Committee for Risk Assessment

RAC

Annex 1

Background document

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

Terbuthylazine

EC Number: 227-637-9
CAS Number: 5915-41-3

CLH-O-0000001412-86-66/F

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

Adopted

05 June 2015

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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Annex II - Terbuthylazine – Position on Leydig cell tumours in Sprague Dawley-Derived rats
Annex III – Additional fate and ecotoxicity information for terbuthylazine degradants

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

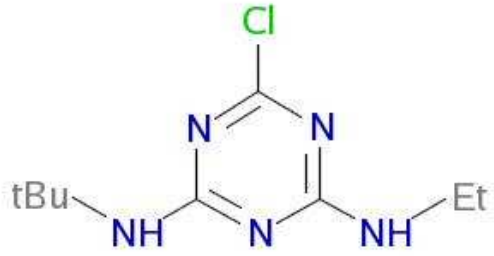
1 IDENTITY OF THE SUBSTANCE

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:

<u>Propazine</u>

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 ^{o}C 175.7 ^{o}C	R Das 1999a Moller 2007	OECD 102 Purity 99.4% DSC Purity 99.6%
Boiling point	Decomposition observed at 224 ^{o}C before boiling point reached Decomposition observed at 230 ^{o}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 ^{o}C (extrapolated) 1.52 x 10^{-4} Pa at 22 ^{o}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 ^{o}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 ^{o}C) 6.64 mg/l at pH 7 (20^{o}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 ^{o}C) Log Pow = 3.41 (20 ^{o}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

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

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

(1) Acute oral toxicity of Terbuthylazine in rats:

Summary of the acute oral toxicity studies:

Rat Strain	Sub. purity	LD_{50} (mg/kg bw/day)	Study
Tif:RAIf	99%	> 2000	Hartmann (1989), OECD 401, GLP
OFA.SD(IOPS Caw)	96.4%	1000 - 1590	Mercier (1991), FIFRA 81-1, GLP
Crl:CD(SD)BR	97%	> 6400	Gardner (1988) OECD 401, GLP

The DS proposed Acute Tox. 4; H302 on the basis of the LD_{50} results in the study by Mercier (1991).

(2) Acute inhalation toxicity of Terbuthylazine in rats:

An inhalation LC_{50} of > 5.3 mg/L over 4 hours (nose only) was derived from the Hartmann (1989) study conducted with rats (OECD 403, GLP). No deaths occurred during the study period.

The DS did not propose classification for the inhalation route.

(3) Acute dermal toxicity of Terbuthylazine:

Summary of the acute dermal toxicity studies:

Animal Strain	Sub. purity	LD_{50} (mg/kg bw/day)	Study
OFA.SD(IOPS Caw) rats	96.4%	> 2000 (no deaths)	Mercier (1991), OECD 402 (1987), GLP
New Zealand White rabbits	97%	> 2000 (no deaths)	Kynoch *et al*., (1988) EPA FIFRA 81-2, GLP

The DS did not propose classification for the dermal route.

Comments received during public consultation

Three Member States (MS) commented during the public consultation. All supported the acute oral toxicity classification proposals of the DS.

Assessment and comparison with the classification criteria

(1) Acute Oral Toxicity:
In general, the lowest LD_{50} value from a study associated with one sex is used to determine whether that study supports classification or not. The lowest reported acute oral LD_{50} value was 1000-1590 mg/kg in Sprague-Dawley (SD) derived rats (Mercier, 1991).

According to CLP, the LD_{50} values for acute oral toxicity, category 4 are from 300 to 2000 mg/kg bw. It was considered appropriate for the classification to be based on the lowest LD50 value available, in consistence with the Guidance on the application of the CLP criteria (CLP Guidance). The RAC agrees with the DS conclusion that terbuthylazine warrants classification as Acute Tox. 4 - H302 (Harmful if swallowed).

(2) Acute Inhalation Toxicity:
The acute inhalation LC_{50} was > 5.3 mg/L/4h for Tif:RAIf rats.

According to CLP, the LC_{50} values for acute inhalation, category 4 are from 1.0 to 5.0 mg/L for dusts/mists. The RAC agrees with the DS that no classification is justified.

(3) Acute Dermal Toxicity:
The LD_{50} values for dermal toxicity are above the threshold value of 2000 mg/kg bw for triggering classification. No classification is required.

Supplemental information - In depth analyses by RAC

(1) Acute oral toxicity of Terbuthylazine in rats:

Summary of the lethality data:

(a) Hartmann (1989)

Dose (mg/kg bw/d)	Males	females
2000	0/5	1/5

(b) Mercier (1991)

Dose (mg/kg bw/d)	Males	females
0	0/5	0/5
1000	2/5	1/5
1590	3/5	3/5
2510	2/5	4/5

(c) Gardner (1988)

Dose (mg/kg bw/d)	Males	females
4000	0/5	1/5
5000	2/5	0/5
6400	2/5	2/5

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.

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

Summary of the Dossier submitter's proposal

The toxicity seen after single oral, dermal and inhalation exposure to terbuthylazine were indicative of non-specific, general acute toxicity. There was no clear evidence of specific toxic effects on any target organ or tissue, no signs of respiratory tract irritation or narcotic effects. The DS did not propose classification for specific target organ toxicity (single exposure).

Comments received during public consultation

No comments were received relating to STOT SE

Assessment and comparison with the classification criteria

No classification is required.

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

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS summarised the details of two eye irritation studies (Ligget, 1988; Mercier, 1990, both used 6 animals/study) performed in rabbits and described in table 13 of the CLH report. Both were standard guideline and GLP compliant studies. In one study (Liggett, 1988), diffuse crimson colouration of the conjunctivae, with some swelling, was observed in one animal at the one hour reading only. Some mild conjunctival irritation was observed in the other five animals. Although the Mercier (1990) study was terminated at 72 hours, before the effects had fully resolved, the results of the Liggett (1988) study showed the effects to be fully reversible within 3 days.

The DS did not propose classification.

Comments received during public consultation

No comments were received relating to eye corrosion/irritation.

Assessment and comparison with the classification criteria

The criteria for Category 2: In at least 2 out of 3 tested animals (4 out of 6 under CLP guidance), corneal opacity or iritis score ≥ 1 or conjunctival redness or edema score ≥ 2, calculated as the mean scores following grading at 24, 48 and 72 hours following installation and which fully reverse within the observation period of 21 days.

The individual animal eye irritation scores do not exceed the CLP trigger values and therefore do not meet the criteria for classification as irritating to the eyes. RAC supports the DS conclusion that no classification is required for this hazard class.

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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The DS summarised two skin irritation studies (Ligget, 1988; Mercier, 1990; both used 6 animals/study), performed in rabbits and described in table 12 of the CLH report. Both were standard guideline-compliant, GLP studies. There was no evidence of corrosion or scarring or any damage to the dermal surface. The only sign of irritation was slight erythema observed in one study (Mercier, 1990) in two out of six animals (mean severity score 0.33) at the one hour time point.

The DS did not propose classification.

Comments received during public consultation

No comments were received relating to skin corrosion and/or irritation.

Assessment and comparison with the classification criteria

Relevant average scores for erythema and oedema were 0 and thus below the trigger

value of 2.3 (as specified in the CLP criteria). Any effects observed were not sufficiently severe or persistent in any individual animal. No classification is required under CLP.

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

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal Skin sensitisation was investigated in three Guinea pig maximisation tests (GPMT) performed according to OECD TG 406 and GLP (Hagemann, 1991; Mercier, 1991 and Kynoch & Parcell, 1988). The results are summarised in table 15 of the CLH report. All studies were conclusive (2 were negative with respect to sensitisation, the 3rd showed a weak response of 20%), and supported non-classification. The DS did not propose classification. **Comments received during public consultation** No comments were received relating to skin sensitisation. **Assessment and comparison with the classification criteria** *Criteria for skin sensitisation on the basis of the GPMT:* If a test substance is present at > 1% for intradermal induction and the incidence of sensitisation is ≥ 30% then potency is judged to be moderate and the predicted sub-category should be 1B. The findings from the relevant studies are assessed below. (1) Hagemann (1991): an approximately 5% response rate (1/19) with an intradermal induction of 5% terbuthylazine. The CLP criteria for classification are not fulfilled. (2) Mercier (1991): a zero response rate was observed with an intradermal induction concentration of 0.00085%. The DS rightly commented on the extremely low concentration of the test substance and this raises concern over the quality of this particular study. The CLP criteria for classification are not fulfilled. (3) Kynoch & Parcell (1988): a 20% response rate (4/20) was observed with an intradermal induction of 50% terbuthylazine. The CLP criteria for classification are not fulfilled. The sensitisation response was < 30 % in all guinea-pig maximisation studies. Therefore, no classification is required under the CLP Regulation.

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

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

Summary of the Dossier submitter's proposal

Repeated dose toxicity has been investigated extensively via the oral route in mice, rats, dogs and rabbits and via the dermal route in rats and rabbits.

There is clear evidence for significant, consistent and identifiable toxic effects in experimental animals across all species tested. According to the DS, because the effects on body weight (the primary effect) 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.

Short-term Studies:

No clear target organ or tissue was identified from the short-term toxicity studies with terbuthylazine. However, a consistent set of effects on body weight parameters are noted from short-term oral and dermal administration including reduced body weights, reduced body weight gains and decreased food consumption.

Severe reductions in body weight indices were seen at high dose levels. Poor palatability of diets containing the test material has not been convincingly demonstrated. Indeed, it is notable that reduced weight gain and food consumption were seen in dermal toxicity studies (occlusive, 6 hours on 5 days/week over 4 weeks) in the rat and rabbit where palatability of the test material was not an issue. Recovery of body weight was seen following withdrawal of dermally applied test material. There was no evidence of local dermal irritation at the application sites.

There were some generalised effects, particularly at the highest dose levels – altered red blood cell parameters in oral studies in the rat and mouse, effects on white blood cells in rats and some effects on liver, kidney and ovarian weights. Increased blood urea nitrogen (BUN) with urinalysis effects in a number of studies indicated possible renal toxicity. Non-specific signs of toxicity were observed in the rabbit following oral administration at comparatively low dose levels; reduced thymus weights were noted at high dose levels. Clinical signs and reduced thymus weight were also observed in dermal studies in the rabbit. Effects in the rat following dermal administration were limited to reduced body weights and food consumption. Reduced ovary weight was also noted at a high dose level.

Lethality and early termination were also documented in the CLH report at high dose levels in both rabbits and rats.

Chronic Studies:

The chronic toxicity and carcinogenicity of terbuthylazine was investigated in three studies in the rat and three studies in the mouse. Reduced weight gain and food consumption were seen in all studies. Chronic administration of terbuthylazine did not increase mortality. Signs of toxicity were non-specific in nature across multiple organ systems.

In the three chronic rat studies available, the main effect was a significant reduction in body weight observed from low doses (> 1 mg/kg bw/d). 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/d 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/d (Ramesh, 2001).

In all the three chronic studies, a reduction in body weight was the main effect. In two of the three studies, effects on body weight were observed at doses < 20 mg/kg bw/d (Kumar (2000), Frankhauser (1999)), whereas in the other study effects were only noted at the top dose (79 mg/kg bw/d; Gfeller, 1982). Other effects observed included a dose-

related increase in pituitary weight in females (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/d in males/females; Kumar, 2000).

Comments received during public consultation

Two MS commented specifically on the classification for STOT RE 2 – H373.

In both cases the comments either questioned the validity of classification based solely on body weight effects or considered that the data presented did not justify a STOT RE classification. There was general agreement that the decreases in food consumption, body weight and body weight gain were treatment related in all species.

The DS argued in their response to the comments that the severity of the body weight effects could not be ignored and therefore they were considered relevant for classification.

Assessment and comparison with the classification criteria

The oral guidance cut-off values for a classification for STOT RE in category 2 under CLP are: ≤ 300 mg/kg bw/d from subacute studies in rat (28 days), ≤ 100 mg/kg bw/day from subchronic studies in rat (90 days), ≤ 25 mg/kg bw/day from one year studies and ≤ 12.5 mg/kg bw/day from long term studies. If dermal studies are used then the cut-off values are 2-fold greater.

A substance should be classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity following repeated exposure by the oral, dermal or inhalation routes at or below the guidance values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

A summary of all the short-term and long-term study data with respect to body weight parameters is presented below. See also table 17c in the CLH report for a direct comparison of significant weight changes with classification trigger values. The reductions in body weight parameters as a consequence of exposure to terbuthylazine determines the LOAEL in many cases.

Table: Summary of relevant NOAELs and LOAELs from repeated dose short-term toxicity studies. Values in bold are below the cut-off criteria for STOT RE 2.

Study	Notifier	NOAEL	LOAEL	Effects at LOAEL (M/F)	Reference
		mg/kg bw/d			
Oral studies					
Rat 28-day	Oxon	M: -	**35**	↓ body weight (27/18%), ↓ weight gain (55/50%) ↓ food consumption (25/24%)	Hopkins, 1988
		F: -	**39**		
Rat 60-day	Oxon	M: 10.7	**38.1**	↓ body weight (22%) & organ weight effects	Ramesh, 1999
		F: 2	**5.0**	↓ weight gain (14%)	
Rat	Oxon	M: 4	**8.0**	↓ weight gain (19%)	Kirk, 1990

90-day		F: 9	**18.0**	↓ weight gain (16%)	
Rat **90-day**	Syngenta	M: 2.08 F: 2.13	**7.11** **7.18**	↓ body weight wk 13 (16/11%) haematology & clinical chemistry	Bachmann, 1995
Mouse 14-day	Oxon	M: 75.4 F: 88.7	**320** **304**	↓ body weight gain (40/48%) & food consumption (19/26%)	Krishnappa, 1999
Mouse 28-day	Oxon	M: 120 F:-	315 **43.2**	↓ body weight (11%) & body weight gain (27%) & clinical chemistry (↑ BUN 30%, ↑ Cholesterol 17%) ↑spleen weight (69%)	Suresh, (1996)
Dog **52-week**	Syngenta	M: 0.4 F: 0.4	**1.8** **1.6**	↓ body weight gain (36/48%) & ↓ food consumption (13/17%)	Cope, 1992
Rabbit 28-day	Syngenta	M: - F: -	**20.0*** **20.0***	↓ body weight (18/9%) & ↓ food consumption (30/6.5%)	Seifert, 1984a
Rabbit 28-day	Syngenta	M: 5.0 F: 5.0	- -	No adverse effects	Schiavo *et al*, 1987a
Dermal studies					
Rat **28-day**	Syngenta	10	**100**	↓ body weight (12/6%) & ↓ food consumption (16/4%)	Marty, 1994
Rabbit 28-day	Syngenta	-	**5**	Clinical signs, ↓ body weight (16/13%)& ↓ body weight gain (86/73%) ↓ food consumption 15/8%)	Seifert, 1984
Rabbit 28-day	Syngenta	0.5	**500**	Mortality, clinical signs, ↓ body weight (9/18%) & ↓ food consumption (16/18%)	Schiavo, 1987b

* LOAEL considered more appropriate by RAC

Table: Summary of relevant NOAELs and LOAELs from repeated long-term toxicity studies. Values in bold are below the cut-off criteria for STOT RE 2.

