Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

paclobutrazol (ISO); (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol

EC Number: -
CAS Number: 76738-62-0

CLH-O-0000001412-86-213/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

8 June 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Paclobutrazol

EC Number: **Not assigned**

CAS Number: **76738-62-0**

Index Number: **Not listed**

Contact details for dossier submitter: **UK Competent Authority**

Chemicals Regulation Directorate

Health and Safety Executive

United Kingdom

Version number: **2** **Date: March 2017**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Paclobutrazol**
EC number:	**Not listed**
CAS number:	**76738-62-0**
Annex VI Index number:	**Not listed**
Degree of purity:	**≥ 93% as a 1:1 ratio of the (2*S*,3*S*)- and (2*R*,3*R*)- enantiomers**
Impurities:	**There are a number of impurities, these have been taken into account and are not considered to impact on the proposed classification. Further information is available in the IUCLID dossier.**

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 listed.
Current proposal for consideration by RAC	Acute Tox 4; H302 - Harmful if swallowed Acute Tox 4: H332 - Harmful if inhaled Eye Irrit 2; H319 - Causes serious eye irritation Repr 2; H361d - Suspected of damaging the unborn child Aquatic Acute 1; H400 - Very toxic to aquatic life, acute M factor = 10 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects, chronic M factor = 10

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PACLOBUTRAZOL (ISO); (2*RS*,3*RS*)-1-(4-CHLOROPHENYL)-4,4-DIMETHYL-2-(1H-1,2,4-TRIAZOL-1-YL)PENTAN-3-OL

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4; H302 - Harmful if swallowed Acute Tox 4: H332 - Harmful if inhaled Eye Irrit 2; H319 - Causes serious eye irritation Repr 2; H361d - Suspected of damaging the unborn child Aquatic Acute 1; H400 - Very toxic to aquatic life, acute M factor = 10 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects, chronic M factor = 10

1.3 Proposed harmonised classification and labelling

1.4 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 - Harmful if swallowed**	Not applicable	Not classified	-
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	**Acute Tox 4; H332 - Harmful if inhaled**	Not applicable	Not classified	-
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	**Eye Irrit 2; H319 - Causes serious eye irritation**	Not applicable	Not classified	-
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	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	**Repr 2; H361d - Suspected of damaging the unborn child**	Not applicable	Not classified	-
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	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
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 - Very toxic to aquatic life** **Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects,**	**M = 10** **M = 10**	Not classified	-
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

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

Labelling:

Pictogram(s): GHS07, GHS08 and GHS09

Signal word: Warning

Hazard statements:

H302+H332;	Harmful if swallowed of if inhaled
H319;	Causes serious eye irritation
H361d;	Suspected of damaging the unborn child
H410;	Toxic to aquatic life with long lasting effects

Precautionary statements: 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

Paclobutrazol is an active substance approved under Directive 91/414/EEC. Paclobutrazol does not have an existing entry in Annex VI of CLP and has not been considered for harmonised classification and labelling previously in the EU.

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

2.2 Short summary of the scientific justification for the CLH proposal

The review of the active substance concluded that the following classification should be considered (refer to EFSA conclusion; EFSA Journal 2010;8(11):1876); Xn R20/R22, Repro Cat 3; R63, R50-53. Which would translate to Acute Tox 4; H302+H332, Repr 2; H361d, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 under CLP. The classification in the EFSA conclusion is supported in this CLH proposal, with the addition of Eye Irrit 2; H319. Further details are provided below and full details are given in the relevant sections of this report.

The available data do not support classification of paclobutrazol for physical hazards.

In the acute studies, the oral LD_{50} values ranged from 490 - 1219 in mice to 1336 - >2000 mg/kg in rats, the dermal LD_{50} was >1000 mg/kg and the inhalation 4-hour LC_{50} was 3.13-4.79 mg/l for a dust aerosol. Therefore, the criteria for classification with **Acute Tox 4: H302 + H332 – Harmful if swallowed or if inhaled** are met. There was no clear evidence of specific toxic effects on a target organ or tissue following acute exposure and no signs of respiratory tract irritation or narcotic effects. Therefore, no classification for specific target organ toxicity (single exposure) is proposed. Mild skin irritation was observed but all scores were below those relevant for classification. In the eye, corneal opacity (score of 1), which resolved by the end of the observation period was observed in 2/3 tested rabbits in one study. Whilst only mild effects were seen in an earlier study with 6 animals (i.e., corneal opacity with scores of <1 in 5/6 animals) the criteria for classification with **Eye Irrit 2; H319 – Causes serious eye irritation** are considered to have been met. Paclobutrazol does not meet the criteria for classification as a skin sensitiser.

The repeated dose toxicity of paclobutrazol has been investigated in rats, mice and dogs. The dog was found to be the most sensitive species, with testicular toxicity observed in the 90 day study, and liver toxicity in the 1-year study. In rats, the liver was the critical target organ, with liver changes observed in both the 90-day and lifetime studies. However, none of the observed changes were regarded of sufficient severity to support classification with STOT-RE or they occurred outside the (adjusted) guidance values for classification. Therefore, no classification is proposed.

Paclobutrazol tested negative *in vitro* and *in vivo*, and no classification for germ-cell mutagenicity is proposed.

The carcinogenic potential of paclobutrazol has been investigated in standard studies in rats and mice. No evidence of tumour induction was observed and therefore, no classification for carcinogenicity is proposed.

The potential for paclobutrazol to adversely affect fertility was investigated in rats. No treatment-related adverse effects on fertility were observed. Whilst testicular toxicity was observed in the 90-day study *(*marked decreases in absolute and relative testes and epididymides weights associated with an absence of spermatozoa from the epididymidal ducts, and the testes were described as immature),

it is considered that these changes may reflect retardation of sexual maturity. This is supported by the lack of testicular toxicity in the 1 year dog study. In the absence of any other findings, these effects in the dog are not considered sufficient to support classification for fertility.

The potential for paclobutrazol to adversely affect development has been investigated in rats and rabbits. Rats appear to be the most sensitive species with bone and kidney variations noted at doses of 10 mg/kg/day and above, with malformations (cleft palate) and severe maternal toxicity observed at 250 mg/kg/day, the highest dose tested. Cleft palate was observed in 3 foetuses/2 litters at the highest dose (compared to 0 in controls) of 250 mg/kg/day; a dose that caused severe maternal toxicity (1 dam died and 4 sacrificed *in extremis*). There was no evidence of other malformations. This dose of 250 mg/kg/day was also associated with widespread skeletal variations, mostly delayed ossification and supernumerary ribs (increased 14th bilateral). At lower non-maternally toxic doses, paclobutrazol caused retardation of skeletal development, increased supernumerary ribs and visceral variations (kidney and urinary tract). Cleft palate is a very rare malformation (relevant background rate of 0) in rats and the observation of this malformation in 3 foetuses from 2 litters is of high concern for human health. Additional concern comes from the increases in skeletal and visceral variations, observed at doses below those that cause malformations and severe maternal toxicity. No evidence of developmental toxicity was observed in rabbits even at maternally lethal dose levels. That cleft palate is only induced in one species at maternally lethal doses reduces the overall level of concern for human health, with only skeletal retardations and variations observed at non-maternally toxic doses. Therefore, classification with **Repr 2; H361d – Suspected of damaging the unborn child** is proposed.

Paclobutrazol is considered hydrolytically stable and the potential for aquatic photolysis is likely to be limited. Overall, the degradation information does not provide sufficient data to show paclobutrazol is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, paclobutrazol is considered non-rapidly degradable for the purpose of classification and labelling. The log K_{ow} is below the CLP trigger value of ≥ 4 and the whole fish BCF for parent paclobutrazol (or TRR) is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

Aquatic acute toxicity data on paclobutrazol are available for fish, invertebrates, algae and aquatic plants. Aquatic plants are the most acutely sensitive trophic group, with *Lemna gibba* exhibiting the most acute sensitivity. The two *Lemna gibba* E_rC_{50} values of 0.0283 and 0.0237 mg/l are in the range 0.01 to 0.1 mg/l. On this basis paclobutrazol should be classified as **Aquatic Acute 1; H400 – Very toxic to aquatic life, with an acute M-factor of 10.**

At present there are no valid chronic toxicity data on fish. Based on current data, fish are the least sensitive species in acute studies. Adopting the surrogate approach using available acute data would not result in a more stringent classification than the chronic classification proposal below. This is partially supported by the NOEC from a prolonged fish toxicity study (to OECD TG 204)

Adequate chronic toxicity data on paclobutrazol are available for invertebrates, algae and aquatic plants. Data are available for two *Lemna* species with *Lemna gibba* exhibiting the most chronic sensitivity. The two *Lemna gibba* NOE_rC values of 0.002 and 0.00151 mg/l are in the range 0.001 to 0.01 mg/l. Given this and because the substance is also considered non-rapidly degradable, paclobutrazol should be classified as **Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects, with a chronic M-factor of 10.**

2.3 Current harmonised classification and labelling

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

Paclobutrazol is not currently listed in Annex VI of CLP.

2.4 Current self-classification and labelling

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

At the time of submission the following entries were included in the C&L Inventory

Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)	Number of notifiers
Acute Tox. 4 Eye Irrit. 2 Acute Tox. 4 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H319 H332 H361 H400 H410	H302 H319 H332 H361 H410		GHS07 GHS09 GHS08 Wng	45
Flam. Sol. 1 Acute Tox. 4 Acute Tox. 4	H228 H302 H312	H228 H302 H312		GHS07 GHS02 Dgr	24
Acute Tox. 4	H302	H302		GHS07 Wng	18
Acute Tox. 4 Eye Irrit. 2 Acute Tox. 4 Aquatic Chronic 2	H302 H319 H332 H411	H302 H319 H332 H411		GHS07 GHS09 Wng	4
Acute Tox. 4 Acute Tox. 4 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H332 H361 H400 H410	H302 H332 H361 H410	EUH401	GHS07 GHS09 GHS08 Wng	3
Acute Tox. 4 Eye Irrit. 2 Acute Tox. 4	H302 H319 H332	H302 H319 H332		GHS07 Wng	1

RAC general comment
Paclobutrazol (ISO) (CAS 76738-62-0) is an active substance used in plant protection products as a plant growth regulator (fungicide) on winter oilseed rape. It has been approved in the EU under Directive 91/414/EEC. Paclobutrazol does not have an existing

entry in Annex VI of CLP Regulation and has not previously been considered for harmonised classification and labelling.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Paclobutrazol is a pesticide active substance and CLH is required in accordance with Article 36(2) of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

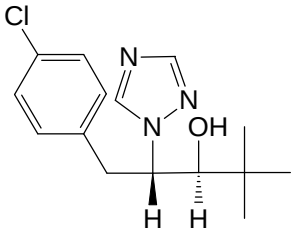
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

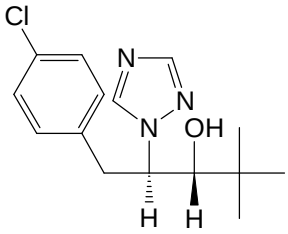
Table 4: Substance identity

EC number:	Not listed
EC name:	Not listed
CAS number (EC inventory):	Not listed
CAS number:	76738-62-0
CAS name:	1H-1,2,4-Triazole-1-ethanol, β-[(4-chlorophenyl)methyl]-α-(1,1-dimethylethyl)-, (αR,βR)-rel-
IUPAC name:	(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol
CLP Annex VI Index number:	Not listed
Molecular formula:	$C_{15}H_{20}ClN_3O$
Molecular weight range:	293.8

Structural formula:



(2S,3S)



(2R,3R)

1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Paclobutrazol	c.a., 96%	> 93% - < 100%	-

Current Annex VI entry: Not listed

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Current Annex VI entry: Not listed

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not relevant

1.2.1 Composition of test material

The tested material is considered to be equivalent to that outlined above.

1.3 Physico-chemical properties

Studies are taken from the Draft Assessment Report (DAR) – Volume 3, Annex B.2 – Physical and chemical properties – July 2006. All studies were conducted to GLP and are considered relevant and reliable for CLH.

Table 8: 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/beige fine granular solid	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.7/8	Visual Purity 99.7%
Melting/freezing point	164 °C	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.1	EEC method A 1 (DSC) Purity 99.7%
Boiling point	384 °C	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.2	EEC method A 2 (DSC) Purity 99.7%
Relative density	1.23 at 20 °C	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.4	EEC method A 3 (pycnometer) Purity 99.7%
Vapour pressure	1.9×10^{-6} Pa at 20 °C	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.5	EEC method A 4 (effusion method: vapour pressure balance) Purity 99.7%
Surface tension	66.0 mN/m at 20.5 °C	Woolley, S.M., 2001 DAR B.2.1.24	EEC method A 5 Purity (not stated)
Water solubility	2.29×10^{-2} g/l (purified water) 1.72×10^{-2} g/l (pH 5) 2.48×10^{-2} g/l (pH 7) 2.41×10^{-2} g/l (pH 9)	Cuthbert, J.E., Mullee, D.M., 2001 DAR B.2.1.11	EEC method A 6 (flask method) Purity 99.7%
Partition coefficient n-octanol/water	Log P_{ow} = 3.11 at 23 °C No evidence of pH dependence.	Cuthbert, J.E., Mullee, D.M., 2001 B.2.1.13	EEC method A 8 (Shake flask method) Purity 99.7%
Flash point	Not applicable, substance is a solid with a melting point of 164 °C		

Flammability	Material ignited but did not propagate combustion. Experience in handling and use indicates that the material is not pyrophoric and does not emit flammable gases on contact with water.	Woolley, S.M., 2001 B.2.1.20	Flammability: EEC method A 10 Purity 95.1%
Explosive properties	No evidence of shock, friction or thermal sensitivity.	Woolley, S.M., 2001 B.2.1.22	EEC method A 14 Purity 95.1%
Self-ignition temperature (Auto-flammability)	No evidence of self-ignition below the melting point of 159°C	Woolley, S.M., 2001 B.2.1.20	EEC method A 16
Oxidising properties	Ignition but no propagation with all mixtures of cellulose. Slow burning rates, ca 200mm in 4 mins; significantly lower than the reference mixture.	Woolley, S.M., 2001 B.2.1.23	EEC method A 17 Purity 95.1%
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	Solubility (g/L) in organic solvents at 20°C xylene 5.67 n-heptane 0.199 acetone 72.4 ethyl acetate 45.1 n-octanol 29.4 methanol 115 1,2 dichloroethane 51.9	Woolley, S.M., 2001 DAR B2.1.12	EEC method A 6 (flask method) Purity 95.1%
Dissociation constant	No significant differences in UV-vis spectra at different pH. Low solubility of active substance in water rendered usual methods unsuitable. Structure of molecule such that dissociation not expected. Most acidic proton is 2° alcohol, which is weak acid, having pKa of 15. Triazole group weakly basic (pKa ca 2). Molecule of similar structure have shown low pKa values (1,2,4-triazole and flutriafol pKa = 2.3).	Cuthbert, J.E., Mullee, D.M., 2001	
Viscosity	Not relevant, solid		

2 MANUFACTURE AND USES

2.1 Manufacture

Paclobutrazol is manufactured outside of the EU.

2.2 Identified uses

Paclobutrazol is used as a pesticidal active substance within the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 Physical Hazards

In a standard flammability study (EEC, A10), paclobutrazol ignited but did not propagate combustion. Experience in handling and use indicates that the material is not pyrophoric and does not emit flammable gases on contact with water. There was no evidence of self-ignition below the melting point of 159°C

In a standard explosivity study (EEC, A14) there was no evidence of shock, friction or thermal sensitivity.

In a standard oxidizing study (EEC, A17) all mixtures of paclobutrazol/cellulose were found to ignite, but did not propagate combustion. Slow burning rates, ca 200mm in 4 mins; significantly lower than the reference mixture were observed.

3.1.1 Summary and discussion of physical hazards

See above.

3.1.2 Comparison with criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. There was no evidence of shock, friction or thermal sensitivity when paclobutrazol was tested in a standard explosivity study. Therefore, given that all results were negative, the criteria for classification are not met.

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. Paclobutrazol ignited but did not propagate combustion and therefore, the criteria for classification as a flammable solid are not met.

Experience in handling and use indicates that paclobutrazol is not pyrophoric and does not emit flammable gases on contact with water. Therefore, the criteria for classification in these hazard classes are not met.

A substance is classified as an oxidising solid when the burning time of a sample-to-cellulose mixture is less than or equal to the burning time of the appropriate reference sample. Mixtures of paclobutrazol-cellulose were found to ignite but did not propagate combustion. The burning rates were all significantly slower that the reference mixtures. Therefore, the criteria for classification are not met.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification of paclobutrazol for physical hazards based on the following physico-chemical assessment and test results:

- Paclobutrazol ignited but did not propagate the combustion in a standard flammability study (EEC, A10);
- Experience in handling and use of paclobutrazol indicates that the material is not pyrophoric and does not emit flammable gases in contact with water;
- There was no evidence of self-ignition of paclobutrazol below the melting point of 159 °C;
- There was no evidence of shock, friction or thermal sensitivity in a standard EEC, A14 study;
- All mixtures of paclobutrazol/cellulose were found to ignite, but did not propagate combustion in a standard oxidizing study (EEC, A17).

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS's proposal that the available data do not support classification of paclobutrazol for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

The following summary is based upon that in the Pesticide Draft Assessment Report (DAR), 2006 made for the review under Regulation Directive 91/414/EEC and the Additional Report, 2010.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetics of paclobutrazol has been well investigated in rats, in single and repeated oral dosing studies, and in a more limited study in dogs following a single oral dose.

4.1.2 Human information

There are no data to inform on the potential toxicokinetics of paclobutrazol in humans.

4.1.3 Summary and discussion on toxicokinetics

Rat

In the rat, absorption was rapid and extensive (88-95%) and was not saturated at high doses. Absorbed paclobutrazol was readily oxidized to paclobutrazol diol, which was excreted or further oxidized to a carboxylic acid. Biotransformation was limited to the tertiary butyl moiety, with no metabolism of the triazole of chlorophenyl rings. A small proportion of radioactivity equilibrated into the tissues and was subsequently eliminated. The highest concentrations of radioactivity were seen in the liver after a lot or high dose. There was no evidence of bioaccumulation.

Excretion at a low dose was relatively high, with more than 70% of radioactivity excreted within 48-hours. The delay in excretion in high dose animals (>70% not achieved until >72-hours post dose) and the significant amount in the faeces (well beyond normal transit-time) was due to significant enterohepatic recirculation. In cannulated rats, biliary excretion at a low dose represented 50 – 70% of the administered dose in females and males respectively. In bile-duct cannulated rats, only 5% was excreted as unchanged parent.

Kinetics in dogs

Following a single oral low dose, radioactivity was rapidly absorbed reaching peak concentrations in plasma and blood within 1 hour and declined to below the limits of detection by 72 hours. Most of the radioactivity was associated with plasma. Elimination was faster than for rats with >75% of radioactivity was eliminated in urine and faeces within 24 hours, and at 168 hours after dosing, there was almost a complete absence of radioactivity in all tissues examined (with the exception of liver in one animal). There was no evidence of bioretention of paclobutrazol or its metabolites in dogs.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

<table>
<tr><th colspan="3">Oral</th></tr>
<tr><th>Method</th><th>LD_{50}/LC_{50}</th><th>Remarks</th></tr>
<tr><td>Similar to OECD TG 401
5 or 10 Alderley Park rats/sex/dose
As an aqueous suspension in 0.5% Lissitan at doses of 400, 500, 640, 800, 1000, 1260, 1600, 2000, 3200, 4000 and 5000 mg/kg by gavage

Purity 97%</td><td>1954 mg/kg in males
1336 in females</td><td>Deaths occurred at ≥500 mg/kg paclobutrazol. Full mortality data is shown in the table below;
<table>
<tr><td></td><td colspan="11">Dose (mg/kg)</td></tr>
<tr><td></td><td>400</td><td>500</td><td>640</td><td>800</td><td>1000</td><td>1260</td><td>1600</td><td>2000</td><td>3200</td><td>4000</td><td>5000</td></tr>
<tr><td>M</td><td>0/5</td><td>2/5</td><td>1/5</td><td>1/5</td><td>3/10</td><td>3/5</td><td>5/10</td><td>6/10</td><td>1/5</td><td>8/10</td><td>3/5</td></tr>
<tr><td>F</td><td>0/5</td><td>0/5</td><td>2/5</td><td>0/5</td><td>6/10</td><td>3/5</td><td>8/10</td><td>6/10</td><td>4/5</td><td>8/10</td><td>3/5</td></tr>
</table>
All deaths occurred within 4 days of dosing.

Clinical signs of toxicity were apparent one hour after dosing and were seen at all dose levels, These signs included subdued behaviour, unsteady gait, and loss of righting reflex, hypothermia, coma, piloerection, respiratory difficulties and urinary incontinence. Survivors appeared normal nine days after dosing.

There were no indications of specific target organ toxicity.

[redacted] *(1982)*</td></tr>
<tr><td>OECD TG 425 Limit Test
5 female Sprague-Dawley
2000 mg/kg suspension in distilled water

Purity 95.77%</td><td>>2000</td><td>No Mortalities or clinical signs of toxicity. There were no indications of specific target organ toxicity.

[redacted] *(2006a)*</td></tr>
<tr><td>Similar to OECD TG 401
5/10 Alderley Park albino mice, sex/dose

As an aqueous suspension in 0.5% Lissitan , by gavage

Males were administered doses of 250, 320, 400, 500, 640 and 800 mg/kg paclobutrazol, and females administered doses of 400, 500, 640, 800, 1000, 1260, 2000, 2500</td><td>490 mg/kg in males
1219 mg/kg in females</td><td>Deaths occurred in males at doses of ≥320 mg/kg paclobutrazol, and in females at all dose levels tested (≥ 400 mg/kg paclobutrazol). Full mortality data is shown in the table below;
<table>
<tr><td></td><td colspan="11">Dose (mg/kg)</td></tr>
<tr><td></td><td>250</td><td>320</td><td>400</td><td>500</td><td>640</td><td>800</td><td>1000</td><td>1260</td><td>2000</td><td>2500</td><td>3200</td></tr>
<tr><td>M</td><td>0/5</td><td>1/5</td><td>2/5</td><td>1/5</td><td>4/5</td><td>5/5</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>F</td><td></td><td></td><td>1/10</td><td>1/5</td><td>4/10</td><td>7/10</td><td>5/10</td><td>1/10</td><td>9/10</td><td>4/5</td><td>2/5</td></tr>
</table>
All deaths occurred within three days of dosing.

Clinical signs of toxicity were seen at all dose levels one hour after dosing and included subdued behaviour, piloerection, unsteady gait, hypothermia and coma. Most of the surviving animals appeared normal six days after dosing.
There were no indications of specific target organ toxicity.

[redacted] *(1982)*</td></tr>
</table>

<table>
<tr><td>and 3200 mg/kg paclobutrazol.

Purity 97%</td><td></td><td></td></tr>
<tr><td>Similar to OECD TG 401
5/Dunkin-Hartley Guinea Pigs, sex/dose

As an aqueous suspension in 0.5% Lissitan at doses of 320, 400, 500 or 640 mg/kg paclobutrazol by gavage. A further group of five male guinea pigs received 800 mg/kg

Purity 97%</td><td>542 mg/kg in males
400-640 mg/kg in females</td><td>Full mortality data is shown in the table below;
<table><tr><td></td><td colspan="5">Dose (mg/kg)</td></tr><tr><td></td><td>320</td><td>400</td><td>500</td><td>640</td><td>800</td></tr><tr><td>M</td><td>0/5</td><td>1/5</td><td>3/5</td><td>2/5</td><td>5/5</td></tr><tr><td>F</td><td>0/5</td><td>0/5</td><td>3/5</td><td>5/5</td><td>-</td></tr></table>
Clinical signs of toxicity were observed at all dose levels, and were apparent within three hours of dosing and included subdued behaviour and unsteady gait.

There were no indications of specific target organ toxicity.

[redacted] (1982)</td></tr>
<tr><td>Similar to OECD TG 401
5 New Zealand White rabbits, sex/dose

As an aqueous suspension in 0.5% Lissitan , by gavage at doses of 250, 500, 1000 and 2300 mg/kg

Purity 97%</td><td>835 mg/kg in males
937 mg/kg in females</td><td>All rabbits dosed at 2300 mg/kg, 1 male and 2 females at 1000 mg/kg and 3 males and 1 female at 500 mg/kg l died.

Clinical signs of toxicity were seen at all dose levels within one hour after dosing and included subdued behaviour and unsteady gait. Most of the surviving animals appeared normal 12 days after dosing.

