted with Wistar rats.

Known causes of Leydig cell tumours

Leydig cell tumours occur spontaneously at a high incidence in Fischer 344 rats and therefore such findings in this strain generally do not inform on the carcinogenic potential of the substance. However, a similar spontaneous incidence has not been established for Sprague-Dawley rats or Wistar rats. Leydig tumours are also known to be caused by dopamine and GnHR agonists and tumours caused by these agents are not considered relevant to humans (EU Specialised Experts Report, 2004). There is no evidence that terbuthylazine is a dopamine of GnHR agonist and, therefore, the tumours can not be dismissed on these grounds.

Effect of the study design on tumour incidence

The study with Sprague-Dawley rats (Gfeller, 1983a) was a non-standard study. The animals were dosed for 2 years and then the study continued until survival was 20 % in one group (interim groups at one year 2 year were also included). In this case the study was terminated in males after 779 days. This is in contrast to a standard study, which is terminated after a maximum of 2 years, or if there is high toxicity, when survival in any group has reduced to 25 %. It has been argued by Industry that this is of significance as, due to the effects on bodyweight, a higher proportion of animals from the 750 mg/kg bw/day dose group survived to termination than in the control group (see Table c, reproduced from Industry's position paper on Leydig tumours (see Annex II)).

Table c. Body weight gain, body weight, survival and Leydig cell tumour incidence in Sprague-Dawley rats

Dose (ppm)	Body Weight Gain (% of Control) Week 1-105*	Body Weight (% of Control) Week 105*	Survival Time – 75th Percentile (days)	Leydig Cell Tumour Incidence
0	100	100	642	3/79 (3.8%)
30	91	93	647	4/79 (5.1%)
150	78	81	660	2/80 (2.5%)
750	54	59	740	10/80 (12.5%)

*: Animals received test item for 24 months, after which all groups were placed on control diet until survival in one group dropped to ~20% (in this case, the control group). Therefore, the measurements taken at week 105 are the best for evaluating effects of the test item on body weight gain and body weight.

Table d sets out when the Leydig cell tumours occurred (see below).

Table d. Leydig cell tumour incidence in Sprague-Dawley rats

	Number of animals				Number of leydig cell tumours			
Dose level [ppm]	0	30	150	750	0	30	150	750
Animals found dead or killed moribund day 0-677	31	32	25	8	0	0	0	0
Animals found dead or killed moribund 678-779	17	12	11	16	2	1	0	3
Total	**48**	**44**	**36**	**24**	**2**	**1**	**0**	**3**
Scheduled sacrifice of year 1	10	10	10	10	0	1	0	1
Scheduled sacrifice of year 2	10	10	13	16	1	2	1	2
Scheduled terminal sacrifice	11	15	21	30	0	0	1	4
Total sacrifices	**31**	**35**	**44**	**56**	**1**	**3**	**2**	**7**
Total of all animals	79	79	80	80	3	4	2	10

Table d shows that the main increase in the incidence of Leydig cell tumours in top dose animals was between the scheduled sacrifice after 2 years and the termination of the study (7 tumours compared to 2 in the control animals). This table supports the argument that the increase in the incidence of Leydig cell tumours is a consequence of the increased survival in the high dose groups and not a treatment related effect.

Relevance of historical control data on tumour incidence

Laboratory historical control data from similarly conducted studies has been provided. This control data suggest the incidence of tumours in the Gfeller study is above the historical control range (Historical control range: 0 – 7.5 %); however, as discussed for the mammary tumours, the use of this historical control data is confounded by the difference in durations and, therefore, can not be used confidently to inform on the relevance of these tumours as survival rates may have been different.

Relevance of toxicity on results

According to the CLP guidance (page 307), the highest dose should induce minimal toxicity, such as characterised as a 10 % reduction in bodyweight gain (maximal tolerated dose). In the Gfeller (1983a) study, bodyweight gain was significantly reduced (17-50 % lower than controls) suggesting the maximal tolerated dose (MTD) had been exceeded, further reducing concern for these tumours.

Summary

In summary, an increased incidence of Leydig cell tumours was observed in the top dose of a carcinogenicity study conducted in Sprague-Dawley rats, but not Wistar (conducted at lower dose levels). The incidence of these tumours was above the historical control level; however, interpretation is confounded by the varied study durations of this study and the studies used to provide historical control range. The increase was only observed at a dose level exceeding the MTD, reducing concern for these tumours. Moreover, there was a higher survival rate in the high dose group compared to the other dose groups with the majority of tumours developing after the standard 2-year dosing period had ended. Since Leydig cell tumours are considered spontaneous age-related tumours, it is considered probable that the increased incidence in Leydig cell tumours is a consequence of the increased survival in the top dose group and not a treatment related effect. Overall, it is considered there were no treatment related carcinogenic effects in Leydig cells of rats of potential concern to human health.

Mouse

As shown in table 19, in the three available mouse studies, no signs of carcinogenicity were observed.

Summary

In conclusion, in the three available mouse carcinogenicity studies, terbuthylazine was not carcinogenic.

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available

4.10.2 Human information

No data available

4.10.3 Other relevant information

No data available

4.10.4 Summary and discussion of carcinogenicity

There are three carcinogenicity studies available in the rat and three studies available in the mouse. Carcinogenic effects were observed in the mammary gland and testes of rats; no carcinogenic effects were observed in the mouse.

Mammary gland

In rats, terbuthylazine was shown to have a marginal carcinogenic effect of potential relevance to humans in the mammary gland of female Wistar rats (mammary gland carcinoma). Similar effects observed in Sprague-Dawley rats were dismissed based on their being within the overall historical control incidence and read-across from atrazine where the response in this strain of rat was not considered of relevance to humans.

Leydig cells

The increase in benign Leydig cell tumours was dismissed as an artefact of the increased survival rate of rats in the high dose group as compared to the controls.

4.10.5 Comparison with criteria

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

Since terbuthylazine was not genotoxic in *in vivo* studies and increased tumours were only observed in rats, a simple argument for Category 2 classification can be made. This is supported by the fact that the tumour incidence was only increased at the top dose; a dose that strictly exceeded the MTD. It is also possible that the tumours were the result of a mode of action not relevant to humans; however, this has yet to be established adequately.

In view of these considerations, the available evidence is deemed to match the criteria for classification as a category 2 carcinogen. There are no grounds to draw attention to a particular route of exposure on the label.

4.10.6 Conclusions on classification and labelling

Carc 2; H351

4.11 Toxicity for reproduction

There are two 2-generation studies and one 1-generation study available investigating the effects of terbuthylazine on reproduction.

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Reference
2-generation study OECD 416 Oral (diet) Rat (32/sex/dose) Sprague-Dawley 0, 6, 60 and 300 ppm equivalent to 0.4, 4, 20 mg/kg bw/day in males and 0, 0.4, 5, 22 mg/kg bw/day in females of the F0 generation and 0, 0.5, 5, and 24 mg/kg bw/day in males and 0.5, 5, and 26 mg/kg bw/day in females of the F1 generation SG 6925 (96.7 % purity)	*Parental toxicity* 300 ppm F0: 30/32 % ↓ pre-mate weight gain (males/females), 10/16 % ↓ food intake (males/females) F1: 22/25 % ↓ pre-mate weight gain (males/females), 13/16 % ↓ food intake (males/females) 60 ppm F0: 12 % ↓ pre-mate weight gain (males), 5/6 % ↓ food intake (males/females), F1: 12/16 % ↓ pre-mate weight gain (males/females), 6/8 % ↓ food intake (males/females), 6 ppm No adverse effects in either F0 or F1 generation *Reproductive effects* 300 ppm F0: ↓ % pregnant (78.1 % compared to 96.7 % in controls), no indication of mating in 4/7 non-pregnant females, reduced/absent corpora lutea in 4/7 non-pregnant females. F1: ↓ % pregnant (78.6 % compared to 85.7 % in controls), no indication of mating in 5/6 of the non-pregnant females, 2 successfully littered on re-mating, four failed to become pregnant following two matings. Reduced corpora lutea was noted in 3 pregnant females. Absent corpora lutea was noted in 4/6 non-pregnant females. 60 and 6 ppm No adverse effects observed in either F0 or F1 generation Control F0: 1 non-pregnant female, with indication of mating F1: 4 non-pregnant females showing no indication of mating. 2 successfully littered on re-mating *Offspring effects* 300 ppm F1: 8 % ↓ pup weight day 0 increasing to 19 % ↓ pup weight by day 21, slight delay in sexual maturation (day 43.6 compared to day 42 in controls) F2: 8 % ↓ pup weight day 0 increasing to 17 % ↓ pup weight by day 21, slight delay in sexual maturation (day 36.2 compared to day 33.8) 60 ppm and 6 ppm No adverse effects observed in either the F0 or F1 generation A reproductive NOAEL of 4.5 mg/kg bw/day was derived for males and females due to reduced fertility at 23 mg/kg bw/day. A parental NOAEL of 0.4 mg/kg bw/day is derived for parental animals, based on bodyweight effects at ≥ 4.5 mg/kg bw/day and an offspring NOAEL of 4.5 mg/kg	Masters et al (1992)

	bw/day was derived based on effects on pup survival at the top dose.	
One-generation reproductive toxicity study OECD 415 Oral (diet) Rat (15/sex/dose) Wistar 0, 50, 100 and 350 ppm equivalent to 4, 7, 25 mg/kg bw/day in males and 0, 5, 10, 36 mg/kg bw/day in females 453/990/96 (96.6 % purity)	*Parental toxicity* 350 ppm Males: 14 % ↓ week 14 bodyweight, 20% ↓ week 0-14 bodyweight gain Females: Pre-mating; 11% ↓ 10 week bodyweight Gestation: 13/14 % ↓ day 0/day 20 bodyweight, 16 % ↓ bodyweight gain days 0-20, 13 % ↓ food consumption Lactation: 13/18 % ↓day 1/day 21 bodyweight, 44 % ↓ bodyweight gain days 1-21, 14 % ↓ food consumption 100 ppm Males: 11 % ↓ week 14 bodyweight, 17% ↓ week 0-14 bodyweight gain Females: Pre-mating; 7 % ↓ week 10 bodyweight Gestation: 9/11 % ↓ day 0/day 20 bodyweight, 15 % ↓ bodyweight gain Lactation: 11/10 % day 4/day 21 bodyweight, 34 % bodyweight gain days 1-21, 11 % ↓ food consumption 50 ppm No significant adverse effects Reproductive effects No effect on fertility was observed. Corpora lutea (range): 16.9 (8-21), 16.3 (6-20),15.7 (11-23), 15.5 (13-19), in the control, low, mid and high doses, respectively *Offspring effects* 350 ppm 13 % ↓ female pup weight on day 1 increasing to 29 % ↓female pup weight by day 21, 13 % ↓ male pup weight on day 1 increasing to 21 % by day 21. 22/32 % ↓ bodyweight gain day 1-21 (male pups/female pups) 100 ppm 11 % ↓ bodyweight day 1 in female pups, 12/11 % ↓ bodyweight gain day 1-21 (male pups/female pups) 50 ppm 10 % ↓ bodyweight gain day 1-21 (female pups) A reproductive NOAEL of 350 ppm was derived for males and females. A parental NOAEL of 50 ppm is derived for both sexes and an offspring NOAEL of 50 ppm was derived.	Gainger (1999)
2-generation study OECD 416 Oral (diet) Rat (30/sex/dose) Wistar	*Parental toxicity* *200 ppm* *Males* F0: 11 % ↓ male pre-mating bodyweight by week 16. 17 % ↓ bodyweight gain weeks 0-16 F1: 20 % ↓ pre-mating bodyweight by week 16, 19 % ↓ bodyweight gain weeks 0-16 100 ppm:	Krishnappa (1998)