Study	**Notifier**	**NOAEL**	**LOAEL**	**Body weight effects at LOAEL (M/F)**	**Reference**
		mg/kg bw/d			
Rat	Syngenta	M: -	**1.2**	↓ body weight (9/9%),	Gfeller,

2-year		F: -	**1.4**	↓ weight gain (11/21%)	1983a
Rat 2-year	Syngenta	M: 0.35 F: 0.36	**1.6** **1.6**	↓ body weight (4/12%) & organ weight effects	Gfeller, 1983b
Rat 2-year	Oxon	M: 0.4 F: 0.6	**1.7** **2.4**	No effect on body weight at LOAEL	Ramesh, 2001
Mouse 2-year	Syngenta	M: 15.8 F: 3	76.3 15.1	No effect on body weight at LOAEL	Gfeller, 1982
Mouse 18-month	Syngenta	M: 5.6 F: 5.2	58 56	↓ body weight (7/18%) & ↓ weight gain (20/35%) & ↓ food consumption (1/17%)	Frankhauser 1999
Mouse 18-month	Oxan	M: 14.6 F: 15.5	36.7 39.7	↓ body weight (1/3%) & ↓ weight gain (12/8%) &	Kumar, 2000

Cut-off for category 2 (applying Haber's rule): 2-year studies: ≤ 12.5 mg/kg bw/day; 18-month studies: ≤ 16.7 mg/kg bw/day

In addition, the rat sub-chronic (90-day) neurotoxicity study by Moxon (2003), reported in the DAR for terbuthylazine, showed only minor reductions on body weight (< 9%) at a maximum oral dietary dose of 100 ppm (7 and 8 mg/kg bw/d for males and females respectively).

Criteria for Specific Target Organ Toxicity – Repeated Exposure:

Category 1:
"Substances are classified… on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and /or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations."

The criteria for category 1 are not considered fulfilled. There were no human data for terbuthylazine to substantiate this classification. Furthermore, data from animal experiments were unconvincing. In some studies, the DS considered that body weight was significantly reduced at dose levels relevant for classification in STOT RE category 1 (particularly in the rabbit); however, the severity of these effects was marginal so it is very doubtful that the criteria for severe toxic effects are truly met in this instance.

What remains is a consideration of category 2 or no classification.

Category 2:
"Substances are classified… on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations."

As the DS has indicated under section 4.7.1.8 of the CLH report, there is clear evidence of significant, consistent and identifiable toxic effects in experimental animals across all the species tested. The primary effects were on body weight parameters, including absolute body weight, body weight gain and food consumption. Reductions in these

parameters in many cases were observed below the relevant cut-off for classification in all species and in all study durations (except for the short-term mouse studies).

However, no clear target organ or tissue was identified for the repeated dose toxicity of terbuthylazine. There is no data as to the mechanism responsible for the significant reductions in body weight parameters. Unpalatability seems unlikely to be the sole determinant of these effects because they are also observed following oral gavage and dermal (occlusive) administration of the test substance to rabbits.

According to the DS, because the effects on body weight 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.

RAC considers that there is no doubt that the decrease in food consumption, body weight and body weight gain are treatment related in all species and that the effects are observed following dietary, oral gavage and dermal exposures.

According to Annex 1: 3.9.2.9.4, CLP: "The decision to classify at all can be influenced by reference to the dose/concentration guidance values at or below which a significant toxic effect has been observed". In this regard a significant toxic effect is clearly demonstrated in a consistent manner across all species tested, by both the oral and dermal routes. Accordingly RAC concluded that classification as STOT RE 2 – H373 is justified.

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	- 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	Seeberg (1988a)

50 - 800 ug/plate Batch 29 (97 % purity)		Positive controls included	
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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Terbuthylazine was tested in a wide battery of *in vivo* and *in vitro* studies. All studies were performed according to appropriate contemporary guidelines, with the exception of the *in vitro* UDS assay by Seeburg (1988b). All of the submitted studies were negative, with the exception of the mammalian cell mutagenicity (HGPRT) study by Mosesso (1988a) in the absence of metabolic activation. However, the positive response in the study by Mosesso (1988a) was at terbuthylazine concentrations of 600 - 800 µg/mL associated with cytotoxicity. The (HGPRT) mammalian cell mutagenicity study by Hertner, (1995b) was clearly negative. The maximum concentration of terbuthylazine used in the Hertner (1995b) study was 380 µg/mL, which was stated to be the limit concentration and was associated with less marked cytotoxicity. A brief summary is presented below, the CLH report details each specific study in tables 18a and 18b.

Table: Summary of genotoxicity tests

Study	Notifier	Test system	Result	Reference
In vitro				
Bacterial mutagenicity	Syngenta	TA98, TA100, TA102, TA1535, TA1835; *E. coli* WP2*uvrA*	Negative	Hertner, 1995a
	Oxon	TA1535, TA1537, TA1538,	Negative	Forster, 1988

		TA98, TA100		
	Oxon	TA1535, TA1537, TA 98, and TA100; *E. coli* WP2*urvA*	Negative	Bowles, 2009
Mammalian cell mutagenicity	Syngenta	V79 (HGPRT locus)	Negative	Hertner, 1995b
	Oxon	V79 (HGPRT locus)	Positive (-S9) Negative (+S9)	Mosesso, 1988a
Clastogenicity	Syngenta	CHO cells	Negative	Hertner, 1995c
	Oxon	CHO cells	Negative	Seeberg, 1988a
UDS	Oxon	HeLa cells (Study unacceptable*)	Negative	Seeburg, 1988b
In vivo				
Micronucleus	Syngenta	Mouse (Tif:MAGf) bone marrow	Negative	Hertner, 1989
	Oxon	Mouse (Swiss CD-1) bone marrow	Negative	Mosesso, 1988b

* This study deviated from the relevant OECD TG (1986) because 3 (rather than 6) cultures were used, and adequate assay sensitivity was not obtained using the positive control benzo(a)pyrene in the presence of S9.

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

The DS concluded that terbuthylazine was not mutagenic *in vitro* or *in vivo*. No classification was proposed.

Comments received during public consultation

No comments were received relating to germ cell mutagenicity.

Assessment and comparison with the classification criteria

No human data are available for terbuthylazine, therefore a classification with Muta. 1A is not supported. Terbuthylazine is not positive in *in vivo* somatic cell mutagenicity tests in mammals and thus does not trigger classification as Muta. 1B.

Despite the single positive *in vitro* result (mammalian cell mutagenicity; Mosesso, 1988a) the two respective *in vivo* studies and remaining *in vitro* studies showed a clear negative outcome, hence a classification in category 2 is precluded.

The RAC agrees with the conclusion of the DS that 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)

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Neoplastic findings
<table>
<tr><th colspan="9">Neoplastic findings</th></tr>
<tr><td></td><td colspan="4">Male</td><td colspan="4">Female</td></tr>
<tr><td>Dose</td><td>0</td><td>30</td><td>150</td><td>750</td><td>0</td><td>30</td><td>150</td><td>750</td></tr>
<tr><td>Group size (interim and terminal)</td><td>79</td><td>79</td><td>80</td><td>80</td><td>80</td><td>80</td><td>80</td><td>80</td></tr>
<tr><td colspan="9">Mammary gland</td></tr>
<tr><td>Carcinoma</td><td>2</td><td>1</td><td>2</td><td>0</td><td>4</td><td>9</td><td>3</td><td>14*</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control: Range 4/80 – 17/80</td></tr>
<tr><td>Fibroadenoma</td><td>2</td><td>0</td><td>1</td><td>0</td><td>16</td><td>17</td><td>9</td><td>8</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control: Range 19/80-37/80</td></tr>
<tr><td>Adenoma</td><td>0</td><td>1</td><td>0</td><td>0</td><td>3</td><td>4</td><td>2</td><td>1</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control: Range 0/80 – 4/80</td></tr>
<tr><td>Carcinosarcoma</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td></td><td colspan="8">Laboratory historical control: no data</td></tr>
<tr><td>Total</td><td>4</td><td>2</td><td>3</td><td>0</td><td>23</td><td>30</td><td>15</td><td>23</td></tr>
<tr><td>Leydig cell tumours</td><td>3</td><td>4</td><td>2</td><td>10</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="8">Laboratory historical control: 0-7.5 %</td></tr>
</table>
* 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)
</td>
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<tr>
<td>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</td>
<td>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.</td>
<td>Gfeller (1983b)</td>
</tr>
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<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).

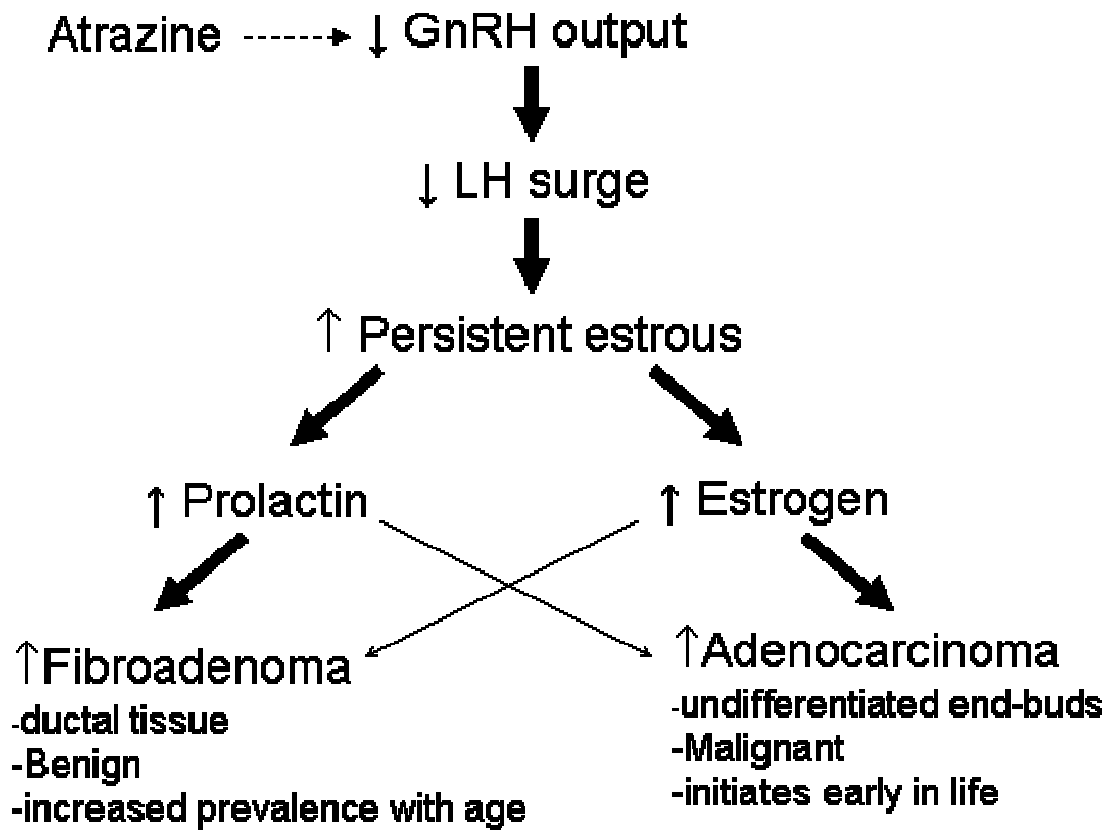


Figure 1. Key events associated with the earlier appearance and increased incidence of mammary tumours in atrazine-treated female SD rats. Figure produced from Simpkins et al (2011).

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))

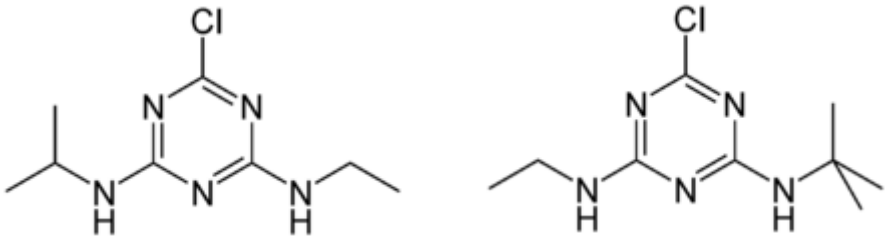
Parameter	*SD rat*	*Women*
Start of senescence (% of normal lifespan)	30-40%	60-70%
Principle cause of senescence	Hypothalamic failure to stimulate LH/FSH	Depletion of ovarian follicle content
LH surge capability	Lost	Maintained
Predominant cycle pattern	Persistent oestrus	Menopause
Oestrogen/progesterone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



Atrazine 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

RAC evaluation of carcinogenicity
Summary of the Dossier submitter's proposal
The chronic toxicity and carcinogenicity of terbuthylazine was investigated in two studies in the rat (a third study was supplementary to the Gfeller 1983a study and cannot be considered suitable for the determination of carcinogenic potential by itself because of the limited number and low doses of terbuthylazine investigated), and three studies in the mouse. In common with the short-term studies, reduced weight gain and food consumption

were observed in all studies. Chronic administration of terbuthylazine did not increase mortality; signs of toxicity were non-specific and no single or specific target organ or tissue was identified. The DS has summarised the key data for all six long-term studies in table 19 of the CLH report.

There was no evidence of carcinogenicity in any of the three mouse studies.

The two primary chronic toxicity and carcinogenicity studies in rats (Gfeller, 1983a, SD-derived strain, and Ramesh, 2001, Han Wistar strain) had findings of increased incidences of mammary gland tumours. In addition, the Gfeller (1983a) study alone showed an increased incidence of Leydig cell tumours in SD rats. The Gfeller (1983b) study was designed to complement the previous study by Gfeller (1983a) and to establish a NOAEL for the SD-derived rat strain exposed to lower levels of terbuthylazine than those used in the original study.

(1) Relevant Historical Control Data

The incidences of the observed tumours implied a treatment related response. Laboratory historical control data were available for both primary rat studies.

Mammary gland tumours:
Mammary gland tumours are a common spontaneous tumour in female SD rats (NTP, 2005) and the incidences in the Gfeller (1983a) study were reported to be within the laboratory historical control range (top dose of 53 mg/kg bw/day with 14/80 relative to 4/80 with concurrent controls; historical control range: 4/80 – 17/80, mean 8.7/80 from seven studies initiated between 1979 - 1986).

The incidence of mammary gland tumours in the Han Wistar rats of the Ramesh (2001) study was above the laboratory historical control range at the top female dose of 7.6 mg/kg bw/d (16% vs 2% in concurrent controls; historical control mean 4.9% and range 0-12%); see table with historical control data below. On this basis, the DS originally concluded that a treatment related effect could not be ruled out.

Table: Historical control data for female Han Wistar Rats presented in the DAR for the Ramesh (2001) study (G1 = controls, G2 = 0.6, G3 = 2.4 and G4 = 7.6 mg/kg bw/d)

Mammary tumour incidence

1807 (Present study)

	G1		G2		G3		G4	
No. of rats examined	50		49		49		49	
	A	B	A	B	A	B	A	B
1. Adenomas	1	2	3	6	1	2	1	2
2. Fibroadenomas	6	12	7	14.3	10	20.4	6	12.2
3. Adenocarcinomas	1	2	4	8.2	4	8.2	8+	16.3+
4. Total mammary tumours	8	16	14	28.6	15	30.6	15	30.6

A: Incidence B: Percentage incidence
+: Significantly higher(+) than the control group

In house historical data: (Number examined 350)

	Incidence (%)	Range(%)
1. Adenomas	0.9	0 - 2
2. Fibroadenomas	8.9	0 - 18
3. Adenocarcinomas	4.9	0 - 12
4. Total mammary tumours	15.4	4 - 23

Published historical data:

1. Bayer[1] - (Number examined 4060)

	Incidence (%)	Range(%)
1. Adenomas	1.0	0 - 10
2. Fibroadenomas	6.8	0 - 32
3. Adenocarcinomas	3.3	0 - 22
4. Total mammary tumours	11.1	0 - 50

2. Parke-Davis[2] - (Number examined 685)

	Incidence (%)	Range(%)
1. Adenomas	Not available	2 - 6
2. Fibroadenomas	25.3	Not available
3. Adenocarcinomas	13.1	Not available
4. Total mammary tumours	42.8	Not available

References:

1. Eiben R. and Bomhard EM. : Trends in mortality, body weights and tumour incidences of Wistar rats over 20 years. Exp Toxic Pathol 1999;51:523-536
2. Walsh KM and Poteracki J. : Spontaneous neoplasms in control Wistar rats. Fundam Appl Toxicol 1994 Jan ; 22(1): 65-72.