There were no indications of specific target organ toxicity.

[redacted] (1982)</td></tr>
</table>

Inhalation		
Method	**LD_{50}/LC_{50}**	**Remarks**
OECD TG 403 5/10 Alderley Park rats/sex/concentration At concentrations of 0, 0.54, 1.84, 3.70 and 5.19 mg/l, nose only for 4-hours to a dust aerosol Purity 91.4%	4.79 mg/l in males 3.13 mg/l in females	One female exposed to 1.84 mg/l, four females and one male exposed to 3.70 mg/l, and three females and three males at 5.19 mg/l paclobutrazol died or were sacrificed *in extremis* by day two of the study. Treatment related clinical signs observed immediately after treatment at 1.84, 3.70, 5.19 mg/l paclobutrazol, were reduced response to sound, increased breathing depth and reduced breathing rate. These effects were accompanied in some animals by gasping, 'reduced stability', and abnormal respiratory noise (indicative of respiratory irritancy). Animals exposed to 0.54 mg/l showed only a slight reduction in their response to sound which became apparent in the latter half of the exposure period. No other signs were sign in the 0.54 mg/l animals. No clinical signs consistent with respiratory tract irritation were observed. There were no indications of specific target organ toxicity. [redacted] *(1988)*
OECD TG 403 5 Han Wistar rats/sex/ At concentration of 2.02 mg/l, nose only for 4-hours to a dust aerosol MMAD 2.61 μm Purity 95.7%	>2.02 mg/l	No mortalities of clinical signs of toxicity were observed. No clinical signs consistent with respiratory tract irritation were observed. There were no indications of specific target organ toxicity. [redacted]*(2006)*

Dermal		
Method	**LD_{50}/LC_{50}**	**Remarks**
Similar to OECD TG 402 5 Alderley Park rats/sex/dose As an aqueous suspension in propylene glycol at a single concentration of 1000 mg/kg under an occlusive dressing. Purity 97%	>1000 mg/kg	The applied dose was 1000 mg/kg, compared to 2000 mg/kg, which is the limit dose in a standard OECD TG 402 study. There were no deaths. Clinical signs of toxicity were seen 24 hours after dosing and included urinary incontinence/ and upward curvature of the spine. All of the animals appeared normal five days after dosing. Signs of slight skin irritation (desquamation and small scattered scabs) were seen during the study. There were no indications of specific target organ toxicity. [redacted] *(1982)*
Similar to OECD TG 402 New Zealand White rabbits, 4//sex/dose As an aqueous suspension in propylene glycol at a single concentration of 1000 under an occlusive dressing. Purity 97%	>1000 mg/kg	The applied dose was 1000 mg/kg not 2000, which is the limit in a standard OECD TG 402 study. There were no deaths. None of the rabbits showed any signs of systemic toxicity. There were indications of specific target organ toxicity. [redacted] *(1982)*
OECD TG 402 Rat Sprague Dawley (5/sex/dose) Limit Test, 2000 mg/kg Occlusive Purity 95.7%	>2000 mg/kg	There were no deaths. None of the animals showed any signs of systemic toxicity. There were indications of specific target organ toxicity. [redacted] *(2006b)*

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Data are available from acute oral dosing studies in rats, mice, rabbits and guinea pigs. The LD_{50} values for paclobutrazol ranged from the lowest value of 490 - 1219 mg/kg in mice to the highest value of 1336-1954 mg/kg in rats, all supporting classification for acute oral toxicity. In a further limit test, conducted in rats with technical material manufactured in China in 2006, no mortalities or clinical signs of toxicity or mortalities were observed at doses of 2000 mg/kg. The differing results may be due to the low solubility of paclobutrazol in water and the fact that the original rat study used surfactants in the vehicle which may have increased the bioavailability. This was not the case with the latter study which used distilled water only. However, the results of the original study cannot be dismissed and are considered relevant for classification.

4.2.1.2 Acute toxicity: inhalation

The four-hour LC_{50} of paclobutrazol in rats was calculated to be 4.79 mg/l for males and 3.13 mg/l for females, supporting classification for acute inhalation toxicity.

4.2.1.3 Acute toxicity: dermal

The acute dermal toxicity of paclobutrazol has been well investigated in studies in rats and rabbits, at concentrations of up to 1000 mg/kg, and in an additional study in rats at a concentration of 2000 mg/kg and no deaths or clinical signs of toxicity were observed. No classification is proposed.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

There is no information to inform on the acute toxicity potential of paclobutrazol in humans.

4.2.3 Summary and discussion of acute toxicity

See section 4.2.1

4.2.4 Comparison with criteria

For a single oral dose, LD_{50} values of 490 - 1219 (in mice) to 1336-1954 mg/kg or > 2000 mg/kg (in rats) were observed. Taking account of the earlier studies, the criteria for classification with Acute Tox 4 ($300 < ATE \leq 2000$ mg/kg) under the CLP Regulation are met.

For a single dermal exposure the LD_{50} was >1000 mg/kg. It is not possible to estimate where the dermal LD_{50} lies. Therefore no classification is proposed for acute dermal toxicity under the CLP Regulation.

Following single inhalation exposure, a 4-hour LC_{50} of 3.13-4.79 mg/l was identified in rats for a dust aerosol of paclobutrazol. Classification is required if the LC_{50} is $\geq 1 \leq 5$ mg/l for dusts and mists under the CLP Regulation. Therefore classification with Acute Tox 4 under the CLP Regulation is proposed.

4.2.5 Conclusions on classification and labelling

Acute Tox 4; H302 + H332 - Harmful if swallowed or if inhaled

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of paclobutrazol as acute toxicity Category 4 via the oral route (H302: Harmful if swallowed) on the basis of several studies with LD_{50} values of 490-1 219 (in mice) to 1 336-1 954 mg/kg bw or > 2 000 mg/kg bw (in rats) after a single oral dose.

The DS proposed no classification of paclobutrazol for acute dermal toxicity since it was not possible to estimate the LD_{50} by the dermal route based on three different studies where single doses of paclobutrazol of 1 000, 1 000 and 2 000 mg/kg bw were administered.

The DS proposed classification of paclobutrazol as acute toxicity Category 4 via the inhalation route (H332: Harmful if inhaled), since following a single inhalation exposure a 4-hour LC_{50} of 3.13-4.79 mg/L was identified in rats for a dust aerosol of paclobutrazol.

Comments received during public consultation

Two different Member States Competent Authorities (MSCAs) supported the DS's proposal for classification of paclobutrazol as acute toxicity Category 4 by both oral and inhalation routes.

One MSCA questioned the DS's proposal of classification by oral route, arguing that the vehicle used to increase the bioavailability of paclobutrazol in some studies may be toxic. This MSCA supported its position based on that other toxicity studies performed without surfactant reported LD_{50} values higher than 2 000 mg/kg bw. The DS replied that they welcomed a discussion on the potential effect of the vehicle in the oral toxicity studies, but did not provide any additional information about the acute toxicity of the vehicles used in the acute oral toxicity studies.

The same MSCA also questioned the DS's proposal to classify for acute toxicity via the inhalation route arguing that some of the animals sacrificed in extremis might have suffered from non-specific toxicity due to high dust concentration rather than substance-specific toxicity. The DS replied that no information indicated why the animals sacrificed in extremis were experiencing severe effects and therefore it is possible that these secondary non-specific effects might be due to exposure to the dust. However, without further information, these effects should be regarded as treatment-related deaths, thus supporting classification for acute inhalation toxicity.

Assessment and comparison with the classification criteria

The three tables below summarise the available acute toxicity studies by oral, dermal and inhalation routes, respectively.

Table: Summary of the acute oral toxicity studies with paclobutrazol

Study	Dose level	Results	Reference
Similar to OECD TG 401 Rats, Alderley Park 5 or 10 animals/sex and dose Purity 97 %	As an aqueous suspension in 0.5 % Lissitan at doses of 400, 500, 640, 800, 1 000, 1 260, 1 600, 2 000, 3 200, 4 000 and 5 000 mg/kg bw of paclobutrazol, by gavage	Clinical signs of toxicity (subdued behaviour, unsteady gait, and loss of righting reflex, hypothermia, coma, piloerection, respiratory difficulties and urinary incontinence) were apparent one hour after dosing and were seen at all dose levels. Deaths occurred at doses from 500 mg/kg bw and up in males, and from 640 mg/kg bw and up in females, within 4 days of dosing. Survivors appeared normal nine days after dosing.	Report No CTL/P/748 (1982)

<table>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><td colspan="3">Full mortality data recorded in this study</td></tr>
<tr><td>mg/kg bw</td><td>Males</td><td>Females</td></tr>
<tr><td>400</td><td>0/5</td><td>0/5</td></tr>
<tr><td>500</td><td>2/5</td><td>0/5</td></tr>
<tr><td>640</td><td>1/5</td><td>2/5</td></tr>
<tr><td>800</td><td>1/5</td><td>0/5</td></tr>
<tr><td>1 000</td><td>3/10</td><td>6/10</td></tr>
<tr><td>1 260</td><td>3/5</td><td>3/5</td></tr>
<tr><td>1 600</td><td>5/10</td><td>8/10</td></tr>
<tr><td>2 000</td><td>6/10</td><td>6/10</td></tr>
<tr><td>3 200</td><td>1/5</td><td>4/5</td></tr>
<tr><td>4 000</td><td>8/10</td><td>8/10</td></tr>
<tr><td>5 000</td><td>3/5</td><td>3/5</td></tr>
</table>

LD_{50} in males: 1 954 mg/kg bw
LD_{50} in females: 1 336 mg/kg bw
</td>
<td></td>
</tr>
<tr>
<td>OECD TG 425 Limit Test

Rats, Sprague-Dawley

5 females

Purity 95.77 %</td>
<td>2 000 mg/kg bw of paclobutrazol, suspension in distilled water</td>
<td>No mortalities or clinical signs of toxicity.

LD_{50} higher than 2 000 mg/kg bw</td>
<td>Syngenta T016891-04 (2006a)</td>
</tr>
<tr>
<td>Similar to OECD TG 401

Albino mice, Alderley Park

5/10 animals/sex and dose

Purity 97 %</td>
<td>As an aqueous suspension in 0.5 % Lissitan at doses of 250, 320, 400, 500, 640 and 800 mg/kg bw of paclobutrazol in males and 400, 500, 640, 800, 1 000 and 1 260 mg/kg bw in females.

In both males and females</td>
<td>Clinical signs of toxicity (subdued behaviour, piloerection, unsteady gait, hypothermia and coma) were apparent one hour after dosing and were seen at all dose levels. Deaths occurred at doses from 320 mg/kg bw and up in males, and at all tested doses in females within 3 days of dosing.

Survivors appeared normal six days after dosing.
<table>
<tr><td colspan="3">Full mortality data recorded in this study</td></tr>
<tr><td>mg/kg bw</td><td>Males</td><td>Females</td></tr>
<tr><td>250</td><td>0/5</td><td>1/10</td></tr>
<tr><td>320</td><td>1/5</td><td>1/5</td></tr>
<tr><td>400</td><td>2/5</td><td>4/10</td></tr>
<tr><td>500</td><td>1/5</td><td>7/10</td></tr>
</table>
</td>
<td>Report No CTL/P/748 (1982)</td>
</tr>
</table>

	paclobutrazol was administered by gavage.	640 4/5 5/10 800 5/5 1/10 1 000 - 9/10 1 260 - 4/5 2 000 - 2/5 2 500 - - 3 200 - - LD_{50} in males: 490 mg/kg bw LD_{50} in females: 1 219 mg/kg bw	
Similar to OECD TG 401 Guinea Pigs, Dunkin-Hartley 5 animals/sex and dose Purity 97 %	As an aqueous suspension in 0.5 % Lissitan at doses of 320, 400, 500 or 640 mg/kg bw of paclobutrazol by gavage. A further group of five male Guinea pigs received 800 mg/kg bw.	Clinical signs of toxicity (subdued behaviour and unsteady gait) were apparent three hours after dosing and were seen at all dose levels. Deaths occurred at doses from 400 mg/kg bw and up in males and from 500 mg/kg bw and up in females. Full mortality data recorded in this study mg/kg bw / Males / Females 320 0/5 0/5 400 1/5 0/5 500 3/5 3/5 640 2/5 5/5 800 5/5 - LD_{50} in males: 542 mg/kg bw LD_{50} in females: 400-640 mg/kg bw	Report No CTL/P/748 (1982)
Similar to OECD TG 401 Rabbits, New Zealand White 5 animals/sex and dose Purity 97 %	As an aqueous suspension in 0.5 % Lissitan at doses of 250, 500, 1 000 and 2 300 mg/kg bw of paclobutrazol by gavage.	Clinical signs of toxicity were seen at all dose levels within one hour after dosing and included subdued behaviour and unsteady gait. Full mortality data recorded in this study mg/kg bw / Males / Females 250 0/0 0/0 500 3/5 1/5 1000 1/5 2/5 2300 5/5 5/5 Most of the surviving animals appeared normal 12 days after dosing. LD_{50} in males: 835 mg/kg bw LD_{50} in females: 937 mg/kg bw	Report No CTL/P/748 (1982)

Table: Summary of the acute dermal toxicity studies with paclobutrazol

Study	Dose level	Results	Reference
Similar to OECD TG 402 Rats, Alderley Park 5 animals/sex and dose Purity 97 %	As an aqueous suspension in propylene glycol at a single concentration of 1 000 mg/kg bw of paclobutrazol under an occlusive dressing.	There were no deaths. Clinical signs of toxicity were seen 24 hours after dosing and included urinary incontinence and upward curvature of the spine. All of the animals appeared normal five days after dosing. Signs of slight skin irritation (desquamation and small scattered scabs) were seen during the study. LD_{50} higher than 1 000 mg/kg bw.	Report No CTL/P/748 (1982)
Similar to OECD TG 402 Rabbits, New Zealand White 4 animals/sex and dose Purity 97 %	As an aqueous suspension in propylene glycol at a single concentration of 1 000 mg/kg bw of paclobutrazol under an occlusive dressing.	There were no deaths. None of the rabbits showed any signs of systemic toxicity. LD_{50} higher than 1 000 mg/kg bw.	Report No CTL/P/748 (1982)
OECD TG 402 Rats, Sprague Dawley 5 animals/sex and dose Purity 95.7 %	Limit Test: 2 000 mg/kg bw of paclobutrazol under an occlusive dressing.	None of the animals showed any signs of systemic toxicity. LD_{50} higher than 2 000 mg/kg bw.	Syngenta T005846-05 (2006b)

Table: Summary of the acute inhalation toxicity studies with paclobutrazol

Study	Dose level	Results	Reference
OECD TG 403 Rats, Alderley Park 5/10 animals/sex and concentration	0, 0.54, 1.84, 3.70 and 5.19 mg/L of paclobutrazol, nose only for 4 hours to a dust aerosol MMAD (µm) = 2.89, 3.25, 5.40 and 4.53 for 0.54,	Animals exposed to 0.54 mg/L showed only a slight reduction in their response to sound which became apparent in the latter half of the exposure period. No other signs were sign in the 0.54 mg/L animals. Treatment-related clinical signs observed immediately after treatment at 1.84, 3.70, 5.19 mg/L, were reduced response to sound, increased breathing depth, reduced breathing rate, hunched posture and piloerection. These effects were accompanied in some animals by	Report No CTL/P/2072 (1988)

<table>
<tr>
<td>Purity 91.4 %</td>
<td>1.84, 3.70 and 5.19 mg/L, respectively</td>
<td>gasping, ‘reduced stability’, and abnormal respiratory noise (indicative of respiratory irritancy). Piloerection and respiratory noise persisted in survivor animals.

Small dose-related increase in lung/body weight ratio (statistically significant only in females).

<table>
<tr><td colspan="3">Full mortality data recorded in this study</td></tr>
<tr><td>mg/L</td><td>Males</td><td>Females</td></tr>
<tr><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.54</td><td>0</td><td>0</td></tr>
<tr><td>1.84</td><td>0</td><td>1</td></tr>
<tr><td>3.70</td><td>1</td><td>4</td></tr>
<tr><td>5.19</td><td>3</td><td>3</td></tr>
</table>

LC_{50} in males = 4.79 mg/L
LC_{50} in females = 3.13 mg/L</td>
<td></td>
</tr>
<tr>
<td>OECD TG 403

Rats, Han Wistar

5 animals/sex

Purity 95.7 %</td>
<td>2.02 mg/L of paclobutrazol, nose only for 4 hours to a dust aerosol

MMAD = 2.61 µm</td>
<td>No mortalities or clinical signs of toxicity were observed.

LC_{50} higher than 2.02 mg/L.</td>
<td>HR2542-REG (2006)</td>
</tr>
</table>

There are available data for acute oral toxicity in four different species (rats, mice, Guinea pig and rabbits). The LD_{50} values for paclobutrazol vary substantially, ranging from 490 mg/kg bw in male mice to 1 954 mg/kg bw in male rats.

RAC notes that in some studies paclobutrazol was administered in the presence of 0.5 % formaldehyde-naphthalenesulfonic acid condensate sodium salt (CAS number 9084-06-4) as a vehicle, whereas other studies used water as a vehicle although paclobutrazol is nearly insoluble in water (2.29×10^{-2} g/L, purified water). The use of 0.5 % formaldehyde-naphthalenesulfonic acid condensate sodium salt might have influenced the LD_{50} values in rats. This might be explained either by an increase of bioavailability of paclobutrazol (which is likely but not known) or by a toxic effect of the vehicle itself. However, RAC notes that, according to the C&L Inventory this substance is self-classified by notifiers only for skin and eye irritation, and not for acute toxicity. Therefore, RAC considers that the presence of the vehicle in some available acute toxicity studies does not diminish their relevance for classification purposes.

According to the Guidance on the Application of the CLP Criteria, if there are different LD_{50} values from tests using different vehicles, generally the lowest valid value would be the basis for classification. Therefore, the LD_{50} of 490 mg/kg bw recorded in male mice should be

considered for classification by the oral route, warranting a classification in Category 4 (300 mg/kg bw ≤ LD_{50} ≤ 2 000 mg/kg bw).

RAC notes that the difference in LD_{50} values for male and female mice seems to point to a difference in sensitivity that is not obvious in other species. RAC also notes that the LD_{50} values for female mice is 2.5 times higher than the LD_{50} values for male mice, probably influenced by the mortality rate at 800 mg/kg bw, which is atypically low when comparing to the lower and higher doses. Therefore, taking this into consideration, RAC proposes the LD_{50} detected in male mice (490 mg/kg bw) as ATE instead of the classical approach of combined LD_{50}.

In the available acute dermal toxicity studies, paclobutrazol did not induce mortalities in rats at either 1 000 or 2 000 mg/kg bw, or in rabbits at 1 000 mg/kg bw. The highest LD_{50} value for considering a substance for classification is 2 000 mg/kg bw and therefore, with the available data RAC agrees with the DS that classification of paclobutrazol for acute dermal toxicity is not warranted.

Following a single inhalation exposure of 4 hours for a dust aerosol of paclobutrazol in rats with LC_{50} of 3.13-4.79 mg/L, classification in Category 4 (1.0 mg/L ≤ LC_{50} ≤ 5.0 mg/L) is warranted. For the ATE, RAC proposes 3.13 mg/L.

In conclusion, RAC supports the DS's proposal for classification of paclobutrazol as acute toxicity Category 4; H302 (Harmful if swallowed), with an ATE of 490 mg/kg bw and acute toxicity Category 4; H332 (Harmful if inhaled), with an ATE of 3.13 mg/L (dust and mist).

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 10.

All clinical signs were considered to be non-specific signs of general acute toxicity. No adverse effects were noted in surviving animals. No effects attributable to specific target organ toxicity were observed for any relevant route of exposure.

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 paclobutrazol 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) 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 DS proposed no classification of paclobutrazol for STOT SE since all the effects were considered non-specific. Comments received during public consultation No comments were received during public consultation. Assessment and comparison with the classification criteria RAC notes that the acute toxicity studies did not reveal indications of (non-lethal and severe) specific target organ toxicity after a single exposure, which is a requirement for classification

as either STOT SE 1 or 2. No narcotic effects were reported and there were no robust indications of respiratory tract irritation; only gasping and abnormal respiratory noise (potential indicators of respiratory irritancy) were described (without observing a dose-response relationship) in three females (belonging to three different dosing groups) and two males (belonging to two different dosing groups). Hence, classification as STOT SE 3 is not warranted.

In conclusion, RAC supports the DS's proposal for no classification of paclobutrazol for STOT SE.

4.4 Irritation

4.4.1 Skin irritation

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks
Similar to OECD TG 404 Rabbits New Zealand White (n=6 females) Purity 97 % [redacted] *(1982)*	Mean 24-72 hour individual animal scores intact scores: Erythema 1,1,1.5,1,1,1.5 Oedema 0,0,0,0,0,0	The study was conducted in 1977 and pre-dates the advent of the OECD TGs. No 48-hour time point investigation was conducted, but this is not considered to confound interpretation of the study. No eschar scores were reported
OECD TG 404 Rabbits New Zealand White (n=3, 2 females and 1 male) Purity 95.7 % [redacted] *(2006c)*	Mean 24-72 hour individual animal scores intact scores: Erythema 0.3, 0.6, 0 Oedema 0,0,0	

4.4.1.1 Non-human information

The skin irritation potential of paclobutrazol has been investigated in both a standard and a non-standard study in rabbits. Paclobutrazol caused slight erythema in the first study, with mean scores of 1 or 1.5 in 6/6 tested rabbits. In the standard study, erythema was observed in 2/3 tested rabbits with mean individual scores of 0.3 and 0.6. No oedema was observed in either study.

4.4.1.2 Human information

There is no information on the skin irritation potential of paclobutrazol in humans.

4.4.1.3 Summary and discussion of skin irritation

See section 4.4.1.1.

4.4.1.4 Comparison with criteria

In the standard study, slight skin reactions (erythema) were observed, which were insufficient to support classification (i.e., all individual mean scores were < 2.3 and had resolved by the end of the observation period). Effects were not severe in any individual animal. Therefore the criteria for classification are not met.

Erythema was observed in the non-standard study (conducted with six animals), but the individual mean scores in all animals was <2.3 and had resolved by the end of the observation period.

Effects were not severe in any individual animal. Therefore, the criteria for classification are not met.

4.4.1.5 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 proposed no classification of paclobutrazol for skin corrosion/irritation on the basis of the following findings: i) one non-standard study in rabbits reporting slight erythema with mean 24 and 72 hour scores of 1.0 in four animals and 1.5 in two animals and no oedema; and, ii) one standard study in rabbits reporting slight erythema with mean 24-72 hour scores of 0.3, 0.6 and 0 in three animals and no oedema.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies.

Table: Summary of skin corrosion/irritation studies with paclobutrazol

Study	Dose level	Results	Reference
Similar to OECD TG 404 Rabbits, New Zealand White	500 mg paclobutrazol moistened with 0.5 mL of olive oil Occlusive	<u>Mean 24-72-hour individual animal scores:</u> Erythema: 1, 1, 1.5, 1, 1, 1.5 Oedema: 0, 0, 0, 0, 0, 0 No eschar scores were reported.	Report No CTL/P/741 (1982)

6 females Purity 97 %	dressing for 24 hours	The study was conducted in 1977 (before OECD TG was available). No 48-hour time point investigation was conducted, but this is not considered to confound interpretation of the study.	
OECD TG 404 Rabbits, New Zealand White 3 animals (2 females and 1 male) Purity 95.7 %	500 mg paclobutrazol moistened with water Occlusive dressing for 4 hours	<u>Mean 24-72-hour individual animal scores:</u> Erythema: 0.3, 0.6, 0 Oedema: 0, 0, 0	Syngenta T005847-05 (2006c)

RAC notes that the slight skin reactions (erythema) observed in the two available studies were insufficient to support classification since the mean scores were always lower than 2.3 (the minimum erythema score for triggering classification). Therefore, RAC supports the DS's proposal for no classification of paclobutrazol for skin irritation and corrosion.

4.4.2 Eye irritation

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks
Similar to OECD TG 405, New Zealand White rabbits (n=6 females) Purity 97% [redacted] *(1982)*	Mean 24-72 hour individual animal scores: Corneal Opacity 0.66, 0.33, 0.66, 0, 0.66, 0.33 Iris 0, 0 ,0, 0, 0,0 ,0 Conjunctival Redness 1.25, 1.25, 1, 1, 1.25, 1 Conjunctival Chemosis 0.33, 0.33, 0.66, 0, 33.66, 0.33	The study was conducted in 1977 and pre-dates the advent of the OECD TGs., and included 28, 48 and 72 hour time points. All effects reversed by the end of the observation period.
Similar to OECD TG 405, New Zealand White Rabbits (n=3 females) Purity 95.7% [redacted] *(2006d)*	Mean 24-72 hour individual animal scores: Corneal Opacity 1, 1, 0 Iris 0.33, 0.33, 0.33 Conjunctival Redness 1, 1, 1 Conjunctival Chemosis 0, 0.0 0.33	All effects reversed by the end of the observation period.