0, 50, 100 and 200 ppm equivalent to 4, 7, 15 mg/kg bw/day in males and 0, 5, 9, 18 mg/kg bw/day in females of the F0 generation and 0, 4, 9, and 19 mg/kg bw/day in males and 6, 11, and 24 mg/kg bw/day in females of the F1 generation 453/990/96 (96.6 % purity)	F0 9% ↓ in pre-mating bodyweight gain weeks 0-16 F1: 9 % ↓ in pre-mating bodyweight and 9 % pre-mating bodyweight gain weeks 0-16 50 ppm No significantly adverse effects *Females* 200 ppm F0: Pre-mating: 10 % ↓ bodyweight, 20 % ↓ bodyweight gain (up to week 10). Gestation: 14 % ↓ bodyweight (day 20), 21 % ↓ bodyweight gain (day 0-20). Lactation: 10 % ↓ bodyweight (day 21) F1: Pre-mating: 13% ↓ bodyweight, Gestation, 11 % ↓ bodyweight (day 20), 8 % ↓ bodyweight gain. Lactation 67 % ↑ bodyweight gain (day 20) 100 ppm F0: Gestation: 6 % ↓ bodyweight (day 20), 7 % ↓ bodyweight gain (day 0-20). Lactation: 6 % ↓ bodyweight (day 21), 23 % ↓ bodyweight gain (day 1-21) F1: Gestation: 7 % ↓ bodyweight (day 20), 9 % ↓ bodyweight gain (day 0-20). Lactation: 77 % ↑ bodyweight gain (day 1-21) 50 ppm Changes not considered toxicologically significant *Reproductive effects* No effect on mating performance or number of pregnancies was observed in any treatment group in any generation. Variation in proportion of implantations in F0 (92.23, 93.2, 96.9 and 88.8 % - control to high dose) and F1 (92.8, 89.6,89.1,88.7 % - control to high dose) generations Variation in Proportion of pre-implantation loss in F0 females (7.7, 6.8, 3.1 and 11.2 %- control to high dose) and F1 females (7.2 10.4, 10.9 and 11.3 – control to high dose) Variation in proportion of post-implantation loss in F0 (21.1, 23.4, 8.5, 17.5 % - control to high dose) and F1 females (6.9,14.1,18.3,15.6 % - control to high dose) *Offspring effects* 200 ppm F1: 11 pups born dead, ↓ day 4 viability index (90.8 % v 97.3 % in controls), ↓ lactation index (80.3 % v. 92.3 % in controls), ↓ male pup weight on days 7 - 21 (14 % decreasing to 6 % on day 21), ↓ females pup weight day 1-21 (14-18 %) F2: ↓ day 4 viability index (92.3 % v. 97.1 % in controls), ↓ lactation index (84.2 % v 92.9 % in controls), ↓ male pup weight on day 7 and 21 (16 and 14 %, respectively), ↓ female pup weight on day 7 and 21 (15 and 18 %, respectively) 100 ppm: F1: ↓ lactation index (83.3 % v. 92.3 % in controls) F2: 7 pups born dead, ↓ day 4 viability index (93.7 % v. 97.1 % in controls),	

	↓ lactation index (83.2 % v. 92.9 % in controls) 50 ppm F1: No effects F2: ↓ day 4 viability index (88.5 % v 97.1 % in controls), ↓ lactation index (84.9 % v. 92.9 % in controls), ↓ male pup weight on days 1-7 (7% increasing to 12 %) No reproductive NOAEL of 200ppm was derived. A parental NOAEL of 50 ppm and an offspring NOAEL of 50 ppm was derived.	

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The effects of terbuthylazine on fertility has been investigated in one 2-generation study in Sprague-Dawley rats and one 1-generation study and one 2-generation study in Wistar rats.

Sprague Dawley rats

In the 2-generation study conducted in Sprague-Dawley derived rats (Masters et al, 1992) effects on reproductive performance were observed. In the high dose from the F0 generation, 7 out of 32 (compared to 1 out of 32 controls) failed to become pregnant. Of these, 4 showed no indication of mating. Reduced or absent corpora lutea was also observed in 4 out of the 7 females that failed to conceive, but not in any of the fertile females. Similar effects were not observed in the controls, low or mid dose groups. In the F1 generation, 6 out of 30 high dose females failed to fall pregnant. In addition, 4 out of 30 control animals also failed to become pregnant. No indication of mating was observed in 5/6 of the high dose females. Following re-mating with proven males, 2 females from both the control and high dose group successfully produced litters. In this generation, corpora lutea was absent in the four high dose females that failed to conceive; however reduced corpora lutea was also observed in 3 females who successfully conceived. No effects on corpora lutea were noted at any other dose level. At the top dose level, parental bodyweight gain was lower than control during the pre-mating period (30-32 % in F0 generation and 22-25 % in the F1 generation) in both sexes at 300 ppm and was accompanied by a reduction in food consumption (between 10-16 % in both generations). The extent of the parental toxicity was marked and reductions in fertility in the presence of reduced bodyweight have been observed in Sprague-Dawley rat (Terry *et al*, 2005). As such, the effects on fertility and corpora lutea number are likely to be a secondary, non-specific, consequence of maternal toxicity and, therefore not relevant for classification.

Effects on offspring were also noted at 300 ppm (reduced pup weight and delayed sexual maturation) in both generations. Given the extent of the general toxicity observed at this dose in the F0 generation (bodyweight gain was reduced by between 20- 32 % at time of mating), it is likely these offspring effects were a secondary, non specific consequence of maternal toxicity and not a specific effect on development.

Wistar rats

The effect of terbuthylazine on fertility has also been investigated in a 1-generation study and a 2-generation study in Wistar rats.

In the 2-generation study (Krishnappa, 1998), no effect on mating performance, number of pregnant animals or number of corpora lutea was observed. The proportion of implantations and pre-implantations varied with dose and generation; however, these changes are likely to be artefacts caused by slight but opposite changes in the number of corpora lutea and implantations at any one dose, rather than a treatment related effect. Similarly, the increase in the proportion of post-implantation loss observed at the top dose in the F2 generation was lower than that observed in the control group of the F1 generation and is, therefore, unlikely to be treatment related.

In this study, pup viability was reduced in both F1 and F2 pups. In the F1 generation, viability was reduced in the top dose group on day 1 and 4 and in both the mid and high dose on day 21. In the F2 generation, pup viability was lower in all dose groups on both day 4 and day 21. Pup weight was also reduced at these dose levels. In this study, maternal bodyweight was significantly reduced at the top dose (> 10 %); however, bodyweight was not significantly affected in the mid or low dose groups indicating these effects cannot be dismissed as a secondary non-specific consequence of maternal toxicity. Effects on mortality (as a consequence of reduced bodyweight) were also observed in the repeat dose studies, for which classification has been proposed. On this basis, it is considered likely that the reduced viability is a repeated dose effect and not a specific developmental effect.

In the one-generation study in Wistar rats (Gainger, 1999), no effects on fertility were observed up to the top dose (25/ 36 mg/kg bw/day). This study was generally to guideline, except that group sizes were lower than required. There was a small decrease in average corpora lutea number at the top two doses; however, since the range was similar to the control at all doses it is not considered treatment-related. Effects in offspring were limited to reductions in bodyweight gain. This was most pronounced at the top two doses and is likely to be a secondary non-specific consequence of the significantly lower bodyweights (> 10 %) of females throughout the treatment period. Female pup weight gain was lower (10 %) in the lowest dose group; however male pup weight was unaffected.

4.11.1.2. Human information

No information available

4.11.2 Developmental toxicity

The developmental toxicity of terbuthylazine has been investigated in three developmental studies in rat and four developmental studies in rabbit.

Table 21: Summary of relevant developmental toxicity studies

Method	Results	Reference
Developmental toxicity Oral (gavage) OECD 414 (1981) Rat, RAIF 24/group 0, 1, 5 or 30 mg/kg bw/day on days 6-15 of gestation Vehicle: aqueous starch suspension Batch: SG 6925 (96.4 % purity)	Dams 30 mg/kg bw/day 13 % ↓ bodyweight gain (days 6-21), 22.7 % ↓ food consumption (day 6-11) and 12 % ↓ food consumption (days 11-16) 5 and 1 mg/kg bw/day No effects observed Foetuses No substance related malformations noted at any dose level The total % litter incidence of skeletal anomalies increased marginally with dose (36 % of litters at 30 mg/kg bw/day compared to 25 % in controls) and, at the top dose, included ↓ ossification of the occipital and no ossification of metacarpal 5. Total skeletal variance ↓ with dose. Individual skeletal variations were higher at ≥ 5 mg/kg bw/day and were mainly due to reduced or absent ossification of digits, whose incidence was well within the historical control range. Those findings that weren't, included an ↑ incidence of no ossification of metatarsal 1 in the top two doses and, at the top dose level, ↓ ossification of anterior digit proximal phalanx, and no ossification of the distal phalanx. A maternal NOAEL of 5 mg/kg bw/day. A developmental NOAEL of 5 mg/kg/bw/day	Fitzgerald (1990)
Developmental toxicity Oral (gavage) OECD 414 (1981) Rat /BR 10/group 0, 100, 200 or 400 mg/kg bw/day on days 6-15 of gestation Vehicle: 1% aqueous methylcellulose Batch 18 (96.4 % purity)	Maternal toxicity Reduced body tone, unsteady gait/tip-toe gait, intervals of temporary collapse, ptosis, excessive washing, hunched posture, respiratory distress and salivation at all doses. Top dose animals were sacrificed on day 9 due to severe weight loss and reduced food consumption. Necropsy revealed multiple punctuate crater-like depressions of forestomach epithelium (9/10 animals) and thickened and oedematous forestomach in 3/10 animals. Marked weight loss and reduced food consumption was observed at 100 and 200 mg/kg bw/day at the start of treatment, with animals recovering from day 8-9 onwards. Foetal effects No effects on litter parameters were noted at a dose level up to 200 mg/kg bw/day; no gross abnormalities were observed in foetuses at these dose levels.	Brooker (1995a)
Developmental toxicity Oral (gavage) OECD 414 (1981) Rat /CD 25/group 0, 5, 25 or 125	Maternal toxicity Signs of toxicity observed at ≥ 25 mg/kg bw/day included intervals of temporary collapse, excessive grooming and ptosis; 125 mg/kg bw/day unsteady gait and post-dose salivation At the top dose, bodyweight loss was observed on days 6-8 followed by 17 % ↓ bodyweight gain on days 8-16, food consumption was 15 % ↓ between days 6-15. In the mid dose there was a 55 % ↓ in body weight gain between day 6-8. 10 % ↓in food consumption during the same period.	Brooker (1995b)

mg/kg bw/day on days 6-15 of gestation Vehicle: 1% methylcellulose Batch 18 (96.4 % purity)	Foetal effects No malformations were observed. ↑ incidence of squat foetus syndrome was observed at the top dose (1.5 % foetal incidence compared to 0.6 % in controls), The incidence of small interventricular septal defect ↑ in a dose related manner in the top two doses (1.2 (8.3) and 1.8 (12.0) % foetal (% litter) compared to none in control). The number of foetuses with 14 ribs increased in all treated groups (Foetal incidence; 1.2, 17.4, 17.4 and 32.3 % with increasing dose). A maternal NOAEL of 5 mg/kg bw/day and a developmental NOAEL of 25 mg/kg bw/day was proposed.	
Developmental toxicity Oral (gavage) OECD 414 (1983) Rabbit New Zealand White (16-22/group) 0.05, 1.5 or 4.5 mg/kg bw/day between days 7-19 Vehicle: aqueous methylcellulose Batch 16727 (98.5 % purity)	Maternal toxicity No adverse effects observed Foetuses No adverse effects observed A maternal NOAEL of 4.5 mg/kg bw/day and a developmental NOAEL of 4.5 mg/kg bw/day was determined	Bottomley, et al (1983)
Developmental toxicity Oral (gavage) OECD 414 (1981) Rabbit/Russian Chbb 0, 0.5, 1.5 and 5 mg/kg bw/day on days 7-19 of gestation Vehicle: aqueous methylcellulose Batch SG 8201 (96.8 % purity)	Maternal toxicity Deaths and body weight loss was observed in all dose groups, but was more pronounced in the top two dose groups. Food consumption was significantly ↓ throughout the dosing period in the top dose and ↓ during days 16-20 of the mid dose group. Bodyweights recovered after the dosing period finished. 1 dam in top dose aborted. Foetal toxicity Dose related ↓ in early resorptions (23.7, 18, 13, and 5% - control to high dose), ↑ in late resorptions (0, 1.9, 2.3 and 6.1 % - control to high dose), dose related ↓ post-implantation loss (23.7, 19.9, 15.3 and 11 % - control to high dose). ↑ % forelimb flexure at top dose.	Khalil (1996)
Developmental toxicity Oral (gavage) OECD 414 (1981)	Maternal toxicity In appetence and reduced faecal output were observed at high dose. All groups (including controls) showed bodyweight loss or no bodyweight gain during first four days of dosing. Weight loss was most pronounced in the top dose group and continued throughout dosing. Food consumption was markedly reduced at the top dose level (69 %) during the dosing period.	Brooker (1995c)

Rabbit/New Zealand White 6/group 0, 2.5, 5 and 7.5 mg/kg bw/day on days 6-18 of gestation Vehicle: 1 % methylcellulose Batch SG 8201 (96.4 % purity)	Foetal toxicity No malformations were observed. The number of late in utero deaths were slightly increased (1.2, 0.5, 0.6 and 2.2 in 0, 2.5, 5 and 7.5 mg/kg bw/day), resulting in a slightly increased number of total in utero deaths in the top groups (2.7 compared to 2 in controls). A dose related reduction in mean foetal weight was seen in all groups (23 % at top dose). The proportion of males was also reduced in the top dose group (34.9 % compared to 50 % in controls)	
Developmental toxicity Oral (gavage) OECD 414 Rabbit/ New Zealand White 16/group 0, 0.8, 2.4 and 7 mg/kg bw/day on days 6-18 of gestation Vehicle: 1% methylcellulose Batch 18 (96.4 % purity)	Maternal toxicity Inappetence and reduced faecal output observed at ≥ 2.4 mg/kg bw/day. One top dose dam aborted after termination of treatment; this dam exhibited severe bodyweight effects. Weight loss was observed at the beginning of the dosing period at ≥ 2.4 mg/kg bw/day and weight gain was also reduced throughout the treatment period, increasing thereafter. Foetal toxicity No malformations observed. Slight increase in late in utero deaths in the high dose (1.2 compared to 0.7 in controls) A maternal NOAEL of 0.8 mg/kg bw/ and a developmental NOAEL of 7 mg/kg bw/day were derived.	Brooker (1995d)

4.11.2.1 Non-human information

The developmental toxicity of terbuthylazine has been investigated in three developmental studies in rats and four developmental studies in rabbits.