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Testicular Leydig Cell tumours:

The incidence of Leydig Cell tumours in the SD rats of the Gfeller (1983a) study was above the laboratory historical control range at the top male dose of 42 mg/kg bw/d (12.5% vs 3.8% in concurrent controls; historical control range 0-7.5%), as shown in the table below. Higher incidences have been reported in other laboratories and in the Registry of Industrial Toxicology Animal (RITA) database.

Table: Comparison of Gfeller Leydig Cell Tumour incidenses with historical controls and other sources.

Data source	Range of Leydig cell tumour incidence (%)
750 ppm terbuthylazine (Gfeller, 1983a)	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

(2) Mammary Gland Tumours

Mammary tumours were observed in two carcinogenicity studies, one in SD rats (Gfeller, 1983a) and one in Han Wistar rats (Ramesh 2001).

Gfeller, (1983a) Study – SD Rats
In a chronic toxicity and carcinogenicity study with SD-derived rats (Gfeller, 1983a), an increased incidence of mammary gland adenocarcinoma was noted for female rats receiving the highest dose of terbuthylazine tested (53 mg/kg bw/d). The Gfeller (1983a) study was a non-guideline 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). The incidences were reported to be within the laboratory historical control range (top dose 14/80; historical control range: 4/80 – 17/80).

Ramesh, (2001) Study – Han Wistar Rats
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 (7.6 mg/kg bw/d). The incidence of mammary gland tumours in Wistar rats was above the laboratory historical control range at the top dose (16%; historical control mean = 4.9 % with range 0-12 %).

The DS dismissed the tumours in the SD-derived rats as being non-relevant to humans on the basis of chemical structural similarity of terbuthylazine to other chlorotriazines and in particular from read-across with atrazine and the well characterised mode of action (MoA) of atrazine in disturbing the hypothalamic-pituitary-ovary axis in this strain of rat.

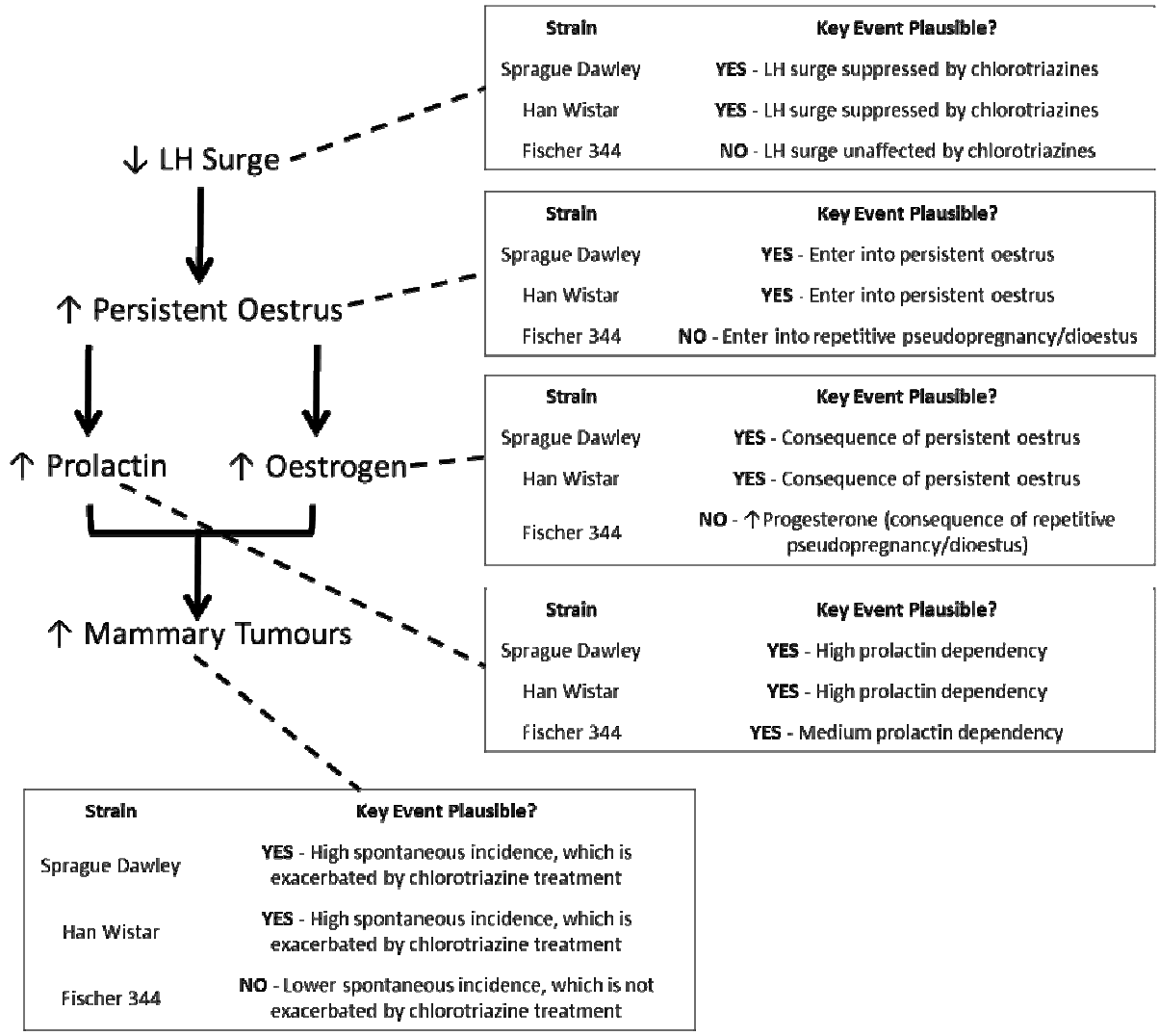
Originally the DS could not dismiss the findings observed for the Wistar rat tumours due to only limited data being available at the time of the drafting of the CLH report showing that chlorotriazines might have a comparable effect on the hypothalamic-pituitary-ovary axis in Wistar rats as in SD rats. This position of the DS changed with the introduction of new data reported subsequent to completion of the CLH report and submitted during public consultation.

Mode of Action

The MoA for mammary gland tumours in the rat involves 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 oestrogen and prolactin, and development of tumours in response to these prolonged hormone exposures.

This MoA assumes that there are significant species and/or strain differences with respect to (1) sensitivity and magnitude of the LH response to terbuthylazine and (2) differences in the process of reproductive senescence. New data supports this proposed mechanism of action for both terbuthylazine and atrazine in SD and Wister rats, but not in Fischer 344 rats. An outline of the MoA is presented in the figure below. See also Annex I of the CLH report.

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



Human Relevance of the Rodent Mammary Gland Tumours:

The MoA of atrazine (Simpkins *et al*., 2011) and terbuthylazine in producing mammary tumours is considered not relevant to humans because the preovulatory LH surge mechanism is different in humans and other primates compared to rodents when at normal reproductive age and at reproductive senescence:

1. In rats, a brief LH surge leads to ovulation. This occurs during a critical 2-hour period on the afternoon of proestrus when GnRH surges in response to increasing plasma oestrogen levels. GnRH is deterministic in this case.

2. By contrast, ovulation in humans occurs in the absence of a GnRH surge, in response to an oestrogen-stimulated increase in pulsatile GnRH/LH release from the pituitary lasting for 2–3 days. The role of GnRH is permissive with respect to ovulation in humans, allowing the pituitary gland to respond to circulating oestrogens.

3. In rodents, the GnRH surge is deterministic in the timing of the LH surge. Ageing rats lose the ability to mount an LH surge, but ageing humans retain the ability to produce GnRH and pituitary gonadotropins such as LH and FSH.

4. The ovary in the ageing woman becomes unresponsive due to the lack of responsive follicles, and oestrogen production falls.

5. Ageing rat ovaries continue to produce oestrogen.

Suppression of the LH surge (via effects on GnRH release) is a crucial key event for the induction of mammary tumours in SD 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). Both SD and Han Wistar rats primarily enter into a state of constant oestrus following atrazine and terbuthylazine exposure (and this may be viewed as akin to early-onset of reproductive senescence in these rat strains, i.e. similar to hypothalamic failure to stimulate pituitary release of LH). Therefore, greater oestrogenic exposure in these strains from an earlier timepoint that is usually associated with normal reproductive senescence (i.e. < 12 months of age), is considered a causal key event contributing to the development of mammary tumours.

There are several reasons to suggest that the disruption of the LH surge as outlined for selected rat strains and resulting in an increased incidence of mammary tumours when treated with terbuthylazine is not relevant to humans:

1. In rats, alterations by triazines in the LH surge appear to be the result of an effect on the hypothalamus, reducing the release of GnRH. In humans, the signal for initiating the LH surge is a feedback mechanism driven by oestradiol released by the ovary, and therefore reductions in GnRH are unlikely to impact on LH in humans in the same way as they do for the rat.

2. Atrazine for example, does not directly affect LH secretion from the pituitary in the rat. In humans, the ovary and pituitary play a more central role in the hormonal control of the menstrual cycle, disruption of hypothalamic signalling may not have the same effect as in rats. For example, women respond to reduced levels of LH by reductions in levels of oestrogen, not increases as seen in the rat.

3. The causes of reproductive senescence are different between rats and humans. In rats, exposure to atrazine for example, leads to a reduction in GnRH signalling, and suppresses the LH surge. The failure to ovulate results in persistent secretion of oestrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin. As a result, atrazine accelerates the normal reproductive ageing process in female SD rats and is characterised by persistent exposure to oestrogen and prolactin. The persistent oestrus in dams leads to premature reproductive failure. In humans, reproductive senescence is not due to changes in GnRH signalling or LH but to reduced numbers of eggs and follicles in ageing ovaries and the lack of hormonal feedback to the pituitary.

4. The timing and control of ovulation in humans is not linked to a circadian signal

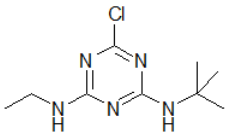
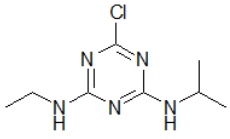
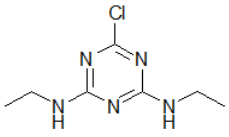
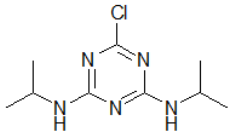
like it is in the rat and the primary site for feedback from oestradiol is the pituitary, not the preoptic area of the hypothalamus which is the feedback site in rats.

There were no data to determine whether there is general relevance for the human hormonal cycle at different life stages with exposure to terbuthylazine.

Relevance of Structural Similarity Amongst the Chlorotriazines:

Chlorotriazine herbicides share a very high degree of structural similarity (see table below):

Table: An illustration of the high degree of structural similarity across all the chlorotriazines.

Chlorotriazine	Terbuthylazine	Atrazine	Simazine	Propazine
Structure				

The comparison of terbuthylazine with other chlorotriazines such as atrazine is important because Industry originally proposed to read-across the information available for atrazine to terbuthylazine in support of no classification. This was based on there being a substantial body of evidence from studies conducted with atrazine that suggest effects such as mammary tumours are of low or questionable significance to humans. Investigations of other MoA did not provide any evidence that atrazine had intrinsic oestrogenic activity or that it increased aromatase activity *in vivo*. However, it must be pointed out that an indepth evaluation of the atrazine data is beyond the scope of this opinion document. A brief look at the published data indicates that atrazine decreases the latency of mammary adenocarcinomas and fibroadenomas, increases the incidence of these tumours in female SD rats, has no effect in female F344 rats or in mice (C57BL/6, CD-1, C3H/Anf) and is not genotoxic, nor does it display any significant oestrogen receptor binding.

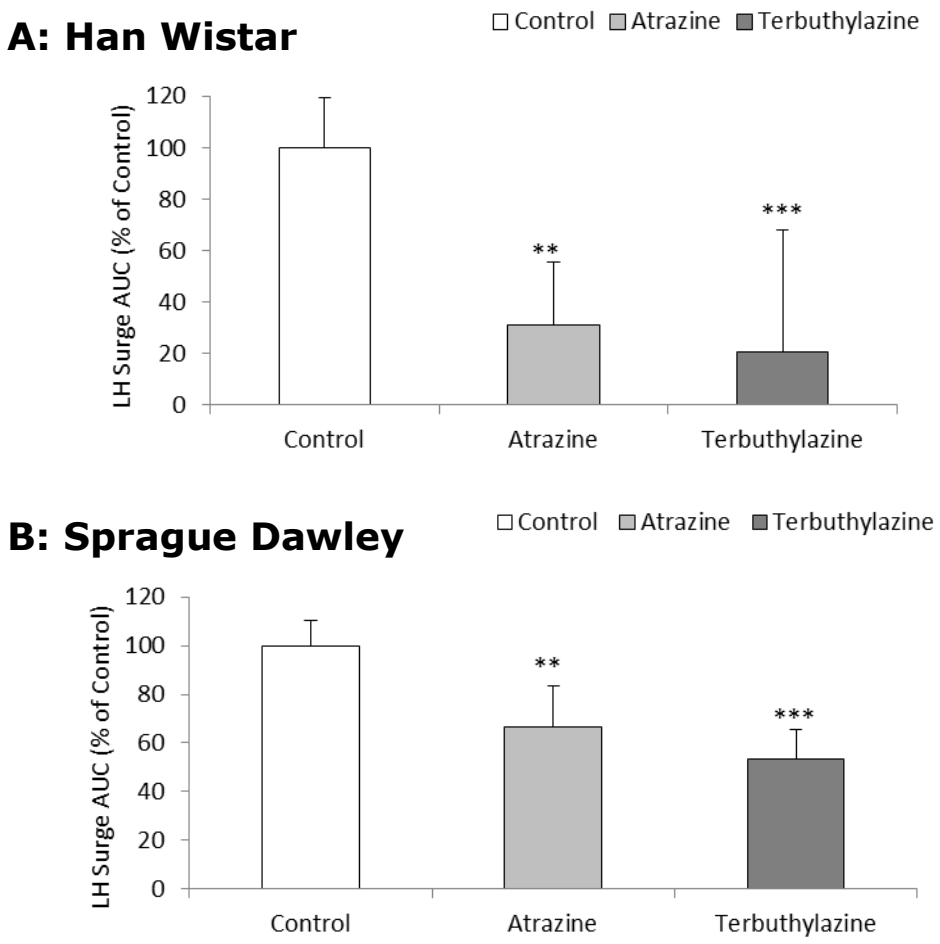
It is because of a number of shared features (herbicidal MoA, similar toxicology, chemical structural similarity), that chlorotriazines can be reasonably considered as a common mechanism group with respect to their biological toxicity and tumourigenicity; indeed this has been accepted by the US EPA. The tumours in SD rats (Gfeller, 1983a) were dismissed by the DS due to the similarities in the effects between terbuthylazine and atrazine. In addition, the tumour incidences for terbuthylazine fell within the historical control range for this particular rat strain. The DS originally argued in favour of classification for carcinogenicity, stating that this was warranted based on residual concern for mammary gland tumours in Wistar rats (Ramesh, 2001) and due to the limited data available supporting the assertion that chlorotriazines have a comparable effect on the hypothalamic-pituitary-ovary axis in Wistar rats to that in SD rats.

New Mechanistic Data:

New mechanistic studies have been conducted with terbuthylazine and atrazine in both ovariectomized SD and Han Wistar rats that support a common mechanism of action involving the perturbation of the hypothalamic-pituitary-ovary axis. These studies were not assessed by the DS in the CLH report because they became available during public consultation. The DS urged a careful consideration of the results in the RCOM document and considered that terbuthylazine has a similar effect on the LH surge as atrazine in both SD and Wistar rats. The DS concluded that the new data support the argument that the tumours in Wistar rats also occur by a mechanism not relevant to humans. The data are summarised below.