4.4.2.1 Non-human information

The eye irritation potential of paclobutrazol has been investigated in both a standard and a non-standard study in rabbits.

In the standard study, corneal opacity was observed in 2/3 rabbits with a mean 24-72 hour individual score of 1). No iritis was observed. Conjunctival redness (3/3 rabbits mean score grade 1) and chemosis (1/3 rabbits mean score 0.33) were observed. All effects reversed by the end of the observation period.

In the non-standard study, five of the six animals had slight corneal opacity and all of the animals had moderate redness of the conjunctivae with some chemosis and discharge. No iritis was observed in any of the rabbits. All of the observed effects had resolved by day 7 post instillation.

4.4.2.2 Human information

There is no information to inform on the eye irritation potential of paclobutrazol in humans.

4.4.2.3 Summary and discussion of eye irritation

See section 4.4.2.1.

4.4.2.4 Comparison with criteria

The positive findings in the standard study; 2/3 animals with a corneal opacity score of 1 meet the CLP criteria for classification as a Category 2 eye irritant. That is if, when applied to the eye of an animal, a substance produces (in at least in 2 of 3 tested animals) a positive response of:

- corneal opacity ≥ 1 but < 3 and/or
- iritis ≥ 1 but < 1.5, and/or
- conjunctival redness ≥ 2 and/or
- conjunctival oedema (chemosis) ≥ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

As the non-standard 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 is greater than the cut off values (stated above) in 4 out of the 6 animals. No individual animal average was greater than the cut-off values.

Overall, the results of the standard eye irritation study indicate that placlobutrazol meets the criteria for classification as a Category 2 eye irritant. The criteria for Category 1 (serious eye damage) were not met.

4.4.2.5 Conclusions on classification and labelling

Eye Irrit 2; H319 - Causes serious eye irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of paclobutrazol as eye irritant Category 2; H319 (Causes serious eye irritation) on the basis of one study showing reversible corneal opacity grade 1 in 2/3 animals.

Comments received during public consultation

Three different MSCAs supported the proposal for classification as eye irritant Category 2; H319.

Assessment and comparison with the classification criteria

The table below summarises the available eye corrosion/irritation studies.

Table: Summary of eye corrosion/irritation studies with paclobutrazol

Study	Dose level	Results	Reference
Similar to OECD TG 405 Rabbits, New Zealand White 6 females Purity 97 %	100 mg paclobutrazol instilled into the conjunctival sac and after 30 seconds the eye was gently washed	Mean 24-72-hour individual animal scores: Corneal opacity: 0.66, 0.33, 0.66, 0, 0.66, 0.33 Iris: 0, 0, 0, 0, 0, 0, 0 Conjunctival redness: 1.25, 1.25, 1, 1, 1.25, 1 Conjunctival chemosis: 0.33, 0.33, 0.66, 0.33, 0.66, 0.33 All effects were reversed by the end of the observation period.	Report No CTL/P/741 (1982)
Similar to OECD TG 405 Rabbits, New Zealand White 3 females Purity 95.7 %	100 mg paclobutrazol instilled into the conjunctival sac	Mean 24-72-hour individual animal scores: Corneal opacity: 1, 1, 0 Iris: 0.33, 0.33, 0.33 Conjunctival redness: 1, 1, 1 Conjunctival chemosis: 0, 0, 0.33 All effects were reversed by the end of the observation period.	Syngenta T005848-05 (2006d)

The first study (Report No CTL/P/741, 1982) showed slight reversible corneal opacity, conjunctival redness and chemosis in 6/6 animals but not severe enough to trigger classification. A second study (Syngenta T005848-05, 2006d) also showed slight reversible iritis (score 0.33 in 3/3 animals), conjunctival redness (score 1 in 3/3 animals) and chemosis (score 0.33 in 1/3 animals) not reaching the scores triggering classification. However, corneal opacity score 1 was reported in 2/3 animals, meeting the criteria for classification as eye irritant Category 2.

Since positive results that are adequate for classification should not be overruled by negative findings, RAC supports the DS's proposal for classification of paclobutrazol for Eye Irrit. 2; H319 (Causes serious eye irritation).

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

There is no information from single (Section 4.2) and repeated inhalation exposure (Section 4.7.1.2) studies in experimental animals to indicate that paclobutrazol is a respiratory tract irritant.

4.4.3.2 Human information

There is no information on the respiratory tract irritation potential of paclobutrazol in humans.

4.4.3.3 Summary and discussion of respiratory tract irritation

There is no information in humans, or from studies in experimental animals to indicate that paclobutrazol is a respiratory tract irritant.

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification

4.5 Corrosivity

Paclobutrazol is not a skin irritant, see section 4.4.

4.5.1 Non-human information

4.5.2 Human information

There are no data available on the skin corrosivity of paclobutrazol.

4.5.3 Summary and discussion of corrosivity

See section 4.5.1

4.5.4 Comparison with criteria

No evidence of skin corrosivity was observed in the skin irritation study.

4.5.5 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification.

4.6 Sensitisation

4.6.1 Skin sensitisation

The skin sensitisation potential of paclobutrazol has been well investigated in a LLNA and a Guinea Pig Maximisation Test.

Table 13: Summary table of relevant skin sensitisation studies

Method	Results	Remarks
OECD TG 429 LLNA Mouse (4/concentration CBA/ca/ola/has strain/) Vehicle DMF Purity 95.7% [redacted] *(2006)*	SI 10% - 0.8 25% - 0.7 65% 0.9 **Positive control,** hexylcinnamanic aldehyde 6.5	Negative
Similar to OECD TG 406 maximisation study Guinea-pig/ Dunkin Hartley strain 20 test 10 negative control 92.4% purity [redacted] *(1982)*	Negative 1/20 test animals died **50%** 3/19 grade 1 and 1/19 grade 2 at 24 hours 1/19 grade 1 at 48 hours **25%** 1/19 grade 1 at 24 hours **10%** No adverse skin reactions **Negative Controls** 2/8 grade 1 after 24 hours at 50% 1/8 grade 1 at 25%	<u>Induction:</u> Intradermal: 1% in dimethylformamide/corn oil Skin responses not reported Topical: 75% in dimethylformamide Skin responses not reported <u>Challenge</u>: 10, 25 and 50% in dimethylformamide assessed at 24 and 48 hrs No positive control animals were included in the study

4.6.1.1 Non-human information

In the LLNA, groups of 4 mice were tested with 0, 10, 25 and 65% paclobutrazol in dimethylformamide. The maximum concentration was selected following a preliminary study, and a positive control substance, hexylcinnamonaldehyde was included. All animals were treated daily for 3 consecutive days, after which the animals were sacrificed and the draining lymph nodes excised for further analysis. Stimulation indices for paclobutrazol were below 1 for all 3 test concentrations. The positive control produced a SI of 6.5. Overall, paclobutrazol tested negative.

The guinea pig maximisation study was broadly similar to OECD TG 406. The most important deviation from the guideline was the lack of a positive control group. Clear negative responses were observed in this study which employed challenge concentrations of 10, 25 and 50% paclobutrazol. It should be noted the induction concentration was low raising some concerns as to the quality of this study. Slight positive skin reactions were observed in 4/19 (21%) test animals after 24 hours, reducing to 1/19 after 48 hours. .

4.6.1.2 Human information

There is no information to inform on the skin sensitisation potential of paclobutrazol in humans.

4.6.1.3 Summary and discussion of skin sensitisation

Paclobutrazol tested negative in a standard LLNA (all SI < 3) and also in a non-standard guinea pig test for assessment of skin sensitisation potential.

4.6.1.4 Comparison with criteria

A stimulation index (SI) of 3 or more is considered a positive response in the LLNA. The SI values for paclobutrazol were below 1 for all test concentrations in the LLNA. In an adjuvant type study a response in at least 30% of animals is considered to be a positive result. The sensitisation response was <30 % in the maximisation test with paclobutrazol. Therefore, the criteria for classification are not met.

4.6.1.5 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of paclobutrazol for skin sensitisation on the basis of a mouse local lymph node assay (LLNA) showing stimulation indexes lower than 1, and a maximisation study in Guinea pigs (GPMT) showing that paclobutrazol-challenged animals did not suffer adverse skin reaction with incidences higher than the respective negative controls.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The table below summarises the available skin sensitisation studies.

Table: Summary of skin sensitisation studies with paclobutrazol

Study	Dose level	Results	Reference
OECD TG 429 (LLNA) Mice, CBA/Ca/Ola/Has 4 animals/concentration	25 µl of 10, 25 and 65 % paclobutrazol in dimethylformamide during 3 consecutive days Positive control: 25 % hexylcinnamanic aldehyde	<u>Stimulation index:</u> Paclobutrazol 10 % = -0.8 Paclobutrazol 25 % = -0.7	T005837-05 (2006)

Purity 95.7 %		Paclobutrazol 65 % = 0.9 Positive control = 6.5 Global result: negative	
Similar to OECD TG 406 (GPMT) Guinea Pigs, Dunkin Hartley 20 animals for the test and 10 for negative control Purity: 92.4 %	Induction: Intradermal: 1 % in dimethylformamide/corn oil Topical: 75 % in dimethylformamide (occlusive dressing during 2 days) Challenge: In each animal: 10% (lower left flank), 25 % (right flank) and 50 % (upper left flank) in dimethylformamide under occlusive dressing during 24 hours (assessed at 24 and 48 hours) No positive control.	One animal died during the study and the occlusive dressings for challenge application slipped (no assessment was performed on these animals). The skin responses during induction were not reported. Positive responses: Paclobutrazol 50 %: 4/16 at 24 hours and 1/16 at 48 hours Paclobutrazol 25 %: 1/16 at 24 hours Paclobutrazol 10 %: 0/16 Negative control at 50 %: 2/7 after 24 hours and 0/7 after 48 hours Negative control at 25 %: 1/7 after 24 hours and 0/7 after 48 hours	Report No CTL/P/741 (1982)

The two available skin sensitisation tests showed negative results with the stimulation index in the mouse LLNA being lower than 3 and the skin reactions in the GPMT occurring in below 30% of the test animals. In addition, RAC notes certain deficiencies in the GPMT (no positive control and no report about the skin responses during the induction treatment) that limits the relevance of this study for classification purposes.

In conclusion, RAC agrees with the DS that the criteria for classification for skin sensitisation are not met.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data are available. However, paclobutrazol gave negative results in two skin sensitisation studies.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

Not classified, data lacking.

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal The DS proposed no classification of paclobutrazol for respiratory tract irritation since there are no relevant data to substantiate such classification. Comments received during public consultation No comments were received during public consultation. Assessment and comparison with the classification criteria RAC supports the DS's proposal of no classification of paclobutrazol for respiratory tract irritation due to the absence of data.

4.7 Repeated dose toxicity

The repeated dose toxicity of paclobutrazol has been investigated in standard 90-day and lifetime dietary studies in rats, in a lifetime dietary study in mice, in 90-day and 1 year capsule studies in dogs, and in a 3-week repeated dermal application study, also in rats.

Table 14: Summary table of relevant repeated dose toxicity studies

Method	Results (±*)
90-day study Rat (20/sex/dose Alderley Park, Wistar derived) OECD TG 408 Oral, diet 0, 50, 250 and 1250 ppm equivalent to 0, 3.7, 19 or 93 mg/kg /day in males and 0, 4.4, 22 and 107 mg/kg /day in females 92.4% purity Dose level relevant for classification (guidance value for 90-day rat study) 100 mg/kg bw/d [redacted] *(1983a)*	There were no deaths or treatment-related clinical signs of toxicity observed in any dose group. With the exception of activated partial thromboplastin clotting time, there were no treatment-related changes in any haematology parameter investigated, see below. **<u>1250 ppm (93 mg/kg/day in males and 107 mg/kg/day in females)</u>** ***Males*** 40 % ↑ ALT at week 4 and by 15% at study termination 8% ↑ in relative liver weight Liver; Hydropic change – minimal (11/20) moderate, (2/20) 10% ↑ aminopyrene-N-demethylase activity ***Females:*** 1-9% ↓ Food consumption and 7% ↓body weight gain 20% ↑ activated partial thromboplastin clotting time at week 4 and by 13% at study termination ↑ in absolute (16%) and relative (19%) liver weight Liver; Hydropic change – minimal (10/20) moderate, (1/20) 33% ↑ aminopyrene-N-demethylase activity, **<u>250 ppm (19 mg/kg/day in males and 22 mg/kg/day in females)</u>** ***Males:*** Liver; Hydropic change – minimal (9/20), moderate (1/20) ***Females:*** ↑ absolute (7%) and relative (6%) liver weight. Liver; Hydropic change – minimal (4/20) , moderate (0/20) 11% ↑ aminopyrene-N-demethylase activity **<u>50 ppm (3.7 mg/kg/day in males and 4.4 mg/kg/day in females)</u>** ***Males:*** Liver, Hydropic change – minimal (5/20), moderate (2/20) ***Females:*** Liver, Hydropic change – minimal (2/20), moderate (0/20) **<u>Control</u>** ***Males*:** Liver, Hydropic change – minimal (8/20), moderate (2/20) ***Females:*** Liver, Hydropic change – minimal (7/20), moderate (0/20) *A NOAEL of 19-22 mg/kg/day, was identified, based on liver weight increases in males and females, at 93-107 mg/kg/day the highest dose tested.

2-year study Sprague-Dawley rats (50/sex/dose) OECD TG 453 Dosed for 24 months, via the diet Interim sacrifice (12 months): 10/sex/group 0, 50, 250 and 1250 ppm paclobutrazol Equivalent to 0, 2.2, 11, and 54; and 0, 2.8, 14 and 72 mg/kg/day in males and females respectively Purity 92.4% Dose level relevant for classification (calculated from the guidance value for 90-day rat study) 12 mg/kg bw/d [redacted] *(1986b)*	There were no differences in mortality rates between treated animals and controls. **<u>1250ppm (54 mg/kg/day in males and 72 mg/kg/day in females)</u>** ***Males:*** No changes in body weight, body weight gain or food consumption ↑ absolute (14%) and relative (12%) liver weight Hepatic steatosis 32/50 ***Females***: ↓ body weight (16 % at terminal sacrifice and 21% at interim sacrifice) 22% ↓ body weight gain 30% ↑ relative liver weight Hepatic steatosis 34/50 **<u>250 ppm (11 mg/kg/day in males and 14 mg/kg/day in females)</u>** ***Males:*** Hepatic steatosis 8/50 ***Females***: 13% ↓ body weight gain **<u>50ppm (2.2 mg/kg/day in males and 2.8 mg/kg/day in females)</u>** No toxicologically significant changes observed *A NOAEL of 2.2-2.8 mg/kg/day was identified, the lowest dose tested, based on hepatic hypertrophy/steastosis in males and decreases in body weight gain in females, at doses of 11-14 mg/kg/day and above
2-year study OECD TG 453 Mouse (CD-1 strain) (63/sex/dose) Dosed for 24 months, Interim sacrifice (12 months): 12/sex/group 0, 25, 125 and 750 ppm paclobutrazol Equivalent to 0, 2.6, 14, and 81,; and 0, 3, 16 and 89mg/kg/day in males and females respectively Purity 92.4% Dose level relevant for classification (calculated from the guidance value for 90-day rat study) 12 mg/kg bw/d [redacted] *(1986a)*	There were no differences in mortality rates between treated animals and controls, and no changes in food consumption. **Non Neoplastic changes** **<u>750 ppm (81 mg/kg/day in males and 89 mg/kg/day in females)</u>** ***Males:*** 42% ↓ Cholesterol at week 104 36% and 31% ↑ Triglycerides at weeks 52 and 104 respectively ↑ absolute (10%) and relative (18%) liver weight at week 52. ↑ absolute (29%) and relative (31%) liver weight at week 104. Hepatic hypertrophy/steatosis severity : Total incidence 37/52; Severity grade 1 - (0/52), grade 2 - (3/52), grade 3 - (10/52), grade 4 - (12/52) and grade 5 - (12/52). ***Females* :** ↑ body weight (16% at terminal sacrifice and 21% at interim sacrifice) 21% ↑ body weight gain **<u>125ppm (14 mg/kg/day in males and 16 mg/kg/day in females)</u>** ***Males:*** Hepatic hypertrophy/steatosis: total incidence 34/52; Severity grade 1 - (4/52), grade 2 - (10/52), grade 3 - (14/52), grade 4 - (6/52) and grade 5 - (0/52)

	<u>25 ppm (2.6 mg/kg/day in males and 3 mg/kg/day in females)</u> ***Males:*** Hepatic hypertrophy/steatosis : total incidence 29/51; Severity, grade 1 - (3/51), grade 2 - (7/51), grade 3 - (14/51), grade 4 - (4/51) and grade 5 - (1/51) **<u>Control</u>** ***Males:*** Hepatic hypertrophy/steatosis ; total incidence 30/52; severity grade 1 - (1/52), grade 2 - (12/52), grade 3 - (12/52), grade 4 - (2/52) and grade 5 - (3/52) ***Females:*** ↑ absolute (18%) and relative (25%) liver weight at week 104 *A NOAEL of 14-16 mg/kg/day was identified, based on clinical chemistry changes and liver weight increases observed at a dose of 81-89 mg/kg/day, the highest dose tested.
90-days week oral capsule. Broadly consistent with OECD TG 409 Beagle dogs 4/sex/dose Doses of 0, 3, 15 and 450 mg/kg/day Purity 95.6% Dose level relevant for classification (based on the guidance value for 90-day rat study) 100 mg/kg bw/d [redacted] *(1987a)*	There were no deaths or treatment-related clinical signs of toxicity observed at any dose level. **<u>450mg/kg/day</u>** ***Males:*** 6% ↓ body weight 8.5 fold ↑ Alkaline phosphatase 2.3 fold ↑Hepatic aminopyrene-N-demethylase activity ↑ absolute (35%) and relative (40%) liver weights hepatocyte fine fat deposition (4/4 compared to 1/4 in controls) 18% ↑ relative kidney weights Testes: ↓ absolute (51%) and relative (48%) weights Giant spermatid cells (3/4 compared to 0/4 in controls) Immature testes (4/4 compared to 0/4 in controls). Epididymides: ↓ absolute (32%) and relative (31%) weights No spermatozoa in epididymides (3/4 compared to 0/4 in controls), ***Females*:** 5 fold ↑Alkaline phosphatase 2.6 fold ↑ Hepatic aminopyrene-N-demethylase activity ↑ absolute (36%) and relative (46%) liver weights ↑ absolute (19%) and relative (27%) kidney weights **<u>15 mg/kg/day and 3 mg/kg/day</u>** No adverse effects noted *A NOAEL of 15 mg/kg/day was identified, based on clinical chemistry changes, testicular weight decreases, and abnormal testicular histopathology observed at a dose of 450 mg/kg/day, the highest dose tested.

1-year oral capsule. Broadly consistent with OECD TG Beagle dogs 6/sex/dose Doses of 0, 15, 75 and 300 mg/kg /day Purity 92.4% Dose level relevant for classification (calculated from the guidance value for 90-day rat study) 24 mg/kg bw/d [redacted], *(1984)*	There were no deaths or treatment-related clinical signs of toxicity observed at any dose level. **<u>300mg/kg/day</u>** ***Males :*** 44% ↓ body weight gain 41% ↑ Alkaline phosphatase 73% ↑ triglycerides 2.38 fold ↑Hepatic aminopyrene-N-demethylase activity ↑ absolute (38%) and relative (42%) liver weights Mild hepatocellular swelling (2/6 compared to 0/6 in controls) 13% ↑ relative kidney weights ***Females*:** 44% ↑Alkaline phosphatase 80% ↑ triglycerides 1.9 fold ↑ Hepatic aminopyrene-N-demethylase activity ↑ absolute (29%) and relative (31%) liver weights focal ballooned hepatocytes minimal (2/6 compared to 3/6 in controls) and focal ballooned hepatocytes slight (3/6 compared to 0/6 in controls) **<u>75 mg/kg/day</u>** ***Males:*** 13%↑ Alkaline phosphatase 1.5 fold ↑Hepatic aminopyrene-N-demethylase activity ↑ absolute (24%) and relative (25%) liver weights ***Females*:** 17% ↑ Alkaline phosphatase 1.5 fold ↑Hepatic aminopyrene-N-demethylase activity Focal ballooned hepatocytes minimal (4/6 compared to 3/6 in controls) and focal ballooned hepatocytes slight (2/6 compared to 0/6 in controls) **<u>15 mg/kg/day</u>** ***Males:*** 1.1 fold ↑ Hepatic aminopyrene-N-demethylase activity *It was not possible to identify a clear NOAEL as hepatic aminopyrene-N-demethylase activity was elevated at doses of 15 mg/kg/day and above, the lowest dose tested. A LOAEL of 15 mg/kg/day is proposed.

± Values are reported as increased (↑) or decreased (↓) compared to controls

*** NOAEL/NOEL/LOAEL values are taken from the DAR and provided for information only.**

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rats

In a 90-day study groups of rats (Alderley Park Wistar derived 20 sex/dose) were administered paclobutrazol in the diet at concentrations of 50, 250 and 1250 ppm (equivalent to 3.7, 19, 93 and 4.4,

22, 107 mg/kg day for males and females respectively). There were no deaths or treatment-related clinical signs of toxicity.

Body weight and food consumption were statistically significantly decreased, when compared to controls, in females at the top dose only throughout the study period. There were no treatment-related changes observed in males.

The following changes were noted at 93-107 mg/kg/day: increased plasma alanine transaminase (ALT) activity in males at weeks 4 (40%) and 13 (15%). Minor changes in ALT activity are not regarded as being toxicologically significant, but they may reflect slight alterations in liver function. Hepatic aminopyrene-N-demethylase activity was statistically significantly increased compared to controls, by 10 and 33% in males and females respectively at the top doss, and in females receiving 22 mg/kg/day, by 11%. The toxicological significance of this change is unclear.

Compared to controls, absolute and relative liver weights were increased in females at doses of 22 mg/kg/day and above (by 7 and 6 % absolute and relative respectively at 22 mg/kg/day, and 16 and 19% absolute and relative respectively at 107 mg/kg/day). In males, relative liver weight was statistically significantly increased at the top dose, by 8%. The only histopathological finding at necropsy was hepatic hydropic change, which occurred with the same incidence in control and treated animals, and is therefore considered to be a spontaneous finding, not treatment-related. There were no other treatment related histological changes observed doses of up to 93-107 mg/kg/day, including reproductive tissues.

In a lifetime study; groups of rats, (Sprague-Dawley strain 12 interim +50/main study - sex/dose) were administered paclobutrazol in the diet for 2-years at concentrations of 0, 50, 250 and 1250 ppm (equivalent to 0, 2.2, 11, 54 and 0, 2.8, 14, 72 mg/kg day for males and females respectively). There were no deaths or treatment-related clinical signs of toxicity.

No treatment-related changes were observed in food consumption, body weight, body-weight gain or mortality rates in males. In females, no treatment-related changes were observed in food consumption, or mortality rates. However, body weight gain was statistically significantly decreased, compared to controls, by 13 and 22% at doses of 14 mg/kg/day and above, and body weights; interim (21%) and terminal (16%) sacrifice at the highest dose of 72 mg/kg/day. Organ weight changes were confined to increased absolute (14%) and relative (12%) liver weights in high dose males, and increased relative liver weights (30%) in high dose females, at terminal sacrifice. No toxicologically significant clinical chemistry changes were observed.

At terminal sacrifice the incidence of hepatic steatosis with hypertrophy was statistically significantly increased at the top dose, in males (32/50 compared to 1/51 in controls) and females (34/50 compared to 0/50 in controls.). It was also noted in 8/50 males administered 11 mg/kg/day at terminal sacrifice. There is no reason not to discount these changes as being relevant for human health. No other toxicologically significant changes were observed.

Mouse

CD-1 strain mice were administered paclobutrazol at concentrations of 0, 25, 125 and 750 ppm (equivalent to 0, 2.6, 14, 81 and 0, 3, 16, 89 mg/kg day for males and females respectively) via the diet for 104 weeks. There were no treatment-related changes in food consumption. Body weight, and body-weight gain were increased in females at 89 mg/kg/day..