Rats

In the first rat study (Fitzgerald (1990)), no malformations were observed. The incidence of incomplete ossification (↓ ossification of occipital; no ossification of metacarpal 5; ↓ or absent ossification of digits) was increased at the top dose. These effects are considered indicative of developmental delay as a result of marked maternal toxicity (↓ bodyweight gain) and not a direct effect on development.

In the second rat study (Brooker (1995a)), no treatment-related effects were noted at doses causing severe maternal toxicity (bodyweight loss).

In the third rat study (Brooker (1995b)), no treatment-related malformations were observed. An increased foetal incidence of foetus squat syndrome (all five affected foetuses were from the same litter) was observed in the high dose group. There was also an increase in the

number of foetuses with small interventricular septal defect (an anomaly) in the top two doses. These effects were only observed in the presence of marked maternal toxicity (significant ↓ in bodyweight gain, temporary collapse) and similar effects were not noted in the other Brooker study, conducted at higher doses. As such, they are considered to be a secondary non specific consequence of the maternal toxicity and not a direct effect on development. There was also an increased incidence of foetuses with 14 ribs in all treatment groups; as this effect is a variation it is not considered severe enough to warrant classification.

Rabbits

In the first study (Bottomley (1983)), no adverse effects were observed in dams or foetuses up to the top dose (4.5 mg/kg bw/day).

In the second study (Khalil (1996)), maternal deaths and bodyweight loss was observed in all dose groups. The deaths were considered unrelated to treatment. The extent of the bodyweight loss increased in severity in the top two dose groups. There was a dose related increase in late resorptions; however, the significance of this result is difficult to judge given that post-implantation loss decreased in a dose-related manner. No malformations were observed and the increased foetal incidence of forelimb flexure, observed in the top dose, was considered the result of overcrowding due to a larger than average litter size.

In the third study (Brooker (1995c)), no malformations were observed. The number of late *in utero* deaths was slightly increased in the top dose (2.2 compared to 1.2 in the controls). Foetal weight was also reduced, as was the proportion of males in the top dose group. It is likely the increased deaths and decreased foetal weight are a non-specific consequence of maternal toxicity, as weight loss was observed at all dose levels and was most pronounced at the highest dose level. The reduced proportion of males is probably a chance-finding, unrelated to treatment, as a similar finding was not noted in any other study.

In the final study (Brooker (1995d)), no malformations were observed. A slight increase in late in utero deaths was observed; however, again this was only observed at doses with severe maternal toxicity (body weight loss).

4.11.2.2 Human information

No data

4.11.3 Other relevant information

No data

4.11.4 Summary and discussion of reproductive toxicity

Fertility

Effects on fertility were investigated in two 2–generation studies and one 1-generation study.

In one 2-generation study, conducted in Sprague-Dawley rats, a number of females at the top dose level (300 ppm) from both the F0 and F1 generation did not conceive. In several of these animals there was no sign of mating. In the F1 generation, the effect was less clear as a number of control animals also failed to become pregnant, raising a question as to whether

reduced fertility was as a result of the high level of background variation in mating performance in these particular animals and not a treatment related effect. Although the results suggest that terbuthylazine may have an adverse effect on fertility, it should be noted that reduced bodyweight in Sprague-Dawley rats has previously been shown to affect fertility adversely (including leading to a reduction in corpora lutea numbers) (Terry *et al*, 2005). Furthermore, no effects on fertility were noted in either a 2-generation study or a 1-generation study conducted in Wistar rats at similar dose levels. Overall, it is considered probable that the findings are either due to a high variation in the background incidence in mating performance in these animals or are secondary to the general toxicity observed (effects on bodyweight).

Development

The developmental toxicity of terbuthylazine has been investigated in three studies in rat and four studies in rabbits.

In none of the studies were any malformations of concern noted and the foetal findings observed were considered to be a secondary non specific consequence of the maternal toxicity and not a direct effect on development.

Reduced pup viability was observed in both generations of one multigeneration study, conducted in Wistar rats. However, this is considered likely to be a repeated dose effect rather than a specific effect on development.

Overall, the results show that terbuthylazine does not affect development.

4.11.5 Comparison with criteria

Fertility

No effects were observed in the absence of marked toxicity that provides sufficient evidence to cause a strong suspicion of reduced fertility.

Developmental toxicity

No effects were observed in the absence of marked toxicity that provides sufficient evidence to cause a strong suspicion of impaired developmental toxicity.

4.11.6 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.12. Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

There is one sub-chronic neurotoxicity study available in rats.

Table 22: Summary of relevant neurotoxicity studies

Method	Results	Reference
Neurotoxicity, oral, diet OECD 424 (1997) Sprague-Dawley rats (12/sex/group) 0, 6, 30 and 100 ppm equivalent to 0, 0.4, 2.1 and 7 mg/kg bw/day in males and 0.5, 2.4 and 8 mg/kg bw/day in females for 90 days Batch SG 8201 (96.8 % purity)	**100 ppm** *Bodyweight and food consumption:* bodyweight ↓ (< 8 %) in both sexes. Food consumption was ↓ during the first 2 weeks of the study in both sexes **30 and 6 ppm** No treatment related effects observed	Moxon (2003)

There was no evidence of neurotoxicity or treatment- related neuropathology observed in a guideline sub-chronic neurotoxicity study in rats.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Terbuthylazine undergoes minimal degradation in the environment – where available, data on the degradants have also been included in a separate annex to this report (Annex III). This includes terbutryn (MT26) [CAS: 886-50-0, EC: 212-950-5] which is also a herbicide.

Table 23: Summary table of relevant degradation studies

Method	Results	Remarks	Reference
US EPA Guideline N 161-1	Hydrolysis DT_{50} at 25°C: pH 5 = 73 days pH 7 = 205 days pH 9 = 194 days	-	Doyle, 1991
EU C7 (Directive 92/69/EEC),	Hydrolysis at 50°C: pH 4 - 76% by day 5 pH 7 - <10% pH 9 - <10%	Insufficient data to calculate DT_{50}	Flack, 1995d
SETAC	Hydrolysis DT_{50} at 20°C: pH 4 = > 1 year	-	Slangen, 2001a
ECETOC Technical Report No. 12	Photolysis DT_{50} at 50°N: ≥ 240 days		Zetsch & Palm, 1993
Various: Dir. 95/36/EC and EPA	No significant photodegradation	-	Mamouni, 2002
SETAC	Photolysis DT_{50} natural summer sunlight at 40°N: 29.5 days	-	Slangen, 2001b
OECD Guideline 301B	2-3 % biodegradation Not readily biodegradable	-	Bader, 1990
OECD Guideline 301B	3-9 % biodegradation Not readily biodegradable	-	Desmares-Koopmans, 2001
SETAC water sediment simulation	Whole system DT_{50}: 20°C = 33-73 days 9°C = 224-136 days	-	Mamouni, 1998, 1999, 2004
BBA Guideline Part IV, 5-1	Whole system DT_{50}: 20°C = 83.52 to 118.54 days	-	Mamouni, 1995

5.1.1 Stability

Hydrolysis

Terbuthylazine

Three aqueous hydrolysis studies are available showing that terbuthylazine is hydrolytically stable at environmentally relevant temperatures and pH values for the purposes of classification.

Study 1 (Doyle, 1991)
Using ^{14}C-terbuthylazine (purity 96.4 %) and following GLP and US EPA guideline N 161-1, hydrolysis was assessed at pH 5, 7 and 9 at 25°C over 50 days in the dark. The following half-lives were calculated: 73 days at pH 5, 205 days at pH 7 and 194 days at pH 9. One

degradant (2-hydroxy terbuthylazine / MT13) was observed reaching a maximum of 15.6 % by day 50 at pH 5.

Study 2 (Flack, 1995d)
Following GLP and EU C7 (Directive 92/69/EEC), hydrolysis of terbuthylazine (purity 99.5 %) was assessed at pH 4, 7 and 9 over 5 days at 50°C in the dark. Less than 10 % hydrolysis was observed at pH 7 and 9. At pH 4 16 % hydrolysis was observed after 2.4 hours and 76 % by day 5. However, there was insufficient data to calculate a DT_{50}.

Study 3 (Slangen, 2001a)
In a follow up study to Flack 1995d radio-labelled ^{14}C-terbuthylazine (purity 99.0 %) was used to assess hydrolysis at pH 4 and 20°C over 30 days. The study was considered GLP compliant and followed SETAC guidelines. One degradant (2-hydroxy terbuthylazine / MT13) was observed at a maximum of 4.1 % Applied Radioactivity (AR) by day 30. The calculated DT_{50} was greater than 1 year.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

Photolysis

Terbuthylazine

Three aqueous photolysis studies are available showing that terbuthylazine undergoes limited photodegradation and is considered photolytically stable under environmentally relevant conditions for the purposes of classification. Photolysis is not an issue for interpretation of the algal toxicity tests.

Study 1 (Zetsch & Palm, 1993)
Following GLP and ECETOC Technical Report No. 12, the photolysis of terbuthylazine (purity 99.4 %) was assessed. The test substance was dissolved in filtered methanol to aid solubility (this was not considered to affect light absorbance). Considering a UV spectrum to 330 nm and an assumed quantum yield of 1, the programme GCSOLAR (Zepp and Cline) was used to calculate a half-life of ≥ 240 days at 50°N in surface water.

Study 2 (Mamouni, 2002)
Following GLP and various guidelines (Directive 95/36/EC, US EPA 540/9-82-021 and 540/09-90-078) the photolysis of ^{14}C-terbuthylazine (purity 98.5 %) was assessed over 10 days at ~24°C and pH 7 in simulated natural water. The 12 hours light/12 hours dark irradiation cycle was considered equivalent to 13.4 days natural mid-summer sunlight at 30/40°N. No significant photo-degradation was observed.

Study 3 (Slangen-, 2001b)
Following GLP and SETAC guidelines the photolysis of ^{14}C- terbuthylazine (purity 99 %) was assessed over 30 days at ~25°C and pH 7 in sterile buffer solutions. The constant irradiation was considered equivalent to 49.2 days summer natural sunlight at 40°N. The photolytic DT_{50} was 29.5 days natural summer sunlight at 40°N. Two degradants were observed: 2-hydroxy terbuthylazine / MT13 (maximum 38.9 % AR) and desethyl-terbuthylazine / MT1 (maximum 11.4 % AR). The quantum yield was considered to be 3×10^{-6}.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Terbuthylazine

Study 1 (Bader, 1990)
A ready biodegradation test following GLP and OECD Guideline 301B resulted in 2-3 % degradation (based on theoretical carbon dioxide) at day 28. On this basis, terbuthylazine is considered not readily biodegradable.

Study 2 (Desmares-Koopmans, 2001)
A ready biodegradation test following GLP and OECD Guideline 301B resulted in 3-9 % degradation (based on theoretical carbon dioxide) at day 28. On this basis, terbuthylazine is considered not readily biodegradable.

5.1.2.3. Simulation tests

Terbuthylazine

Aquatic/Sediment system

Study 1 (Mamouni, 1998, Amendment 1 Mamouni, 1999 and Amendment 2 Mamouni 2004)
A GLP water/sediment study using [^{14}C-terbutylazine (purity 99.4 %) following BBA (1990) and EPA subdivision N540/9-82-021, and Dutch Registration Guideline Section G.2. is available. Two laboratory water/sediment systems (Rhine river system and Ormalingen pond system) were used to assess the fate of terbuthylazine at 9^{o}C and 20^{o}C in the dark over 182 and 365 days. As microbial populations were viable the extension of the study timescale was considered acceptable. The Rhine system used a sandy loam sediment and water pH 8.2-8.4. The Ormalingen system used a silty loam sediment and water pH 7.7-8.4. In both systems the water phase was aerobic and the sediment phase anaerobic.

In terms of Applied Radioactivity (AR) distribution, less than 1 % carbon dioxide was observed for both systems/temperatures indicating significant mineralisation did not occur. Aquatic concentrations of terbuthylazine declined as the substance partitioned from the water phase to the sediment phase with a subsequent decline in sediment concentrations. Three degradants were identified in water and sediment: 2-hydroxy-tertbuthylazine (MT13), desethyl-terbuthylazine (MT1), and terbutryn (MT26).