Terbuthylazine suppresses the LH surge in both SD and Han Wistar rats:
Suppression of the LH surge (via effects on GnRH release) is a crucial key event for the induction of mammary tumours in SD rats by chlorotriazines such as atrazine, with a lack of an effect on this parameter being the reason why chlorotriazines do not induce mammary tumours in for example, Fischer 344 rats (Simpkins *et al*., 2011). Mode of action work with atrazine has demonstrated that it can inhibit the LH surge in both SD (Simpkins *et al*., 2011) and Han Wistar (Foradori *et al*., 2009) rats, but not in Fischer 344 rats (Simpkins *et al*., 2011).
Furthermore, recently completed MoA studies have demonstrated that both atrazine and terbuthylazine suppress the LH surge in both SD and Han Wistar rats and that the levels of suppression are comparable when the two chlorotriazines are dosed in equimolar quantities (100 mg/kg bw/d and 106.5 mg/kg bw/d for atrazine and terbuthylazine, respectively) (Handa, 2014; Stump, 2014). A summary of these data are presented in the figure below. Further detail of the Handa, (2014) and Stump, (2014) studies are presented under "Additional Key Elements" in the background document.

Figure: Atrazine and terbuthylazine both suppress the LH surge in (A): Han Wistar (Handa, 2014) and (B): SD (Stump, 2014) rats.



Atrazine and terbuthylazine were dosed at molar equivalent doses (100 and 106.5 mg/kg bw/d for atrazine and terbuthylazine, respectively).

Data are area under the curve (AUC) values presented as % of Control.

** and *** statistically significantly different from control with $p<0.01$ and $p<0.001$, respectively

No statistically significant differences were observed between atrazine and terbuthylazine treated groups.

In summary, the MoA for chlorotriazine-induced mammary tumours in female SD rats can be extrapolated to Han Wistar rats, based on their shared sensitivity to chlorotriazine-mediated suppression of the LH surge. The similarities and differences amongst rat strains are briefly summarised in the table below.

Table: 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).

	SD	**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

(3) Leydig Cell Tumours:

An increased incidence of Leydig cell tumours was observed in top dose SD male rats (42 mg/kg bw/d). However, there was no such increase in the study conducted with Wistar rats (conducted at lower dose levels). The DS clearly outlined their view that the apparent increase in Leydig cell tumours in male SD rats was an artefact of increased survival in the high dose group. Details of the notifiers' position which was in agreement with that of the DS, were given in Annex II of the CLH report. In table d of section 4.10.1.1 of the CLH report, the DS shows when Leydig Cell tumours occurred during the *Gfeller*, (1983a) study. This table shows two important pieces of information:

1. Increasing dose leads to increased survival of animals to scheduled sacrifice, and
2. The majority of Leydig Cell tumours observed in top dose animals occurred at or beyond the two year time point (6 tumours vs. 1 in controls).

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.

Human Relevance of Leydig Cell Tumours:

The incidence of these tumours was above the historical control level. However, interpretation is confounded by the difference in study durations of this study and the studies used to provide historical control range data. A revised statistical analysis (Annex II, Appendix 1 of the CLH report) confirms that 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. 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 benign tumours, their increased incidence in this case is a consequence of the increased survival in the top dose group and not a treatment related effect. The DS considers there were no treatment related carcinogenic effects in Leydig cells of rats and no potential concern to human health.

(4) Summary:

The DS proposed classification as Carc. 2; H351 because of the high incidence of mammary tumours at the top dose of the Ramesh, (2001) study that used the Han Wistar strain of rat. It was only during public consultation that new data became available supporting the assertion that terbuthylazine has a comparable effect to that of atrazine on the hypothalamic-pituitary-ovary axis in Wistar rats and in SD rats.

Comments received during public consultation

Several comments were received from a variety of sources, including industry, trade associations, three MS and a statement from a group of individual scientists originally asked by Syngenta to review the original study reports. There was no support for classification amongst most of the comments. Of the three MS who commented, two did not support classification, and one MS supported the original DS proposal for classification but apparently without knowledge of the new data supporting the same MoA for mammary tumours in Wistar and SD rats upon treatment with terbuthylazine. These new data has been summarised under the "Summary of the dossier submitter's proposal" above (under the heading New mechanistic data in the section Mammary gland tumours).

Additional key elements

Detailed summary of the studies by Stump (2014) and Handa (2014), submitted during public consultation, are presented below.

(1) Stump, 2014.

This is an investigative study with no applicable guidelines. The study was conducted in compliance with the United States EPA GLP Standards (40 CFR Parts 160 and 792), 16 October 1989 and 18 September 1989, respectively; the OECD Principles of GLP [C(97) 186/Final], 26 November 1997. The hormone analysis conducted by The University of Arizona Phoenix was not conducted according to GLP standards.

Justification for Test System Selection: The SD rat was used as the test system on this study. This species and strain of animal is recognized as appropriate for reproduction studies as well as for toxicity studies with atrazine. The number of animals selected was selected to ensure a final minimum number of at least 15 blood samples per group for LH surge assessment. This was the minimum number of samples required for meaningful analysis of the hormone and plasma analysis data.

Four previous studies conducted at WIL Research (Sawhney Coder 2010, 2011a, 2011b, 2011c) have demonstrated that atrazine administered by oral gavage at doses of 50 or 100 mg/kg/day for 4 days suppressed the oestrogen-induced LH surge in intact as well as ovariectomized, oestrogen-induced female SD rats (approximately 13 week old). Foradori *et al.* (2009) reported that 4 days of atrazine administration in approximately 13-week old female Wistar rats also suppressed the oestrogen-progesterone induced LH surge. As the purpose of the current study was to assess the effects of terbuthylazine administered for 4 days on the estrogen-induced LH surge in ovariectomized young-adult SD rats, atrazine was used as the positive control substance for comparison.

The selected route of administration for this study was oral (gavage) because this is a potential route of exposure for humans. Historically, this route has been used extensively for studies of this nature.

SUMMARY

In a study to assess the effects on the hormone-induced LH surge in ovariectomized female rats, three groups of 23, 20 and 21 ovariectomized female SD rats were dosed by oral gavage with 1% carboxymethylcellulose in deionized water (vehicle control group), 100 mg atrazine per kg bw/d (positive control group) or 106.5 mg terbuthylazine per kg bw/d (molar equivalent of 100 mg/kg atrazine), respectively. The animals were ovariectomized and a subcutaneous silastic capsule containing 4 mg/mL of estradiol benzoate in sesame oil was implanted to induce afternoon light-entrained, LH surges. On the fourth day following ovariectomy, plasma samples were taken (over the course of 12 hours) and analysed for LH levels using radioimmunoassay.

Treatment of ovariectomized female SD rats with terbuthylazine resulted in a statistically significant reduction in the estradiol-induced LH surge with a significant main effect of treatment, time of day, and an effect of interaction between time of day and treatment. The peak LH level of the terbuthylazine treated group was reduced to approximately 47% of the control levels while the peak LH level of the atrazine treated group was reduced to approximately 69% of the control level. Both terbuthylazine and atrazine significantly decreased the area under the curve (AUC) of the LH surge.

Adverse effects, including increased mean body weight losses, lower mean food consumption, and lower mean body weights, were noted in the 106.5 mg/kg bw/d terbuthylazine group. Similar effects on body weight loss, food consumption, and body weight were noted for females in the 100 mg/kg bw/d atrazine group.

RESULTS AND DISCUSSION

Effects on the luteinizing hormone surge: Terbuthylazine at the same molar equivalent dose as atrazine (positive control) showed a significant reduction in the oestrogen-induced LH surge. Two-way ANOVA (treatment x time) analysis of plasma LH using all data points showed significant main effects of treatment [$F_{2,308}$ = 9.976, $p < 0.0001$], and time [$F_{5,308}$ = 22.71, $p < 0.0001$], and an interaction effect [$F_{10,308}$ = 3.649, $p = 0.0001$]. A significant decrease in LH was observed for atrazine and terbuthylazine compared to the vehicle control at the 16.00 h time point based upon a Bonferroni post-hoc analysis (see table below). There was also a significant decrease at the 18:00 h sampling time point for both atrazine and terbuthylazine compared to the vehicle control.

Table: Summary of plasma luteinizing hormone (LH) levels (ng/mL)

Treatment group		**Time of day**					
		11:00	**14:00**	**16:00**	**18:00**	**20:00**	**23:00**
Vehicle	Mean	0.15	0.40	1.71	1.31	0.81	0.18
	SEM	0.01	0.05	0.28	0.23	0.08	0.01

	N	20	20	20	20	20	17
100 mg/kg bw atrazine	Mean	0.14	0.22	0.72***	0.74*	1.03	0.17
	SEM	0.01	0.03	0.23	0.18	0.26	0.01
	N	19	19	18	18	18	14
106.5 mg/kg bw terbuthylazine	Mean	0.14	0.16	0.56***	0.64**	0.77	0.16
	SEM	0.004	0.02	0.09	0.11	0.13	0.01
	N	17	17	18	18	17	15

Bonferroni Post-hoc Analysis - statistically significance * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

Peak LH level of the terbuthylazine treated group was approximately 47% of the vehicle control group and the atrazine-positive control group peak LH was approximately 69% of the vehicle control group. There was a significant reduction in peak LH amplitude between treatment groups compared to vehicle controls group [$F_{2,54} = 5.47$, $p = 0.0192$]. Post hoc analysis using Bonferroni's multiple comparison tests showed significant reductions ($p<0.05$) in the terbuthylazine group compared to vehicle control but no significant difference between the vehicle control and atrazine groups.

Terbuthylazine as well as atrazine significantly reduced the LH AUC compared to the vehicle group [$F_{2,46} = 97.40$, $p = 0.0023$]. Post-hoc analysis with Bonferroni's multiple comparison tests showed significant reductions in LH AUC in the atrazine ($p<0.05$) and terbuthylazine ($p<0.01$) groups versus vehicle controls with no difference between the terbuthylazine and atrazine groups (see table below).

Table: Summary of plasma luteinizing hormone (LH) level (ng/mL): LH peak and LH AUC

Treatment group	**Peak LH amplitude**		**LH area under the curve**	
Vehicle	Mean	2.04	Mean	9.36
	SEM	0.29	SEM	0.99
	N	20	N	20
100 mg/kg bw atrazine	Mean	1.41	Mean	6.24*
	SEM	0.32	SEM	1.04
	N	18	N	18
106.5 mg/kg bw terbuthylazine	Mean	0.96*	Mean	4.99**
	SEM	0.11	SEM	0.61
	N	17	N	17

One-way ANOVA - statistical significance* ($p<0.05$), ** ($p<0.01$)

Mortality and clinical observations:

No terbuthylazine-related deaths, moribundity, or clinical observations were noted (one female was found dead and two females were euthanized *in extremis*). Clinical findings of pale and cool extremities, a cool and pale body, red material around the nose, and yellow material around the urogenital area were observed. The death, moribundity and clinical findings were attributed to the surgical procedure.

No atrazine-related death or moribundity was noted.Two females were found dead due to the surgical procedure. Clinical findings were noted infrequently and/or at similar frequencies to the control group.

Body weight and food consumption:

Terbuthylazine- or atrazine-related greater mean body weight losses, with corresponding lower mean food consumption, were noted for females in the 106.5 mg/kg bw/d terbuthylazine and 100 mg/kg bw/d atrazine groups, when compared to the control group

during the first 2-3 days of treatment (see table below). As a result, lower mean body weights (up to 7.2% and 4.9%, respectively) were recorded.

Table: Summary of body weight changes and food consumption

Treatment group	**Mean body weight change (g): days 0-4**		**Mean food consumption (g/rat/day): days 0-4**	
Vehicle	Mean	-11	Mean	16
	SD	6.1	SD	1.6
	N	20	N	20
100 mg/kg bw atrazine	Mean	-23**	Mean	13
	SD	9.4	SD	2.5**
	N	18	N	18
106.5 mg/kg bw terbuthylazine	Mean	-28**	Mean	10**
	SD	11.3	SD	3.6
	N	17	N	17

* statistical significance * ($p<0.05$), ** ($p<0.01$)

Necropsy:
For all females, the ovaries were absent, the estradiol capsule was present, and the femoral vein catheter was in place at the time of euthanasia.

CONCLUSION: Terbuthylazine reduced the magnitude of the LH surge in female SD rats when administered for 4 days by oral gavage at a dose of 106.5 mg/kg/day. This effect was comparable to that observed in SD rats administered atrazine for 4 days at the molar equivalent dose of 100 mg/kg/day.

(2) Handa R, 2014.
This was an investigative study with no applicable guidelines. The study was not conducted in compliance with the current US EPA 40 CFR Part 160 GLP regulations. Analytical method validation and dose formulation analyses were performed under EPA 40 CFR Part 160 GLP.

Justification for Test System Selection: The rat was selected as the test species as it is recognized by international guidelines as a preferred test species. The number of animals used was considered to be the minimum required to meet the scientific and regulatory objectives of the study.

Previous studies have shown that the chlorotriazine herbicide, atrazine, can block the oestrogen and/or progesterone induced LH surge in ovariectomized Wistar, SD, or Long Evans (LE) rats when treated with high doses (Foradori *et al*., 2009, McMullin *et al*., 2004). Consequently, the objective of this study was to determine if terbuthylazine could block the hormone-induced LH surge in Wistar rats. The preovulatory LH surge was induced in adult ovariectomized female rats by the timed administration of estradiol and progesterone. Previous studies have demonstrated that this is an effective procedure that induces a preovulatory–like surge of LH (Foradori *et al*., 2009, 2011).

SUMMARY
In a study to assess the effects on the hormone-induced LH surge in ovariectomized female rats, three groups of 8, 11 and 10 ovariectomized female Wistar rats were dosed via oral gavage with 1% carboxymethylcellulose sodium salt in saline (vehicle control group), 100 mg atrazine per kg bw/d (positive control group) or 106.5 mg terbuthylazine per kg bw/d (molar equivalent of 100 mg/kg atrazine), respectively. The animals were ovariectomized and stimulated with estradiol + progesterone to induce an afternoon LH surge. On the fourth day following ovariectomy, plasma samples were taken (once hourly for 8 hours) and analysed for LH levels.

Treatment of ovariectomized female Wistar rats with either terbuthylazine or atrazine resulted in a statistically significant reduction in the LH surge. The peak LH level of the terbuthylazine treated group was reduced to approximately 26% of the control levels while the peak LH level of the atrazine treated group was reduced to approximately 33% of the control level. In addition, treatment with terbuthylazine or atrazine resulted in a significant reduction in body weight.

RESULTS AND DISCUSSION

Effects on the luteinizing hormone surge:

Terbuthylazine or atrazine caused a significant reduction in the estradiol + progesterone-induced LH surge. The results of a two-way ANOVA (treatment × time) analysis of plasma LH levels revealed a significant main effect of treatment [$F_{2,206} = 50.12$; $p < 0.0001$], time [$F_{7,206} = 12.49$, $p < 0.0001$] and interaction effect [$F_{14,206} = 3.77$, $p < 0.001$]. Post-hoc analysis showed that there was a significant difference between the vehicle and TBA groups at 1700h ($p < 0.05$) and between 1800h to 2100h (all $p < 0.001$). Similarly, when vehicle control and atrazine groups were compared, there were significant differences at 1700h ($p<0.05$), 1800-2000h ($p < 0.001$), and 2100h ($p < 0.01$).