The only clearly treatment-related clinical chemistry changes were in triglyceride and cholesterol in males; cholesterol was statistically significantly decreased (by 42% compared to controls) at the top dose at study termination, and triglyceride levels were statistically significantly decreased at week 52 and 104 (compared to controls by 36 and 31% respectively).

Compared to controls, a statistically significant increase in liver weight was observed in males given 81 mg/kg/day at week 52 (10% absolute and 18% relative) and week 104 (29% absolute and 31% relative). No clearly treatment-related hepatic histopathological changes were observed. It is noteworthy that hepatic steatosis was present at similar incidence in control and treated animals, suggesting that this change may be a spontaneous pathology in this strain of mouse. No other treatment-related toxicologically significant changes were observed.

Dogs

Beagle dogs (4/sex/ dose) were administered paclobutrazol at doses 0, 3, 15 and 450 mg/kg/day via capsule for 90-days. The age of the dogs on commencement of the study was 20-23 weeks. The range of in-life and study termination investigations was comparable with those expected for a standard OECD TG 409 study.

Isolated instances of decreases in body weight gain were noted in top dose animals, but these are not regarded as toxicologically significant. The terminal body weight of high dose males was found to be statistically significantly decreased, by 6% only.

No treatment-related effects were noted in any haematological parameter measured.

With the exception of marked increases in alkaline phosphatase activity (8.5 and 5 fold in males and females respectively compared to controls) at the top dose, no treatment-related clinical chemistry changes were observed. It is probable that the small group size contributed to the large increases in alkaline phosphatase activity not achieving statistical significance. Hepatic aminopyrine-N-demethylase activity was increased at least two-fold in males and females at the top dose.

At necropsy, absolute and relative liver weights were statistically significantly increased, compared to controls (by 35 and 40% in males and 36 and 46% in females) at 450 mg/kg/day, the highest dose tested. Histopathological examination found hepatocyte fine fat deposition in 4/4 high dose males.

Marked decreases in absolute and relative testes (by 51 and 48% compared to controls) and epididymides (by 32 and 31% compared to controls) weights were observed at the top dose. The testes were immature and spermatozoa absent from the epididymal ducts in all high dose animals. These changes are treatment-related and probably reflect a slight retardation in attainment of sexual maturity of these animals.

Kidney weights were statistically significantly increased in males (relative weight by 18%) and females (absolute and relative by 19 and 27% respectively) at 450 mg/kg/day; however, the increases were not associated with any histopathological changes.

Beagle dogs (6/sex/dose) were administered paclobutrazol at doses 0, 15, 75 and 300 mg/kg/day via capsule for1-year. The age of the dogs on commencement of the study was 20-23 weeks. The range of in-life and study termination investigations was comparable with those expected for a standard OECD TG 409 study.

There were no changes in food consumption in males or females, and body weight gain in females. In males receiving 300 mg/kg/day, body weight gain was statistically significantly decreased throughout the study (decreased by 44% compared to controls at study termination).

No treatment-related effects were noted in any haematological parameter measured.

Clinical chemistry investigations found statistically significant increases in alkaline phosphatase levels at 75 (13 and 17% in males and females respectively) and 300 mg/kg/day (41 and 44% in males and females respectively), and increased triglycerides at the top dose only (73 and 80% in males and females respectively). These changes may reflect perturbations in liver function. Minor decreases (<10%) in total protein, albumin and calcium levels were noted, but are not regarded as toxicologically significant. Hepatic aminopyrine-N-demethylase activity was statistically significantly increased in all treated males (by 1.1-2.4 fold) and females at doses of 75 mg/kg/day and above (1.2-1.9 fold).

At necropsy, absolute and relative liver weights were statistically significantly increased, compared to controls, in males at 75 (by 24 and 25% absolute and relative respectively) and 300 mg/kg/day (by 38 and 42% absolute and relative respectively). Increased absolute and relative liver weights were also observed in females at the top dose only (29 and 31% respectively). Focal hepatocyte ballooning was observed in females at 75 (minimal 4/6 compared to 3/6 in controls and slight 2/6 compared to 0/6 in controls) and 300 mg/kg/day (minimal 2/6 compared to 3/6 in controls and slight 3/6 compared to 0/6 in controls), see table below. No other histopathological changes were observed, in the liver or other organs, including the testes.

Table showing hepatic histopathological changes

Sex	Males				Females			
Dose	0	15	75	150	0	15	75	150
Mild hepatocellular swelling	0	0	0	2	0	0	0	0
Ballooned hepatocytes (minimal focal)	0	0	0	0	3	3	4	2
Ballooned hepatocytes (slight focal)	0	0	0	0	0	0	2	3

4.7.1.2 Repeated dose toxicity: inhalation

There are no studies available

4.7.1.3 Repeated dose toxicity: dermal

There is only one repeated dermal application study available, a non-standard study, conducted in rabbits with a 21-day exposure period. The study is limited in terms of design, compared to modern test guidelines, particularly the study period and the use of animals with abraded skin, more usual in older skin irritation studies.

Method	Results (±*)
3-week dermal toxicity study Non standard Rabbits (New Zealand White) 10/sex/dose) 5/sex/dose with abraded skin and 5/sex/dose without Doses of 0, 10, 100 and 1000 mg/kg/day 6-hours a day, 5-days per week for 3 weeks. Purity 97% Dose level relevant for classification (calculated from the guidance value for 90-day rat study) 600 mg/kg bw/d [redacted] *(1980)*	Two control males and 3 males and 3 females receiving 100 mg/kg/day via abraded skin died during the study, but these deaths are not considered to be treatment-related. No treatment-related systemic effects were reported at any dose level. The only changes observed related to local irritation.

± Values are reported as increased (↑) or decreased (↓) compared to controls

In the only repeated dermal application study available, no systemic effects were observed. However, the study design is limited such that this study provides no useful information on the potential of paclobutrazol to cause toxicity following repeated dermal application. A systemic NOAEL of 1000 mg/kg/day was identified, the highest dose tested.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

There is no information in humans to inform on the potential of paclobutrazol to cause repeated toxicity by any route of exposure.

4.7.1.6 Other relevant information

No other relevant information.

4.7.1.7 Summary and discussion of repeated dose toxicity

The repeated dose toxicity of paclobutrazol has been investigated in standard 90-day and lifetime dietary studies in rats, in a lifetime study in mice, in 90-day and 1 year capsule studies in dogs, and in a single 3-week repeated dermal application study, also in rats.

The dog was found to be the most sensitive species, with testicular toxicity observed in the 90 day study, and liver toxicity in the 1-year study. In rats, the liver was the critical target organ, with liver changes observed in both the 90-day and lifetime studies.

Liver Toxicity

Liver toxicity was thoroughly investigated in a 1-year study, in dogs. At the lowest dose of 15 mg/kg/day a relatively minor increase in hepatic aminopyrine-N-demethylase was observed which is of uncertain toxicological significance as no other indicators of liver toxicity were observed at this dose. At 75 mg/kg/day increased alkaline phosphatase and hepatic aminopyrine-N-demethylase activity were observed in both males and females. These changes were associated with absolute and relative liver weight increases in males only, and focal hepatocyte ballooning in females. At the highest dose, the previously noted enzyme activities were elevated, along with an increase in triglyceride levels. Absolute and relative liver weight increases were observed in males and females, with focal hepatocyte ballooning observed in females only. There is no reason to discount these liver changes as not being relevant for human health.

In rats, the most thorough investigation of liver toxicity was conducted in a 2-year study at doses of 2.2-2.8 mg/kg/day and above. In this study, no treatment-related changes were observed at the lowest dose of 2.2-2.8 mg/kg/day or in females administered 11 mg/kg/day. In males administered 14 mg/kg/day, the only adverse effect was hepatic steatosis with hypertrophy. At the highest dose of 54-72 mg/kg/day, toxicologically significant liver weight increases were observed in male and females. This increase in liver weight was associated with an increased incidence of hepatic steatosis with hypertrophy in males, which was also prevalent in females at this dose. There is no reason to discount these liver changes as not being relevant for human health. Although these changes occurred around the guidance values for classification, when revised to take account of study duration, the liver changes are not regarded of sufficient severity to support classification.

Testicular Toxicity

In a 90-day study in dogs, testicular toxicity was only observed at a dose of 450 mg/kg/day, the highest dose tested. Testicular toxicity, manifested as marked decreases in absolute and relative testes and epididymides weights associated with an absence of spermatozoa from the epididymidal ducts, and the testes were described as immature. Testicular toxicity was associated with increased liver and kidney weights, and fine fat deposition in the hepatocytes. However, the observed testicular toxicity is unlikely to be a secondary consequence of kidney and liver changes.

The observation of aspermatazoic testes in the 90-day study at a dose which did not cause marked body weight decreases suggests that the testicular changes are treatment-related. It is possible that the testicular changes reflect retardation of sexual maturity, as male dogs usually attain sexual maturity around 3-months of age. Some support for this view comes from the 1-year dog study in which no testicular toxicity was observed. The lack of testicular toxicity in the 1-year study may indicate that paclobutrazol-treated male dogs are able to overcome this retardation in attainment of sexual maturity.

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

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

Refer to section 4.7.1.7

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

None of the observed changes discussed above are regarded of sufficient severity to support classification with STOT-RE or they occurred outside the estimated guidance values for an equivalent 90-day study, as discussed above.

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

Not classified. Conclusive, but not sufficient for classification.

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

Summary of the Dossier Submitter's proposal

The DS assessed repeated dose toxicity studies in rats (90-d and two-year studies by oral route), mice (two-year study by oral route), dogs (90-d and one-year studies by oral route) and rabbits (3-week study by dermal route). The DS identified liver as a potential target organ of paclobutrazol. However, the DS proposed no classification of paclobutrazol for STOT RE because either the observed effects were regarded to not be of sufficient severity to support classification, or the effects occurred outside the CLP guidance values.

Comments received during public consultation

One MSCA proposed classification of paclobutrazol as STOT RE Category 2 on the basis of the hepatotoxicity reported in the: i) one-year oral dog study at 15 mg/kg bw/d; ii) two-year rat study at 54 and 72 mg/kg bw/d; iii) 90-d rat study at 22, 93 and 107 mg/kg bw/d; and iv) two-year study in mice at 14 and 81 mg/kg bw/d. The DS replied that most of the reported findings appeared at doses above the CLP guidance values for classification in category 2 or were not considered to be severe enough to cause significant hepatotoxicity or impairment of liver function. One MSCA also stated that: *"although the DS states that there is "no testicular toxicity in the 1 year dog study", there were in fact degenerative changes in the seminiferous epithelium (1-0-2-2), moderate chronic inflammatory cell infiltration (0-0-0-1), seminiferous debris (1-0-0-2), atrophic prostate (0-0-0-1) and dilated acini of the prostate (0-0-0-1). The testicular toxicity might be taken into consideration for STOT RE, but it is argued in the CLH dossier that it would rather suggest a delay in sexual maturation. Such an offspring effect would justify classification as Repr. 2; H361f."* The DS replied that indeed some testicular changes were observed in the one-year study, but these changes were not dose-related and could easily be chance findings.

Assessment and comparison with the classification criteria

The table below summarises the available oral repeated dose toxicity studies.

Table: Summary table for oral repeated dose toxicity studies with paclobutrazol

Method	Results	Reference
OECD TG 408 90-d study Rats, Alderley Park (Wistar derived) 20 animals/sex and dose Oral, diet 0, 50, 250 and 1 250 ppm equivalent to 0, 3.7, 19 or 93 mg/kg bw/d in males and 0, 4.4, 22 and 107 mg/kg bw/d in females Purity: 92.4 % Guidance value for classification in category 2: 100 mg/kg bw/d	There were no deaths or treatment-related clinical signs of toxicity observed in any dose group. <u>1 250 ppm (93 mg/kg bw/d in males and 107 mg/kg bw/d in females)</u> <u>Males</u> 40 % ↑ ALT at week 4 and 15% at study termination 8 % ↑ in relative liver weight Hydropic changes in liver: minimal (11/20), moderate (2/20) 10 % ↑ aminopyrene-N-demethylase activity <u>Females</u> 1-9 % ↓ Food consumption and 7 % ↓ body weight gain 20 % ↑ activated partial thromboplastin clotting time at week 4 and 13 % at study termination ↑ in absolute (16 %) and relative (19 %) liver weight Hydropic changes in liver: minimal (10/20), moderate (1/20) 33 % ↑ aminopyrene-N-demethylase activity <u>250 ppm (19 mg/kg bw/d in males and 22 mg/kg bw/d in females)</u> <u>Males</u> Hydropic changes in liver: minimal (9/20), moderate (1/20) <u>Females</u> ↑ absolute (7 %) and relative (6 %) liver weight Hydropic changes in liver: minimal (4/20), moderate (0/20) 11 % ↑ aminopyrene-N-demethylase activity <u>50 ppm (3.7 mg/kg bw/d in males and 4.4 mg/kg bw/d in females)</u> <u>Males</u> Hydropic changes in liver: minimal (5/20), moderate (2/20) <u>Females</u> Hydropic changes in liver: minimal (2/20), moderate (0/20) <u>Control</u> <u>Males</u> Hydropic changes in liver: minimal (8/20), moderate (2/20) <u>Females</u> Hydropic changes in liver: minimal (7/20), moderate (0/20) NOAEL = 19-22 mg/kg bw/d LOAEL = 93-107 mg/kg bw/d (liver changes)	Report No CTL/P/760 (1983a)

OECD TG 453 Two-year study Rats, Sprague-Dawley 50 animals/sex and dose Oral, diet Interim sacrifice (12 months): 10 animals/sex and group 0, 50, 250 and 1250 ppm equivalent to 0, 2.2, 11, and 54; and 0, 2.8, 14 and 72 mg/kg bw/d in males and females, respectively Purity 92.4 % Guidance value for classification in category 2: 12.5 mg/kg bw/d	There were no differences in mortality rates between treated animals and controls. <u>1 250 ppm (54 mg/kg bw/d in males and 72 mg/kg bw/d in females)</u> <u>Males</u> ↑ absolute (14 %) and relative (12 %) liver weight Hepatic steatosis 32/50 <u>Females</u> ↓ body weight (16 % at terminal sacrifice and 21 % at interim sacrifice) 22 % ↓ body weight gain 30 % ↑ relative liver weight Hepatic steatosis 34/50 <u>250 ppm (11 mg/kg bw/d in males and 14 mg/kg bw/d in females)</u> <u>Males</u> Hepatic steatosis 8/50 <u>Females</u> 13 % ↓ body weight gain <u>50 ppm (2.2 mg/kg bw/d in males and 2.8 mg/kg bw/d in females)</u> No toxicologically significant changes observed NOAEL = 2.2-2.8 mg/kg bw/d LOAEL = 11-14 mg/kg bw/d (liver changes)	Report No 5055-72/273 / CTL/C/1763A (1986b)
OECD TG 453 Two-year study Mice, CD-1 63 animals/sex and dose Oral, diet Interim sacrifice (12 months): 12 animals/sex and group 0, 25, 125 and 750 ppm, equivalent to 0, 2.6, 14, and 81; and 0, 3, 16 and 89 mg/kg bw/d in males and females, respectively Purity: 92.4 % Guidance value for classification in	There were no differences in mortality rates between treated animals and controls. <u>750 ppm (81 mg/kg bw/d in males and 89 mg/kg bw/d in females)</u> <u>Males</u> 42 % ↓ cholesterol at week 104 36 % and 31 % ↑ triglycerides at weeks 52 and 104, respectively ↑ absolute (10 %) and relative (18 %) liver weight at week 52 ↑ absolute (29 %) and relative (31 %) liver weight at week 104 Hepatic hypertrophy/steatosis: grade 1: 0/52; grade 2: 3/52; grade 3: 10/52; grade 4: 12/52; grade 5: 12/52. <u>Females</u> ↑ body weight (16 % at terminal sacrifice and 21 % at interim sacrifice) 21 % ↑body weight gain <u>125 ppm (14 mg/kg bw/d in males and 16 mg/kg bw/d in females)</u> <u>Males</u> Hepatic hypertrophy/steatosis: grade 1: 4/52; grade 2: 10/52; grade 3: 14/52; grade 4: 6/52; grade 5: 0/52	Report No 5014-72/274 / CTL/C/1759A (1986a)

category 2: 12.5 mg/kg bw/d	25 ppm (2.6 mg/kg bw/d in males and 3 mg/kg bw/d in females) Males Hepatic hypertrophy/steatosis: grade 1: 3/51; grade 2: 7/51; grade 3: 14/51; grade 4: 4/51; grade 5: 1/51 Control Males Hepatic hypertrophy/steatosis: grade 1: 1/52; grade 2: 12/52; grade 3: 12/52; grade 4: 2/52; grade 5: 3/52 Females ↑ absolute (18 %) and relative (25 %) liver weight at week 104 NOAEL = 14-16 mg/kg bw/d LOAEL = 81-89 mg/kg bw/d (liver changes)	
Broadly consistent with OECD TG 409 90-d Dogs, Beagle 4 animals/sex and dose Oral capsule Doses of 0, 3, 15 and 450 mg/kg bw/d Purity: 95.6 % Guidance value for classification in category 2: 100 mg/kg bw/d	There were no deaths or treatment-related clinical signs of toxicity observed at any dose level. 450 mg/kg bw/d Males 6 % ↓ Body weight 8.5 % ↑ Alkaline phosphatase 2.3 % ↑ Hepatic aminopyrene-N-demethylase activity ↑ Absolute (35 %) and relative (40 %) liver weights Hepatocyte fine fat deposition (4/4 compared to 1/4 in controls) 18 % ↑ Relative kidney weights ↓ Absolute (51 %) and relative (48 %) testes weights Giant spermatid cells (3/4 compared to 0/4 in controls) Immature testes (4/4 compared to 0/4 in controls). ↓ Absolute (32 %) and relative (31 %) weights of epididymides No spermatozoa in epididymides (3/4 compared to 0/4 in controls) Females 5 % ↑ Alkaline phosphatase ↑ Absolute (19 %) and relative (27 %) kidney weights 2.6 % ↑ Hepatic aminopyrene-N-demethylase activity ↑ Absolute (36 %) and relative (46 %) liver weights 15 mg/kg bw/d and 3 mg/kg bw/d No adverse effects noted. NOAEL = 15 mg/kg bw/d LOAEL = 450 mg/kg bw/d (liver and testicular changes)	Report No CTL/P/1496 (1987a)
Reported as “Broadly consistent with OECD TG” one-year Dogs, Beagle 6 animals/sex and dose	There were no deaths or treatment-related clinical signs of toxicity observed at any dose level. 300 mg/kg bw/d Males 44 % ↓ Body weight gain 41 % ↑ Alkaline phosphatase 73 % ↑ Triglycerides 2.38 ↑ Hepatic aminopyrene-N-demethylase activity	Report No CTL/P/958 (1984)

Oral capsule Doses of 0, 15, 75 and 300 mg/kg bw/d Purity: 92.4 % Guidance value for classification in category 2: 25 mg/kg bw/d	↑ Absolute (38 %) and relative (42 %) liver weights Mild hepatocellular swelling (2/6 compared to 0/6 in controls) 13 % ↑ Relative kidney weights <u>Females</u> 44 % ↑ Alkaline phosphatase 80 % ↑ Triglycerides 1.9 ↑ Hepatic aminopyrene-N-demethylase activity ↑ Absolute (29 %) and relative (31 %) liver weights Focal ballooned hepatocytes: minimal (2/6 compared to 3/6 in controls) and slight (3/6 compared to 0/6 in controls) <u>75 mg/kg bw/d</u> <u>Males</u> 13 %↑ Alkaline phosphatase 1.5 ↑ Hepatic aminopyrene-N-demethylase activity ↑ Absolute (24 %) and relative (25 %) liver weights <u>Females</u> 17 % ↑ Alkaline phosphatase 1.5 ↑ Hepatic aminopyrene-N-demethylase activity Focal ballooned hepatocytes: minimal (4/6 compared to 3/6 in controls) and slight (2/6 compared to 0/6 in controls) <u>15 mg/kg bw/d</u> <u>Males</u> 1.1 fold ↑ Hepatic aminopyrene-N-demethylase activity <u>LOAEL = 15 mg/kg bw/d (liver changes)</u>	

In addition to the studies summarised in the table above, the CLH report also includes a repeated dose toxicity study by the dermal route in rabbits dosed with 0, 10, 100 and 1 000 mg paclobutrazol/kg bw/d (6 hours/d, 5 d/week for 3 weeks). This study reported only local irritation with no treatment-related systemic toxicity at any dose level.

The table below summarises all adverse effects seen at dose levels below the guidance values in the CLP criteria for classification as STOT RE.

Table: Summary of adverse effects reported in the repeated dose toxicity studies with paclobutrazol, potentially relevant for classification as STOT RE

Study	Effect	Dose (mg/kg bw/d)	Guidance value for STOT RE classification (mg/kg bw/d)
90-d study in rats	15 % ↑ ALT 8 % ↑ in relative liver weight Hydropic changes in liver: minimal (11/20), moderate (2/20) 10 % ↑ aminopyrene-N-demethylase activity	93	Cat 1 ≤ 10 10 ≤ Cat 2 ≤ 100

90-d study in rats	Hydropic changes in liver (males + females): minimal (13/40), moderate (1/40) ↑ absolute (7 %) and relative (6 %) liver weight (females) 11% ↑ aminopyrene-N-demethylase activity (females)	19-22	Cat 1 ≤ 10 10 ≤ Cat 2 ≤ 100
90-d study in rats	Hydropic changes in liver (males + females): minimal (7/40), moderate (2/40)	3.7-4.4	Cat 1 ≤ 10 10 ≤ Cat 2 ≤ 100
Two-year study in rats	Hepatic steatosis 8/50 in males (not reported in females) ↓ 16 % body weight and 22% bodyweight gain ↓ 13 % bodyweight gain	11 72 14	Cat 1 ≤ 1.25 1.25 ≤ Cat 2 ≤ 12.5
Two-year study in mice	Hepatic hypertrophy/steatosis in males (not reported in females): grade 1: 3/51; grade 2: 7/51; grade 3: 14/51; grade 4: 4/51; grade 5: 1/51	2.6	Cat 1 ≤ 1.25 1.25 ≤ Cat 2 ≤ 12.5
One-year study in dogs	1.1 fold ↑ Hepatic aminopyrene-N-demethylase activity ↓ 44 % body weight gain	15 300	Cat 1 ≤ 2.5 2.5 ≤ Cat 2 ≤ 25

Significant bodyweight and bodyweight gain reductions were reported in the two-year study in rats at 72 and 14 mg/kg bw/d and in the one-year study in dogs at 300 mg/kg bw/d. These bodyweight and bodyweight gain reductions were considered significant by EFSA in the paclobutrazol peer review report. However, RAC notes that, despite the severity of the reductions (between 13 and 44 %), these reductions always appear at doses above the guidance values for warranting STOT RE classification.

Minimal to moderate hydropic changes (cellular swelling) in liver were reported in the 90-d toxicity study in rat at all tested doses. However, this effect was also reported in control animals with a high background incidence (18 and 5 % incidence for minimal and moderate severity, respectively, in males and females combined). There was no clear increased severity with dose. A dose-related increase in the incidence of minimal hydroponic changes was observed at 250 ppm (11 and 14 mg/kg bw/d in males and females, respectively) and at the top dose of 1 250 ppm in males (17 % at a dose of 93 mg/kg bw/d). Other hepatocellular effects reported in this study were an increase of 15 % of ALT, a maximum increase of 8 and 6% in absolute and relative liver weights and an increase of 11% in the aminopyrene-N-demethylase activity. RAC considers the above effects (specifically considered in Annex I: 3.9.2.8.1 of Guidance on the Application of the CLP Criteria) not severe enough to support classification.

Hepatic steatosis was also reported in both rat and mouse two-year carcinogenicity studies. The table below summarises the incidences and the severity of hepatic steatosis in the mouse study. The steatosis was characterised by vacuolation of the hepatocyte cytoplasm. The only treatment-related effect was on the severity of steatosis in the male liver. This was scored on a scale of 1-5 based on the degree of cytoplasmic vacuolation. All animals had steatosis to some degree, but the amount was highly variable, even in controls, ranging from minor cytoplasmic vacuolation (grade 1) to prominent vacuolation throughout the lobule (grade 5). Steatosis in males given 25 and 125 ppm was similar to controls. In females, the degree of

steatosis was equally variable, but there was no appreciable difference between any of the treatment groups and the control groups.