MT13 was the most significant degradant with a maximum of 20 and 14.5 % AR (total water and sediment) in the Rhine and Ormalingen systems at day 365 at 20^{o}C. At the lower 9^{o}C temperature, 4.1 and 5.7 % AR (total water and sediment) was observed in the Rhine and Ormalingen systems at day 182.

MT26 (terbutryn) was observed in the water and sediment phase of both systems at 20^{o}C. The maximum was 7.4 % AR (total water and sediment) in the Ormalingen pond system by day

365. Less than 2 % AR was observed in total water and sediment phases of both systems at the lower 9°C temperature by study termination at day 182. In both systems/temperatures, the levels of terbutryn increased with time and may not have peaked by study termination.

Whole system DT_{50} values were 33 to 73 days at 20°C and 136 to 224 days at 9°C.

Study 2 (Mamouni, 1995)
A GLP water/sediment study using ^{14}C-terbuthylazine (purity 97.4 %) following BBA Part IV, 5-1 (1990) guideline is available. Two laboratory water/sediment systems (Rhine river system and Anwil pond system) were used to assess the fate of terbuthylazine at 20°C in the dark over 110 days. The Rhine system used a loamy sand sediment and water pH 8.2. The Anwil system used a clay loam sediment and water pH 8.26. In both systems the water phase was aerobic and the sediment phase anaerobic.

In terms of Applied Radioactivity (AR) distribution less than 1% carbon dioxide was observed for both systems/temperatures indicating significant mineralisation did not occur.

Terbuthylazine aquatic concentrations declined with the substance partitioning from the water phase to the sediment phase and a subsequent decline in sediment concentrations. Seven degradants were identified – the most significant were 2-hydroxy-tertbuthylazine (MT13) and desethyl-terbuthylazine (MT1). MT1 reached a maximum of 6.0-7.3 % AR (total water and sediment) at study termination. MT13 reached a maximum of 9.1-9.8 % AR (total water and sediment) at study termination. The degradant MT26 (terbutryn) was not identified.

Whole system DT_{50} values were 83.52 to 118.54 days at 20°C.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.1.3 Summary and discussion of degradation

Terbuthylazine is considered hydrolytically and photolytically stable at environmentally relevant temperatures and pH values.

Terbuthylazine reached a maximum of 9 % degradation in a ready biodegradation study and is considered not readily biodegradable.

Mineralisation of terbuthylazine to carbon dioxide was minimal (<1 %) in two aquatic water/sediment studies over 100 days or more. Calculated total system DT_{50} values were between 33 and 118.5 days at 20°C. These are greater than 16 days (i.e. less than 70 % degradation is expected within 28 days). On this basis terbuthylazine is not considered to undergo rapid ultimate degradation and is considered not rapidly degradable for classification purposes. Due to this stability, classification of terbuthylazine should be based on parent substance data only.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Terbuthylazine

Study 1 (Phaff, 2000b)

The adsorption of ^{14}C-terbuthylazine (purity 99.1 %) in four soils was assessed following OECD Guideline 106 and GLP. Adsorption values were in the range 187 to 318 ml/g Kfoc (loam with 2 % organic carbon to sandy clay loam with 1.8 % organic carbon).

Study 2 (Muller, 1991)
The adsorption of ^{14}C-terbuthylazine (purity 99 %) in three soils was assessed following OECD Guideline 106 and GLP. Adsorption values were in the range 312 to 333 ml/g Kfoc (sand with 0.1 % organic carbon to loamy sand with 1.48 % organic carbon).

Study 3 (Morgenroth, 1995)
The adsorption of ^{14}C-terbuthylazine (purity 97.8 %) in four soils was assessed following OECD Guideline 106 and GLP. Adsorption values were in the range 151 to 246 ml/g Kfoc (sandy loam with 1.57 % organic carbon and sandy loam with 1.2 % organic carbon).

Summary
The corresponding log K_{oc} values (based on Freundlich adsorption coefficients (K_f, K_{foc})) from all studies and soils range from 0.88 to 0.98 (mean 0.93) indicating a low adsorption potential.

5.2.2 Volatilisation

Two studies (Widmer, 1999 and Bacher, 2004) indicate the measured vapour pressure of terbuthylazine is between 9.0 x 10^{-5} Pa at 25^{o}C and 1.52 x 10^{-4} Pa at 22^{o}C. Calculated Henry's Law Constants range between 2.3 x 10^{-3} Pa m^3/mol at 25^{o}C (Burkard, 2000) and 4.18 x 10^{-3} Pa m^3/mol at 20^{o}C (Görg, 2004). These values indicate terbuthylazine is unlikely to partition significantly from the aquatic environment to air.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

A measured octanol-water partition coefficient and two bioaccumulation in fish studies are available:

Table 24: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD Guideline 107	Log K_{ow} at 25°C = 3.4	-	Kettner, 1999
OECD Guideline 107	Log K_{ow} at 20°C = 3.41	-	Howes, 1994
OECD Guideline 305	*Lepomis macrochirus* BCF(whole fish) = 34	no lipid normalization to 5%	Baranowski, 1990
OECD Guideline 305	*Oncorhynchus mykiss* BCF(whole fish) = 19	no lipid normalization to 5%	Van Dijk, 1997

5.3.1.1 Bioaccumulation estimation

Following GLP and OECD Guideline 107 (shake flask method), two measured log K_{ow} values are available for terbuthylazine: 3.4 at 25°C (Kettner, 1999) and 3.41 at 20°C (Howes, 1994).

5.3.1.2 Measured bioaccumulation data

Terbuthylazine

Study 1 (Baranowski, 1990)
Following GLP and OECD Guideline 305 the bioaccumulation of ^{14}C-terbuthylazine (purity 98 %) in fish was assessed using bluegill sunfish (*Lepomis macrochirus*) in a 42 day study (28 day uptake, 14 day depuration). A stock solution was prepared with the aid of acetone solvent and TWEEN 80 (0.0012 %) resulting in a single exposure concentration of 0.4 mg a.s./l. Exposure concentrations were renewed daily with a mean measured concentration based on ^{14}C-activity of 0.402 ± 0.047 mg/l. Rapid elimination was observed with a half life of 0.68 to 0.93 days. The calculated whole fish steady state BCF was 34. Given this low value, lipid normalisation has not been performed.

Study 2 (Van Dijk, 1997)
Following GLP and OECD Guideline 305 the bioaccumulation of ^{14}C-terbuthylazine (purity 95.6 %) in fish was assessed using rainbow trout (*Oncorhynchus mykiss*) in a 21 day study (7 day uptake, 14 day depuration). Using a flow through design two exposure concentrations were assessed: 0.005 mg/l and 0.05 mg/l. Measured exposure concentrations were 0.0048 to 0.005 mg/l and 0.0489 to 0.0519 mg/l. Rapid elimination was observed with half life of 0.2 to 0.6 days. The calculated whole fish BCF was 19 ± 2. Given this low value, lipid normalisation has not been performed.

5.3.2 Summary and discussion of aquatic bioaccumulation

On the basis of two bioaccumulation in fish studies with BCFs less than 500 (and 100), terbuthylazine is not considered bioaccumulative for classification purposes.

5.4 Aquatic toxicity

Tables 25a-c present a summary of key ecotoxicity information for terbuthylazine. Further details of reliable studies are provided in each sub-section.

5.4.1 Fish

Table 25a: Summary of relevant information on aquatic toxicity to fish

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Terbuthylazine (96.8 %)	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	2.2 mg a.s./l	Static Nominal	Swarbrick and Maynard, 2002
Terbuthylazine (96.8 %)	*Cyprinus carpio*	OECD 203	96-h LC_{50}	>5.7 mg a.s/l	Static Mean measured	Wallace and Woodyer, 2002
Terbuthylazine (97 %)	*Oncorhynchus mykiss*	OECD 203	96-h LC_{50}	2.4 mg a.s./l See data quality note below	Semi-static Nominal	Douglas *et al*, 1988a
Terbuthylazine (96.8 %)	*Oncorhynchus mykiss*	OECD 210	90-d NOEC (based on growth)	0.09 mg a.s./l	Flow-through Mean measured	Rufli, 1996

5.4.1.1 Short-term toxicity to fish

Terbuthylazine

Three valid GLP acute toxicity to fish studies using terbuthylazine are available. Two further acute toxicity to fish studies are available and reported in the DAR. However, due to study deficiencies, principally relating to analytical support, the results are not considered valid and the studies are not reported here.

Study 1 (Swarbrick and Maynard, 2002)

The acute toxicity to fish was assessed following OECD Guideline 203 and rainbow trout (*Oncorhynchus mykiss*). The study used terbuthylazine with a purity of 96.8 % and nominally 0.56, 1.0, 1.8, 3.2, and 5.6 mg a.s./l exposure concentration range. Under static conditions measured concentrations were 82 to 106 % of nominal. Based on nominal concentrations, the 96-h LC_{50} was 2.2 mg a.s./l. Sub-lethal effects were observed at all exposure concentrations (0.56 to 5.6 mg a.s./l) and a 96-h NOEC could not be determined.

Study 2 (Wallace and Woodyer, 2002)

The acute toxicity to fish was assessed following OECD Guideline 203 and carp (*Cyprinus carpio*). The study used terbuthylazine with a purity of 96.8 %. Under static conditions a single nominal 8.5 mg a.s./l exposure concentration was used. This corresponded to a mean measured concentration of 5.7 mg a.s./l. Based on mean measured data, the 96-h LC_{50} was > 5.7 mg a.s./l. Sub-lethal effects were observed at all exposure concentrations and a 96-h NOEC could not be determined.

Study 3 (Douglas *et al*, 1988a)

The acute toxicity to fish was assessed following OECD Guideline 203 and rainbow trout (*Oncorhynchus mykiss*). The study used terbuthylazine with a purity of 97 %. Under semi-static conditions with daily renewal, the nominal exposure concentration range was 0.32, 0.56, 1.0, 1.8, and 3.2 mg a.s./l. Measured concentrations were within 20 % of nominal except at the two lowest exposure concentrations of 0.32 and 0.56 mg a.s./l which were 180 to 18 % nominal and 123 to 101 % nominal respectively. Mortality (of 10%) was only observed at the highest exposure concentration of 3.2 mg a.s./l, and the study 96-h LC_{50} was therefore >3.2 mg a.s./l based on nominal concentrations. Sub-lethal effects were observed at all exposure concentrations and a 96-h NOEC could not be determined. It is not ideal that the LC_{50} is based on nominal concentrations but the two exposure concentrations which resulted in 0 and 100 % mortality were within 20 % of nominal. Therefore recalculation using mean measured concentrations is not anticipated to result in a lower $L(E)C_{50}$ than observed for the most sensitive aquatic species (algae).

Two prolonged acute fish toxicity tests are also available (Ritter, 1990 and Bell 1994a) but since they do not address endpoints that are relevant for classification, they are not included in this dossier.

Degradants

While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.4.1.2 Long-term toxicity to fish

Rufli (1996)

Following GLP and OECD Guideline 210 a long-term toxicity to fish (Fish Early Life Stage) study is available for terbuthylazine (purity 96.8 %). Under flow-through conditions the following nominal exposure concentration range was employed, 0.0031, 0.0063, 0.013, 0.025, 0.05 and 0.1 mg a.s./l. Analytical measurements were 81 to 100 % nominal. Embryo viability, time of hatching or hatching success were not affected at any exposure concentration. Based on mean-measured concentrations, the 90 day NOEC was 0.09 mg a.s./l based on weight.

5.4.2 Aquatic invertebrates

Table 25b: Summary of relevant information on aquatic toxicity to invertebrates

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Terbuthylazine (96.8 %)	*Daphnia magna*	OECD 202	48-h EC_{50}	>69.3 mg a.s./l	Static Mean measured	Van der Kolk (1996)
Terbuthylazine (97 %)	*Daphnia magna*	OECD 202	48-h EC_{50}	11 mg a.s./l (see summary for data quality note)	Static Nominal	Douglas *et al* (1988c)
Terbuthylazine (96.8 %)	*Daphnia magna*	OECD 202, Part II 21-d	21-d NOEC	0.019 mg a.s/l	Semi-static Nominal	Shillabeer *et al*, 2002
Terbuthylazine (96.5 %)	*Daphnia magna*	OECD 202, Part II 21-d	21-d NOEC	0.17 mg a.s/l	Semi-static Mean measured	Bell, 1995
Terbuthylazine (99 %)	*Chironomus riparius*	BBA (1995)	27-d NOEC	0.5 mg a.s/l	Static Nominal	Memmert, 1998

5.4.2.1 Short-term toxicity to aquatic invertebrates

Terbuthylazine

Study 1 (Van der Kolk, 1996)
The acute toxicity of terbuthylazine (purity 96.8 %) to *Daphnia magna* was assessed following GLP and OECD Guideline 202 in a static system. Exposure solutions (6.3, 12.5, 25.0, 50.0 and 100 mg a.s./l nominal) were prepared with the aid of acetone and a solvent control was included. The water solubility of terbuthylazine is 9 mg/l at 25°C, pH 7.4 and therefore all but the lowest exposure concentration are considered above the water solubility. Analytical measurements were 3 to 80 % nominal and some undissolved material was observed in exposure solutions. No immobilisation was observed at any exposure concentration. Based on mean measured concentrations, the 48-h EC_{50} was >69.3 mg a.s./l reflecting the saturated solution and above the water solubility.