Table: Summary of plasma leuteinizing hormone levels (ng/mL)

Treatment group		**Time of day**							
		14:00	**15:00**	**16:00**	**17:00**	**18:00**	**19:00**	**20:00**	**21:00**
Vehicle	Mean	1.52	1.85	3.47	13.36	25.24	22.74	25.08	20.44
	SEM	0.38	0.50	0.81	3.75	4.83	5.83	6.53	4.95
	N	8	8	8	8	8	8	7	7
100 mg/kg bw atrazine	Mean	0.75	1.66	1.90	2.88	6.60***	7.20***	6.41***	6.32**
	SEM	0.27	0.46	0.47	0.87	1.98	2.47	2.12	2.17
	N	11	11	11	11	11	11	11	11
106.5 mg/kg bw terbuthylazine	Mean	0.96	0.45	0.69	1.56*	5.37***	6.20***	3.60***	3.66**
	SEM	0.50	0.22	0.30	0.47	3.90	3.22	1.72	1.28
	N	10	10	10	10	10	10	10	10

* statistically significant ($p<0.05$), **statistically significant ($p<0.01$), *** statistically significant ($p<0.001$)

Peak LH level of the terbuthylazine and atrazine-treated groups was approximately 26% and 33% of the vehicle control group values, respectively. There was a significant reduction in peak LH amplitude between treatment groups compared to vehicle controls group [$F_{2,26} = 8.51$, $p = 0.0014$]. Post hoc analysis using Bonferonni's multiple comparison test showed significant reductions ($p<0.01$) in the terbuthylazine and atrazine groups compared to vehicle controls but no difference between the terbuthylazine and atrazine groups. Similarly, both terbuthylazine and atrazine reduced the LH AUC compared to the vehicle group [$F_{2,26} = 11.63$, $p = 0.0002$]. Post-hoc analysis with Bonferroni's multiple comparison test showed significant reductions in LH AUC in the terbuthylazine and atrazine groups versus controls ($p < 0.01$) with no difference between the terbuthylazine and atrazine groups (see table below).

Table: Summary of plasma leuteinizing hormone levels (ng/mL): LH peak and LH AUC

Treatment group	**Peak LH amplitude**		**LH area under the curve**	
Vehicle	Mean	31.80	Mean	97.38
	SEM	5.51	SEM	18.91
	N	8	N	8
100 mg/kg bw	Mean	10.13**	Mean	30.17**

atrazine	SEM	2.49	SEM	7.36
	N	11	N	11
106.5 mg/kg bw terbuthylazine	Mean	8.19***	Mean	20.18***
	SEM	3.91	SEM	9.502
	N	10	N	10

* statistically significant (p<0.05), ** statistically significant (p<0.01), *** statistically significant (p<0.001)

Data were recalculated with the peak LH surge aligned to visualize the dynamics of the LH surge. Two-way ANOVA (treatment x time) showed a significant main effect of treatment ($F_{2,145} = 44.23$, $p<0.0001$) and time ($F_{6,145} = 4.23$, $p=0.0006$). There was no interaction effect ($p=0.5007$). Bonferroni post-hoc analysis showed significant decreases at the -1 hr relative to peak ($p<0.01$), 0 (designated as the peak; $p<0.001$) and +1 hr relative to peak ($p<0.01$) for terbuthylazine and atrazine treatment (see table below).

Table: Summary of plasma leuteinizing hormone levels (ng/mL): LH peak and LH AUC

Treatment group		**Time relative to peak**						
		-3	**-2**	**-1**	**0**	**1**	**2**	**3**
Vehicle	Mean	7.06	11.46	20.17	30.91	22.70	18.80	19.22
	SEM	4.08	3.96	5.36	5.67	5.30	4.18	5.83
	N	8	8	8	8	7	4	4
100 mg/kg bw atrazine	Mean	2.19	2.40	5.58**	9.37***	6.97**	4.70	3.97
	SEM	0.53	0.81	1.95	2.63	2.10	1.80	3.78
	N	11	11	11	11	9	3	3
106.5 mg/kg bw terbuthylazine	Mean	0.97	0.98	1.25***	6.29***	4.63***	3.11*	1.94*
	SEM	0.53	0.37	0.44	4.29	3.31	1.60	1.53
	N	9	9	9	9	9	9	6

* statistically significant (p<0.05), ** statistically significant (p<0.01), *** statistically significant (p<0.001)

Body weight:

A significant effect of treatment on body weight change was observed when all treated animals were analysed ($F_{2,32} = 26.17$, $p<0.0001$) and when only animals with a blood sample were included in the analysis ($F_{2,26} = 19.66$, $p<0.0001$). Post-hoc analysis revealed significant decreases in body weight in the terbuthylazine and atrazine groups ($p<0.001$), when compared to vehicle-treated animals (see table below).

Table: Summary of body weight changes (g)

Treatment group	**Final body weight change: all animals**		**Final body weight change: sampled animals only**	
Vehicle	Mean	-7.00	Mean	-5.63
	SEM	2.31	SEM	3.15
	N	12	N	8
100 mg/kg bw atrazine	Mean	-22.18**	Mean	-22.18**
	SEM	2.33	SEM	2.33
	N	11	N	11
106.5 mg/kg bw terbuthylazine	Mean	-26.43***	Mean	-26.30***
	SEM	1.29	SEM	1.56
	N	12	N	10

* statistically significant (p<0.05), ** statistically significant, (p<0.01), *** statistically significant

(p<0.001)

CONCLUSION: At the dose level tested (106.5 mg/kg bw/d), terbuthylazine reduced the magnitude of the LH surge in female Wistar rats, analogous to the effects shown with molar equivalents of atrazine.

Assessment and comparison with the classification criteria

(1) In accordance with the criteria in the CLP Regulation a classification in Category 1A is not appropriate because there is no epidemiological/human evidence for the carcinogenicity of terbuthylazine in humans.

(2) It is therefore necessary to decide whether a classification of terbuthylazine in category 1B or category 2 is justified. Since an increase in tumour incidence has been observed in one species only (rat, but in two strains) and the available genotoxicity data on terbuthylazine do not support a genotoxic MoA for tumour induction, classification in category 1B is inappropriate.

(3) Category 2 may be considered on the basis of increased tumours only observed in rats at the top dose, at a dose that appeared to exceed the MTD. However, it seems probable that the tumours were the result of a MoA not relevant to humans but common to other chlorotriazines such as atrazine and are relevant to SD and Han Wistar rat strains.

The new data suggest that consideration of no classification is appropriate for terbuthylazine.

Consideration of no classification:
Originally the DS proposed classification in category 2 for terbuthylazine on the basis of the mammary gland tumours seen in Han Wistar rats from the Ramesh (2001) study. The DS had made a valid point about read across from other chlorotriazines such as atrazine because limited data was available supporting the assertion chlorotriazines have a comparable effect on the hypothalamic-pituitary-ovary axis in Wistar rats and in SD rats at the time the CLH report was drafted. However since then Industry have submitted additional studies that add weight to the argument that the mammary tumours in Wistar rats occured via the same MoA as those in SD rats which have been dismissed as not relevant for humans.

The weight of evidence indicates that Terbuthylazine acts in a similar manner to atrazine in both SD and Wistar rat strains and that the primary feature of both substances in long-term administration studies in females is a suppression of the pre-ovulatory LH surge. This is a crucial key event for the induction of mammary tumours in SD rats by chlorotriazines such as atrazine which is considered to be not relevant to humans. Accordingly RAC concludes that no classification with respect to carcinogenicity is warranted for terbuthylazine.

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

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal **(1) Fertility:** The effects of terbuthylazine on fertility have been investigated in two two-generation studies and one one-generation study in rats. The DS summarised the data from these studies in table 20 of the CLH report. *(i) Two-generation study by Masters et al. (1992)* The two-generation study by Masters *et al.,* (1992) utilised SD rats. There were no treatment-

related mortalities. Apparent effects on mating, pregnancy and corpora lutea were noted for F0 and F1 female rats administered terbuthylazine via the diet at 300 ppm (22–26 mg/kg bw/d). No similar effects were noted in females receiving 6 or 60 ppm. These data are summarised in the table below, along with data for body weight gain and food consumption.

Table: Summary of mating and pregnancy parameters, *corpora lutea*, body weight gain and food consumption in female SD rats receiving terbuthylazine

	Dose (ppm)	No. females mated	No. pregnant	Total no. not pregnant	Pregnancy rate (%)	Reduced/ Absent *Corpora Lutea*	Pre-mate body weight gain (% of control)	Pre-mating food consumption (% of control)
F0	0	32	31	1	96.9	0	100	100
	6	32	30	2	93.8	0	103	97
	60	32	30	2	93.8	0	94	94
	300	32	25	7	78.1	4	68	84
F1	0	28	24	4	85.7	0	100	100
	6	28	24	4	85.7	0	100	99
	60	28	26	2	92.9	0	84	92
	300	28	22	6/4[1]	78.6/85.7[1]	7	75	84

[1] Initially 6 females were found not pregnant; however, 2 of these animals became pregnant after mating with a male proven to be fertile.

A number of effects were noted by the DS in the Masters *et al.,* (1992) study:

1. At the top dose level, parental body weight gain was lower than in controls during the pre-mating period (30-32 % in the 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).
2. Prior to mating, mean body weight loss for females in the high dose F0 and F1 generations was 13.6% and 25% respectively (not statistically significant).
3. In the F0 high dose group 7 out of 32 females (compared to 1 out of 32 controls) failed to become pregnant (4 showed no indication of mating). Reduced or absent corpora lutea were also observed in 4 out of the 7 females that failed to conceive.
4. 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. Corpora lutea were absent in the four high dose females that failed to conceive; however, reduced numbers of corpora lutea were also observed in 3 females who successfully conceived.
5. Effects on offspring were also noted at 300 ppm (reduced pup weight and slightly delayed sexual maturation) in both generations.

(ii) Two-generation study by Krishnappa (1998)

No treatment-related deaths occurred and no clinical signs of toxicity were observed in Wistar rats in the study of Krishnappa (1998). The highest dose level in this study was 200 ppm (18–24 mg/kg bw/d; females). Many body weight parameters were significantly lowered in both sexes at the top dose level.

Mating, fertility and gestation indices were unaffected by treatment. There were no effects on the numbers of corpora lutea. The proportion of implantations at the top dose level was slightly

(but significantly) lower; this finding correlates with a slightly higher post-implantation loss but is not considered to be clearly treatment-related in the absence of any effect on litter size. Gross necropsy did not reveal any treatment-related findings.

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 at 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. The DS considered that the reduced viability was a repeated dose effect and not a specific developmental effect. Table B.6.67 from the original DAR which details the offspring findings from the Krishnappa study is reproduced in the table below.

Table: Wistar Rat 2-generation study, Krishnappa, (1998): offspring findings

Parameter /Time point			**Dose level** (ppm)							
			F1				**F2**			
			0	**50**	**100**	**200**	**0**	**50**	**100**	**200**
		Litters (#)	26	27	30	29	29	28	27	27
		Litter size (#)	11.3	11.0	12.3	11.2	12.0	12.5	11.4	11.6
		Born dead (#)	3	4	3	11*	-	3	7*	2
		Live birth index	99.0	98.7	99.2	96.6*	100	99.1	97.7	99.4
		Viability index	97.3	97.6	96.7	90.8*	97.1	88.5*	93.7*	92.3*
		Lactation index	92.3	90.0	83.3*	80.3*	92.9	84.9*	83.2*	84.2*
Number of pups[1]		Day 1	11.2	10.9	12.2	10.9	12.0	12.4	11.1	11.5
		Day 4 (pre)	11.2	10.9	12.2	10.9	12.0	12.4	11.1	11.5
		Day 4 (post)	10.9	10.6	11.8	9.9	11.7	11.0	10.4	10.6
		Day 7	7.0	7.4	7.5	7.1	7.7	7.2	6.7	7.2
		Day 14	6.9	6.3	6.9	6.4	7.3	6.5	6.2	6.4
		Day 21	7.6	7.0	6.6	5.9	7.2	6.4	6.1	6.3
			M				**F**			
			0	**50**	**100**	**200**	**0**	**50**	**100**	**200**
Pup weight (g)	F1	Day 1	5.5	5.7	5.6	5.3	5.7	5.5	5.5	4.9*
		Day 4	7.6	7.9	7.5	7.3	7.6	7.7	7.2	6.8*
		Day 7	11.6	11.5	10.7	10.0*	11.1	11.2	10.3	9.2*
		Day 14	22.3	22.9	20.1	19.6*	21.0	22.3	18.9	17.3*
		Day 21	32.7	34.5	31.1	30.7*	32.0	33.5	28.9	26.6*
Weight gain (g)		Day 1-21	27.2	28.8	25.5	25.4	26.3	28.0	23.4	21.4
Pup weight (g)	F2	Day 1	5.9	5.5*	5.7	5.6	5.6	5.2	5.4	5.4
		Day 4	8.1	7.3*	7.7	7.6	7.8	7.1	7.4	7.0
		Day 7	12.2	10.7*	11.0	10.3*	11.7	10.6	11.1	9.9*
		Day 14	23.4	21.8	21.8	21.4	22.3	21.0	22.1	20.2
		Day 21	35.5	33.5	32.7	30.5*	35.3	32.5	33.1	28.9*
Weight gain (g)		Day 1-21	29.6	28	27	24.9	29.7	27.3	27.7	23.5

[1]not analysed statistically

*significantly different to controls $p<0.05$ (Dunnett's test)

In the other reproduction studies with terbuthylazine (Masters *et al*., 1992; Ganiger, 1999) there was no evidence of any effect on pup survival.

(iii) One-generation study by Ganiger (1999)

No deaths occurred and no signs of toxicity were observed during the study period. Acceptable as a range-finding study, this one-generation study in the Wistar rat had no effects on fertility up to the top dose (25-36 mg/kg bw/d). However, the group size was too small to yield 20 pregnant females/dose and this could impact on the ability to discern subtle effects. There was a small reduction in the mean number of corpora lutea, although ranges were comparable in treated and control animals and fertility was unaffected.

Significantly lower body weight gains and mean body weights were seen in both sexes at ≥100 ppm (7–10 mg/kg bw/d) during the pre-mating period; food consumption was also slightly lower in these groups. Mean body weights of females at ≥100 ppm were also significantly lower than controls throughout gestation and lactation; body weight gains during lactation and gestation were also clearly lower in these groups, but the values did not attain statistical significance.

Mean body weights of pups at the top dose level were lower than controls throughout the lactation period. Gross necropsy of pups did not reveal any treatment-related findings.

In all the rat multi-generation studies the primary parental and offspring effects were reduced body weight, reduced body weight gain and food consumption. Several published studies have investigated the effect of reduced body weight on SD (CD) female rats and reproductive function (Chapin *et al*., 1993; Carney *et al*., 2004; Terry *et al*., 2005). These studies utilised restricted caloric diets to achieve weight loss and show effects on the reproductive performance of SD rats. Reduced pre-mating body weight generally resulted in a reduced pregnancy rate, reduced numbers of corpora lutea, and reduced offspring body weight with delays in puberty compared to controls.

The DS considered that the apparent effects on fertility in SD rats at the top dose level (300 ppm) are not attributable to a direct effect of terbuthylazine on mating or fertility *per se*. The findings reflected a combination of background variation in mating performance in the animals (historical control incidences for fertility rate in SD rats ranges from 84–100% for the F0 generation and 36-100% for the F1 generation) and/or were secondary to the general systemic toxicity observed, specifically lower body weight parameters. The DS did not propose classification for fertility.

(2) Development:

The developmental toxicity of terbuthylazine has been investigated in three studies in rats and four studies in rabbits (briefly summarised below). In none of the studies were any malformations of concern noted and the foetal findings observed were considered by the DS to be a secondary non-specific consequence of the maternal toxicity and not a direct effect on development.

(i) Fitzgerald (1990)
RAIF rats: No evidence of teratogenicity was seen in this study. Increased incidences of absent ossification of the anterior digit proximal phalanges at the top dose level were considered to be treatment-related and likely to be a result of developmental delay secondary to maternal toxicity. Increased incidences of absent ossification of the posterior digit proximal phalanges at ≥5 mg/kg bw/d were all within the historical control range and were not considered to be clearly related to treatment. Further analysis by the notifier is presented under "Additional Key Elements".