Table: Incidence of hepatic steatosis in the two-year toxicity study in mouse. The dose below the reference value for warranting classification as Category 2 (1.25 < C ≤ 12.5 mg/kg bw/d) is shaded in grey.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
MALES					
Control group 1	1	2	2	5	0
Control group 2	1	2	5	1	2
2.6 mg/kg bw/d	1	2	5	3	1
14 mg/kg bw/d	0	5	4	2	0
81 mg/kg bw/d	0	0	1	3	7
FEMALES					
Control group 1	0	2	3	4	2
Control group 2	0	2	4	5	1
3 mg/kg bw/d	3	0	4	2	1
16 mg/kg bw/d	0	0	5	4	3
89 mg/kg bw/d	0	1	1	5	3

In the two-year study in rats, a centrilobular hypertrophy in the liver sometimes accompanied by a minor degree of steatosis in both sexes in animals given the highest dose was reported. The hypertrophy was generally low grade and was slightly more evident in females than in males. There was no hypertrophy/steatosis in animals given lower doses. The table below shows the incidences of liver steatosis, necrosis and hypertrophy in this two-year study in rats.

Table: Incidences of liver steatosis, necrosis and hypertrophy in the two-year toxicity study in rat. The dose below the reference value for warranting classification as Category 2 (1.25 < C ≤ 12.5 mg/kg bw) is shaded in grey.

	Steatosis			Necrosis			
	Focal	Zonal	Lobal	Focal	Zonal	Lobal	Hypertrophy
MALES							
Control	1	8	0	1	1	0	0
2.2 mg/kg bw/d	0	2	1	1	1	0	0
11 mg/kg bw/d	1	5	0	2	0	1	0
54 mg/kg bw/d	1	8	1	1	0	0	1
FEMALES							
Control	2	10	0	1	1	0	0
2.8 mg/kg bw/d	0	10	0	2	0	0	0
14 mg/kg bw/d	1	5	0	1	1	0	0
72 mg/kg bw/d	0	7	0	2	1	0	12

The historical control data of the performing facility from three contemporary chronic toxicity studies conducted in the same strain of rats shows that hepatic steatosis is a relatively common finding in rats with incidences up to 30-39 % (addendum to the Draft Assessment Report, publicly available). The overall incidence of hepatic steatosis reported in male rats at 11 mg/kg bw/d is clearly within the historical control data.

The EFSA peer review report about paclobutrazol considered steatosis as adverse stating that: *"The historical control data (30-39 %) refer to the overall incidence of all forms of steatosis (zonal, focal, lobar, single cell, vacuolated and hypertrophic) in the male rat and not only to a*

form of steatosis associated with hypertrophy, as seen in the Shaw (1986) study. Therefore, the 25 % incidence of steatosis (steatosis/hypertrophy) observed at 250 ppm cannot be directly compared with the historical control data (30-39 %) provided by the applicant for all forms of steatosis. However, when adding up the incidences of all forms of steatosis seen in the 250 ppm rats, a total of 52 % is obtained, which is significantly above the historical control ranges (30-39 %) for all forms of steatosis".

This information (the details about historical control data) was not available to RAC for assessment but nevertheless RAC also notes that the incidences of steatosis, necrosis and hypertrophy at doses below 12.5 mg/kg bw/d (the guidance value for classification) are low and do not dose-dependent. In conclusion, RAC does not consider the liver effects reported in the two-year rat and mouse studies as relevant for classification for STOT RE.

An increase of 10 % in hepatic aminopyrene-N-demethylase activity was reported in the one-year toxicity study in dog at a dose of 15 mg/kg bw/d that could warrant classification as STOT RE category 2 (guidance values: 2.5 < Cat 2 ≤ 25). However, RAC does not consider this effect relevant for classification purposes because it is very mild and a small change in clinical biochemistry is not severe enough for supporting classification.

In conclusion, RAC considers that the repeated toxicity studies indicate liver as the main target organ for paclobutrazol. However, the significant alterations either appear at doses that do not warrant classification or appear with incidences and severities that do not compromise the functionality of the organ.

Therefore, RAC supports the DS proposal for no classification of paclobutrazol as STOT RE.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 15: Summary table of relevant *in vitro* mutagenicity studies

Method	Results	Remarks
Ames (OECD 471 *S. typhimurium* TA98, TA100, TA1535 TA 1537 and 1538 Five concentrations between 1.6-5000 µg/plate 94.2% purity *Callander, R.D (1982)*	- S9: Negative + S9: Negative	Positive controls were included, and gave the expected results In experiment 2, a slight dose-related increase was observed in strain TA1537 with S9; however, this increase was not statistically significant and did not exceed the twice-background limit criteria. In strain TA98, an increase in the number of revertants was observed with and without S9. Although these increases did not exceed 2x background and failed to follow a dose-response relationship, in view of the statistical significance of the results (with S9), a repeat experiment was performed, which gave negative results. In all three experiments, toxicity was observed at the highest concentration (5000 µg/plate).
Mammalian cell gene mutation (TK) (OECD 476 Mouse lymphoma 1-100 ug/plate in the first experiment and 60-140 ug/plate in the second Purity not specified *Mcgregor, D., Riach, C (1982)*	- S9: Negative + S9: Negative	Positive controls were included , and gave the expected results The selected concentrations were shown to extend into the cytotoxic range during a pre-study cytotoxicity assay.
In vitro cytogenetics (OECD TG 743 Human lymphocytes (1m and 1f) 50, 250 and 500 µg/ml Purity 98.8% *Mackay, J.M (1990)*	- S9: Negative + S9: Negative	Positive controls were included, and gave the expected results Mitotic index was assessed by examining 1000 lymphocytes per culture and. One hundred cells in metaphase were analysed. Extended analysis of an additional 100 metaphases per culture was conducted for the male solvent controls and the 500 µg/ml dose level without metabolic activation. A repeat study was performed using only the male donor cells at a concentration of 500 µg/ml without metabolic activation. A small (4 compared to 0.5 in negative controls) but statistically significant increase in aberrant cells was recorded for the 500 µg/ml concentration in the male donor cultures treated in the absence of S9-mix. It was not reproducible and was not considered to be biologically significant. To confirm this, a further limited repeat assay was conducted. No statistically or biologically significant increases in chromosomal damage were observed,

Table 16: Summary table of relevant *in vivo* mutagenicity studies

Method	Results	Remarks
Bone Marrow chromosomal aberration - broadly consistent with OECD 475 0, 30, 150 and 300 mg/kg via, gavage in corn oil Rats (Alderley Park strain) 8/sex/group 92.4% [redacted] *(1984a)*	Negative	The positive controls responded as expected Paclobutrazol induced a statistically significant increase in the percentage of cells with abnormalities, including and excluding gaps, in males at 300 mg/kg at the 12-hour harvest time (3.0 with gaps compared to 1.78 in controls and 0.83 without gaps compared to 0.33 in controls). Abnormalities were also increased in females but the increases did not achieve statistical significance. These findings were considered not to be biologically significant, no treatment-related effects were observed at later harvest times.
Bone Marrow chromosomal aberration 0 and 250 mg/kg/day mg/kg via, gavage in corn oil, for 5-days Rats (Alderley Park strain) 6 m and 6 f per group 92.4% [redacted] *(1984b)*	Negative	The positive controls responded as expected The dose level was selected on the basis of a sighting study, in which 259 mg/kg/day was found to be the MTD. No treatment-related effects were observed in test animals.
Bone Marrow micronucleus, OECD 474 Mice - (5/sex/group, C57/BL strain) 0, 233 and 373 mg/kg , in corn oil, via gavage 92% purity [redacted] *(1983)*	Negative	The positive controls responded as expected One male at 233 mg/kg was killed *in extremis* approximately 4 hours after dosing and 3 males at 373 mg/kg were killed *in extremis* approximately 22 hours after dosing. Two females at 373 mg/kg were killed *in extremis* after dosing (after 4 and 20 hours, respectively). No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, compared to vehicle controls were observed.
Bone Marrow micronucleus, Broadly consistent with OECD 474 Mice - C57/BL strain (5/sex/group) 0, 87.5 and 140 mg/kg , in corn oil, IP route 92.4% purity [redacted] *(1991)*	Negative	Positive controls responded as expected. Dose levels were selected on the basis of a sighting study in which 5 male mice were administered a single IP dose of 100, 250, 500, 750 or 1000 mg/kg/. Mortalities were observed at doses of 250 mg/kg and above (4/5. 4/5/4/5 and 5/5). Doses used were 80 and 50% of the LD_{50} value. A statistically significant increase in the frequency of micronuclei at 140 mg/kg at the 24-hour time was noted. The individual incidence of micronuclei for mice treated with paclobutrazol was within the control range of 0 to 12, even at the time interval (24 hours) which showed the statistically significant increase. No statistically significant increases in the incidence of micronuclei were noted at 87.5 mg/kg at 24 hours or with both treatment levels at later sampling times of 48 and 72 hours. The apparent positive results at 140 mg/kg at the 24-hour sampling time are considered to reflect a high

		variation in background incidence of micronuclei rather than evidence of clastogenicity.
In Vivo UDS OECD TG 482 0, 40, 200 and 400 mg/kg via, gavage in corn oil Rats (Alderley Park strain) 5/sex/group 92.4% purity [redacted] *(1986)*	Negative	Positive controls responded as expected. There is no information available to inform on dose selection.
OECD TG 478 Dominant Lethal Test Mice (CD-1 strain) - 20 males dosed at 0, 25, 100 and 300 mg/kg/day for 5-days via, gavage in corn oil 15 were mated with untreated females 92.4% purity [redacted] *(1983)*	Negative	The positive control substance gave the expected results One of the males at 300 mg/kg /day died on Day 4 of the dosing period. Mortality was not observed at any other dose level. Clinical signs of toxicity consisting of piloerection, urinary incontinence and tremors were noted in males at 300 mg/kg /day. Statistically significant reductions in fertility were noted in pregnant females at 100 mg/kg /day in Weeks 4 and 8, and in females at 25 mg/kg /day in Week 8. This effect was considered not to be treatment related because reductions were not dose related and were not observed in any other week. No treatment-related effects on the mean number of implantations per pregnancy at any dose level were noted. There was no evidence of an effect on the number of early deaths or the percentage of implantations that were late or early deaths.

4.9.1 Non-human information

4.9.1.1 In vitro data

The *in vitro* genotoxicity of paclobutrazol has been well investigated in an Ames test, a mammalian cell gene mutation test (TK) and an *in vitro* cytogenetics test using human lymphocytes. The appropriate positive controls were included and gave the expected results.

In the Ames test, a slight dose-related increase was observed in strain TA1537 with S9; however, this increase was not statistically significant and did not exceed the twice-background limit criteria. In strain TA98, an increase in the number of revertants was observed with and without S9. A repeat experiment was performed, which showed unequivocal negative results. Paclobutrazol gave negative results in a mammalian cell gene mutation test, at the TK locus.

In the *in vitro* chromosome aberration test, a small but statistically significant increase in aberrant cells was recorded for the 500 µg/ml dose level in the male donor cultures treated in the absence of S9-mix. This was a small increase in only one donor at a dose level which was at the limit of solubility. It was not reproducible and was not considered to be biologically significant. To confirm this, a further limited repeat assay was conducted. No statistically or biologically significant increases in chromosomal damage were recorded for the male donor cultures treated at 500ug/ml in the absence of metabolic activation in the repeat assay. Overall, it can be concluded that paclobutrazol is not genotoxic *in vitro*.

4.9.1.2 *In vivo* data

The *in vivo* genotoxicity of paclobutrazol has been extensively investigated in two rat bone marrow cytogenetics tests, two mouse bone marrow micronucleus tests (one gavage the other ip) a rat liver UDS test and a mouse dominant lethal assay. The appropriate positive controls were included and gave the expected results. In all studies, the highest dose was based on a preliminary study, conducted to establish the maximum tolerated dose.

Paclobutrazol tested negative in a bone marrow cytogenetics test, in which animals were dosed for 5-days at 250 mg/kg/day, in a gavage mouse micronucleus test at doses of up to 375 mg/kg, in a rat liver UDS test at doses of up to 400 mg/kg and in a mouse dominant lethal test.

In the single dose bone marrow chromosomal aberration test, paclobutrazol induced a statistically significant increase in the percentage of cells with abnormalities, including and excluding gaps, in males at 300 mg/kg at the 12-hour harvest time only. Abnormalities were also increased in females but the increases did not achieve statistical significance. No evidence of any treatment-related effect on the incidence of aberrations was noted at 30, 150 or 300 mg/kg in either sex. Also, at the 48-hour harvest, no treatment-related effects on the incidence of aberrations were noted in males or females at 300 mg/kg.

In an ip mouse micronucleus test, a wide variation in the mean incidence of micronuclei was observed in the control groups 2.8 at the 24-hour sampling time and 4.8 at the 72-hour sampling time with individual values ranging from 0 to 12. A statistically significant increase in the frequency of micronuclei at 140 mg/kg at the 24-hour time was noted. No statistically significant increases in the incidence of micronuclei were noted at 87.5 mg/kg at 24 hours or with both dose levels at the other sampling times. The positive results at 140 mg/kg at the 24-hour sampling time are considered to reflect variation in the background incidence of micronuclei rather than a clastogenic effect of paclobutrazol.

Paclobutrazol has been well investigated for genotoxicity *in vitro* and *in vivo*, and it can be concluded that paclobutrazol is not genotoxic.

4.9.2 Human information

No data available

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

The available data indicate that paclobutrazol is not mutagenic *in vitro* or *in vivo*

4.9.5 Comparison with criteria

Paclobutrazol tested negative *in vitro* and *in vivo*, and no classification for germ-cell mutagenicity is proposed.

4.9.6 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of paclobutrazol for germ cell mutagenicity on the basis of three *in vitro* studies (one Ames test, one mammalian cell gene mutation test and one cytogenetic test) and six *in vivo* studies (two bone marrow chromosomal aberrations tests, two bone marrow micronucleus tests, one unscheduled DNA synthesis test and one dominant lethal test) all showing negative results.

Comments received during public consultation

One MSCA alerted about a mistake in the references and authorship of two of the studies that was cited by the DS in the CLH report. The DS recognised the mistakes and they were corrected by RAC in this opinion.

Assessment and comparison with the classification criteria

The two tables below summarise the results of the all available mutagenicity and genotoxicity tests.

Table: Summary table of relevant in vitro mutagenicity studies with paclobutrazol.

Method	Test system	Tested concentrations	Results	Remarks	Reference
OECD TG 471 Ames test	S. typhimurium TA98, TA100, TA1535 TA 1537 and 1538	Five concentrations between 1.6-5 000 µg/plate. Purity: 94.2 %	- S9: Negative. + S9: Negative.	Positive controls were included and gave the expected results. Three different experiments. Toxicity was observed at the highest concentration.	Callander, R.D (1982)
OECD TG 476 Mammalian cell gene mutation (TK)	Mouse lymphoma	1-100 µg/plate in the first experiment and 60-140 µg/plate in the second. Purity not specified.	- S9: Negative. + S9: Negative.	Positive controls were included and gave the expected results. The selected concentrations were shown to extend into the cytotoxic range during a pre-study cytotoxicity assay.	Mcgregor and Riach (1982)
OECD TG 743 *In vitro* cytogenetics	Human lymphocytes (1m and 1f)	50, 250 and 500 µg/mL Purity 98.8 %	- S9: Negative. + S9: Negative.	Positive controls were included and gave the expected results.	Mackay (1990)

				A small (4 compared to 0.5 in negative controls) but statistically significant increase in aberrant cells was recorded for the 500 µg/mL concentration in the male donor cultures treated in the absence of S9-mix. It was not reproducible in a second assay and was not considered to be biologically significant.	

Table: Summary table of relevant in vivo mutagenicity studies with paclobutrazol

Method	Species, strain, number of animals	Tested concentrations	Results	Remarks	Reference
Broadly consistent with OECD TG 475 Bone Marrow chromosomal aberration	Rats, Alderley Park 8 animals/sex and group	0, 30, 150 and 300 mg/kg bw via, gavage in corn oil Purity: 92.4 %	Negative.	The positive controls responded as expected. Paclobutrazol induced a statistically significant increase in the percentage of cells with abnormalities in males at 300 mg/kg bw at the 12-hour harvest time but not at later harvest times.	Report No CTL/P/891 (1984a)
Bone Marrow chromosomal aberration	Rats, Alderley Park 6 animals/sex and group	0 and 250 mg/kg bw/d via gavage in corn oil for 5 days Purity: 92.4 %	Negative.	The positive controls responded as expected. 259 mg/kg bw/d was determined as the maximum tolerable dose.	Report No CTL/P/926 (1984b)
OECD TG 474	Mice, C57/BL	0, 233 and 373 mg/kg bw in corn oil via gavage	Negative.	The positive controls responded as expected.	Report No CTL/P/3216 (1991)

Bone Marrow micronucleus	5 animals/sex and group	Purity: 92 %		One and five animals were sacrificed in extremis at 233 and 373 mg/kg bw, respectively.	
Broadly consistent with OECD TG 474 Bone Marrow micronucleus	Mice, C57/BL 5 animals/sex and group	0, 87.5 and 140 mg/kg bw in corn oil by i.p. route Purity: 92.4 %	Negative.	Positive controls responded as expected. An apparent positive at 140 mg/kg bw at 24 hours (but not at 48 or 72 hours) was attributed to high background variability.	Report No CTL/P/848 (1983)
OECD TG 482 *In Vivo* Unscheduled DNA Synthesis	Rats, Alderley Park 5 animals/sex and group	0, 40, 200 and 400 mg/kg bw via gavage in corn oil Purity: 92.4 %	Negative.	Positive controls responded as expected.	Report No CTL/P/1608 (1986)
OECD TG 478 Dominant Lethal Test	Mice, CD-1 20 males dosed (15 were mated with untreated females)	0, 25, 100 and 300 mg/kg bw/d for 5 days via gavage in corn oil Purity: 92.4 %	Negative.	The positive control substance gave the expected results. One of the males at 300 mg/kg bw/d died on day 4 of the dosing period. Clinical signs (piloerection, urinary incontinence and tremors) in males at 300 mg/kg bw/d.	Report No CTL/P/922 (1983)

The available database suggests that paclobutrazol is not mutagenic *in vitro* or *in vivo*. Therefore, RAC supports the DS's proposal for no classification for germ cell mutagenicity.

4.10 Carcinogenicity

The carcinogenic potential of paclobutrazol has been well investigated in standard studies, in rats and mice. Although these studies do not have a GLP certificate, they do appear to conform to the relevant OECD TG and were conducted in a reputable CRO. The tumour findings are discussed in this section. Discussion of significant repeated dose effects can be found in the repeated dose section (section 8.7).

Table 17: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
CD TG 453 Rat Sprague-Dawley (50/sex/dose) Dosed for 24 months, via the diet Interim sacrifice (12 months): 10/sex/group Purity 92.4% [redacted] *(1986b)*	0, 50, 250 and 1250 ppm paclobutrazol Equivalent to 0, 2.2, 11, and 54; and 0, 2.8, 14 and 72 mg/kg/day in males and females respectively	**Neoplastic changes** No toxicologically significant increases in tumour incidence were observed
OECD TG 453 Mouse (CD-1 strain) (63/sex/dose) Dosed for 24 months, Interim sacrifice (12 months): 12/sex/group Purity 92.4% [redacted] *(1986a)*	0, 25, 125 and 750 ppm paclobutrazol Equivalent to 0, 2.6, 14, and 81; and 0, 3, 16 and 89 mg/kg/day in males and females respectively	**Neoplastic changes** No toxicologically significant increases in tumour incidence were observed

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat

Sprague-Dawley rats (male and female 50+10 sex/dose) were administered paclobutrazol at doses of up to 54 and 72 mg/kg/day, in males and females respectively for up to 104 weeks. No treatment-related changes were observed in food consumption, body weight, body-weight gain or mortality rates in males. In females, no treatment-related changes were observed in food consumption, or mortality rates. However, body weight gain was statistically significantly decreased, compared to controls, by 10 and 21% at doses of 14 mg/kg/day and above, and body weights; interim (21%) and terminal (16%) sacrifice at the highest dose of 72 mg/kg/day.

The repeated dose toxicity effects observed in this study are reported and evaluated in the repeated dose section (4.7.1.1). The most prominent adverse effects observed were increased liver weight, accompanied by steatosis and hepatocyte hypertrophy, seen in both males and females at the top dose. Liver weights were also increased in males at the middle dose.

No treatment-related increases in tumour incidence were observed in either sex, at interim or terminal sacrifice at doses of up to 54-72 mg/kg/day, the highest dose tested.

Mice

CD-1 strain mice (male and female 50+10 sex/dose) were administered paclobutrazol at doses of up to 81-89 mg/kg/day, for up to 104 weeks. There were no treatment-related changes in food consumption, body weight, body-weight gain or mortality rates.

No toxicologically significant non neoplastic changes were observed in this study. The repeated dose effects are reported and evaluated in the repeated dose toxicity section (4.7.1.1).

No treatment-related increases in tumour incidence were observed in either sex, at interim or terminal sacrifice at doses of up to 81-89 mg/kg/day the highest dose tested..

4.10.1.2 Carcinogenicity: inhalation

No data are available

4.10.1.3 Carcinogenicity: dermal

No data are available

4.10.2 Human information

There is no information in humans to inform on the carcinogenic potential of paclobutrazol.

4.10.3 Other relevant information

No data are available

4.10.4 Summary and discussion of carcinogenicity

The carcinogenic potential of paclobutrazol has been investigated in standard studies in rats and mice, and no evidence of tumour induction was observed. No classification for carcinogenicity is proposed.

4.10.5 Comparison with criteria

Paclobutrazol does not meet the criteria for classification for carcinogenicity.

4.10.6 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS noted that no treatment-related increases in tumour incidence were observed in either sex, at interim or terminal sacrifice at doses of up to 54-72 mg/kg bw/d in a rat carcinogenicity study. Similarly, the DS also noted that no treatment-related increases in tumour incidence were observed in either sex, at interim or terminal sacrifice at doses of up to 81-89 mg/kg bw/d in an acceptable and reliable mouse carcinogenicity study. Therefore, the DS proposed no classification of paclobutrazol for carcinogenicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The non-neoplastic findings reported in both two-year carcinogenic studies in rats and mice were summarised in the corresponding table in the section "RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)". No toxicologically significant increases in tumour incidences were observed in any of these two studies. Therefore the substance does not meet the criteria for classification for carcinogenicity and RAC supports the DS's proposal for no classification of paclobutrazol for carcinogenicity.

4.11 Toxicity for reproduction

There are two studies available, conducted to investigate the potential of paclobutrazol to adversely affect fertility, both conducted in rats. One is a 2-generation study and the other a 1-generation screening study.

4.11.1 Effects on fertility

Table 18: Summary table of relevant reproductive toxicity studies - Fertility

Method	Results (±*)
2-generation study OECD 416 Oral (diet) Rat (15 male and 30 female, Alderley Park, Wistar derived) 0, 50, 250 and 1250 ppm equivalent to 4.9, 24 and 108.4 mg/kg/day in males and 0, 5.1, 25.9 and, 126.2 mg/kg/day in females of the F0 generation and 4.7, 23.2, and 116.9 mg/kg/day in males and 5.1, 24.8, and 124.1 mg/kg/day in females of the F1 generation Purity 92.4% [redacted] *(1987a)*	**Parental toxicity** ***1250 ppm*** *F0* ↑ Absolute (22.7%) and relative (26%) liver weight in females. Centrilobular fatty change (23/30), cytoplasmic eosinophillia of centrilobular hepatocytes (14/30) and inflammatory cell infiltrate (11/30) in females. *F1* ↑ Absolute (7%) and relative (7%) liver weight in females ***250 and 50 ppm*** No toxicologically significant changes **Reproductive effects** No toxicologically significant adverse effects on reproduction were observed **Offspring effects** ***1250 ppm*** F1A: ↑ Absolute and relative liver weights in males and female by ~ 20% Centrilobular fatty change 3/6 and 4/5 in males and females respectively, compared to 0/5 in controls 16% ↓ number of pups/litter (post-partum day 5) F1B: ↑ Absolute and relative liver weights in males and females by 14-16% and 20-23% respectively F2A: ↓ Pup weight gain (~11-14%), during lactation Centrilobular fatty change 9/12 and 5/11 in males and females respectively, compared to 0/14 in controls ↑ Absolute and relative liver weights in males and females by 10-12% and 20% respectively ***250 ppm*** F1A: 14% ↑ Absolute liver weights in males only. 18% ↓ number of pups/litter post-partum day 1 21% ↓ number of pups/litter post-partum day 5: F1B and F2A: No adverse effects observed.