Study 2 (Douglas *et al*, 1988c)
The acute toxicity of terbuthylazine (purity 97 %) to *Daphnia magna* was assessed following GLP and OECD Guideline 202 in a static system. Exposure solutions of 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 mg a.s./l were prepared with the aid of TWEEN 80 and a solvent control was included. Analytical measurement of new (0 hrs) solutions was undertaken at 0.32, 1.0, 3.2, 10 and 32 mg a.s./l and were 17 to 123 % of nominals. Analytical measurements were taken for all concentrations at termination (48 hrs) and were 63 to 139 % of nominals. It was reported thatsome undissolved material was observed in exposure solutions. The terbuthylazine water solubility is 9.0 mg/l at 25°C, pH 7.4 and it is likely the upper range of exposure solutions contained undissolved material in excess of the water solubility.

Based on nominal concentrations, the 48-h EC_{50} was 11 mg a.s./l. It is not ideal that the EC_{50} is based on nominal concentrations as there is uncertainty regarding actual terbuthylazine dissolved concentrations (at higher test concentrations settlement was considered likely) and

this value is above the quoted water solubility. However immobilisation was observed in 1.8 mg a.s./l exposure solutions and above (which were within 20 % of nominal concentrations) and recalculation using mean measured data is not anticipated to result in a lower $L(E)C_{50}$ than observed for most sensitive aquatic species (algae). The study was used in the DAR but is not considered the critical study for classification.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Terbuthylazine

Study 1 (Shillabeer *et al*, 2002)
The chronic toxicity of terbuthylazine (purity 96.8 %) to *Daphnia magna* was assessed following GLP and OECD Guideline 202, Part II in a semi-static system. Nominal exposure solutions were 0.006, 0.019, 0.061, 0.2, 0.63, and 2 mg a.s./l. With the exception of two values of 79 and 167 % of nominal, analytical measurements were within 20 % of nominal. Based on nominal concentrations, the 21-d NOEC was 0.019 mg a.s./l based on number of offspring. This equates to a mean measured concentration 0.020 mg a.s./l.

Study 2 (Bell, 1995)
The chronic toxicity of terbuthylazine (purity 96.5 %) to *Daphnia magna* was assessed following GLP and OECD Guideline 202, Part II in a semi-static system. Nominal exposure solutions of 0.18, 0.56, 1.8, 5.6 and 18 mg a.s./l were prepared with solvent and a solvent control was included. Measured concentrations ranged from 77 to 230 % and 63 to 142 % of nominal for fresh and expired solutions. The variability was considered to be due to difficulties with dispersing the test substance in test medium. However, the majority of measurements were within 20 % of nominal and in relation to the NOEC, the 0.18 and 0.56 mg a.s./l analytical results were within 20 % of nominal. Based on mean measured concentrations, the 21-d NOEC was 0.17 mg a.s./l based on reproduction.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.4.3 Algae and aquatic plants

Table 25c: Summary of relevant information on aquatic toxicity to algae and aquatic plants

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
Terbuthylazine (96.6 %)	*Pseudokirchneriella subcapitata* [1]	OECD 201	72-h E_rC_{50} 72-h NOE_rC	0.028 mg a.s./l 0.0012 mg a.s./l	Static Mean measured	Kelly, 1996
Terbuthylazine (96.4 %)	*Desmodesmus subspicatus*[2]	OECD 201	72-h E_rC_{50} 72-h NOE_rC	>0.03 mg/l 0.0011 mg/l	Static Nominal	Grade, 1993a [3]
Terbuthylazine (96.4 %)	*Microcystis aeruginosa*	ASTM E1218-90	96-h E_rC_{50} 96-h NOE_rC	0.018 mg/l 0.0037 mg/l	Static Nominal	Grade, 1993b [3]
Terbuthylazine (96.4 %)	*Navicula pelliculosa*	ASTM E1218-90	96-h E_rC_{50} 96-h NOE_rC	>0.03 mg/l 0.01 mg/l	Static Nominal	Grade, 1993c [3]
Terbuthylazine (98 %)	*Anabaena flos-aquae*	OECD 201	48-120h E_rC_{50} [4] 48-120h NOE_rC [4]	0.052 mg/l 0.02 mg/l	Static Mean measured	Migchielsen, 2002a
Terbuthylazine (98 %)	*Microcystis aeruginosa*	OECD 201	48-120h E_rC_{50} [4] 48-120h NOE_rC [4]	0.102 mg/l 0.0396 mg/l	Static Mean measured	Migchielsen, 2002b
Terbuthylazine (96.4 %)	*Lemna gibba*	US EPA FIFRA 122-2	14-d EC_{50} (frond no.) 14-d $NOEC_{(frond\ no.)}$	0.019 mg a.s./l 0.0022 mg a.s./l	Static Mean measured	Hoberg, 1993
Terbuthylazine (97.7 %)	*Lemna gibba*	OECD 221 draft	7-d EC_{50} (frond no.) 7-d $NOEC_{(frond\ no.)}$	0.0128 mg a.s./l 0.0029 mg a.s./l	Semi-static Nominal	Dengler, 2001

[1] Formerly known as *Selenastrum capricornutum*
[2] Formerly known as *Scenedesmus subspicatus*
[3] Updated data from industry for the purpose of this dossier – personal correspondence February 2012
[4] 48-120h reflects period of exponential growth

Terbuthylazine

Six reliable studies assessing the toxicity of terbuthylazine to various algae and diatom species are available.

Study 1 (Kelly, 1996)
A 72-hour, GLP, static algal growth inhibition study is available using the unicellular green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) using terbuthylazine (purity 96.6 %) and following OECD guideline 201. Exposure solutions of 0.001, 0.0022, 0.0046, 0.010, 0.022, 0.046 and 0.10 mg a.s./l were prepared with the aid of acetone and a solvent control was included. Analysis was undertaken at 0 and 72 hours – at 0 hours measured concentrations were 92 to 109 % of nominal and at 72 hours 95 to 128 % of nominal. Based on mean measured concentrations the study 72-h E_rC_{50} was 0.028 mg a.s./l and NOEC 0.0012 mg a.s./l.

Study 2 (Grade, 1993a)

A 72-hour, GLP, static algal growth inhibition study is available using unicellular green algae *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*) (purity 96.4 %). The study followed OECD guideline 201. The nominal exposure concentrations were 0.000123, 0.00037, 0.0011, 0.0033, 0.01 and 0.03 mg/l. Measured concentrations by HPLC were 73 to 91 % nominal, but one was 61 %. Only biomass endpoints were presented, so the NOE_rC was calculated from the raw data as 0.0011 mg/l nominal – at this exposure concentrations measured concentrations at 0 h and 72 h were 0.001 mg/l (91 % of nominal). Limited growth inhibition was observed in the study and the 72-h E_rC_{50} exceeded the highest tested concentration of 0.03 mg/l.

Study 3 (Grade, 1993b)
A 96-hour, GLP, static algal growth inhibition study is available using blue green algae *Microcystis aeruginosa* and terbuthylazine (purity 96.4 %). The study followed ASTM Guideline E1218-90 which is considered broadly similar to OECD guideline 201. The nominal exposure concentrations were 0.00123, 0.0037, 0.011, 0.033, 0.1 and 0.3 mg/l. Analytical measurements were 81 to 142 % of nominal. Only biomass endpoints were presented so, based on nominal concentrations and calculations from the raw data, the 96-h E_rC_{50} was determined to be 0.018 mg a.s./l and NOE_rC 0.0037 mg a.s./l.

Study 4 (Grade, 1993c)
A 96-hour, GLP, static algal growth inhibition study is available using freshwater diatoms *Navicula pelliculosa* and terbuthylazine (purity 96.4 %). The study followed ASTM Guideline E1218-90 which is considered broadly similar to OECD guideline 201. The nominal exposure concentrations were 0.000123, 0.00037, 0.0011, 0.0033, 0.01 and 0.03 mg/l. A solvent control was included based on 96 % DMF and 4 % alkylphenol-polyglycolether). At 0 h analytical concentrations were 72.7 to 90.9 % nominal. Growth data were re-analysed; limited growth inhibition was observed and effects were only observed in the two highest concentrations. The E_rC_{50} exceeded the highest tested concentration of 0.03 mg/l and the NOEC is considered to be 0.01 mg/l nominal. Analytical measurements at these two highest concentrations were 69 to 87 % of nominal at the end of the study.

Study 5 (Migchielsen, 2002a)
A 120-hour, GLP, static algal growth inhibition study is available using cyanobacteria *Anabaena flos-aquae* and terbuthylazine (purity 98 %) following OECD guideline 201. Exposure solutions were prepared with dilutions of a 5 µm filtrate of nominal 100 mg a.s./l. Analysis was undertaken at 0 and 120 hours. The nominal exposure concentrations were 0.0041, 0.0089, 0.02, 0.041, 0.089, 0.197, 0.411 mg/l. Analytical measurement was undertaken for the 0.0041, 0.041 and 0.411 mg/l treatments with measured values extrapolated for the remaining test concentrations. The study results are based on the exponential growth phase between 48 and 120 hours. Based on mean measured concentrations the study 48 to 120-h E_rC_{50} was 0.052 mg a.s./l and NOEC 0.02 mg a.s./l.

Study 6 (Migchielsen, 2002b)
A 120-hour, GLP, static algal growth inhibition study is available using blue-green algae *Mycrocystis aeruginosa* and terbuthylazine (purity 98 %) following OECD guideline 201. Exposure solutions were prepared with the aid of acetone and a solvent control was included. The nominal exposure concentrations were 0.004, 0.008, 0.016, 0.032, 0.056, and 0.128 mg/l, Analysis was undertaken at 0 (117 to 139 % nominal) and 120 hours (70 to 99 % nominal). The study results are based on the exponential growth phase between 48 and 120 hours. Based on mean measured concentrations the study 48 to 120-h E_rC_{50} was 0.102 mg a.s./l and NOEC 0.0396 mg a.s./l.

Two reliable studies assessing the toxicity of terbuthylazine to aquatic plant species are available.

Study 1 (Hoberg, 1993)
A 14-day, GLP, static toxicity to *Lemna gibba* using terbuthylazine (purity 96.4 %) and following US EPA FIFRA 122-2 (considered similar to OECD guideline 221) is available. The nominal exposure concentrations were 0.0031, 0.0063, 0.013, 0.025, 0.050 and 0.1 mg a.s./l. Analysis was undertaken at 0 (73 to 100 % nominal) and 14 (35 to 57 % nominal) days. Based on mean measured concentrations the study 14-d $EC_{50\ (frond\ number)}$ was 0.019 mg a.s./l and the 14-d $NOEC_{(frond\ number)}$ was 0.0022 mg a.s./l. Based on mean measured concentrations and growth rate calculations from the raw data, the 14-d E_rC_{50} was 0.086 mg a.s./l and the 14-d NOE_rC was 0.0022 mg a.s./l.

Study 2 (Dengler, 2001)
A 7-day, GLP, semi-static toxicity to *Lemna gibba* using terbuthylazine (purity 97.7 %) and following draft OECD guideline 221 is available. Exposure solutions (nominally 0.0009, 0.0029, 0.0093, 0.0298, 0.0954, 0.3052, 0.9766, 3.125, 10 and 100 mg a.s./l) were prepared in acetone and a solvent control was included. Based on the study LOQ (0.1 mg a.s./l) analysis of fresh and expired media was undertaken for exposure solutions 0.3052 to 10 mg a.s./l. Fresh exposure solutions were 51.5 to 123 % nominal and expired exposure solutions were 62.5 to 130 % nominal. Study results were reported as nominal concentrations. Whilst this is not ideal, the lower range exposure solutions were consistently >80% nominal indicating lower exposure concentrations below the water solubility were adequate and stable. Based on nominal concentrations the lowest study 7-d EC_{50} was 0.0128 mg a.s./l based on frond number. The 7-d NOEC was 0.0029 mg a.s./l based on frond number and recalculations of growth rate.

Degradants
While these are not considered relevant to the classification of terbuthylazine, data for these are included in Annex III.