(ii) Brooker (1995a)
Crl:CD.BR rats: A very high dose regimen was employed (100, 200 and 400 mg/kg bw/d). The results of this study indicated severe maternal toxicity associated with local gastric irritation at high dose levels of terbuthylazine. No effects on litter parameters were noted at dose levels up to 200 mg/kg bw/d. No gross abnormalities were observed in foetuses at these dose levels.

(iii) Brooker (1995b)
Crl:CDBR rats: Moderate doses of terbuthylazine were tested (5, 25 or 125 mg/kg bw/d). Litter parameters were unaffected by treatment. Litter size and litter weight were comparable in all dose groups. No evidence of teratogenicity was seen in this study; the incidence of interventricular septum defect was within historical control levels. An increased foetal incidence of foetus squat syndrome (all five affected foetuses were from the same litter) was observed in

the high dose group.

(iv) Bottomley et al. (1983)
New Zealand White rabbits: No adverse effects were observed in dams or foetuses up to the top dose (4.5 mg/kg bw/d).

(v) Khalil (1996)
Russian Chbb:HM rabbits: Deaths occurred in all groups, but the pattern of mortality did not suggest a relationship to treatment. No malformations were observed and the increased foetal incidence of forelimb flexure, observed at the top dose, was considered to be the result of overcrowding due to a larger than average litter size.

(vi) Brooker (1995c)
New Zealand White rabbits (range-finding study): The rabbits were gavaged at dose levels of 5, 7.5 or 10 mg/kg bw/d. No malformations were observed. The number of late *in utero* deaths was slightly increased at the top dose (2.2 compared to 1.2 in the controls). Foetal weight was also reduced, as was the proportion of males at the top dose group.

(vii) Brooker (1995d)
New Zealand White rabbits (main study): The rabbits were gavaged at dose levels of 0, 2.5, 5 or 7.5 mg/kg bw/d. 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).

The DS concluded there were no malformations of concern and that the foetal findings observed were considered to be a secondary non specific consequence of the maternal toxicity and not a direct effect on development. No classification was proposed by the DS. In support of this conclusion, an evaluation by JMPR in 2007 concluded that atrazine was not teratogenic.

Comments received during public consultation

One MS commented extensively on the effects observed following terbuthylazine exposure. They disagreed with the DS who concluded that many of the effects were a secondary, non-specific, consequence of maternal toxicity and therefore not relevant for classification. The MS also remarked on the offspring results from the two-generation study by Krishnappa, (1998), where they consider the pups born dead and the decrease of the viability index should be considered as a developmental effect and not as a repeated dose effect as supported by the DS. Industry supplied a robust and detailed response which is included under "Additional Key Elements".

Individual comments submitted on behalf of scientists who were asked by Syngenta to review the original study reports concluded that the reproductive effects noted in the two-generation study by Masters *et al.,* (1992), were due to maternal toxicity and not to a direct effect of terbuthylazine on the reproductive system. They quoted the publication by Terry *et al.* (2005), where there was a quantitatively similar decrease in corpus luteum numbers associated with feed restriction and weight reduction. They also addressed the decreased pup survival during lactation in the study by Krishnappa (1998). They explained the finding of a decrease in pup survival was an artefact of analysis on a per-pup basis rather than the preferred per-litter basis. Re-analysis of the data on a per-litter basis indicated that there was no effect of treatment on pup survival during lactation.

The company/manufacturer also submitted a position paper on terbuthylazine and reproductive toxicity in SD rats.

Additional key elements

The original notifiers submitted a detailed response to queries raised by the MS during public consultation. This was received late but is included here verbatim for completeness and argues against terbuthylazine having a direct reproductive toxic effect.

The notifiers agree with the UK HSE that terbuthylazine should not be classified for developmental toxicity, and that any apparent effects on pup survival or development reflect the general systemic toxicity of terbuthylazine.

Key Points - Reproduction Studies

Pup and Dam Body weight

- Consistent (across two strains of rat) reduction in body weight and food consumption in dams at high doses.
- Consistent reduction in pup body weight from birth through lactation secondary to the effect on dams early in gestation and to systemic toxicity of the compound once pups begin to consume treated diet.
- Slight developmental delay at high dose (Masters *et al*., 1992) consistent with, and secondary to, reduced pup body weight.

Pup Survival

- Not consistent across studies/strains of rat – seen in 1 of 2 studies in Wistar rats and not seen in SD rats.
- Seen in two-generation study in Wistar rat (Krishnappa, 1998) only. Not clearly dose related and associated with (and likely attributable to) data analysis being conducted on an individual pup basis (rather than litter basis).
- Possible association with treatment day 14-21 *post-partum* consistent with systemic toxicity as pups begin to eat treated diet.

Developmental Toxicity Study (Fitzgerald, 1990)

- As agreed by EFSA, the NOAEL in the study for maternal and developmental toxicity is 5mg/kg.
- The incidences of incomplete ossification are noted only in the presence of significant maternal toxicity, are indicative of developmental delay and are considered to have no consequence postnatally.
- Further evaluation of the data has revealed that all statistically significant instances of delayed ossification are within the wider historical control database when litter incidence is used as the unit of analysis.

Masters et al. 1992 (two-generation study)

The notifiers have previously provided a statement that clarifies that the apparent effects on pregnancy and number of corpora lutea observed in this study in females receiving a high dose of terbuthylazine reflect normal biological variability, excessive systemic toxicity (as evidenced by reduced body weight gain and food consumption) or a combination of both factors and do not represent evidence for a direct effect of terbuthylazine on reproduction (Fleeman *et al*., 2005). Regarding the effects on pup weight, the notifiers would like to clarify that mean pup weight in the 300 ppm F1 generation is not decreasing by 19%; rather it is 19% lower when compared with the control group at day 21 *post-partum*.

At 300 ppm, both F0 and F1 dams showed reduced food consumption (14% less than controls) in the weeks prior to mating. This would have contributed to a significantly lower body weight gain (30-32% F0 and 22-24% F1) prior to mating and would be expected to result in pups with a lower birth weight. Indeed, food restriction during gestation has been shown to result in lower body weight in dams and in pups (Fleeman *et al*., 2005; Camey *et al*., 2004). It is the position of the notifiers that, for both the F1 and F2 generation pups, the reduced mean pup weight at birth is a secondary consequence of the maternal toxicity noted at this dose level. The lower body weight gain in the offspring throughout the study is reflective of this lower body weight at birth which does not recover over time. At later time points (i.e. day 12/13

post-partum onwards) the lower body weight gain is more pronounced and at this point is reflective of general systemic toxicity as the pups begin to eat the treated diet (Redman & Sweney, 1976). Due to the higher food consumption per g of body weight, weaning pups would be expected to receive a substantially higher dose of terbuthylazine than an adult eating the same treated diet, on a mg/kg body weight basis (Hanley & Watanabe, 1985).

Gainger, 1999 (one-generation study)
The notifiers disagree with the MS comment received during PC that there is a dose-related decrease in mean pup weight in this study. Consistently statistically significant differences from control were noted only in animals of the 350 ppm dose. As described for Masters *et al*., 1992 (above), it is the position of the notifiers that the effect on pup weight noted at this dose level and the lower pup body weight gain throughout the study is a secondary effect of the maternal toxicity and general systemic toxicity as the pups begin to eat the treated diet. Although mean pup weight at birth was statistically significantly lower than control in the 100 ppm dose group, it is questionable whether it is related to treatment as it was confined to female pups only and there were no statistically significant differences from control at any other time point in the study (days 4, 7, 14 and 21 *post-partum*). In addition, this data has been evaluated on a pup rather than a litter basis, the consequence of which is an inflation of the degrees of freedom with proneness to incorrect identification of statistical significance. This inappropriate form of analysis is discussed in the OECD Guidance Document 43 (OECD, 2008) which states it is critical that littermates are not treated as independent observations in the statistical analysis and that the litter should be the unit of comparison.

Krishnappa, 1998 (two-generation study)
The notifiers agree with the UK HSE that the reduced pup viability noted in the two-generation study with Han Wistar rats (Krishnappa, 1998) is a repeat dose effect related to the systemic toxicity of terbuthylazine and not a specific developmental effect.

Although a number of statistically significant differences in pup survival were noted between control and treated groups (see table below), it is the position of the notifiers that only the effects noted on day 21 of lactation (F1 generation) can be considered possibly treatment related, with any statistically significant differences noted at other time points attributable to biological variability. In addition, this data has been evaluated on a pup rather than a litter basis which is inappropriate as discussed in the OECD Guidance Document 43 which states that it is critical that litter mates are not treated as independent observations in the statistical analysis and that the litter should be the unit of comparison.

- For the F1 generation, the statistically significant differences noted in the high dose group at days 1 and 4 are primarily due to whole litter losses in 2 dams occurring shortly after birth. The whole litter losses are considered to be a consequence of maternal toxicity which is evidenced at 200 ppm by a statistically significant 25% reduction in body weight gain (15% reduction in weight) compared to concurrent controls during the gestation period. After litter standardisation on day 4 of lactation no statistically significant difference in pup survival was noted on day 7 or 14 of lactation.

- For the F2 generation, the statistically significant differences noted at days 1, 4, 7, 14 and 21 are unlikely to represent real effects of treatment owing to the lack of dose- and time-responses and is considered to be a consequence of the high degree of variability with this endpoint at this laboratory.

Table: Summary of pup survival in a two-generation study in Han Wistar rats (Krishnappa, 1998)

Survival (%)	**F1 Generation**				**F2 Generation**			
	Control	**Low 50 ppm**	**Mid 100 ppm**	**High 200 ppm**	**Control**	**Low 50 ppm**	**Mid 100 ppm**	**High 200 ppm**
Day 1	99.0	98.7	99.2	96.6*	100.0	99.1	97.7*	99.4

Day 4	97.3	97.6	96.7	90.8*	97.1	88.5*	93.7*	92.3*
Day 7	94.3	96.6	94.6	97.2	98.7	95.3*	91.9*	96.0
Day 14	92.3	92.8	88.0	87.8	93.8	86.3*	84.8*	85.6*
Day 21	92.3	90.0	83.3*	80.3*	92.9	84.9*	83.2*	84.2*

* Statistically significantly different from control with p<0.05 (Z test)

The apparent dose-related reductions in viability indices at 100 ppm and 200 ppm on day 21 of lactation (F1 generation) may be dose-related, although the lack of a clear dose-response for similar findings in the F2 generation cast further doubt upon this apparent dose-response in the F1 generation.

However, any treatment-related effects in pups 14 days or older can be considered to represent a repeat dose effect related to the systemic toxicity of terbuthylazine rather than a developmental effect. Rat pups start to consume treated diet from approximately day 11 onwards, with the amount consumed increasing toward the end of the lactation period (personal communication from Advinus [formerly Rallis Research Centre, Bangalore], the laboratory that conducted the Krishnappa, 1998 study). Due to the higher food consumption per g of body weight, weaning pups would be expected to receive a substantially higher dose of terbuthylazine, on an mg/kg body weight basis, than would an adult eating the same treated diet resulting in more significant signs of systemic toxicity (Redman & Sweney, 1976; Hanley & Watanabe, 1985).

Repeat dose toxicity of terbuthylazine is characterized by significant reductions in body weight and increased mortality, consistent with the effects seen in pups and dams in this study, particularly at 200 ppm. At the top dose, significantly reduced body weight (see table below) was noted in both F1 and F2 generation litters and F0/F1 dams.

Table: Summary of dam and pup body weight (g) day 21 *post-partum* (dam gestation day 20) in a 2-generation study in Han Wistar rats (Krishnappa, 1998)

	Males				**Females**			
	0 ppm	**50 ppm**	**100 ppm**	**200 ppm**	**0 ppm**	**50 ppm**	**100 ppm**	**200 ppm**
F1 Generation	32.7	34.5	31.3	30.7*	32.0	33.5	28.9	26.0*
F2 Generation	35.5	33.5	32.7	30.5*	35.3	32.5	33.1	28.9*
Dams P0					341	320	320	292*
Dams F1					327	321	303*	291*

* statistically significantly different from control with p<0.05 (Dunnett's test)

In the other reproduction studies with terbuthylazine (Masters *et al.*, 1992; Gainger, 1999) there was no evidence of any effect on pup survival but a clear reduction in body weight/weight gain compared with the controls at the high dose levels in the presence of maternal toxicity.

In this multigeneration study in the Wistar rat a decrease in pup viability at day 21 of lactation in the high dose group in the F1 generation was not clearly reproducible in the F2 generation and not supported by a similar effect in other reproduction studies. However, if the conservative position that there is a treatment-related effect on pup survival at the top dose, and to a lesser extent at the mid dose, is adopted this can be attributed to systemic toxicity of terbuthylazine rather than a developmental effect.

Fitzgerald, 1990 (Developmental Toxicity Study in the Rat)

The notifiers agree with EFSA and the UK HSE that the NOAEL for both maternal and developmental toxicity in this study is 5 mg/kg bw/d. The incidences of incomplete ossification in the Fitzgerald (1990) study are indicative of a developmental delay as a result of marked maternal toxicity and not a direct effect on development.

As noted in the CLH report the majority of such findings are well within the historical control range. Of the findings noted as being outside the historical control range, a further comparison of the incidence of findings and the control range (found on pages 43–44 of the study report) has confirmed that these findings are within the historical control range for litter incidence and only AD5 proximal phalanx reduced ossification is slightly outside the range for foetal (but not for litter) incidence (see table below).

Table: Historical control data for the delays in ossification – selected findings

Findings	**Dose levels (mg/kg bw/d)**			
% foetal (% litter)	**0**	**1**	**5**	**30**
Metatarsal 1: no ossification	5.8 (30.0)	7.0 (16.7)	12.9* (54.7)	15.8** (45.0)
	Historical control: 5.9 – 23.3 (28.6 – 65.0)			
AD2 proximal phalanx: no ossification	1.3 (10)	-	2.1 (16.7)	6.7* (30.0)
	Historical control: 0.6 – 6.7 (4.5 – 30.0)			
AD5 proximal phalanx: reduced ossification	4.2 (25)	4.9 (16.7)	3.6 (16.7)	10.9* (40.0)
	Historical control: 0.5 – 6.0 (4.3 – 40.0)			

* statistically significantly different from control with p<0.05 (Fisher's Exact test);
** statistically significantly different from control with p<0.01 (Fisher's Exact test).

This further supports the conclusion that the effects on skeletal ossification are minor variations. They are consistent with a slight delay in development as a consequence of severe maternal toxicity and are not indicative of a direct effect on development and these findings have previously been shown to reverse during the early post-natal development period (Collins *et al*., 1987).

Conclusion
In conclusion, the notifiers agree with the UK HSE that terbuthylazine should not be classified for developmental toxicity; with any apparent effects on pup survival or development reflecting the general systemic toxicity of terbuthylazine. This position is well supported by the argumentation and supporting literature described above as well as the lack of consistency of any effect across the studies and strains of rat. It should be noted that owing to the similarity between the Wistar and SD strains in terms of reproductive function (as detailed by Marty *et al*., 2009, who conducted a large retrospective analysis of two-generation reproductive studies using Wistar [16 studies] and SD [27 studies] rats) there is no reason to suspect that the two strains would respond differently to treatment with terbuthylazine.

Assessment and comparison with the classification criteria

Fertility:

According to the CLP Regulation (section 3.7.1.3 of Annex I) "…*any effect of substances that has the potential to interfere with sexual function and fertility has to be regarded for a classification for reproductive toxicity. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*."

Consideration of Category 1A/1B:
Effects are seen in one species (rat). The data do not warrant classification for fertility in Category 1 for the following reasons:

- There is no evidence from humans.
- There is no clear evidence of an adverse effect on sexual function and fertility in rat multi-generation studies.

Consideration of Category 2:
The main effects on fertility were observed in the 2-generation study in rats (Masters *et al*., 1992) at the high dose :

- Decrease in pregnancy rate in F0 and F1 females.
- Reduced or absent Corpora Lutea
- Consistent reduction in pup body weight from birth through lactation.