	50 ppm No adverse effects observed. *A NOAEL of 24.8 mg/kg /day is derived for parental animals, based on liver histopathological changes and liver weight increases observed in females at 124.1 mg/kg /day the highest dose tested. An offspring NOAEL of 23.2-25.5 mg/kg /day is derived based on liver histopathological changes and liver weight increases observed in both sexes at 108-4-126.2 mg/kg /day the highest dose tested. A NOAEL of 108-4-126.2 mg/kg day the highest dose tested is derived for reproductive toxicity.
One-generation reproductive toxicity preliminary study Not conducted to a recognised TG or in a GLP environment Oral (diet) Rat (6 male and 12 females/dose Alderley Park Wistar derived) 0, 100, 500 and 1500 ppm estimated to be equivalent to 0, 10, 50, and 150 mg/kg/day (92.4 % purity) [redacted] *(1987b)*	**<u>Parental toxicity</u>** **1500 ppm** ↑ Absolute and relative liver weight in males (both by 16 %) and females (by 10 and 18% respectively.) Vacuolation of mid-zonal hepatocytes in females (6/12, compared to 0/12 in controls) **500 ppm and 100 ppm** No toxicologically significant changes were observed. **<u>Reproductive effects</u>** No adverse effects on fertility were observed. **<u>Offspring effects</u>** **1500 ppm** ↑ Absolute and relative liver weight in males (by 40 and 50 respectively %) and in females (by 34 and 50% respectively.) Vacuolation of mid-zonal hepatocytes in males (15/20 compared to 0/21 in controls) and females (12/19, compared to 0/21 in controls) Vacuolation of centrilobular hepatocytes in males (4/20 compared to 0/21 in controls) and females (6/19, compared to 0/21 in controls). **500 ppm** ↑ Absolute and relative liver weight in males (by19 and 15 respectively 19%) and in females (by 15 and 10% respectively). **100 ppm** No toxicologically significant changes were observed. *A reproductive NOAEL of 1500 ppm was derived for males and females. A parental NOAEL of 500 ppm is derived for both sexes and an offspring NOAEL of 500 ppm was derived.

± Values are reported as increased (↑) or decreased (↓) compared to controls

*** NOAEL/NOEL/LOAEL values are taken from the DAR and provided for information only.**

4.11.1.1 Non-human information

The effects of paclobutrazol on fertility have been extensively investigated in one 2-generation study and one 1-generation study both conducted in Alderley Park Wistar derived rats.

The potential for paclobutrazol to adversely affect fertility has been well investigated in a standard 2-generation dietary study (OECD TG 416) in rats, at doses of up to 1250 ppm (estimated to be equivalent to 100-125 mg/kg/day). The top dose in this study was selected on the basis of a one-generation preliminary study. Histopathological investigations were confined to control and high dose animals. No toxicologically significant changes in food consumption, body weight, or body weight gain were observed at any dose level tested. In females of both generations, compared to controls, increased liver weights were increased at the top dose (absolute and relative liver weight by 22.7 and 26% respectively). Histopathological examination found these organ weight changes were

accompanied by centrilobular fatty change (23/30), cytoplasmic eosinophilia of centrilobular hepatocytes (14/30) and inflammatory cell infiltrate (11/30).

The number of pups/litter was statistically significantly decreased in the F1A generation only, at doses of 250 ppm and above (see table below). As this change was not observed in the F1B or F2 generations, the pup losses are regarded as a chance observation and not treatment-related. In this study no toxicologically significant adverse effects on reproductive performance were observed at doses of up to 1250 ppm (~100-125 mg/kg/day), the highest dose tested.

Early pup survival

Pup generation	Control (pups/litter (no of litters))	50 ppm (pups/litter (no of litters))	250 ppm (pups/litter (no of litters))	1250 ppm (pups/litter (no of litters))
F1A Day 1 Post-partum	12 (25)	11.9 (27)	9.8 (28)*	10.9 (28)
F1A Day 5 Post-partum	11.9 (25)	11.3 (27)	9.4 (28)*	9.9 (28)*
F1B Day 1 Post-partum	9.2 (21)	11.2 (23)	8.4 (23)	10.2 (24)
F1B Day 5 Post-partum	19 (20)	10.3 (23)	7.7 (23)	9.7 (24)
F2A Day 1 Post-partum	10.4 (27)	10.9 (27)	11.1 (30)	10.1 (26)
F2A Day 5 Post-partum	9.8 (27)	10.7 (27)	10.5 (29)	9.7 (26)

*Statistically significant $p<0.01$

In the preliminary study, groups of 6 male and 12 female rats (Alderley Park Wistar derived) were fed diet containing 0, 100, 500 or 1500 ppm paclobutrazol, estimated to be equivalent to 0, 10 50 and 150 mg/kg/day. After 6 weeks, the animals were mated to produce a single litter to weaning. After weaning, the pups and parents were killed. A limited histopathological examination was conducted on the parental animals, and confined to high dose animals only.

No toxicologically significant changes in food consumption, body weight, or body weight gain were observed at any dose level tested. Absolute and relative liver weights were statistically significantly increased in males (both by 16 %) and females (by 10 and 18% respectively) at the top dose only. Histopathological examination found these organ weight changes were accompanied by vacuolation of mid-zonal hepatocytes in females (6/12, compared to 0/12 in controls) only.

In pups, absolute and relative liver weights were statistically significantly increased compared to controls at the top dose in males (by 40 and 50% respectively) and in females (by 34 and 50% respectively) and at the mid dose in males (by 40 and 50 respectively %) and in females (by 34 and 50% respectively). These liver weight increases were accompanied by; vacuolation of mid-zonal hepatocytes in males (15/20 compared to 0/21 in controls) and females (12/19, compared to 0/21 in controls), and vacuolation of centrilobular hepatocytes in males (4/20 compared to 0/21 in controls) and females (6/19, compared to 0/21 in controls), observed at the top dose only..

In this study no toxicologically significant adverse effects on reproductive performance were observed at doses of up to 150 mg/kg/day, the highest dose tested.

4.11.1.2 Human information

There is no information from humans to inform on the potential of paclobutrazol to adversely affect fertility.

4.11.2 Developmental toxicity

Table 19: Summary table of relevant reproductive toxicity studies - Development

Method	Results(±*)
Developmental toxicity Oral (gavage) OECD 414 (1981) Rat, Wistar strain 24/group 0, 40, 100 or 250 mg/kg /day on days 6-15 of gestation Vehicle: corn oil Purity 92.4% [redacted] *(1983)*	**Dams** No toxicologically significant changes in food consumption, body weight or body weight-gain were observed. **250 mg/kg /day:** One dam died and 4 were sacrificed *in extremis*, no cause of death was provided. **100 and 40 mg/kg /day**: No effects observed **Foetuses** Foetal weights were comparable between test and control groups. **250 mg/kg/day** **Malformations***:* Cleft palate 3 foetuses from 2 litters, one of which had multiple malformations. No other malformations were observed **Variations:** ↑ partial ossification of the transverse process of the 7th cervical vertebra (47 foetuses/15 litters compared to 13/8 in controls). ↑ Incidence of 14th bilateral ribs, (125 foetuses/19 litters compared to 54/16 in controls). ↑ Partially ossified occipital bone (17 foetuses/7 litters compared to 5/5 in controls). ↑Unossified odontoid (36 foetuses/13 litters compared to 9/11 in controls). ↑ 9th centrum partially ossified (8 foetuses/6 litters compared to 2/1 in controls). ↑2nd sternebrae partially ossified (24 foetuses/12 litters compared to 12/8 in controls) **100 mg/kg/day** **Variations**: ↑partial ossification of the transverse process of the 7th cervical vertebra (49 foetuses/17 litters compared to 13/8 in controls). ↑ Incidence of 14th bilateral ribs, (135 foetuses/23 litters compared to 54/16 in controls). ↑Unossified odontoid (36 foetuses/13 litters compared to 9/11 in controls). **40 mg/kg/day:** **Malformations:** Cleft palate 1 foetus **Variations**: ↑ partial ossification of the transverse process of the 7th cervical vertebra (32 foetuses/10 litters compared to 13/8 in controls) *A maternal NOAEL of 100 mg/kg /day, based on severe toxicity and mortalities at 250 mg/kg/day, the highest dose tested. It was not possible to identify a NOAEL for developmental toxicity, a LOAEL of 40 mg/kg/day is identified, the lowest dose tested.

Developmental toxicity Oral (gavage) OECD 414 (1981) Rat, Wistar strain 24/group 0, 2.5, 10, 40 and 100 mg/kg /day on days 6-15 of gestation Vehicle: corn oil Purity 92.4% [redacted] *(1984)*	**<u>Dams</u>** No toxicologically significant changes at any dose level. **<u>Foetuses</u>** There were no treatment-related increases in malformations. **100 mg/kg/day** **Variations:** *Delayed ossification:* ↑ Bilateral partial ossification of the transverse process of the 7th cervical vertebra (14 foetuses/7 litters compared to 3/3 in controls). ↑Unilateral partial ossification of the transverse process of the 7th cervical vertebra (40 foetuses/20 litters compared to 7/6 in controls). ↑ Incidence of 14th bilateral ribs, (86 foetuses/23 litters compared to 24/11 in controls). *Kidney:* Pelvic dilatation moderate-unilateral (6 foetuses/3 litters compared to 0 in controls), Pelvic dilatation slight-unilateral (49 foetuses/19 litters compared to 24/12 in controls), Ureter dilated slight-bilateral unilateral (21 foetuses/8 litters compared to 3/3 in controls), Ureter dilated moderate-unilateral (9 foetuses/3 litters compared to 1/1 in controls), Ureter dilated slight-unilateral (49 foetuses/17 litters compared to 18/9 in controls), Hydroureter-unilateral (8 foetuses/4 litters compared to 0 in controls), Kinked ureter-unilateral unilateral (50 foetuses 19 litters compared to 19/11 in controls). **40 mg/kg/day** **Variations**: *Delayed ossification*: ↑Unilateral partial ossification of the transverse process of the 7th cervical vertebra (35 foetuses/15 litters compared to 7/6 in controls). ↑Incidence of 14th bilateral ribs, (57 foetuses/23 litters compared to 24/11 in controls). **10 mg/kg/day** **Variations**: *Delayed ossification*: ↑Unilateral partial ossification of the transverse process of the 7th cervical vertebra (18 foetuses/15 litters compared to 7/6 in controls). **2.5 mg/kg/day** No toxicologically significant changes *A maternal NOAEL of 100 mg/kg /day the highest dose tested. A NOAEL for developmental toxicity of 10 mg/kg/day was identified.
Developmental toxicity Oral (gavage) OECD 414 (1983) Rabbit New Zealand White (18/group) 0, 25, 75 or 125 mg/kg /day on days 6-18 of gestation Vehicle: corn oil Purity 92.4% [redacted] *(1983b)*	**<u>Maternal toxicity</u>** No treatment related adverse effects observed **<u>Foetuses</u>** No treatment-related adverse effects observed A maternal NOAEL of 75 mg/kg /day and a developmental NOAEL of 125 mg/kg /day was determined

Developmental toxicity Oral (gavage) OECD 414 (1983) Rabbit New Zealand White (18/group) 0, 25, 75 or 125 mg/kg /day on days 6-18 of gestation Vehicle: corn oil Purity 92.4% [redacted] *(1986)*	**Maternal toxicity** No treatment related adverse effects observed **Foetuses** No malformations were observed **Variations:** **125 mg/kg/day** ↑ 7th transverse process partially ossified (8 foetuses from 3 litters, compared to 2/2 in controls) ↑ 5th sternebrae partially ossified (45 foetuses from 13 litters, compared to 30/9 in controls) (Historical control 19-58%) ↑ Extra 13th rib, normal length (62 foetuses from 14 litters, compared to 49/14 in controls) (Historical control 44-78.9%) **75mg/kg/day** ↑ 7th transverse process partially ossified (8 foetuses from 5 litters, compared to 2/2 in controls) ↑ 5th sternebrae partially ossified (47 foetuses from 13 litters, compared to 30/9 in controls) (Historical control 19-58%) **25 mg/kg/day** ↑ 5th sternebrae partially ossified (55 foetuses from 15 litters, compared to 30/9 in controls) (Historical control 19-58%) *A maternal NOAEL of 25mg/kg /day and a developmental NOAEL of 125 mg/kg /day was determined.

± Values are reported as increased (↑) or decreased (↓) compared to controls

* NOAEL/NOEL/LOAEL values are taken from the DAR and provided for information only.

4.11.2.1 Non-human information

The developmental toxicity of paclobutrazol has been well investigated in standard studies in rats and rabbits, and a preliminary study in both rats and rabbits. It is noted that the dosing schedule used in these studies is shorter that that recommended in the current test guideline. However the dosing schedule used was compliant with the OECD TG in use at the time, and is not considered to have had a significant impact on the outcome of these studies.

Rats

In the first study, animals (Alderley Park Wistar derived rats 24/dose) were administered paclobutrazol in corn oil via gavage, at doses of 0, 10, 40 and 125 mg/kg/day on days 6-15 of gestation. Dams were sacrificed on day 20 of gestation and foetuses examined for skeletal and visceral variations and malformations.

There were no toxicologically significant changes in food consumption, body weight or body weight gain, after a statistically significant decrease in maternal body weight gain and food consumption during the first 3 days of treatment at the highest dose. At the top dose, one dam died, and 4 were sacrificed *in-extremis*. No information on the cause of death was provided.

Examination of the foetuses found cleft palate in 3 foetuses from 2 litters at the top dose. It was noted that one of these 3 foetuses had other severe malformations (exencephaly, bilateral anophthalmia and cleft lip). Cleft palate was also observed in 1 foetus at 40 mg/kg/day, the lowest dose tested. No other malformations were observed, including in controls. The concurrent and background incidence

for cleft palate in this strain of rats can be found in the table below. Foetal weights were comparable between treated and control groups.

Historical control incidence of cleft palate.

Study /CTL Report number		Year	No. Foetuses examined	No. Litters examined	Cleft Palate	
					No. Foetuses	% Foetuses
1. CTL/P/576		1980	139	17	0	0
2. CTL/P/656		1980	240	20	0	0
3. CTL/P/756		1982	237	20	0	0
4. CTL/P/875		1982	279	24	0	0
5. CTL/P/842 *	**Group 1**	**1983**	**305**	**24**	**0**	**0**
	Group 2		**297**	**24**	**1**	**0.34**
	Group 3		**283**	**24**	**0**	**0**
	Group 4		**234**	**19**	**3**	**1.28**
6. CTL/P/997		1983	264	22	0	0
7. CTL/P/1039		1983	281	24	0	0
8. CTL/P/1127		1984	255	23	0	0
9. CTL/P/1334		1984	312	24	0	0
10. CTL/P/1332		1984	299	24	0	0

***paclobutrazol study**

The incidence of skeletal variations was statistically significantly increased compared with controls. In particular, at 40 mg/kg/day and above, partial ossification of the transverse processes of the 7th cervical vertebra was noted. At 100 and above there was an increased incidence of unossified odontoid. At 250 mg/kg/day only, retarded ossification of the manus and pes were noted. An increased incidence of 14th bilateral ribs was also observed, at doses of 100 mg/kg/day and above. The complete list of skeletal variations and incidences can be seen in table 19 above.

Overall, paclobutrazol caused some retardation of bone development and an increased incidence of supernumerary ribs at doses of 40 mg/kg/day and above, and cleft palate at the top dose of 250 mg/kg/day. This dose also caused severe maternal toxicity (lethality).

In the second study, dams (Alderley Park Wistar derived rats 24/dose) were administered paclobutrazol in corn oil via gavage, at doses of 0, 2.5, 10, 40, and 100 mg/kg/day on days 6-15 of gestation. Dams were sacrificed on day 20 of gestation and foetuses examined for skeletal and visceral variations and malformations.

There were no toxicologically significant changes in food consumption, body weight or body weight gain, and no animals died or were sacrificed *in-extremis* in this study. No malformations were observed in this study.

A statistically significant and dose-related increase in the incidence of variations of the renal system (characterised as pelvic dilatation and abnormalities of the ureter) was observed at doses of 40 mg/kg/day and above. An increased incidence of partial ossification of the transverse processes of the 7th cervical vertebra and extra (14th) ribs were observed at doses of 40 mg/kg/day and above. The complete list of renal system variations and incidences can be seen in table 19 above.

Overall, paclobutrazol caused a treatment-related increased incidence of kidney and urinary tract variations, and some retardations of bone development.

Rabbits

In the first study, dams (New Zealand White rabbits/18/dose) were administered paclobutrazol in corn oil via gavage, at doses of 0, 25, 75 and 125 mg/kg/day on days 6-18 of gestation. Dams were sacrificed on day 21 of gestation and foetuses examined for skeletal and visceral variations and malformations.

There were no toxicologically significant changes in food consumption, body weight or body weight gain.

Fourteen animals died or were killed *in-extremis* during the study. Seven of these deaths were due to misdosing, two were unrelated to treatment and one was due to abortion. Four deaths at 400 mg/kg /day were considered to be treatment- related. The large number of deaths and the low pregnancy rate in the control and 400 mg/kg /day groups confounds interpretation of this study.

No malformations or variations were observed in this study.

In the second study, rabbits (New Zealand White 18/dose) were administered paclobutrazol in corn oil via gavage, at doses of 0, 25, 75 and 125 mg/kg/day on days 6-18 of gestation. This dosing schedule was compliant with the adopted OECD TG at the time the study was conducted. Dams were sacrificed on day 21 of gestation and foetuses examined for skeletal and visceral variations and malformations.

There were no toxicologically significant changes in food consumption, body weight or body weight gain, and no animals died or were sacrificed *in-extremis*. No malformations were observed in this study. An increased incidence of partial ossification of the 5th sternebrae was observed at all doses, although this finding appears with a high incidence in control foetuses too. An increased incidence of partial ossification of the 7th transverse process was observed at doses of 75 mg/kg/day and above, and extra (13th) ribs were observed at the top dose only. The incidence of these changes is reported in table 19, above.

Overall, paclobutrazol caused some retardation of bone development, and an increased incidence of 13th ribs, at doses of 75 mg/kg/day and above. However, these changes are of uncertain toxicological significance, as these changes were within the background incidence for the test laboratory.

4.11.2.2 Human information

There is no information available on the potential of paclobutrazol to adversely affect development in humans.

4.11.3 Other relevant information

None

4.11.4 Summary and discussion of reproductive toxicity

Fertility

The potential for paclobutrazol to adversely affect fertility has been investigated in a standard 2-generation study in rats, and in a preliminary 1-generation study, also conducted in rats. No treatment-related adverse effects on fertility were observed in the 2-generation study at doses of up to 100-125 mg/kg/day, the highest dose tested. Similarly, no adverse effects on fertility were observed in the sighting study, at doses of up to 150 mg/kg/day, the highest dose tested.

Testicular Toxicity in dogs

In a 90-day study in dogs, testicular toxicity was only observed at a dose of 450 mg/kg/day, the highest dose tested. Testicular toxicity, manifested as marked decreases in absolute and relative testes and epididymides weights associated with an absence of spermatozoa from the epididymidal ducts, and the testes were described as immature. Testicular toxicity was associated with increased liver and kidney weights, and fine fat deposition in the hepatocytes.

It is possible that the testicular changes reflect retardation of sexual maturity, as male dogs usually attain sexual maturity around 3-months of age. Some support for this view comes from the 1-year dog study in which no testicular toxicity was observed. There is no reason to discount these changes as not being relevant for human health. However, in isolation, they are not considered sufficient to support classification for fertility.

Developmental toxicity

The potential for paclobutrazol to adversely affect development has been investigated in standard studies in rats and rabbits, and in preliminary studies in rats and rabbits. Rats appear to be the most sensitive species with bone and kidney variations noted at doses of 10 mg/kg/day and above, with malformations (cleft palate) and severe maternal toxicity observed at 250 mg/kg/day, the highest dose tested.

Paclobutrazol induced cleft palate in 3 foetuses/2 litters at the highest dose (compared to 0 in controls) of 250 mg/kg/day; a dose that caused severe maternal toxicity (1 dam died and 4 sacrificed *in extremis*). There was no evidence of other malformations. This dose of 250 mg/kg/day was also associated with widespread skeletal variations, mostly delayed ossification and supernumerary ribs (increased 14th bilateral). At lower non-maternally toxic doses, paclobutrazol caused retardation of skeletal development, increased supernumerary ribs and visceral variations (kidney and urinary tract) at doses of 40 to 100 mg/kg/day, with only retarded skeletal development noted at a dose of 10 mg/kg/day.

Cleft palate is a very rare malformation (relevant background rate of 0) in rats and the observation of this malformation in 3 foetuses from 2 litters is of high concern for human health. Additional concern comes from the increases in skeletal and visceral variations, observed at doses below those that cause malformations and severe maternal toxicity.

4.11.5 Comparison with criteria

Fertility

No classification is proposed for fertility.

Development

Cleft palate induction in rats is very rare, normally supporting classification for developmental toxicity. This malformation in rats is regarded of high concern, even when observed in the presence of severe maternal toxicity. Therefore, the increased incidence of cleft palate, although at a maternally lethal dose, should be regarded as relevant for human health. Support for classification for developmental toxicity comes from an increased incidence of skeletal and visceral variations, and retardations of skeletal development in rats and rabbits, at doses below those that cause cleft palate and maternal toxicity in rats.

Paclobutrazol only induced cleft palate at maternally lethal doses in rats, with no evidence of cranio-facial or other malformations at lower doses. No evidence of developmental toxicity was observed in rabbits even at maternally lethal dose levels. That cleft palate is only induced in one species at maternally lethal doses reduces the overall level of concern for human health, with only skeletal retardations and variations observed at non-maternally toxic doses. Therefore, classification with Category 2 H361d is proposed.

4.11.6 Conclusions on classification and labelling

Repr 2; H361d - Suspected of damaging the unborn child

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of paclobutrazol for sexual function and fertility on the basis of a two-generation study in rats showing no adverse effects at doses up to 100-125 mg/kg bw/d, and a preliminary one-generation study also conducted in rats showing no adverse effects up to 150 mg/kg bw/d. The DS did not considered the testicular effects found in the 90-d repeated toxicity study in dogs relevant for classification purposes, attributing these effects to problems in sexual maturity of the animals. The latter was supported by the fact that the effects were not reproduced in the one-year repeated dose toxicity study in dogs.

The DS proposed classification of paclobutrazol for development in Category 2 (H361d; Suspected of damaging the unborn child). The DS justified this proposal based on that the cleft palate cases were reported at maternally lethal doses in rats (but not in rabbits) with no evidences of other malformations at lower doses. The DS also considered the increased incidences of skeletal and visceral variations and retardations of skeletal development found in rats and rabbits at doses below those that cause cleft palate at maternal toxicity doses in rats relevant for classification for development in Category 2.

Comments received during public consultation

One MSCA supported classification for development as Repr. 2 (H361d) but highlighted the need for discussion about a more severe classification in light of the reported incidences of cleft palates in rats, other skeletal and soft tissues abnormalities in both rats and rabbits and common pattern of effects among studies and species (e.g. rudimentary cervical and supernumerary lumbar ribs). The MSCA also made a remark that cleft palates and cervical ribs

are abnormalities implying a disturbance in the process of craniofacial morphogenesis commonly observed with triazoles. The DS welcomed further discussion and stated that Category 2 appears most appropriate given the fact that cleft palates were observed in rats at maternally lethal doses and no malformations were observed in rats or rabbits at lower doses.

A second MSCA supported no classification for fertility but considered that category 1B (H360D) might be more appropriate because paclobutrazol not only induced cleft palate at maternally lethal doses in rats, but there was also evidence of cranio-facial malformations, skeletal and visceral variations and retardations of skeletal development at lower doses without maternal toxicity. This MSCA also highlighted that all triazole-induced morphologic alterations are based on an inhibition of certain cytochrome P450 (CYP) isoforms that strongly alter the retinoic acid catabolism and that this mechanism is relevant for humans. The DS replied that Repr. 2 (H361d) might be more appropriate taking into consideration that malformations were only observed at maternally lethal doses in rats and that the observation of a single cleft palate at lower dose might be considered incidental (which is supported, according to the DS's opinion, by the lack of craniofacial malformations at the intermediate dose in the same study and the absence of cleft palate in the second rat study). The DS also replied that there is no evidence to suggest that paclobutrazol is a CYP inhibitor other than structural similarities with other triazoles, and without additional information it is not possible to conclude the mode of action for the cleft palate induction.