5.4.4 Other aquatic organisms (including sediment)

A toxicity to *Chironomus riparius* study is available using ^{14}C terbuthylazine (purity 99 %) an aquatic/sediment system (Memmert, 1998). The GLP study was conducted according to the BBA Guideline (1995) which closely follows OECD Guideline 219 (simulating one off event of pesticide spray drift). Nominal test concentrations were 0.06, 0.12, 0.25, 0.5 and 1.0 mg a.s./l prepared using acetone solvent and DMF. A solvent control was included. The test system employed approximately 2 cm of sediment and 15 cm of reconstituted water. Over the 27 study period terbuthylazine partitioned from the aqueous phase following dosing to sediment. At day 27, 40 % nominal terbuthylazine was measured in the water phase and the degradants M1 and M13 were observed at max. 9 % and max. 4.5 %. The emergence rate was not affected at any exposure concentration. A statistical difference in development rate was observed at the highest exposure concentration of 1.0 mg a.s./l nominal. Therefore the NOEC was considered to be 0.5 mg a.s./l nominal and used in the DAR. While the study NOEC reflects an initial peak concentration and not continuous aquatic exposure, the value is considered relevant for classification and labelling.

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

Terbuthylazine is considered hydrolytically and photolytically stable at environmentally relevant temperatures and pH values. In a ready biodegradation study a maximum of 9 % degradation was observed and terbuthylazine is considered not readily biodegradable.

Mineralisation of terbuthylazine to carbon dioxide was minimal (less than 1 % over 100 days or more) in two aquatic water/sediment studies and calculated total system DT_{50} values were between 33 and 118.5 days. These exceed 16 days (i.e. less than 70 % degradation is expected within 28 days). On this basis terbuthylazine is not considered to undergo rapid ultimate degradation and is considered not rapidly degradable for the purposes of classification.

Fish bioconcentration factors are lower than the trigger values for Regulation EC 1272/2008.

In one water-sediment study the degradant terbutryn (MT26) reached a maximum of 7.4 % AR (total water and sediment) at study termination on day 365 at 20°C. Terbutryn is also a herbicide/algicide and algae/aquatic plants are the most sensitive species. A full set of acute toxicity and a chronic toxicity to *Daphnia* study are available for terbutryn (refer to Annex III). Whilst it is noted that this degradant is an order of magnitude more toxic to algae than the parent terbuthylazine, it is not formed in significant amounts over the period representative of rapid degradation. Therefore terbutryn is not considered relevant to the classification of terbuthylazine. Overall, given the lack of rapid degradation, the classification and labelling proposal for terbuthylazine is based solely on terbuthylazine ecotoxicity.

A full set of valid acute and chronic fish, invertebrate and algae/aquatic plant data is available for terbuthylazine. The key studies presented above are considered sufficiently reliable for classification purposes. Terbuthylazine is a herbicide and as anticipated algae / aquatic plants are the most sensitive trophic group.

Based on available acute and chronic data for terbuthylazine, acute toxicity is observed below the classification threshold of 1 mg/l and the chronic toxicity is observed below the classification threshold of 0.1 mg/l.

Based on acute aquatic toxicity data with $L(E)C_{50}$ values below 1 mg/l, classification with Aquatic Acute 1 is applicable. An acute M-factor of 10 is applicable based on $0.01 < L(E)C_{50} \leq 0.1$ mg/l considering the various algal/*Lemna* E_rC_{50} data in this range for terbuthylazine.

Based on chronic aquatic toxicity data, long-term NOECs for algae and aquatic plants are below 0.1 mg/l and Aquatic Chronic 1 is applicable. A chronic M-factor of 10 is applicable based on $0.001 < NOEC \leq 0.01$ mg/l considering the various algal/*Lemna* NOEC data in this range for terbuthylazine.

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

Aquatic Acute 1; H400 M-factor = 10 based on $0.01 < L(E)C_{50} \leq 0.1$ mg/l

Aquatic Chronic 1; H410 M-factor = 10 based on $0.001 < NOEC \leq 0.01$ mg/l

6 OTHER INFORMATION

This substance has been reviewed under Council Directive 91/414/EEC, with the rapporteur Member State being the United Kingdom. The studies evaluated in this dossier were taken from the pesticide assessment report; where necessary, the full study reports were consulted, but these are generally not publically available. Where other information from additional references has been sourced, this is indicated.

7 REFERENCES

The full reference list can be found in the Pesticide Assessment Report (DAR) – public Version – initial risk assessment provided by the rapporteur Member State United Kingdom for the existing active substance terbuthylazine of the third stage (part B) of the review programme referred to in Article 8 (2) of the Council Directive 91/414/EEC. **Volume 2, Annex A Part 2**

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8 ANNEXES

Annex I – Position paper on mammary tumours in rats

Annex II – Position paper on leydig cell tumours in spragye dawley derived rats.

Annex III – Additional fate and ecotoxicity information for terbuthylazine degradants

oxon ITALIA syngenta		

Annex I: Terbuthylazine – Position on Mammary tumours in Rats

PROVIDED BY SYNGENTA AND OXON

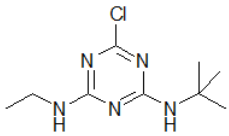
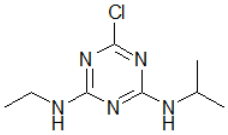
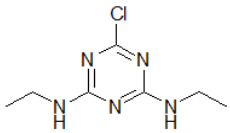
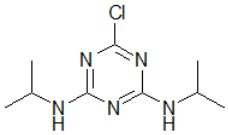
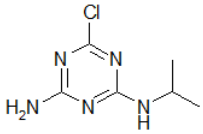
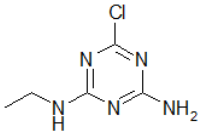
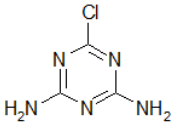
Date: 10th December 2013

Terbuthylazine – Position on Mammary tumours in rats

In a chronic toxicity and carcinogenicity study with Sprague Dawley-derived rats (Gfeller, 1983), an increased incidence of mammary gland adenocarcinoma was noted for female rats receiving the highest dose of terbuthylazine tested (750 ppm).

The mode of action for induction of mammary tumours in Sprague Dawley, but not Fischer 344, rats by chlorotriazine herbicides is well understood and is considered not relevant to humans (summarised in Simpkins *et al.*, 2011). The majority of the relevant mode of action investigations have been conducted using the class exemplar atrazine, with the outcomes extrapolated to the other chlorotriazines (and selected metabolites) owing to a shared herbicidal mode of action and very high degree of structural similarity (see Table 1).

Table 1. Chlorotriazine herbicides (and metabolites) share a high degree of structural similarity

Chlorotriazine	Terbuthylazine	Atrazine	Simazine	Propazine
Structure				
Metabolite	**DEA**	**DIA**	**DACT**	
Structure				

Owing to the shared herbicidal mode of action, similar toxicology databases and very high degree of structural similarity, the United States Environmental Protection Agency (US-EPA, 2002, 2006 and referenced in EFSA 2013) has evaluated this class of chemistry and determined that atrazine, simazine, propazine, DEA, DIA and DACT:

"*will be considered as a common mechanism group for purposes of a cumulative risk assessment and as part of the tolerance reassessment process for triazine pesticides*" (US-EPA, 2002).

Terbuthylazine was not considered in this assessment because it is not registered in the US. However, based on structural similarity, as well as a similar toxicological database, terbuthylazine can be reasonably considered part of this common mechanism group.

A second chronic toxicity and carcinogenicity study was conducted with terbuthylazine, using Han Wistar rats (Ramesh, 2001). An increased incidence of mammary gland adenocarcinoma was noted for female rats receiving the highest dose of terbuthylazine tested (120 ppm).

Although less data regarding the mode of action for induction of mammary gland adenocarcinomas by chlorotriazines in Han Wistar rats, sufficient data are available to support Syngenta/Oxon's position that the mode of action demonstrated for Sprague Dawley rats can be reasonably extrapolated to Han Wistar rats because:

1. **Atrazine suppresses the luteinizing hormone (LH) surge in both Sprague Dawley and Han Wistar rats.** Suppression of the LH surge (via effects on gonadotropin releasing hormone [GnRH] release) is a crucial key event for the induction of mammary tumours in Sprague Dawley rats by chlorotriazines, with a lack of an effect on this parameter being the reason why chlorotriazines do not induce mammary tumours in Fischer 344 rats (Simpkins *et al*., 2011). Mode of action work with the class exemplar atrazine has demonstrated that it can inhibit the LH surge in both Sprague Dawley (Simpkins *et al*., 2011) and Han Wistar (Foradori *et al*., 2009) rats, but not in Fischer 344 rats (Simpkins *et al*., 2011).

2. **The reproductive ageing processes for female Sprague Dawley and Wistar rats are very similar and under similar neuroendocrine control.** And therefore would be expected to respond similarly to suppression of the LH surge. The similarities between the strains are likely a result of their shared ancestry, with Sprague Dawley rats originally derived from Han Wistar rats (White and Lee, 1988). In contrast, the Fischer 344 rat is an inbred strain of non-related origin.

    a. **The primary reason for onset of reproductive senescence is the same for female Sprague Dawley and Wistar Rats.** The primary reason for onset of reproductive senescence in both of these strains is hypothalamic failure to stimulate LH (and follicle stimulating hormone [FSH]). Chlorotriazine-mediated LH suppression therefore mimics the primary reason for onset of senescence in these strains and accelerates the process, leading to earlier onset. This is in contrast to Fischer 344 rats, where the primary reason for onset is a hypothalamic failure to control prolactin surges (US-EPA, 2000a).

    b. **Both Sprague Dawley and Han Wistar rats primarily enter into a state of constant oestrus following onset of reproductive senescence.** Therefore, earlier onset of senescence in these strains will result in greater oestrogenic exposure, which is a causal key contributing to the development of mammary tumours (Simpkins *et al*., 2011). This is in contrast to Fischer 344 rats, which primarily enter into a state of pseudopregnancy/persistent dioestrus following onset of reproductive senescence, which is associated with higher progesterone exposure (US-EPA, 2000a).

    These similarities/differences between different strains of rat have been summarised by US-EPA as part of a Scientific Advisory Panel (SAP) meeting for Atrazine Cancer Risk Assessment (US-EPA, 2000a). The relevant schematic is copied below as Table 2.

Table 2. Summary of the reproductive ageing process in different rat strains.
Taken directly from US-EPA, 2000a (Part B)

Sprague-Dawley, Long Evans, Wistar[1]
▸ Normal cycle is a four to five day cycle with 25% of the time spent in estrus, 25% spent in proestrus and 50% spent in diestrus;
▸ Reproductive aging becomes evident at approximately nine to 12 months;
▸ Reproductive aging is characterized by decreased gonadotropin surges that leads to maintenance of primary, secondary and antral ovarian follicles;
▸ An irregular cycling pattern develops followed by an increase in the days spent in estrus, and prolonged exposure to estrogen;
▸ Pituitary alterations such as increase in pituitary weight, increases in pituitary hyperplasia and pituitary -adenomas become common as the animal ages;
▸ Acyclicity develops in the final months of life
▸ Normal cycling ➜ irregular cycles ➜ prolonged estrus ➜ acyclicity *(begins around 5-6 weeks old)* — *(occurs in the last few months of life ≥ 21 months of age)*
[1]There may be temporal differences between and among strains

F-344
▸ Normal cycle is a four to five days with 25% of the time spent in estrus, 25% spent in proestrus and 50% spent in diestrus;
▸ Reproductive aging becomes evident at approximately 12 months;
▸ Reproductive aging is characterized by increased prolactin surges that leads to maintenance of the corpea lutea;
▸ There is an increase in the days spent in diestrus, and increased exposure to progesterone.
▸ In very aged animals, acyclicity is common

As can be seen from Table 2 above, female rat reproductive ageing in the Han Wistar and Long Evans strains is considered to be similar to the Sprague Dawley.

In US-EPA, 2000a (Part B), the following statement is made:

"*Some rat strains (LE, Wistar and SD included) undergo a similar reproductive aging process which is characterized by the appearance of persistent (or constant) estrus by approximately one year of age and under similar neuroendocrine events. Thus, the LE female rat is considered to be a valid model for evaluating atrazine's mode of action resulting in mammary tumors in SD females*"

Furthermore, in US-EPA (2000b), the following statement is made:

"*Biological plausibility has been established for the mode of carcinogenic activity of atrazine. The rat cancer mode of action (MOA) involves a process consisting of modulation of the gonadotropin releasing hormone (GnRH) pulse, attenuation of pituitary releases of luteinizing hormone (LH), and alteration of ovulatory cycles, expressed as constant estrus, which leads to prolonged exposure of mammary and pituitary tissues to estrogen and prolactin, and development of tumors in response to the prolonged hormone exposures. This MOA essentially accelerates the normal aging process in female SD rats. It would be expected to be operative in other rat strains with a similar reproductive aging process (e.g. Long Evans and Wistar)*"

These statements clearly supports Syngenta/Oxon's position that for strains of rat that undergo similar reproductive ageing processes under similar neuroendocrine control (i.e., Sprague Dawley and Han Wistar [and Long Evans]) it is appropriate to extrapolate the mode of action for chlorotriazine-induced mammary tumours in Sprague Dawley rats to the other strains.

c. **Sprague Dawley and Han Wistar rats have a very similar incidence of spontaneous mammary tumours.** This is a reflection of the fact that reproductive ageing is very similar in both strains. In contrast, control Fischer 344 rats have a lower spontaneous incidence. This is summarised in Table 3.