These effects are considered secondary to the effect on dams early in gestation and to systemic toxicity of the compound once pups begin to consume treated diet. Consideration of no classification is warranted.

Consideration of no classification:
No effects were observed in the absence of maternal toxicity that provides sufficient evidence for suspicion of reduced fertility:

- Effects are minor and consistent with historical control variability. Historical control data from two-generation SD rat studies demonstrate pregnancy rates of 84–100% (F0) and 36–100% (F1).
- Reduced food consumption and body weight are known to affect pregnancy and numbers of corpora lutea in SD rats (Chapin *et al*., 1993; Carney *et al*., 2004; Terry *et al*., 2005). These published studies utilised restricted caloric diets to achieve weight loss and show effects on the reproductive performance of SD rats such as reduced numbers of corpora lutea, and reduced offspring body weight with delays in puberty compared to controls.
- No significant effects on mating, pregnancy or number of corpora lutea were noted in either one-generation (Gainger, 1999) or two-generation (Krishnappa, 1998) studies with Han Wistar rats exposed to terbuthylazine.
- Two-generation reproductive toxicology studies of near-identical design to that used for terbuthylazine were conducted using the same strain of rat in a contemporary time period, but in a different laboratory with two closely structurally-related chlorotriazines: atrazine and simazine. No effects on mating, pregnancy or corpora lutea were noted in these studies.

In summary, there is no clear evidence of an adverse effect on sexual function and fertility. The RAC therefore concludes that terbuthylazine does not meet the criteria for classification for fertility.

Developmental Toxicity:

Possible adverse relevant effects on development regarded as significant and biologically relevant are the following:

- Consistent reduction in pup body weight from birth through lactation is secondary to the effect on dams early in gestation and to systemic toxicity of the compound once pups begin to consume treated diet. This is a known effect for dams displaying significant reductions in body weight (Carney *et al., 2004*).

- Pup deaths in a two-generation study in the Wistar rat only (Krishnappa, 1998). These were not clearly dose related and may be associated with data analysis being conducted on an individual pup basis (rather than on a litter basis). Not consistent across studies/strains of rat – seen in 1 of 2 studies in Wistar rats and not seen in SD rats. For the F1 generation, the statistically significant differences noted in the high dose group at days 1 and 4 are primarily due to whole litter losses in 2 dams occurring shortly after birth. Any treatment-related effects in pups 14 days or older can be considered to represent a repeated dose

effect related to the systemic toxicity of terbuthylazine rather than a developmental effect.

- Incidences of incomplete ossification (Fitzgerald 1990) are noted only in the presence of significant maternal toxicity, and are indicative of developmental delay and are considered to be of no consequence postnatally. All statistically significant incidences of delayed ossification are within the wider historical control database when litter incidence is used as the unit of analysis.

These effects do not warrant classification for development.

According to section 3.7.2.4 of CLP Regulation and the ECHA CLP Guidance, in the interpretation of the developmental outcome to decide classification for developmental effects, it is important to consider the possible influence of maternal toxicity. Adverse developmental effects after terbuthylazine treatment were observed at doses associated with maternal toxicity (i.e. body weight reductions, decreased food consumption). The DS was of the opinion that these effects can be regarded as irrelevant for classification because data exists showing that these types of effects occur when body weight is reduced.

The RAC agrees with this assessment.

The criteria in section 3.7.2.4.3 states *"Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity".* Section 3.7.2.4.2 of the CLP Regulation states *"Classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies"*.

The DS suggests that the effects on pup weight and viability are due to repeated dose toxicity in the offspring rather than a developmental effect.

The RAC concurs with this view. There is no evidence from the animal studies to warrant a classification for terbuthylazine for developmental toxicity.

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^{o}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^{o}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 $\geq$ 240 days at 50^{o}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^{o}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^{o}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^{o}C and pH 7 in sterile buffer solutions. The constant irradiation was considered equivalent to 49.2 days summer natural sunlight at 40^{o}N. The photolytic DT_{50} was 29.5 days natural summer sunlight at 40^{o}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 x 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^{o}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

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Terbuthylazine does not have a current harmonised classification according to the CLP Regulation. Based on the available data on aquatic toxicity and considering the substance is not rapidly degradable, the DS proposed an environmental classification as Aquatic Acute 1 (H400), M=10 and Aquatic Chronic 1 (H410), M=10 according to the CLP Regulation.

Degradation

Hydrolysis

The available hydrolysis studies showed that terbuthylazine is hydrolytically stable at environmentally relevant temperatures and pH values for the purposes of classification. In a study performed following GLP and the US EPA 161-1 guideline, 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, 205 and 194 days, respectively. A single metabolite, 2-hydroxy-terbuthylazine (MT13), was observed.

In a preliminary study (following GLP and Commission Directive 92/69/EEC method C7) hydrolysis was assessed at pH 4, 7 and 9 at 50°C over 5 days in the dark. At pH 4, at the end of the test, 76 % hydrolysis was observed. However, there was insufficient data to calculate a DT_{50}. In the follow-up study (compliant with GLP and following SETAC guidelines), hydrolysis was assessed at pH 4 at 20°C over 30 days with a calculated DT_{50} greater than 1 year. Only the degradant MT13 was observed.

The DS provided two additional studies on terbuthylazine degradants, desethylterbuthylazine (MT1) and MT13, although they were not considered relevant for the purpose of classification. The study results indicated that the degradants are hydrolytically stable.

Photolysis

Three aqueous photolysis studies following GLP are available showing that terbuthylazine undergoes limited photodegradation and was considered photolytically stable under environmentally relevant conditions for the purposes of classification.

Biodegradation

Ready biodegradability was tested with two tests following GLP and OECD TG 301B. Only limited degradation (2-3% and 3-9%) was observed at day 28. On this basis terbuthylazine was considered not readily biodegradable.

Two GLP water/sediment studies showed that mineralisation of terbuthylazine to CO_2 was minimal (< 1%) over more than 100 days. In the first study two water/sediment systems were used (a sandy and a silty loam sediment) to assess the fate of terbuthylazine at 9°C and 20°C in the dark over 182 and 365 days. During the test terbuthylazine partitioned from the water phase to the sediment phase. Whole system DT_{50} values were 33 to 73 days at 20°C and 136 to 224 days at 9°C. Three degradants were identified: MT13, MT1 and terbutryn (MT26). Terbutryn was observed with a maximum of 7.4% AR in the silty loam sediment at 20°C at day 365 and the concentration of the metabolite increased with time and may not have peaked by study termination. In the second study two water/sediment systems were used (a loamy sand and a clay loam sediment) to assess the fate of terbuthylazine at 20°C in the dark over 110 days. During the test terbuthylazine partitioned from the water phase to the sediment phase. Whole system DT_{50} values were 83.52 to 118.54 days at 20°C. Seven degradants were identified, the most significant were: MT13 and MT1 whereas MT26 was not identified.

The DS provided another water/sediment study performed with the degradant MT26 showing whole system DT_{50} values between 178 and 203 days, although degradants are not considered relevant by the DS for the classification of terbuthylazine.

Environmental distribution

Three studies are available where the adsorbtion of terbuthylazine in various soils was assessed according to OECD TG 106 and following GLP. The corresponding log K_{oc} values from all studies and soils ranged from 0.88 to 0.98 (mean 0.93) indicating a low adsorption potential.

Aquatic bioaccumulation

For terbuthylazine two measured log K_{ow} values of 3.4 at 25°C and 3.41 at 20°C (GLP, OECD TG 107) were reported.

Two studies on bioaccumulation in fish are available following GLP and OECD TG 305. In the first study the bioaccumulation of terbuthylazine was assessed using bluegill sunfish (*Lepomis macrochirus*) over 42 days. A single exposure concentration of 0.4 mg a.s./L was used. Rapid elimination was observed and the calculated whole fish steady state BCF was 34 (no lipid normalisation to 5%). In the second study the rainbow trout (*Oncorhynchus mykiss*) over 21 days was used. Two exposure concentrations were assessed: 0.005 mg/L and 0.05 mg/L. Rapid elimination was observed and the calculated whole fish steady state BCF was 19 (no lipid normalisation to 5%).

Aquatic toxicity

Fish

Three acute and one chronic aquatic toxicity tests on fish are available from studies with terbuthylazine, performed according to OECD TG 203 and OECD TG 210 and in compliance with GLP.

Table: Summary of relevant information on aquatic toxicity to fish

Method, Substance and purity	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to aquatic fish					
OECD 203 Terbuthylazine (96.8 %)	*Oncorhynchus mykiss*	Static nom	96-h LC_{50}	**2.2**	Swarbrick and Maynard, 2002
OECD 203 Terbuthylazine (96.8 %)	*Cyprinus carpio*	Static mm	96-h LC_{50}	> 5.7	Wallace and Woodyer, 2002
OECD 203 Terbuthylazine (97 %)	*Oncorhynchus mykiss*	Semi-static nom	96-h LC_{50}	2.4	Douglas et al, 1988a
Long-term toxicity to aquatic fish					
OECD 210 Terbuthylazine (96.8 %)	*Oncorhynchus mykiss*	Flow-through mm	90-d NOEC (based on growth)	**0.09**	Rufli, 1996
mm – mean measured concentration nom – nominal concentration					

Test results indicated that *Oncorhynchus mykiss* as the more sensitive fish species. In the

acute toxicity study the 96-h LC_{50} was 2.2 mg a.s./L based on nominal concentrations as the measured concentrations were 82 to 106 % of nominal. The long-term toxicity study provided a 90-day NOEC of 0.09 mg a.s./L based on weight and mean measured concentrations.

Degradants
The DS did not consider degradants relevant for the classification of terbuthylazine. However, acute toxicity in fish (following GLP) were provided in the CLH report for the following degradants: MT1, MT13, desethylhydroxy-terbuthylazine (MT14), diamino-chloro-triazine (MT20) and MT26. The only LC_{50} value lower than terbuthylazine is for MT26: in a study with *Oncorhynchus mykiss* (according to Commission Regulation (EC) No 440/2008 method C1) the 96-h LC_{50} was 1.1 mg a.s./L (static, mean measured concentration).

Aquatic invertebrates
The DS provided two studies for short-term toxicity and two studies for long-term toxicity to aquatic invertebrates (*Daphnia magna*) – all studies were performed according to OECD guidelines and followed GLP.

Table: Summary of relevant information on aquatic toxicity to invertebrates

Method, Substance and purity	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to aquatic invertebrates					
OECD 202 Terbuthylazine (96.8 %)	*Daphnia magna*	Static mm	48-h EC_{50}	> 69.3	Van der Kolk, 1996
OECD 202 Terbuthylazine (97 %)	*Daphnia magna*	Static nom	48-h EC_{50}	**11**	Douglas et al., 1988c
Long-term toxicity to aquatic invertebrates					
OECD 202, part II , 21-d Terbuthylazine (96.8 %)	*Daphnia magna*	Semi-static nom	21-d NOEC	**0.019**	Shillabeer et al., 2002
OECD 202, part II , 21-d Terbuthylazine (96.5 %)	*Daphnia magna*	Semi-static mm	21-d NOEC	0.17	Bell, 1995
mm – mean measured concentration nom – nominal concentration					

The water solubility of terbuthylazine is 9 mg/L at 25°C, pH 7.4 and therefore all but the lower exposure concentrations were considered above the water solubility, in both short-term toxicity studies with aquatic invertebrates. The lower value from short-term studies resulted in a 48-h EC_{50} = 11 mg a.s./L, based on nominal concentrations. While it is not ideal that the EC_{50} is based on nominal concentrations given some uncertainty regarding actual dissolved concentrations of the test material and the value being above the reported water solubility, immobilisation was observed at 1.8 mg a.s./L exposure solutions and above (which were within 20 % of nominal concentrations). Therefore a recalculated EC_{50} using mean measured data wouldn't be lower than the one for the most sensitive aquatic species (algae).

Other aquatic organisms (including sediment)
In addition, the DS provided one long-term toxicity study on sediment organisms (*Chiromonus riparius*). The GLP study was conducted according to the BBA Guideline (1995) in a static

system, which closely follows OECD Guideline 219 (simulating a one off event of pesticide spray drift). A 27-d NOEC = 0.5 mg a.s./L, based on nominal concentrations, was obtained.

Degradants
Moreover, the DS provided aquatic toxicity data for invertebrates on the most relevant metabolites, although they are not considered relevant for the purpose of terbuthylazine classification. Only the EC_{50} for terbutryn (MT26) is lower than EC_{50} values for terbuthylazine, in a study with *Daphnia magna* following US EPA guideline 660/3-75-00 (static, nominal concentrations) with a 48-h EC_{50} = 2.66 mg a.s./L.

Algae and aquatic plants
Six studies of the toxicity of terbuthylazine to various algae and diatom species and two studies to aquatic plants are available. The algae/aquatic plants is the most sensitive trophic group, terbuthylazine being a herbicide.

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

Method, Substance and purity	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Aquatic toxicity to algae and cyanobacteria					
OECD 201 Terbuthylazine (96.6 %)	*Pseudokirchneriella subcapitata*	Static mm	72-h ErC_{50} 72-h NOErC	0.028 0.0012	Kelly, 1996
OECD 201 Terbuthylazine (96.4 %)	*Desmodesmus subspicatus*	Static nom	72-h ErC_{50} 72-h NOErC	>0.03 **0.0011**	Grade, 1993a
ASTM E1218-90 Terbuthylazine (96.4 %)	*Microcystis aeruginosa*	Static nom	96-h ErC_{50} 96-h NOErC	**0.018** 0.0037	Grade, 1993b
ASTM E1218-90 Terbuthylazine (96.4 %)	*Navicula pelliculosa*	Static nom	96-h ErC_{50} 96-h NOErC	>0.03 0.01	Grade, 1993c
OECD 201 Terbuthylazine (98 %)	*Anabaena flosaquae*	Static mm	48-120h ErC_{50}* 48-120h NOErC*	0.052 0.02	Migchielsen, 2002a
OECD 201 Terbuthylazine (98 %)	*Microcystis aeruginosa*	Static mm	48-120h ErC_{50}* 48-120h NOErC*	0.102 0.0396	Migchielsen, 2002b
Aquatic toxicity to aquatic plants					
US EPA FIFRA 122-2 Terbuthylazine (96.4 %)	*Lemna gibba*	Static mm	14-d EC_{50} (frond n°) 14-d NOEC (frond n°)	0.019 0.0022	Hoberg, 1993
OECD 221 draft Terbuthylazine (97.7 %)	*Lemna gibba*	Semi-static nom	7-d EC_{50} (frond n°) 7-d NOEC (frond n°)	**0.0128** 0.0029	Dengler, 2001
mm – mean measured concentration nom – nominal concentration *reflects period of exponential growth					

The most sensitive species tested in the acute tests were the algae *Microcystis aeruginosa* and

the aquatic plant *Lemna gibba*. In the test with *Lemna gibba* (7 days, semi-static system) an EC_{50} of 0.0128 mg/L based on nominal concentrations was obtained. The blue green algae, *Microcystis aeruginosa* was exposed to terbuthylazine in a static test system for 96 h. The ErC_{50} = 0.018 mg/L (recalculated from the raw data) was based on nominal concentrations (analytical measurements were 81% to 142% of nominal). Regarding chronic toxicity the most sensitive species tested is the algae *Desmodesmus subspicatus (*formerly known as *Scenedesmus subspicatus*) (72 hours, static system) with a NOErC=0.0011 mg/L (recalculated from the raw data), based on nominal concentrations (at these exposure concentrations, mean measured concentrations were 91% of nominal).