A third MSCA requested a more detailed rationale why classification in Category 1B (H360D) was not considered warranted taking into consideration that the cleft palates are commonly observed in reproductive studies at maternally toxic levels in mice but not in rat or rabbit. Furthermore the MSCA noted that a significant increase in foetuses with hydroureter (by the commenting MSCA considered as a malformation; described as extreme dilation of the ureter) was also observed. The DS replied regarding the cleft palates as summarised above to the other MSCAs. Regarding the kidney malformations, the DS noted that in the first rat study, conducted at higher doses than the second one, no kidney changes were reported, and the DS concluded that hydroureters might be a chance finding. However, in order to facilitate the interpretation of this effect, the historical control data for hydroureter and dilates ureter in the performing facility was provided by the DS in the RCOM.

The latter MSCA also highlighted that the absence of effect in the one- and two-generation studies in rats does not exclude that paclobutrazol might have adverse effects on fertility, noting that the testicular effects reported for the 90-d repeated dose toxicity study in dogs and suggesting an additional classification as Repr. 2 (H361f). The DS replied that the testicular toxicity observed at 450 mg/kg bw/d in the 90-d study was not reproduced in the one-year study at 300 mg/kg bw/d; which suggested that the effects in the 90-d study was indeed a transient retardation and not a treatment-related effect. In addition, the testicular effects reported in the one-year study in dogs were not dose-related and could be considered, according to the DS, as incidental.

A manufacturer supported the proposal for classification as Repr. 2 (H361d) and submitted a document providing a summary of all available data relating this endpoint which was welcomed by the DS.

Additional key elements

Industry submitted a paper stating its position on the proposed classification for developmental toxicity of paclobutrazol. This paper contained additional information that were not available in the background document and is described in the following paragraphs.

Preliminary developmental toxicity study in rat

A preliminary study, conducted prior to the main studies, was reported in 1987. According to the industry, this study was not included in the original data submission since it was preliminary and investigative in nature. In this study, paclobutrazol was administered at doses of 0, 80, 160 or 240 mg/kg bw/d in corn oil on days 6-15 of gestation. Cleft palate was observed in 8 foetuses: 1/130 in controls, 1/110 at 80 mg/kg/d, 0/118 at 160 mg/kg/d and 6/85 (all from 1 litter) at 240 mg/kg bw/d. In addition to cleft palate, the foetus at 80 mg/kg bw/d had cleft lip and anophthalmia. There was evidence of maternal toxicity as two out of ten died at 240 mg/kg bw/d (20 % mortality) with surviving animals showing a marginally reduced bodyweight gain and food consumption which persisted on completion of dosing. At 80 mg/kg bw/d, a small reduction in bodyweight gain was seen that reversed after the dosing period.

According to the industry, the single incidence of cleft palate at 80 mg/kg bw/d is considered to be spontaneous as it is within the study control range. However, RAC notes that historical control data of the performing facility was not provided in this position paper.

Published data

Vergieva (1998) published a study that investigated the effects of paclobutrazol on foetal development in the Wistar rat. According to the industry, this paper provides very limited methodology and reporting of data compared to a GLP study, and as such may be of limited reliability. Paclobutrazol was administered at dose levels of 50 and 200 mg/kg bw/d on days 6 through 15 of gestation (repeated dosing), and at 200 and 500 mg/kg bw on days 7, 9, 11 or 13 (single doses). A limited number of dams were included in experimental groups (n=5-12). Animals were sacrificed on day 21 and the following parameters were examined: number of corpora lutea, live foetuses, resorptions, and autolyses. Foetuses were weighed and examined for external abnormalities; two thirds underwent skeletal examinations and the remainder received soft tissue examinations. Not all experimental data were presented in the paper. The table below summarises the main findings of developmental impairments in this study.

Table: Summary of the main developmental impairments reported in Vergieva study according to the position paper submitted by Syngenta.

Dose (mg/kg bw/d)	200	200	500
Day of dosing	6-15	11	11
Open eyes and/or micrognathia	2/116	14/76	-
Cleft palate	2/39	12/25	25/25
Gross anomalies	-	-	21/58

Assessment and comparison with the classification criteria

Fertility

There are two studies available, conducted in rats, investigating the potential of paclobutrazol to adversely affect fertility. The table below lists the main findings of these studies.

Table: Summary table of relevant reproductive toxicity studies.

Method	Results
One-generation reproductive toxicity preliminary study Not conducted according to a recognised TG or GLP Oral (diet) Rats, Alderley Park (Wistar derived) 6 male and 12 females/dose 0, 100, 500 and 1500 ppm estimated to be equivalent to 0, 10, 50, and 150 mg/kg bw/d Purity: 92.4 % purity Report No CTL/P/1967 (1987b)	<u>Parental toxicity:</u> <u>1 500 ppm</u> ↑ Absolute and relative liver weight in males (both by 16 %) and females (by 10 and 18 % respectively) Vacuolation of mid-zonal hepatocytes in females (6/12, compared to 0/12 in controls) <u>500 ppm and 100 ppm</u> No toxicologically significant changes were observed. <u>Reproductive effects:</u> No adverse effects on fertility were observed. <u>Offspring effects:</u> <u>1 500 ppm</u> ↑ Absolute and relative liver weight in males (by 40 and 50 respectively %) and in females (by 34 and 50 % respectively) Vacuolation of mid-zonal hepatocytes in males (15/20 compared to 0/21 in controls) and females (12/19, compared to 0/21 in controls) Vacuolation of centrilobular hepatocytes in males (4/20 compared to 0/21 in controls) and females (6/19, compared to 0/21 in controls) <u>500 ppm</u> ↑ Absolute and relative liver weight in males (by 19 and 15 % respectively) and in females (by 15 and 10 % respectively). <u>100 ppm</u> No toxicologically significant changes were observed.
Two-generation study OECD TG 416 Oral (diet) Rats, Alderley Park (Wistar derived) 15 males and 30 females	<u>Parental toxicity:</u> <u>1 250 ppm</u> *F0* ↑ Absolute (22.7 %) and relative (26 %) liver weight in females. Centrilobular fatty change (23/30), cytoplasmic eosinophilia of centrilobular

0, 50, 250 and 1250 ppm equivalent to: F0 generation: 4.9, 24 and 108.4 mg/kg bw/d in males and 0, 5.1, 25.9 and, 126.2 mg/kg bw/d in females F1 generation: 4.7, 23.2, and 116.9 mg/kg bw/d in males and 5.1, 24.8, and 124.1 mg/kg bw/d in females Purity: 92.4% Report No CTL/P/1496 (1987a)	hepatocytes (14/30) and inflammatory cell infiltrate (11/30) in females *F1* ↑ Absolute (7 %) and relative (7 %) liver weight in females <u>250 and 50 ppm</u> No toxicologically significant changes. <u>Reproductive effects:</u> No toxicologically significant adverse effects on reproduction were observed. <u>Offspring effects:</u> <u>1 250 ppm</u> *F1A* ↑ Absolute and relative liver weights in males and female by ~ 20 % Centrilobular fatty change 3/6 and 4/5 in males and females respectively, compared to 0/5 in controls 16 % ↓ number of pups/litter (post-partum day 5) *F1B* ↑ Absolute and relative liver weights in males and females by 14-16 % and 20-23 % respectively *F2A* ↓ Pup weight gain (~ 11-14 %), during lactation Centrilobular fatty change 9/12 and 5/11 in males and females respectively, compared to 0/14 in controls ↑ Absolute and relative liver weights in males and females by 10-12 % and 20 % respectively <u>250 ppm</u> *F1A* 14 % ↑ Absolute liver weights in males only. 18 % ↓ number of pups/litter post-partum day 1 21 % ↓ number of pups/litter post-partum day 5 *F1B and F2A* No adverse effects observed.

The potential effects of paclobutrazol on fertility and sexual function were assessed in one-generation reproductive toxicity study and in a two-generation reproductive toxicity study, both in rats, where doses up to 150 mg/kg bw/d and up to 100-125 mg/kg bw/d were tested, in the one-generation and two-generation studies, respectively. No treatment-related effects

were found in the one-generation study, while the only alteration potentially significant in the two-generation study was a statistically significant decrease in the number of pups/litter in the F1A generation at 250 and 1 250 ppm.

The table below summarises the early pup survival in this study. RAC notes that this reduction in the number of pups/litter in the F1A generation was not observed in the F1B and F2A generations; which suggests that the effects on F1A generation were incidental. Therefore, RAC does not consider this minor alteration as severe enough to warrant a classification for fertility and sexual function.

Table: Early pup survival in the two-generation reproductive toxicity study (number of pups/litter (number of litters) is shown)

Pup generation	Control	50 ppm	250 ppm	1250 ppm
F1A Day 1 post-partum	12 (25)	11.9 (27)	9.8* (28)	10.9 (28)
F1A Day 5 post-partum	11.9 (25)	11.3 (27)	9.4* (28)	9.9* (28)
F1B Day 1 post-partum	9.2 (21)	11.2 (23)	8.4 (23)	10.2 (24)
F1B Day 5 post-partum	9.0 (20)	10.3 (23)	7.7 (23)	9.7 (24)
F2A Day 1 post-partum	10.4 (27)	10.9 (27)	11.1 (30)	10.1 (26)
F2A Day 5 post-partum	9.8 (27)	10.7 (27)	10.5 (29)	9.7 (26)

* = Statistically significant different form control at $p<0.01$.

RAC notes that severe testicular effects were reported in the 90-d repeated toxicity study in dog. These effects include severe decrease in absolute and relative testes and epididymides weights, giant spermatid cells, immature testes and no spermatozoa in epididymides that appeared at doses of 450 mg/kg bw/d. RAC also notes that the one-year repeated dose toxicity study in dog at doses of 300 mg/kg bw/d did not result in any detectable testicular alteration. The lack of significant effects in the one-year toxicity study suggests that the effects occur at and above a high dose level in dogs and lowers the relevance of the effects reported in the 90-d toxicity study. The severe testicular effects reported might also be transitory or might reflect a retardation of sexual maturity rather than a treatment-related effect. Therefore, RAC does not consider the testicular toxicity found in the 90-d repeated dose toxicity study in dogs relevant for classification purposes.

In conclusion, RAC supports the DS's proposal of no classification for effects on sexual function and fertility.

Development

There are several developmental toxicity studies in rats and rabbits investigating the potential of paclobutrazol to adversely affect development. The table below summarises the main findings of these studies.

Table: Summary table of oral developmental toxicity studies in animals with paclobutrazol

METHOD	RESULT
Developmental toxicity Oral (gavage) OECD TG 414 (1981) Rats, Wistar 24 animals/group	Dams: No toxicologically significant changes in food consumption, body weight or body weight-gain were observed. 250 mg/kg bw/d One dam died and 4 were sacrificed *in extremis*, no cause of death was given. During the dosing period, occasional instances of staining of the pelt in the genital

<table>
<tr>
<td>0, 40, 100 or 250 mg/kg bw/d on days 6-15 of gestation

Vehicle: corn oil

Purity 92.4 %

Report No CTL/P/842 (1983)</td>
<td>and/or ventral areas were noted in 10 out of 19 animals from the 250 mg/kg bw/d dose group. This finding was also observed in the other treated groups and control group but it was less severe and of a shorter duration than seen at 250 mg/kg bw/d.

<u>100 and 40 mg/kg bw/d</u>
No effects observed.

<u>Foetuses:</u>

Foetal weights were comparable between test and control groups.

<u>250 mg/kg/day</u>
Malformations
Cleft palate in three foetuses from two litters, one of which had multiple malformations. No other malformations were observed

Variations
<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>Partial ossification of the 7th cervical vertebra</td><td>47**/15**</td><td>13/8</td></tr>
<tr><td>14th bilateral ribs</td><td>125**/19**</td><td>54/16</td></tr>
<tr><td>Partially ossified occipital bone</td><td>17**/7</td><td>5/5</td></tr>
<tr><td>Un-ossified odontoid</td><td>36**/13</td><td>9/11</td></tr>
<tr><td>9th centrum partially ossified</td><td>8*/6*</td><td>2/1</td></tr>
<tr><td>2nd sternebrae partially ossified</td><td>24**/12</td><td>12/8</td></tr>
<tr><td colspan="3">**Statistically different from control at p<0.01; *Statistically different from control at p<0.05</td></tr>
</table>

<u>100 mg/kg bw/d</u>
Malformations
Not reported.

Variations
<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>Partial ossification of the 7th cervical vertebra</td><td>49**/17**</td><td>13/8</td></tr>
<tr><td>14th bilateral ribs</td><td>135**/23*</td><td>54/16</td></tr>
<tr><td>Un-ossified odontoid</td><td>36**/13</td><td>9/11</td></tr>
<tr><td colspan="3">**Statistically different from control at p<0.01</td></tr>
</table>
</td>
</tr>
</table>

<table>
<tr><td></td><td>
<u>40 mg/kg bw/d</u>
Malformations
Cleft palate in one foetus.

Variations
<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>Partial ossification of the 7th cervical vertebra</td><td>32**/10</td><td>13/8</td></tr>
<tr><td colspan="3">**Statistically different from control at p<0.01</td></tr>
</table>
</td></tr>
<tr><td>Developmental toxicity

Oral (gavage)

OECD TG 414 (1981)

Rats, Wistar

24 animals/group

0, 2.5, 10, 40 and 100 mg/kg bw/d on days 6-15 of gestation

Vehicle: corn oil

Purity 92.4 %

Report No CTL/P/997 (1984)</td><td>
<u>Dams:</u>

No toxicologically significant changes at any dose level.

<u>Foetal malformations:</u>

There were no treatment-related increase in malformations.

<u>Foetal variations:</u>

<u>100 mg/kg bw/d</u>

<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>Bilateral partial ossification of the 7th cervical vertebra</td><td>14*/7</td><td>3/3</td></tr>
<tr><td>Unilateral partial ossification of the 7th cervical vertebra</td><td>40**/20**</td><td>7/6</td></tr>
<tr><td>14th bilateral ribs</td><td>86**/23**</td><td>24/11</td></tr>
<tr><td colspan="3">**Statistically different from control at p<0.01; *Statistically different from control at p<0.05</td></tr>
</table>

<table>
<tr><td colspan="3">Alterations in kidney (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>Moderate-unilateral pelvic dilatation</td><td>6*/3</td><td>0/0</td></tr>
<tr><td>Slight-unilateral pelvic dilatation</td><td>49**/19</td><td>24/12</td></tr>
<tr><td>Slight-bilateral ureter dilatation</td><td>21**/8</td><td>3/3</td></tr>
<tr><td>Moderate-unilateral ureter dilatation</td><td>9*/3</td><td>1/1</td></tr>
<tr><td>Slight-unilateral ureter dilatation</td><td>49**/17*</td><td>18/9</td></tr>
<tr><td>Unilateral hydroureter</td><td>8**/4</td><td>0/0</td></tr>
</table>
</td></tr>
</table>

	<table><tr><td>Unilateral kinked ureter</td><td>50**/19*</td><td>19/11</td></tr><tr><td colspan="3">**Statistically different from control at p<0.01; *Statistically different from control at p<0.05</td></tr></table> 40 mg/kg bw/d <table><tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr><tr><td></td><td>Exposed</td><td>Control</td></tr><tr><td>Unilateral partial ossification of the 7th cervical vertebra</td><td>35**/15*</td><td>7/6</td></tr><tr><td>14th bilateral ribs</td><td>57**/19*</td><td>24/11</td></tr><tr><td colspan="3">**Statistically different from control at p<0.01; *Statistically different from control at p<0.05</td></tr></table> 10 mg/kg bw/d <table><tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr><tr><td></td><td>Exposed</td><td>Control</td></tr><tr><td>Unilateral partial ossification of the 7th cervical vertebra</td><td>18*/11</td><td>7/6</td></tr><tr><td colspan="3">*Statistically different from control at p<0.05</td></tr></table> 2.5 mg/kg bw/d No toxicologically significant changes.
Developmental toxicity Oral (gavage) OECD TG 414 (1983) Rabbits, New Zealand White 18 animals/group 0, 25, 75 or 125 mg/kg bw/d on days 6-18 of gestation Vehicle: corn oil Purity 92.4 % Report No CTL/P/861 (1983b)	Maternal toxicity: No treatment related adverse effects observed. Foetuses: No treatment-related adverse effects observed.
Developmental toxicity Oral (gavage) OECD TG 414 (1983)	Maternal toxicity: No treatment-related adverse effects observed. Foetuses:

<table>
<tr>
<td>Rabbits, New Zealand White

18 animals/group

0, 25, 75 or 125 mg/kg bw/d on days 6-18 of gestation

Vehicle: corn oil

Purity 92.4 %

Report No CTL/P/1460 (1986)</td>
<td>No malformations were observed.

<u>Foetal variations:</u>

<u>125 mg/kg bw/d</u>

<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>7th transverse process partially ossified</td><td>8*/3</td><td>2/2</td></tr>
<tr><td>5th sternebrae partially ossified</td><td>45*/13</td><td>30/9
HC = 19-58 %</td></tr>
<tr><td>Extra 13th rib</td><td>62*/14</td><td>49/14
HC = 44-78.9 %</td></tr>
<tr><td colspan="3">*Statistically different from control at p<0.05
HC= Historical control</td></tr>
</table>

<u>75 mg/kg bw/d</u>

<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>7th transverse process partially ossified</td><td>8*/5</td><td>2/2</td></tr>
<tr><td>5th sternebrae partially ossified</td><td>47*/13</td><td>30/9
HC = 19-58 %</td></tr>
<tr><td colspan="3">*Statistically different from control at p<0.05
HC= Historical control</td></tr>
</table>

<u>25 mg/kg bw/d</u>

<table>
<tr><td colspan="3">Alterations in ossification (number of foetuses affected/number of litters affected)</td></tr>
<tr><td></td><td>Exposed</td><td>Control</td></tr>
<tr><td>5th sternebrae partially ossified</td><td>55*/15</td><td>30/9
HC = 19-58 %</td></tr>
<tr><td colspan="3">*Statistically different from control at p<0.05
HC= Historical control</td></tr>
</table>
</td>
</tr>
</table>

RAC notes the absence of maternal toxicity at the highest dose tested in both studies in rabbits. This is not in line with the dose selection criteria in the test guidelines for developmental toxicity studies, where it is indicated that the highest dose should be chosen to induce some developmental and/or maternal toxicity (OECD TG 414). However, RAC also notes a range-finding study in rabbits presented in the DAR where 100, 200 and 400 mg paclobutrazol/kg bw/d caused four treatment-related deaths at the top dose and an increased incidence of

vaginal bleeding at 200 and 400 mg/kg bw/d, suggesting resorptions or abortions. Animals dosed with the two highest doses also experienced a decrease in body weight during gestation together with haemorrhagic spots and/or white areas on the stomach. All these results suggest that the top dose employed in studying developmental toxicity in rabbits should be lower than 200 mg/kg bw/d because otherwise the interpretation of the results might be very difficult.

The results of the developmental toxicity studies suggest that the following effects may be relevant for classification purposes:

i) malformation (cleft palate) in rats;

ii) variations (alterations in ossification) in rats and rabbits; and

iii) variations (alterations in kidney) in rats.

i) Cleft palates were reported in rats in two different studies and in a publication in the open scientific literature. Cleft palate is a very rare malformation in rats and the observation in different studies and different litters is of concern for human health and therefore also relevant for classification purposes.

ii) Variations (mainly delayed ossification in sternebrae and rib) were consistently reported in three different studies with two different species (rabbits and rats). These variations appeared at doses that induce maternal toxicity but also at doses without maternal toxicity. Therefore, RAC considers these variations relevant as part of the weight of evidence justifying classification.

iii) Kidney effects were reported in one of the studies in rat at doses of 100 mg/kg bw/d. In the RCOM, the DS supplied the historical control data for hydroureter and dilated ureter (extreme) in the Alpk:AP rat at the performing facility between 1983 and 1992. It was noted that hydroureter and dilated ureters are frequently seen in control Alpk:AP rats at the conducting laboratory and are very variable in their occurrence and severity between control groups. RAC notes that the incidences of these impairments in the paclobutrazol study were in general higher than the concurrent and historical controls. However, RAC also notes that the incidences in the concurrent controls were in some cases also higher than the incidences in the historical controls. This suggests high background variability and adds uncertainty to the assessment of the biological relevance of these alterations. It is also noted by RAC that these effects were not reported in one of the two studies in rat at concentrations 2.5 times higher (250 mg/kg bw/d), which reduces the concern. In conclusion, RAC considers these effects (hydroureter and dilated ureters) of minor concern for classification purposes.

There is no information on the potential of paclobutrazol to adversely affect development in humans and therefore classification in Category 1A is not warranted.

Classification for paclobutrazol in Category 1B (presumed human reproductive toxicant) should be largely based on data from animal studies that provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction should be considered not to be a secondary non-specific consequence of other toxic effects.

Category 1B was discussed due to the cases of cleft palates reported in several rat studies. Cases of cleft palates were reported both with and without maternal toxicity. The table below summarises the cases of cleft palate in developmental toxicity studies with paclobutrazol.

Table: Cases of cleft palates in developmental studies with paclobutrazol

Study	Dose level (mg/kg bw/d)	Observations
Preliminary study in rats Report No CTL/P/656 (1980)	0, 80, 160 and 240	240 mg/kg bw/d Maternal toxicity: 20% mortality (2 animals showed on day 8 and 13 thin and/or hunched with excessive lacrimation), small reduction in maternal bodyweight gain. Cleft palate in 6/85 foetuses (all from a single litter). 160 mg/kg bw/d No cleft palates. 80 mg/kg bw/d Small reduction in maternal bodyweight gain; cleft palate (1/110). 0 mg/kg bw/d Cleft palate (1/130).
Main study in rats Report No CTL/P/842 (1983)	0, 40, 100 and 250	250 mg/kg bw/d Maternal toxicity: 20 % mortality. Cleft palate in 3/234 foetuses at 250 (one of the foetuses had multiple malformations exencephaly, anophthalmia, cleft lip). 100 mg/kg bw/d No cleft palates. 40 mg/kg bw/d Cleft palate in 1/297 foetuses. 0 mg/kg bw/d No cleft palate.
Main study in rats Report No CTL/P/997 (1984)	0, 2.5, 10, 40 and 100	No maternal toxicity, no cleft palates.
Published non-GLP study in rats Vergieva (1998)	Repeat dose: 50 or 200 (GD 6-15) Single dose: 200 or 500 on day 7 or 9 or 11 or 13	Repeat dose 200 mg/kg bw/d on days 6-15: Cleft palate in 2/39 foetuses. Single dose 200 mg/kg bw/d on day 11: cleft palate in 12/25 foetuses. 500 mg/kg bw/d on day 9: cleft palate in 4/32 foetuses. 500 mg/kg bw/day on day 11: cleft palate in 25/25 foetuses. 500 mg/kg bw/day on day 13: no cleft palates.
Main study in rabbit Report No CTL/P/861 (1983b)	0, 25, 75 and 125	No cleft palates.
Main study in rabbit CTL/P/1460 (1986)	0, 25, 75 and 125	No cleft palates.

Cases of cleft palate in the absence of maternal toxicity were reported in three studies. In the preliminary study, one case was reported at 80 mg/kg bw/d but a single case was also reported in the control group and no cases were reported at 160 mg/kg bw/d (another dose without maternal toxicity).

In one of the two main studies in rat a single case of cleft palate was reported at 40 mg/kg bw/d; while no cases were reported in the control and 100 mg/kg bw/d groups. The DS provided the historical control data of the performing facility and no cases of cleft palates were found in controls of 10 different studies performed between 1980 and 1984 (including the current study with paclobutrazol).

The third study where a case of cleft palate was found in the absence of maternal toxicity is the study published by Vergieva (1998). However, this study shows several inconsistencies that notably lowers its reliability and relevance for classification. Firstly, in the repeated dose study at 200 mg/kg bw/d, it is impossible from the data presented to ascertain if the malformations in the two animals are spontaneous or treatment-related since there was no information on control animals or historical control data. Secondly, it does not seem logical that in the repeated dose study at 200 mg/kg bw/d, 2/39 foetuses were diagnosed with cleft palate, while a single dose of 200 mg/kg on day 11 produced cleft palate in 12/25 foetuses (noting that in the repeated dose study, treatment occurred on day 11 as well). Also, in the single dose study, cleft palate was reported when dosing occurred on days 9 or 11, but not on day 13 of gestation. Palate closure in the rat typically occurs on about days 16-17, and most compounds that cause cleft palate are active from about days 12 to 17, so it is difficult to understand why a compound that caused 100 % cleft palate on day 11 would be inactive on day 13. Moreover, minimal maternal toxicity was reported in dams administered 200 and 500 mg paclobutrazol/kg bw/d, in contrast to all other studies which demonstrate severe clinical toxicity and mortality at doses greater than 200 mg/kg bw/d. In conclusion, these inconsistencies make the biological plausibility of the reported findings in this study questionable.