Table 3. Spontaneous mammary tumour incidence in various rodent strains. Adapted from US-EPA, 2000a (Part B)

Strain	Spontaneous Mammary Tumour Incidence
Sprague Dawley	~30% Fibroadenoma ~12% Carcinoma
Han Wistar	~25% Fibroadenoma ~13% Carcinoma
Fischer 344	~12% Fibroadenoma ~2% Carcinoma

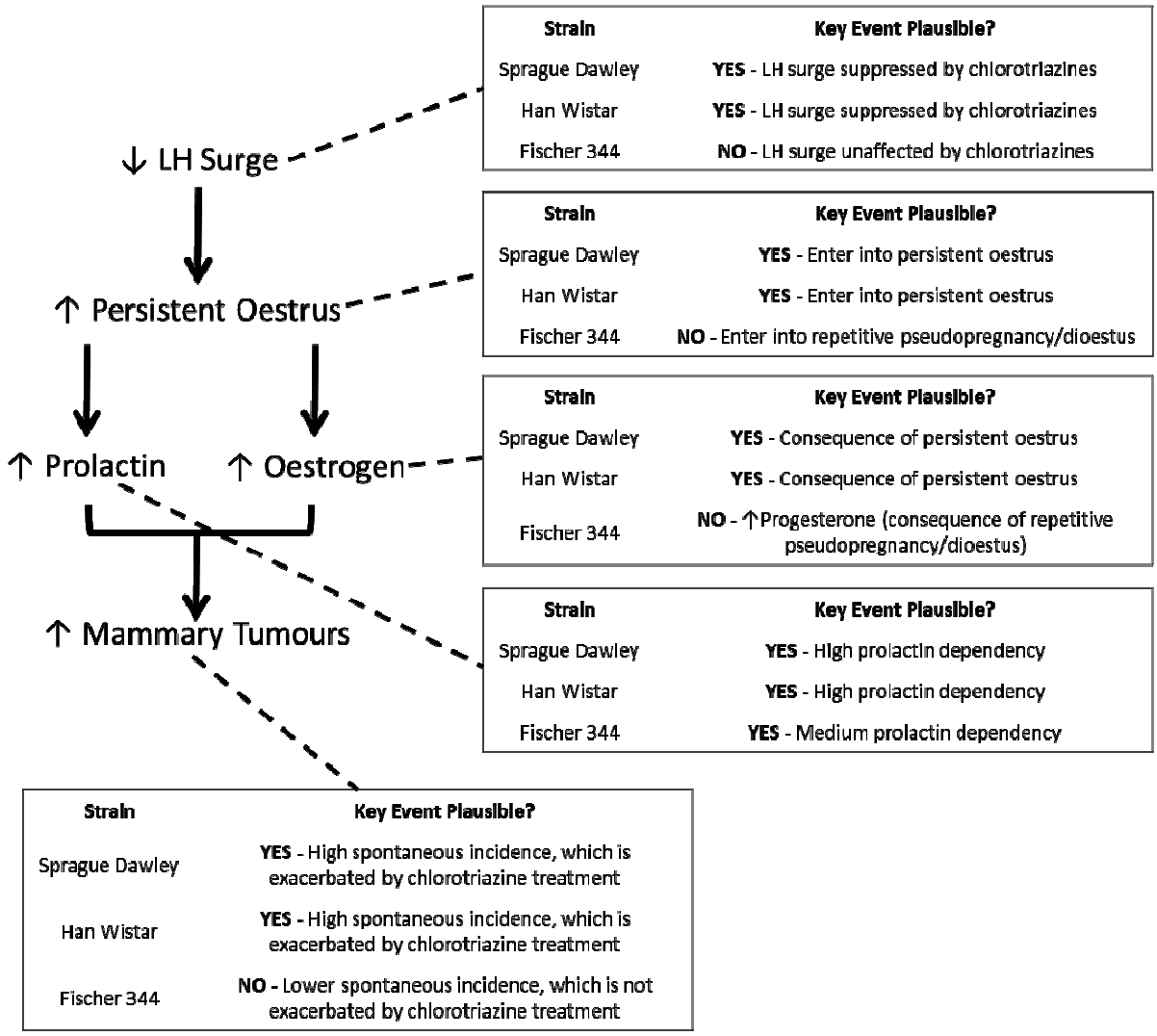
d. **Prolactin is a key factor is reproductive ageing in both Sprague Dawley and Han Wistar rats.** Prolactin is key factor in chlorotriazine-induced mammary tumours (Simpkins *et al*., 2011). In contrast, prolactin is less important in Fischer 344 rats (Simpkins *et al*., 2011; Harleman *et al*., 2012).

In summary, it can be concluded that the well understood, and not relevant to humans, mode of action for chlorotriazine-induced mammary tumours in female Sprague Dawley rats can be extrapolated to Han Wistar rats, based on their shared sensitivity to chlorotriazine-mediated suppression of the LH surge and the overwhelming similarities in their reproductive ageing processes. This is in contrast to the Fischer 344 rat, which ages differently and is not sensitive to chlorotriazine-mediated suppression of the LH surge. These similarities and differences are summarised in Table 4 and overlaid onto the mode of action for chlorotriazine-induced mammary tumours in Figure 1.

Table 4. Summary of rat strain similarities/differences in sensitivity to chlorotriazine-mediated inhibition of the LH surge, reproductive ageing and spontaneous mammary tumour incidence in female rats. Adapted/compiled from Chapin *et al*., 1996; US-EPA, 2000a, 2002, 2006; Simpkins *et al*., 2011 and Harleman *et al*., 2012 [and references therein])

	Sprague Dawley	**Han Wistar**	**Fischer 344**
Sensitivity to Chlorotriazine-Mediated Inhibition of the LH Surge	Yes	Yes	No
Age at which Reproductive Senescence Becomes Evident	~12 months	~12 months	~9-12 months
Principle Cause for Onset of Senescence	Hypothalamic failure to stimulate LH/FSH	Hypothalamic failure to stimulate LH/FSH	Hypothalamic failure to control prolactin surges
LH Surge Capability	Lost	Lost	Maintained
Predominant Cycle Pattern Post-Onset	Persistent oestrus	Persistent oestrus	Repetitive pseudopregnancy/ dioestrus
Oestrogen/Progesterone Ratio	Elevated/prolonged	Elevated/prolonged	Reduced
Prolactin Dependence	High	High	Medium
Spontaneous Mammary Tumour Incidence	~30% Fibroadenoma ~12% Carcinoma	~25% Fibroadenoma ~13% Carcinoma	~12% Fibroadenoma ~2% Carcinoma

Figure 1. Mode of action for chlorotriazine-induced mammary tumours in female rats: Sensitivity of different strains. Mode of action schematic adapted from Simpkins *et al.*, 2011



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Annex II - Terbuthylazine – Position on Leydig cell tumours in Sprague Dawley-Derived rats

PRODUCED BY SYNGENTA AND OXON

Date: 10th December 2013

Terbuthylazine – Position on Leydig cell tumours in Sprague Dawley-Derived rats

In a chronic toxicity and carcinogenicity study with Sprague Dawley-derived rats (Gfeller, 1983), administration of 750 ppm terbuthylazine resulted in an apparent increase in Leydig (interstitial) cell tumours in male rats. Administration of this dose resulted in a marked decrease in body weight gain (in excess of what is considered a suitable Maximally Tolerated Dose [MTD] for a study of this type) and a marked improvement in survival relative to the controls. No increase in Leydig cell tumour incidence was noted in males receiving 30 or 150 ppm. These data are summarised in Table 1.

Table 1. Body weight gain, body weight, survival and Leydig cell tumour incidence

Dose (ppm)	Body Weight Gain (% of Control) Week 1-105*	Body Weight (% of Control) Week 105*	Survival Time – 75th Percentile (days)	Leydig Cell Tumour Incidence
0	100	100	642	3/79 (3.8%)
30	91	93	647	4/79 (5.1%)
150	78	81	660	2/80 (2.5%)
750	54	59	740	10/80 (12.5%)

*: Animals received test item for 24 months, after which all groups were placed on control diet until survival in one group dropped to ~20% (in this case, the control group). Therefore, the measurements taken at week 105 are the best for evaluating effects of the test item on body weight gain and body weight.

It is Syngenta/Oxon's position that the apparent increase in Leydig cell tumours in the 750 ppm group does not represent a direct effect of terbuthylazine because:

1. **Leydig cell tumours are a common spontaneous age-related tumour.** Therefore, the apparent increase noted in the 750 ppm group is attributable to the marked increase in survival (McMartin *et al*., 1992; Nakazawa *et al.,* 2001).

    a. **The marked increase in survival is attributable to the markedly reduced body weight gain.** Studies in which body weight gain is limited (by dietary restriction) have been shown to significantly improve survival in Sprague Dawley rats (Keenan *et al*., 1994, 1997).

    b. **The increase in survival noted in the 750 ppm group is a step-change increase compared to the effects seen at 30 and 150 ppm.** Although statistically significant increases in survival were noted for all treatment groups, that noted at 750 ppm represented a clear step-change compared to the 30 and 150 ppm dose groups. The difference in survival compared to controls at 30 and 150 ppm was not of significant magnitude to affect the incidence of Leydig cell tumours.

    c. **The increase was not statistically significant when appropriate survival-adjusting statistical techniques are used.** In the original study report

(Gfeller, 1983) a Peto trend test was used to test for a statistically significant increase in tumours adjusted for survival. A Peto trend test requires a fatal/non-fatal designation of each tumour by the study pathologist, which was not conducted in this study. Revised statistical analyses using the Poly-k trend test, a statistical technique that adjusts for survival without the requirement for a fatal/non-fatal designation are presented in Appendix 1. The apparent increase in Leydig cell tumours was not statistically significant when using the Poly-k trend test, nor in subsequent pair-wise tests using Fishers exact test.

2. **Higher incidences have been noted in control rats in other studies.** Although slightly higher than the upper limit of the laboratory historical control data, similar/higher incidences have been reported in control Sprague Dawley rats in other laboratories and in the Registry of Industrial Toxicology Animal (RITA) database (see Table 2).

Table 2. Ranges of Leydig cell tumour incidences in control Sprague Dawley rats

Data Source	Range of Leydig Cell Tumour Incidence (%)
750 ppm terbuthylazine	12.5
Laboratory historical control data	0 – 7.5
RITA database	0 – 12
McMartin *et al*., 1992	1.4 – 13.3
Nakazawa *et al*., 2001	22.5 – 27.5

3. **No increase in tumours was noted at 150 ppm, a dose which would have represented a robust MTD for the evaluation of carcinogenicity.** Based on the significant reductions in body weight gain (78% of control over weeks 1 -105) and body weight (81% of control at week 105).

4. **No increase in Leydig cell tumours was noted in a chronic toxicity and carcinogenicity study with Han Wistar rats.** (Ramesh, 2001).

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APPENDIX 1 to Annex II:Statistical Re-Analysis of Leydig Cell Tumour Incidence

Prepared by Ian Pate, Toxstat consultancy Ltd. (31 October 2013)

Introduction

A rat lifetime feeding study for terbuthylazine (GS 13529) was conducted by Ciba–Geigy (Gfeller, 1983). The study consisted of 1 control and 3 treated groups (fed 30, 150 and 750 ppm) each containing 80 animals/sex. Interim kills were scheduled for 10 animals/sex/group at 1 year and 20 animals/sex/group at 2 years. Surviving animals at 2 years were retained on control diet for a further 4 weeks for males and 13 weeks for females. Male survival was statistically significantly increased at 150 and 750 ppm due to marked reductions in body weight and food consumption. Similar but smaller differences were seen in males at 30 ppm and in females but these did not achieve statistical significance.

In the original report it was recognized that the increased survival may affect tumour profiles and Peto analyses were performed for selected tumours. However, a pathological assessment of tumour context (i.e. whether tumours were fatal or incidental) was not formally made and an assumption that all tumours were incidental was used for the Peto analysis.

An alternative statistical analysis (the Poly-k test) which does not require an assessment of tumour context has subsequently been proposed. The purpose of this report is to statistically reappraise tumour profiles using the Poly-k test. The tumour types chosen for statistical re-analyses are those analysed using statistical methods in the original study report.

Statistical Methods

Selected tumours were analysed using the following methods:-

(i) Fisher's Exact Test was used to compare the overall incidence in each treated group with control

(ii) A Cochran-Armitage trend test was used to look for a trend in incidence with group number

(iii) Methods (i) and (ii) provide base analyses which do not take into account survival differences. A Poly-k analysis was used to adjust for survival differences. The Poly-k test is a modification of the Cochran-Armitage test where the denominator of the test is changed. Animals with a tumour or surviving to final termination are given a weight of 1. All other animals are given a weight of their (actual day of death divided by longest survival time) raised to the power k. The weights are then summed across each group to give new denominators for the Cochran-Armitage test. A value of 3 was selected for k as this is most commonly used in reported analyses.