Degradants
The DS did not consider degradants relevant for classification of terbuthylazine. However, aquatic toxicity to algae studies provided for the most relevant degradants, showed that only the EC_{50}/NOEC for terbutryn is lower than the one for terbuthylazine: in the study with *Pseudokirchneriella subcapitata* (OECD 201 guideline) the 72-h ErC_{50} was 0.0036 mg a.s./L and the 72-h NOErC was 0.0002 mg a.s./L (static, mean measured concentration). Whilst it is noted that this degradant is an order of magnitude more toxic to algae/aquatic plants (the most sensitive species) than the parent substance terbuthylazine, the DS concluded that it is not formed in significant amounts over the period representative of rapid degradation and is not considered relevant to the classification of terbuthylazine.

Comments received during public consultation

Four Member States contributed during public consultation stating a general agreement with the proposed environmental classification.

One MS had also a specific comment. It was suggested to add one further acute aquatic invertebrate study conducted with *Mysidopsis bahia* (Ward, G. S., 1988, Report No. 87356-0210-2130) which was not considered in the CLH report but is available for national registration for central zone in Germany. The LC_{50} of 0.092 mg a.s./L (nominal, 96 hours, static system) would support the proposed classification and should be added for completeness, since this represents the most sensitive endpoint for acute toxicity to aquatic invertebrates.
In response, the DS pointed out that although the values obtained from this study are lower than those presented in the CLH report for invertebrates, the proposed classification for terbuthylazine (Acute category 1, acute M-factor = 10), based on studies on algae/aquatic plants, would not be affected.

Assessment and comparison with the classification criteria

Degradation
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 as a consequence terbuthylazine was considered not readily biodegradable. Based on two aquatic water/sediment studies, terbuthylazine is demonstrated to be not ultimately degraded at a level greater than 70% in 28 days (mineralisation was < 1% over more than 100 days, whole system DT_{50} values were between 33 and 118.5 days), therefore terbuthylazine is considered not rapidly degradable for the purposes of classification.

Bioaccumulation
Measured log K_{ow} values are 3.4 at 25°C and 3.41 at 20°C. The measured BCF values from two bioaccumulation studies in fish (34 and 19) are lower than the trigger value of 500 for bioaccumulation stated in the CLP Regulation, therefore terbuthylazine is not considered bioaccumulative for classification purposes.

Aquatic toxicity
Acute aquatic hazard

Acute aquatic toxicity data are available for all three trophic levels. Terbuthylazine is a herbicide and algae/aquatic plants is the most sensitive trophic group, where acute aquatic toxicity is observed below the classification threshold of 1 mg/L.

Chronic aquatic hazard
Adequate chronic aquatic toxicity data are available for all three trophic levels. The most sensitive trophic group is algae/aquatic plants where the chronic aquatic toxicity is observed below the classification threshold of 0.1 mg/L.

Conclusion on classification
Terbuthylazine is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation potential. The lowest acute aquatic toxicity values fall within the range $0.01 < L(E)C_{50} \leq 0.1$ mg/L and the lowest chronic aquatic toxicity values fall in the toxicity range $0.001 < NOEC \leq 0.01$ mg/L. Therefore RAC concludes that terbuthylazine fulfils the CLP criteria for classification as **Aquatic Acute 1 (H400)** with an **M-factor of 10** and **Aquatic Chronic 1 (H410)** with an **M-factor of 10**.

Supplemental information - In depth analyses by RAC

Although terbuthylazine is demostred not to be rapidly degradable, the metabolite terbutryn is an order of magnitude more toxic to algae than the parent substance terbuthylazine. A study performed by Grade (1997) with terbutryn (conducted according to OECD TG 201) showed an E_rC_{50} after 72-h of 0.0036 mg/L (NOE_rC 72-h 0.0002 mg/L) for the freshwater algae *Selenastrum capricornutum*.

In a water/sediment simulation study performed with terbuthylazine (Mamouni, 1998, Amendment 1 Mamouni, 1999 and Amendment 2 Mamouni, 2004), terbutryn was observed with a maximum of 7.4% AR in the silty loam sediment at 20°C at day 365 and the concentration was still rising at experiment termination.

The CLP guideline (section 4.1.3.3.1, last paragraph) states that *"[…] where degradation is slower, it may be possible to test the parent substance and thus generate hazard data in the normal manner. The subsequent degradation may then be considered in determining whether an acute or long-term hazard category should apply. There may be occasions, however, when a substance so tested may degrade to give rise to a more hazardous product. In these circumstances, the classification of the parent compound should take due account of the hazard of the degradation product, and the rate at which it can be formed under normal environmental conditions"*.

RAC acknowledged the importance of the above arguments on this issue. However, due to the low formation rate of terbutryn in the water/sediment simulation study with the parent and the lack of analytical measurements of the degradant in the reported aquatic toxicity studies with terbuthylazine it is the view of RAC that the metabolite terbutryn is not considered relevant for the classification of the parent compound. As a consequence, RAC agreed with the DSs' proposal on the classification of terbuthylazine as stated above.

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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Physical and Chemical Properties

Author(s)	Year	Details
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Das, R.	2000a	Boiling point / boiling range of GS 13529, Novartis Crop Protection AG, Basel, Switzerland, Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland, Report No 76223, GLP, Not Published, Syngenta File N° GS13529/1656
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Author(s)	Year	Details
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Kettner, R.	1999	Octanol / water partition coefficient of GS 13529, Novartis Crop Protection AG, Basel, Switzerland, Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland, Report No 78584, GLP, Not Published, Syngenta File N° GS13529/1637
Burkhard, N.	1975	Gardoprim, Hydrolysis of terbuthylazin under laboratory conditions, Novartis Crop Protection AG, Basel, Switzerland, Ciba-Geigy Ltd., Basel, Switzerland, Report No SPR-22-75, Not GLP, Not Published, Syngenta File N° GS13529/0115
Hoermann, A.	1999	Dissociation constant in water, Novartis Crop Protection AG, Basel, Switzerland Solvias AG, Basel, Switzerland, Report No PP-99/62P.DCW, GLP Not Published, Syngenta File N° GS13529/1633
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Möller M.	2007	TERBUTHYLAZINE. MELTING POINT A.1. (OECD 102). BOILING POINT A.2. (OECD 103), Siemens AG, Oxon Italia S.p.A, Pero, Italy Report-no. 20061225.01, GLP: yes, published: no
Flack I.	1994a	Terbuthylazine (Pure and Technical grades); Physical and Chemical Properties (Part2), Huntingdon Research Centre Ltd., Cambridgeshire, UK, Oxon Italia S.P.A, Pero, Italy, Report-no. OXN 17/932509, GLP: yes, published: no
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Grade, R.	1993c	Report on the growth inhibition test of GS 13529 tech. to Diatoms (Navicula pelliculosa) Novartis Crop Protection AG, Basel, Switzerland, Report No 928433, GLP, Not Published, Syngenta File N° GS13529/1180
Palmer, S, Kendall, T, Krueger, H	2001	A 96-Hour Growth Inhibition Test of GS-26379 (Metabolite of GS-13529) to the Green Alga, Selenastrum capricornutum, Syngenta Crop Protection AG, Basel, Switzerland, Report No 528A-109, GLP, Not Published, Syngenta File N° GS26379/0013
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Vial, A.	1991g	Report on the growth inhibition test of GS 28620 to Green algae (Scenedesmus subspicatus), Novartis Crop Protection AG, Basel, Switzerland, , Report No 918160 GLP, Not Published, Syngenta File N° GS28620/0001
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Grade, R.	1997	Growth inhibition test of GS 14260 tech. to green algae (Selenastrum capricornutum) under static conditions, Novartis Crop Protection AG, Basel, Switzerland, , Report No 961714, GLP, Not Published, Syngenta File N° GS14260/0854

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Hoberg, J.	1993	GS 13529 - Toxicity to Duckweed, Lemna gibba, Novartis Crop Protection AG, Basel, Switzerland, Report No 93-9-4947, GLP, Not Published, Syngenta File N° GS13529/1193
Douglas M.T., Handley J.W., Macdonald I.A.	1988a	The Acute Toxicity of Terbuthylazine to Rainbow Trout (Salmo gairdneri) Oxon Italia S.P.A, Pero, Italy, Report-no. OXN 10(b)/88504, GLP: yes, published: no
Van Dijk A.	1997	Bioconcentration and Elimination of 14C-Terbuthylazine by Rainbow Trout in a Dynamic Flow-Through System, Oxon Italia S.P.A, Pero, Italy, Report-no. 629447 GLP: yes, published: no
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Bell G.	1995	Terbuthylazine: Prolonged Toxicity to Daphnia Magna, Oxon Italia S.P.A, Pero, Italy Report-no. OXN 18(a)/942069, GLP: yes, published: no
Kelly C.	1996	Terbuthylazine Technical Algal Growth Inhibition, Oxon Italia S.P.A, Pero, Italy Report-no. OXN 180/962297, GLP: yes, published: no
Dengler D.	2004a	Testing of Toxic Effects of Desethyl-terbuthylazine on the Single Cell Green Alga Desmodesmus Subspicatus (formerly Scenedesmus Subspicatus), Oxon Italia S.P.A, Pero, Italy, Report-no. 20041034/01-AADs, GLP: yes, published: no
Dengler D.	2004b	Testing of Toxic Effects of 2-Hydoxy-terbuthylazine on the Single Cell Green Alga Desmodesmus Subspicatus (formerly Scenedesmus Subspicatus), Oxon Italia S.P.A, Pero, Italy, Report-no. 20041035/01-AADs, GLP: yes, published: no
Migchielsen M.H.J.	2002a	120-Hour Fresh Water Cyanobacteria Growth Inhibition Test with Terbuthylazine Technical, Oxon Italia S.P.A, Pero, Italy, Report-no. 314055, GLP: yes, published: no
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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

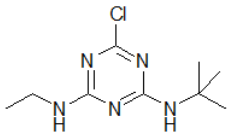
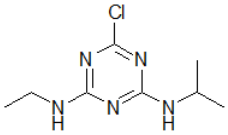
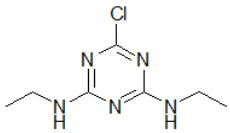
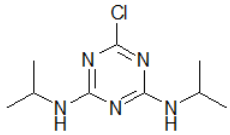
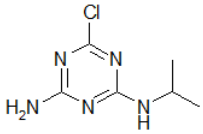
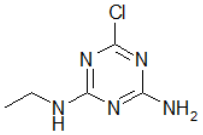
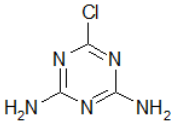
Date: 10th December 2013

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

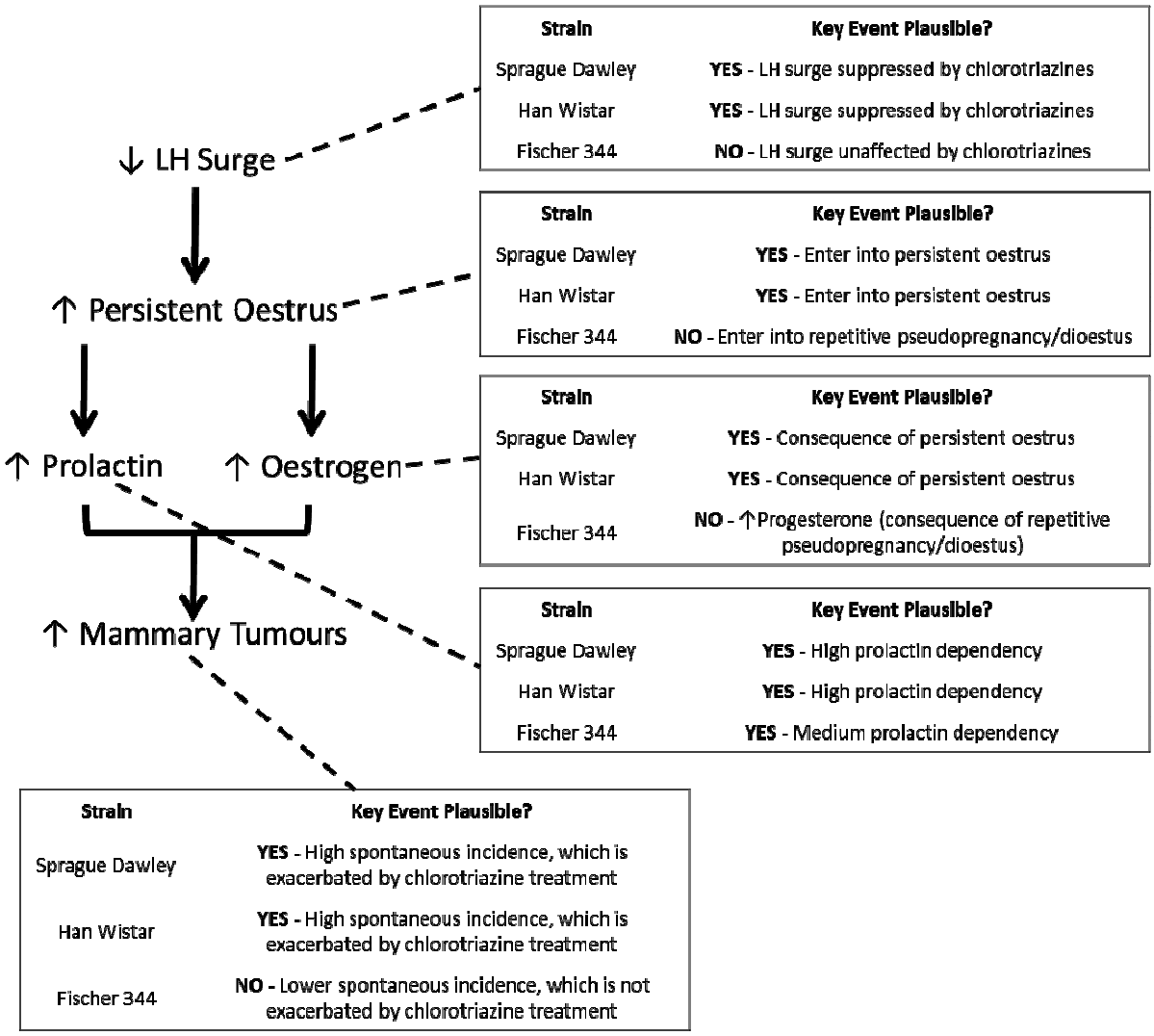
d. **Prolactin is a key factor is reproductive ageing in both Sprague Dawley and Han Wistar rats.** Prolactin is key factor in chlorotriazine-induced mammary tumours (Simpkins *et al*., 2011). In contrast, prolactin is less important in Fischer 344 rats (Simpkins *et al*., 2011; Harleman *et al*., 2012).

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



Strain	Key Event Plausible?
Sprague Dawley	**YES** - LH surge suppressed by chlorotriazines
Han Wistar	**YES** - LH surge suppressed by chlorotriazines
Fischer 344	**NO** - LH surge unaffected by chlorotriazines

Strain	Key Event Plausible?
Sprague Dawley	**YES** - Enter into persistent oestrus
Han Wistar	**YES** - Enter into persistent oestrus
Fischer 344	**NO** - Enter into repetitive pseudopregnancy/dioestus

Strain	Key Event Plausible?
Sprague Dawley	**YES** - Consequence of persistent oestrus
Han Wistar	**YES** - Consequence of persistent oestrus
Fischer 344	**NO** - ↑Progesterone (consequence of repetitive pseudopregnancy/dioestus)

Strain	Key Event Plausible?
Sprague Dawley	**YES** - High prolactin dependency
Han Wistar	**YES** - High prolactin dependency
Fischer 344	**YES** - Medium prolactin dependency

Strain	Key Event Plausible?
Sprague Dawley	**YES** - High spontaneous incidence, which is exacerbated by chlorotriazine treatment
Han Wistar	**YES** - High spontaneous incidence, which is exacerbated by chlorotriazine treatment
Fischer 344	**NO** - Lower spontaneous incidence, which is not exacerbated by chlorotriazine treatment

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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

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

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 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.