As potential differences in tumour incidence were seen as both increases and decreases, all statistical analyses were two-sided. The original reported analyses were one-sided looking only for increases in tumour incidence.

Trend tests were conducted based on group number and not actual dose level as used in the original analysis. The dose levels on the study were chosen to be equally spaced on a logarithmic scale and

using group number reflects this equal spacing. Using actual dose makes the trend test over-dependent on the top dose.

Results

For benign interstitial cell tumours (Table A1), a slightly higher incidence was seen at 750 ppm. This did not achieve statistical significance in the unadjusted analyses but was close. The Poly-k test reduced the statistical significance further indicating that the increased survival is likely to have affected the tumour incidence.

Table A1
Testis Benign Interstitial Cell Tumours
Fisher's Exact Test, Cochran-Armitage and Poly-3 Trend Test Results

Males	0	30	150	750
Benign Interstitial Cell Tumours	3/79	4/80	2/80	10/80
Fishers Exact Test, 2-sided		1.0	0.68	0.08
Cochran-Armitage Trend Test	0.06			
Poly-3 Test Weighted Denominator	49	51	53	62
Poly-3 Trend Test, 2-sided	0.11			

Annex III – Additional fate and ecotoxicity information for terbuthylazine degradants

Degradation

Hydrolysis

Study1(Adam,2002b)
Following GLP and OECD Guideline 111, the hydrolysis of radio-labelled ^{14}C-desethyl-terbuthylazine (MT1) (purity 96.7 %) was assessed at pH 4, 5, 7 and 9, at 50^{o}C in the dark over 5 days. Hydrolysis was observed at pH 4 with a calculated DT_{50} of 135.9 days at 25^{o}C. This equates to a DT_{50} of 215.5 days at 20^{o}C.

Study 2 (van der Gaauw, 2002)
Following GLP and OECD Guideline 111, the hydrolysis of radio-labelled ^{14}C-hydroxy-terbutylazine (MT13) (purity 99.3%) was assessed at pH 4, 7 and 9 at 50^{o}C in the dark over 5 days. Minimal hydrolysis (<10 %) was observed and MT13 is considered hydrolytically stable.

Photolysis

Study 1 (Glänzel, 2002b)
Following GLP and Directive 95/36/EEC guidelines, the photolysis of radio-labelled ^{14}C-desethyl-terbuthylazine (MT1) (purity 97 %) was assessed at pH 5, 7 and 9, at ~20^{o}C over 15 days constant irradiation. Minimal degradation was observed and MT1 is considered photolytically stable.

Study 2 (Hennecke, 2004a)

Following GLP and SETAC 1995 guidelines and OECD draft 2002 guideline on phototransformation f chemicals in water, UV absorbance of desethyl-terbuthylazine (MT1) (purity unknown) was measured at pH 5, 7 and 9. Minimal absorbance was observed and MT1 is considered photolytically stable.

Study 3 (Hennecke, 2004b)

Following GLP and SETAC 1995 guidelines and OECD draft 2002 guideline on phototransformation f chemicals in water, UV absorbance of 2-hydroxy-terbuthylazine (MT13) (purity unknown) was measured at pH 5, 7 and 9. Minimal absorbance was observed and MT13 is considered photolytically stable.

Aquatic/Sediment System

Study 1 (Phaff, 2000)

A water-sediment study for the minor degradant terbutryn (MT26) [CAS: 886-50-0, EC: 212-950-5]is available and described in the DAR Additional Report (2010). The terbutryn whole system DT_{50} ranged between 178 and 203 days.

Soil system

Various aerobic and anaerobic degradation of terbuthylazine in soil studies of differing duration are available (refer to DAR, 2007, section B.8.1) . Within 100 days none show significant mineralisation to carbon dioxide and are not discussed further for classification.

Aquatic toxicity

Fish

Table I Annex III: Summary of relevant information degradant aquatic toxicity to fish

*Analytical support showed concentrations 73 to 79 % at exposure concentration 10 mg/l. Whilst results based on measured data would be preferable, a revised LC50 is not anticipated to lie below the lowest value for the parent

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
MT1 (96 ± 2 %)	*Oncorhynchus mykiss*	EU C1	96-h LC_{50}	18 mg/l	Static Nominal*	Vial , 1991a
MT13 (99 ± 2 %)	*Oncorhynchus mykiss*	OECD 203 – limit test	96-h LC_{50}	> 2.5 mg/l (considered solubility)	Static Mean measured	Peither, 2000
MT14 (84 ± 2 %)	*Oncorhynchus mykiss*	EU C1	96-h LC_{50}	>100 mg/l	Static Nominal	Vial , 1991b
MT20 (98 ± 2 %)	*Oncorhynchus mykiss*	EU C1	96-h LC_{50}	>100 mg/l	Static Nominal	Vial , 1991c
Terbutryn / MT26 (96.6 %)	*Oncorhynchus mykiss*	EU C1	96-h LC_{50}	1.1 mg/l (see summary for data quality note)	Static Mean measured	Sousa *et al*, 1982

terbuthylazine

GLP acute toxicity to fish studies are available for the following degradants: desethyl-terbuthylazine (MT1), 2-hydroxy-terbuthylazine (MT13), desethylhydroxy-terbuthylazine (MT14), diamino-chloro-triazine (MT20) and terbutryn (MT26). The only LC_{50} value lower than terbuthylazine is terbutryn (MT26) which is presented below. The remaining studies are quoted in Table I Annex III for reference but not discussed further.

M26 / Terbutryn (Sousa *et al*, 1982)

The acute toxicity to fish using terbutryn (purity 96.6 %) was assessed following EU guideline C1 (similar to OECD Guideline 203) and rainbow trout (*Oncorhynchus mykiss*) under static conditions. Exposure solutions were prepared with the aid of a solvent and a solvent control was included. Measured concentrations were 71 to 91 % of nominal. Based on initial measured data, the study 96-h LC_{50} was 1.1 mg a.s./l and the NOEC 0.94 mg/l. While oxygen concentrations were above 60 % saturation and considered acceptable until 48 hours, they fell to 39 to 59 % of air saturation by study end (96 hours). This is outside the guideline range of ≥ 60 %. Live fish were observed to be respiring rapidly in all exposure concentrations but not in the blank control / solvent control indicating the respiration effect was related to the test substance. Overall, the lack of oxygen could have resulted in a more conservative LC_{50}. The study was used in the DAR but is not considered the critical study for classification as fish are not the most sensitive species.

Table II Annex III: Summary of relevant information degradant aquatic toxicity to invertebrates

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
MT1 (96 ± 2 %)	*Daphnia magna*	OECD 202	48-h EC_{50}	42 mg/l	Static Nominal	Vial, 1991d
MT13 (99 ± 2 %)	*Daphnia magna*	OECD 202	48-h EC_{50}	> 2.8 mg/l (considered solubility)	Static Mean measured	Grade, 2000a
MT14 (84 ± 2 %)	*Daphnia magna*	EU C2	48-h EC_{50}	>100 mg/l	Static Nominal	Vial, 1991e
MT20 (98 ± 2 %)	*Daphnia magna*	EU C2	48-h EC_{50}	>100 mg/l	Static Nominal	Vial, 1991f
Terbutryn / MT26 (unknown purity)	*Daphnia magna*	US EPA-660/3-75-00	48-h EC_{50}	2.66 mg/l (see summary for data quality note)	Static Nominal	Vilkas and Hutchinson, 1977
Terbutryn / M26 (94 %)	*Daphnia magna*	US EPA protocols, 1975	21-d NOEC	1.3 mg a.s./l	Semi-static Mean measured	Surprenant *et al*, 1982

GLP, acute toxicity to invertebrate studies are available for the following degradants: desethyl-terbuthylazine (MT1), 2-hydroxy-terbuthylazine (MT13), desethylhydroxy-terbuthylazine (MT14), diamino-chloro-triazine (MT20) and terbutryn (MT26). Only the EC_{50} for terbutryn is lower than EC_{50} values for terbuthylazine and presented below. The remaining studies are quoted in III Annex for reference but not discussed further.

MT26/Terbutryn (Vilkas and Hutchinson, 1977)

The acute toxicity of terbutryn (unknown purity) to *Daphnia magna* was assessed following US EPA guideline 660/3-75-00 in a static system. Exposure solutions were prepared with the aid of acetone and a solvent control was included. Analytical measurement was not conducted. Based on nominal concentrations, the study 48-h EC_{50} was 2.66 mg a.s./l. In acute toxicity to fish and algae studies, terbutryn concentrations were observed to decline with a minimum of 69 to 71 % nominal – assuming exposure solutions were dosed adequately, it is not anticipated that an EC_{50} based on analytical concentrations would be below EC_{50} values for algae / aquatic plants. While the study is not valid for the purpose of deriving a classification, the study is reported here as it was included in the DAR and indicates that invertebrates are unlikely to be more sensitive than algae/aquatic plants.

MT26 / Terbutryn (Surprenant *et al*, 1982)

The chronic toxicity of terbutryn (purity 94 %) to *Daphnia magna* was assessed following US EPA Protocols (1975) in a semi-static system. Nominal exposure solutions were prepared with solvent and a solvent control was included. Based on mean measured concentrations, the 21-d NOEC was 1.3 mg a.s./l based on reproduction.

Algae and aquatic plants

Table III Annex III: Summary of relevant information degradant aquatic toxicity to algae and aquatic plants

Substance and purity	Species	Test Guideline	Endpoint	Toxicity value	Conditions	Reference
MT1 (99 ± 2 %)	*Pseudokirchneriella subcapitata*[1]	OECD 201	72-h E_rC_{50} 72-h NOE_rC	0.38 mg/l 0.05 mg/l	Static Mean measured	Palmer *et al*, 2001
MT1 (99.2 %)	*Desmodesmus subspicatus* [2]	OECD 201	72-h E_rC_{50} 72-h NOE_rC	0.471 mg/l 0.128 mg/l	Static Nominal	Dengler, 2004a
MT13 (99 ± 2 %)	*Pseudokirchneriella subcapitata*[1]	OECD 201	72-h E_rC_{50}	> 3.8 mg/l (based on saturated solution filtrate)	Static Mean measured	Grade, 2000b
MT13 (97 %)	*Desmodesmus subspicatus*[2]	OECD 201	72-h E_rC_{50} 72-h NOE_rC	>3.96 mg/l 3.96 mg/l (considered solubility)	Static Nominal	Dengler, 2004b
MT14 (84 ± 2 %)	*Desmodesmus subspicatus*[2]	OECD 201	72-h E_rC_{50}	35.4 mg/l 3.7 mg/l	Static Nominal	Vial, 1991g [3]
MT20 (98 ± 2 %)	*Desmodesmus subspicatus*[2]	EU C3	72-h E_rC_{50} [4] 72-h NOE_rC [4]	>100 mg/l 33 mg/l	Static Nominal[9]	Vial, 1991h [3]
Terbutryn / MT26 (97.4 %)	*Pseudokirchneriella subcapitata*	OECD 201	72-h E_rC_{50} 72-h NOE_rC	0.0036 mg/l 0.0002 mg/l	Static Mean measured	Grade, 1997 [3]

[1] Formerly known as *Selenastrum capricornutum*
[2] Formerly known as *Scenedesmus subspicatus*
[3] Updated data from industry for the purpose of this dossier – personal correspondence February 2012
[4] 48-120h reflects period of exponential growth

Seven toxicity to algae / aquatic plants studies available for the following degradants: desethyl-terbuthylazine (MT1), 2-hydroxy-terbuthylazine (MT13), desethylhydroxy-terbuthylazine (MT14), diamino-chloro-triazine (MT20) and terbutryn (MT26). Where growth rate endpoints were not available from the DAR, the study data were re-analysed. Only the EC_{50} / NOEC for terbutryn is lower than EC_{50} values for terbuthylazine and presented below. The remaining studies are quoted in III Annex III for reference but not discussed further.

MT26 / Terbutryn (Grade, 1997)

A 72 hour, GLP, static algal growth inhibition study is available using the unicellular green algae *Pseudokirchneriella subcapitata* using terbutryn (purity 97.4 %) and following OECD guideline 201. The nominal exposure concentration range was 0.0001, 0.0002, 0.0004, 0.0008, 0.0016, 0.0032, 0.0056 and 0.0128 mg/l. Analysis was undertaken at 0 (69 to 100 % nominal) and 72 hours (69 to 100 % nominal). Based on mean measured concentrations the study report 72-h E_rC_{50} was 0.0036 mg/l and NOE_rC 0.00065 mg/l. During statistical reanalysis in 2012, the registrant proposed a revised NOErC of 0.0002 mg/l.