RAC concludes that all cases of cleft palate reported in the absence of maternal toxicity are of questionable biological relevance. In two of the studies, only a single case was seen at a single dose (40 and 80 mg/kg bw/d, respectively). In one of these studies, there was also one case of cleft palate seen in the control group. In addition, no craniofacial malformations were reported at doses above those causing presumably incidental cases of cleft palate, which would be expected if the cleft palates were attributed to an excess of retinoic acid that might alter the development of neural crest cells, hind brain, cranial nerves and craniofacial structures. It is hence considered that these cases can be attributed to chance and therefore not relevant for classification. The third study where cleft palates were seen without maternal toxicity has several deficiencies and inconsistencies that makes it of limited value for classification purposes.

Cases of cleft palate were also reported in the presence of excessive maternal toxicity (in two different studies at 240 and 250 mg/kg bw/d) that was manifested as 20 % mortality in both cases. RAC notes that the Guidance on the Application of the CLP Criteria establishes in Annex I: 3.7.2.4.4 that: *"Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation"*. Cleft palates were reported concurrently with 20% maternal toxicity and therefore the concern is notably reduced.

Sections 3.7.2.4.1 and 3.7.2.4.2 of Annex I to the CLP Regulation acknowledge that the assessment of whether the development of offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental toxicity.

Section 3.7.2.4.2 of Annex I to the CLP Regulation provides that "the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity". Section 3.7.2.4.3 of Annex I to the CLP Regulation provides that "Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1."

RAC notes that malformations (cleft palate) were reported only in one species and concurrent with high maternal mortality (20 % mortality), or of questionable biological relevance when occurring at non-maternally toxic doses. At doses where no maternal toxicity was seen, the only effect considered to be due to paclobutrazol treatment were variations (mainly retardation in ossification). Therefore, RAC supports the DS's proposal for classification of paclobutrazol as Repr. 2; H361d (Suspected of damaging the unborn child).

4.12 Aspiration Hazard

Classification for aspiration toxicity is intended to apply to liquid substances and mixtures according to point (b) in table 3.10.1 of Annex I of CLP. Paclobutrazol is a granular solid at 20°C with a melting point of 164°C and the criteria for classification are not met

4.12.1 4.12.1 Conclusions on classification and labelling

Not classified. Conclusive, but not sufficient for classification.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of paclobutrazol for aspiration toxicity since this hazard is intended to apply to liquid substances and paclobutrazol is a granular solid with melting point of 164 °C.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC supports the DS's proposal of no classification of paclobutrazol for aspiration toxicity because criteria for classification are not met.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Paclobutrazol (referred to in some test reports as PP333) is approved in the EU as a plant growth retardant which suppresses the plant hormone gibberellin resulting in cell expansion, it also has fungicidal activity. Environmental fate and hazard studies have been considered under Directive 91/414/EEC and summarised in the Draft Assessment Report, 2006 and Additional Report, 2010. The agreed endpoints from the peer review of paclobutrazol under Directive 91/414/EEC are also included in the EFSA Conclusion (EFSA Journal 2010;8(11):1876).

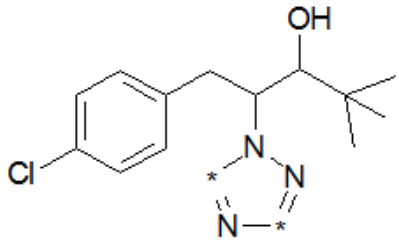
In addition, two further studies on the toxicity to *Lemna* sp. and one ready biodegradation study are available as part of data matching under Directive 91/414/EEC. Although not included in the main Review of paclobutrazol under Directive 91/414/EEC, they are considered reliable for the purpose of classification.

The key information pertinent to determining a classification is presented below.

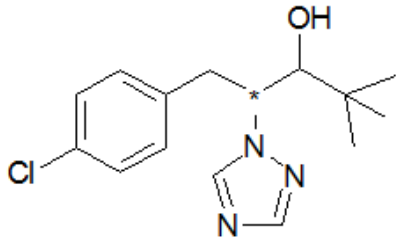
Paclobutrazol is a racemic mixture with two enantiomers. Fate and ecotoxicity testing did not consider individual enantiomers. Consequently endpoints are based on the sum of the two enantiomers, which were tested in similar ratios to that occurring in the marketed technical material.

All radiolabelled studies used ^{14}C-paclobutrazol in a combination of the labels shown in Figure 1.

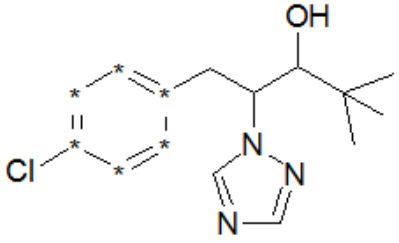
Figure 1: Structure of paclobutrazol indicating positions of the ^{14}C labels.



^{14}C-Triazole



^{14}C-Methine



^{14}C-Phenyl

The measured water solubility of paclobutrazol in distilled water is 22.9 mg/l following the shake flask method. Across the pH range pH 5, 7 and 9 minimal change was observed with water solubilities in the range 17.2 to 24.1 mg/l (Cuthbert and Mullee, 2001).

Paclobutrazol is not anticipated to dissociate (Cuthbert and Mullee, 2001).

Where available information on degradation products is included – details of degradant names and structures are presented in Annex I.

5.1 Degradation

A summary of available valid information on the fate of paclobutrazol is presented in Table 20 below.

Table 20: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis Similar to OECD Test Guideline 111, pre-date GLP	Stable	Valid	Woods and Leahey, 1983a
Aquatic photolysis SETAC guidelines, pre-date GLP	Stable	Valid	Woods and Leahey, 1983a
Aquatic photolysis JMAFF guidelines, GLP	DT_{50}: 38 to 77 days (natural summer sunlight) at latitudes 30-50°N, 24 to 25 °C	Valid	Van der Gauuw, 2004a
Aquatic photolysis OECD Test Guideline 316, GLP	DT_{50}: >64 days at 30°N DT_{50}: >72 days at 40°N DT_{50}: >86 days at 50°N All at room temperature ~22 °C	Valid Additional information	Manoumi, 2008
OECD Test Guideline 301F (Manometric respirometry test), GLP	<5% degradation, day 28	Valid	Wallace and Woodyer, 2002
OECD Test Guideline 301F (Manometric respirometry test), GLP	4% degradation, day 28	Valid Additional information	Neri, 2009
Water/sediment simulation SETAC guidelines, GLP	$DT_{50\ total\ system}$ 167 to 1,378 days <0.7 to 7.4% AR mineralisation after 84 days	Valid	Simmons, 1987
Water/sediment simulation JMFFAF guidelines, GLP	$DT_{50\ total\ system}$ 639 days <0.1 % AR mineralisation after 120 days	Valid Additional information	Van der Gauuw, 2004b
Water/sediment kinetic evaluation according to FOCUS Guidance	$DT_{50\ total\ system}$ 193 days	Valid Reevaluation of Simmons, 1987, data	Harvey, 2009a

5.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Woods and Leahey, 1983a) is available. The study was conducted in 1983 (pre-dating GLP) and followed an in-house method. Review under Directive 91/414/EEC concluded the method was similar to OECD Test Guideline 111 and acceptable. The study used ^{14}C-triazole paclobutrazol (radiochemical purity >98%) at 10.2 mg/l in sterile buffer solutions at pH 4, 7 and 9. Samples were incubated at 25 ^{o}C in the dark for up to 30 days. No hydrolysis was observed and paclobutrazol is considered hydrolytically stable.

Aqueous photolysis

Study 1 (Woods and Leahey, 1983b)
Aqueous photodegradation of paclobutrazol was investigated in 1983 (pre-dating GLP) and following an in-house method. Review under Directive 91/414/EEC concluded the method was similar to SETAC guidelines and acceptable. The study used ^{14}C-triazole paclobutrazol (radiochemical purity >98%) at 10.4 µg/ml in sterile pH 7 buffer solutions at 29 to 40 ^{o}C. Samples were continuously irradiated for 10 days with a xenon arc light considered to mimic natural sunlight > 290 nm wavelength. No degradation was observed.

Study 2 (van der Gaauw, 2004a)
A second aqueous photodegradation of paclobutrazol study is available following GLP and JMAFF[1] Agchem Test Guideline 12. The study used ^{14}C-triazole paclobutrazol (radiochemical purity >98.1%) and two test systems:

- Test I employed small (exposed area of 3.14 cm^2) glass cylindrical test vessels with continuous irradiation for 26 days at ~25 ^{o}C. A control of irradiated sterile natural water was included.
- Test II employed larger (exposed area of 28.26 cm^2) cylindrical test vessels with continuous stirring and irradiation for 20 days at ~24 ^{o}C. A non-irradiated control without aeration was included.

Test substance application solutions were prepared with the aid of the solvent acetonitrile at <0.1 ml/l. The paclobutrazol test concentration range was 1.15 to 1.6 mg/l.

Both tests used sterile natural pond water (pH 8.4) and light excluding radiation <290 nm. Radioactivity was measured by Liquid Scintillation Counting (LSC) with substance concentrations determined by High Performance Liquid Chromatography (HPLC) or Thin Layer Chromatography (TLC).

In Test I paclobutrazol concentrations declined from near nominal at study initiation to 75% AR by day 26. Minimal mineralisation was observed with 0.1 % AR CO_2 by day 26. Various degradants were observed all less than 10% AR. The degradant 1,2-4 triazole was the most significant at 6.4% AR (mean of 2 replicates). Near nominal (96.5% AR) concentrations of paclobutrazol were observed in non-irradiated samples.

In Test II up to 3 times more photodegradation was observed. This was considered due to approximately 3 times more light photons reaching the test solutions due to the increased test vessel size. By day 20 paclobutrazol accounted for 55% AR with 1,2-4 triazole the most significant degradant at 14.4% AR. Minimal mineralisation (0.1% AR CO_2) was also observed. Near nominal

[1] Japanese Ministry of Agriculture, Forestry and Food

(97.5% AR) concentrations of paclobutrazol were observed in non-irradiated, non-stirred control samples.

Photodegradation half-lives were calculated assuming first order degradation kinetics:
Test I DT_{50}: 77 days (natural summer sunlight) at latitudes 30-50ºN, 25 ºC
Test II DT_{50}: 38 days (natural summer sunlight) at latitudes 30-50ºN, 24 ºC

Study 3 (Manoumi, 2008)

The direct and indirect aqueous photolysis of paclobutrazol was investigated according to the OECD 316 guideline. The GLP study calculated half-lives for the latitudes 30ºN, 40ºN and 50ºN as >64, >72 and >82 days at room temperature (~22 ºC).

5.1.2 Biodegradation

Not available.

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Study 1 (Wallace and Woodyer, 2002)
A ready biodegradation study is available following OECD Test Guideline 301F (Manometric respirometry test) and GLP. The study was run at ~15.7 mg/l paclobutrazol. Negligible (<5%) degradation was observed over 28 days. Validation criteria were met.

Study 2 (Neri, 2009)
A second ready biodegradation study is available following OECD Test Guideline 301F (Manometric respirometry test) and GLP. The study was run at ~100 mg/l paclobutrazol. Negligible (4%) degradation was observed over 28 days. Validation criteria were met.

5.1.2.3 Simulation tests

Two GLP water sediment studies are available using radiolabelled paclobutrazol.

Study 1 (Simmons, 1987)
Review under Directive 91/414/EEC concluded the method was similar to SETAC guidelines and acceptable. Two UK aerobic systems were employed: 'Virginia Water' and 'Basing'. The test system had a water to sediment ratio of 100:1 and other test conditions are included in table 21 below. The SETAC test guideline recommendation is for a water:sediment ratio between 4:1 and 10:1. It is unclear if the study ratio influenced the rate of dissipation to sediment.

The study used ^{14}C-triazole and ^{14}C-phenyl paclobutrazol (both >97% purity). The test item was dissolved in methanol before treatment via the water surface with 0.144 to 0.159 µg/l test item.

Table 21: Water-sediment system test conditions

Criteria	Basing, UK	Virginia Water, UK
Water properties	pH: 7.6	pH: 7.62
Sediment properties	51% sand; 29% silt; 20% clay Organic matter: 12.8%	90% sand; 6% silt; 4% clay Organic matter: 4.5%

The study was conducted at 22 °C, in the dark-under aerobic conditions for up to 84 days.

Radioactivity was determined by LSC and if >5% AR was present, additional analysis by TLC. Total mean recoveries for both systems were >90% AR (92.5 to 97.2% AR) for both labels at each sampling point.

Paclobutrazol mainly stayed in the water phase over the study period with 53 to 72% AR in water by day 84. Concentrations of paclobutrazol in sediment peaked on day 21 with 25 to 30% AR. Various degradants were detected in combined water-sediment samples at <10% AR.

Paclobutrazol DT_{50} values were determined based on first-order kinetics.

Water dissipation DT_{50} values combined for both labels were 164 days for the Basing system. However, the eMS notes that the r^2 values were around 0.5 indicating low confidence in the DT_{50} value. It was not possible to determine $DT_{50\ water}$ values for the Virginia Water system as minimal degradation was observed.

Whole system $DT_{50\ totally\ system}$ values for both labels were calculated to be 167 to 226 days for the Basing system. Based on the phenyl label only, the $DT_{50\ total\ system}$ for the Virginia Water system was 1,378 days. The eMS notes the r^2 value for the Virginia Water relationship was 0.7 indicating less confidence than the Basing system values.

Minimal mineralisation was observed with maximums of 7.4% AR and 0.9% AR observed in each system after 84 days.

Subsequent analysis of the data (Harvey, 2009a) using FOCUS (2006) and single first-order (SFO) kinetics, calculated a $DT_{50\ total\ system}$ of 193 days for the Basing System. It was not possible to calculate a DT_{50} for the Virginia Water system as the data did not provide a good statistical fit.

Additional Study (van, der Gaauw A. (2004b)

A second fate study is available following GLP and JMAFF Agchem test guidelines (2-5-1). The study employed ^{14}C-triazole paclobutrazol (98.7% purity) and involved flooding fresh paddy soil with purified water spiked with the test item. While the study is of limited relevance for classification, brief details are included for completeness.

The test item was dissolved in water/acetone (8:2; v/v) to achieve a concentration equating to 0.14 mg a.s./kg dry soil. The soil was a sandy loam with an organic carbon content of 3.02 g/100 dry soil and a pH of 5.47. Test systems were kept in the dark at 25 °C for 120 days. Samples were analysed by LSC and subsequently by HPLC or TLC. Total mean recoveries were >95% AR.

On day 0 paclobutrazol accounted for 4.5% AR in water and 92.7% AR in sediment. This rapid partitioning is considered due to the method where the spiked water phase was mixed with soil after application. Considering this, the eMS feels DT_{50} values should be treated with caution.

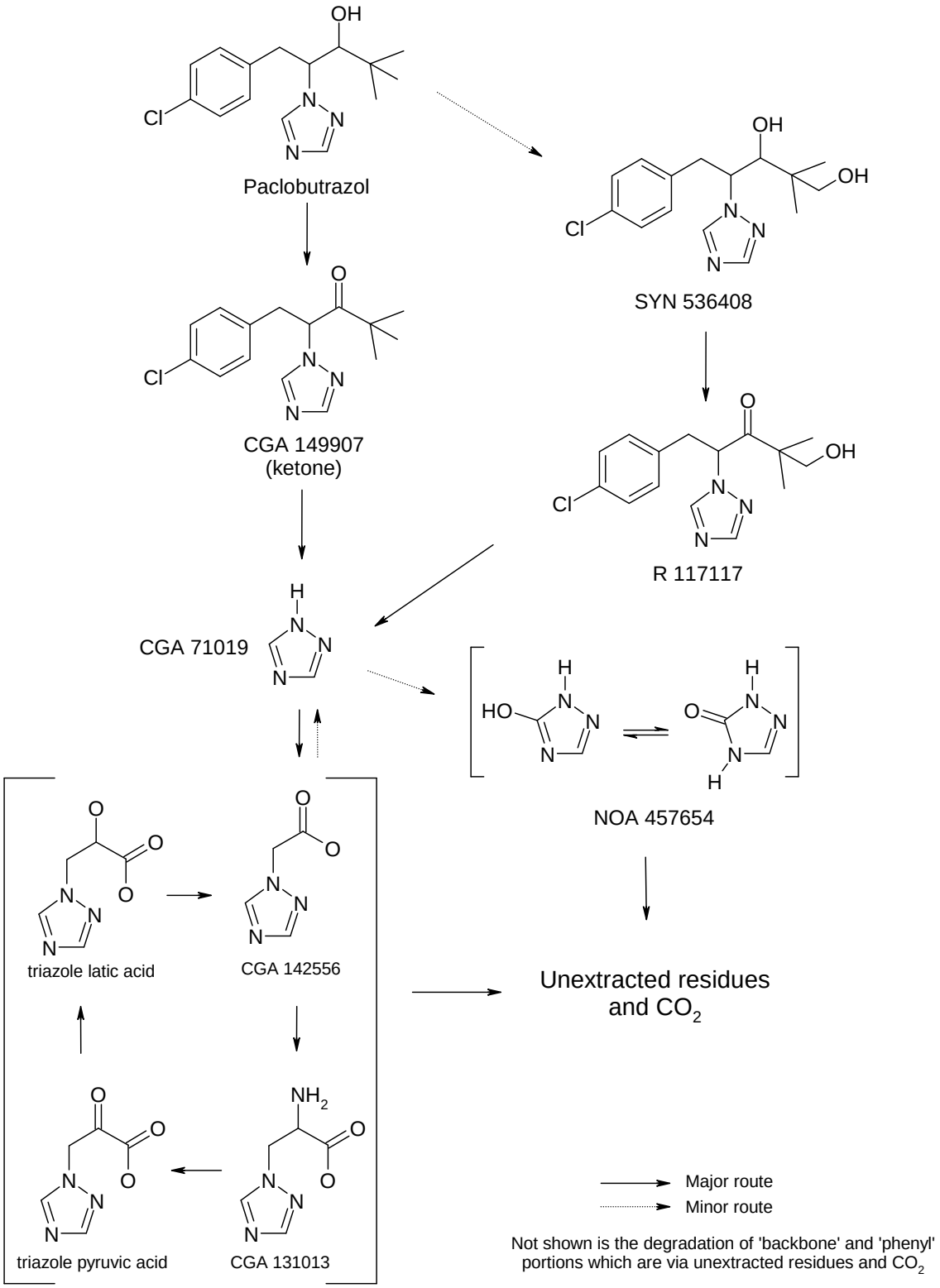
The total system DT_{50} was calculated using Model Maker assuming first order degradation kinetics: $DT_{50\ total\ system}$ 639 days based on an r^2 value of 0.95.

Minimal mineralisation was observed with <0.1% AR observed throughout the study up to termination on day 120.

Miscellaneous:

Based on the DAR, the proposed degradation pathway in water-sediment systems is presented in Figure 2.

Figure 2: Proposed degradation pathway in water-sediment systems



5.1.3 Summary and discussion of degradation

Paclobutrazol is considered hydrolytically stable at pH 7 and 9.

Under experimental conditions paclobutrazol undergoes minimal photodegradation. The experimental DT_{50} in sterile natural water was 38 to 77 days (natural summer sunlight) at latitudes 30-50^{o}N, 24 to 25 ^{o}C. Minimal mineralisation (0.1% AR CO_2) was also observed over 20 days. The actual degree of photodegradation in the aquatic environment depends on local conditions and seasons. Therefore, in reality the potential for aquatic photolysis is likely to be limited.

In a ready biodegradation study minimal (<5%) degradation was observed.

In an aerobic water-sediment study paclobutrazol was observed to dissipate slowly from the water column to sediment in two systems. Limited transformation to degradants was also observed. Estimated whole system DT_{50} values for paclobutrazol were $DT_{50\ total\ system}$ 167 to 1,378 days. Minimal mineralisation was observed with <0.7 to 7.4% AR after 84 days. Subsequent data re-analysis using FOCUS determined a $DT_{50\ total\ system}$ of 193 days.

Overall, the degradation information does not provide sufficient data to show paclobutrazol is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, paclobutrazol is considered non-rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Shaw, 2002

A GLP adsorption/desorption study is available using radiolabelled ^{14}C-triazole paclobutrazol and following OECD Test Guideline 106. Four soils were employed from the USA, Japan and the UK ranging from silty loams to clay loam. K_{oc} values ranged from 40.4 to 263 ml/g indicating paclobutrazol will be mobile in soil.

Additional adsorption studies are available in the DAR for degradants. These are not presented further as they are not considered relevant for classification of paclobutrazol.

5.2.2 Volatilisation

Experimental data (Cuthbert and Mullee, 2001) indicate the vapour pressure for paclobutrazol is low at 1.9 x 10^{-6} Pa at 20 °C.

The Henry's Law Constant (Cuthbert and Mullee, 2001) was calculated to be 2.39 x 10^{-5} Pa m^3 mol^{-1} indicating paclobutrazol is unlikely to partition significantly from the water phase to air.

5.2.3 Distribution modelling

Not relevant for classification and labelling.

5.3 Aquatic Bioaccumulation

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water (shake flask method)	Log K_{ow} 3.11 at 23 °C No evidence of pH dependence		Cuthbert and Mullee, 2001
Experimental aquatic BCF pre-date standard test guidelines and GLP	Paclobutrazol whole fish BCF: 44 l/kg wet weight	Flow through, 14 days exposure, 14 days depuration	[redacted] 1983

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No data available.

5.3.1.2 Measured bioaccumulation data

An experimental aquatic BCF study for paclobutrazol (purity >94.8%) is available which pre-dates GLP and standard test guidelines ([redacted], 1983). It was reviewed under EU Directive 91/414/EEC and considered suitable to fulfil the bioaccumulation in fish endpoint.

The study used ^{14}C-triazole paclobutrazol, a flow-through system with Bluegill Sunfish (*Lepomis macrochirus*) and one exposure concentration; nominally 0.5 mg/l. The exposure period ran for 14 days followed by a 14 day depuration period.

There was no significant increase in ^{14}C-residues after 3 days of exposure. Based on total radioactive ^{14}C residues (TRR considered paclobutrazol equivalents), whole fish BCFs were calculated for days, 1, 3, 7, 10 and 14. This highest value was 44 l/kg on day 10.

During the depuration period, levels of ^{14}C-residues fell rapidly and returned to background levels in all tissues within 7 days.

Data are not available to lipid normalise the BCF. However, given the low BCF value it is unlikely such correction would increase the BCF above the CLP trigger.

While the study has limitations given there was only one test concentration and limited analysis of the test item, it is considered sufficient to indicate the BCF is below the CLP trigger of ≥ 500.

5.3.2 Summary and discussion of aquatic bioaccumulation

The experimental log K_{ow} for paclobutrazol is 3.11 at 23 °C (no pH dependence).

An experimental whole fish BCF was 44 l/kg based on ^{14}C-residues considered as paclobutrazol equivalents.

Overall, the log K_{ow} is below the CLP log K_{ow} trigger value of ≥ 4 and the whole fish BCF for parent paclobutrazol (or TRR) is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of paclobutrazol is presented in Table 23. A summary of valid information for degradants is also included in Annex II, Table 1.

Studies were reviewed under EU Directive 91/414/EEC and considered valid. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the respective test guideline. They are considered reliable and suitable for use in hazard classification.

Two additional studies on the toxicity of technical paclobutrazol to *Lemna* spp. are available following data matching under Directive 91/414/EEC. These are considered reliable for the purpose of classification and details and included below.

Further details are presented for studies conducted on the active substance paclobutrazol but not for its degradants as these are less toxic and not considered further for classification of paclobutrazol.

Paclobutrazol is a racemic mixture of enantiomers (2R,3R and 2S,3S). Ecotoxicity testing did not consider individual enantiomers. Consequently endpoints are based on the sum of the 2.

Paclobutrazol is a plant growth regulator of the triazole group which is mainly taken up by roots. It also has some fungicidal activity. It retards vegetative growth by suppressing gibberellin production resulting in the reduction of cell expansion. It would be expected therefore, that aquatic macrophytes would be sensitive to paclobutrazol and this is borne out by the available data.

Table 23: Summary of relevant information on aquatic toxicity for paclobutrazol

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Acute toxicity to fish Similar to OECD 203, GLP, purity: 92.4%	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Semi-static	96 hours	LC_{50}	23.6 (mm)	[redacted] 1982
Acute toxicity to fish Similar to OECD 203, pre-date GLP, purity: 97%	Rainbow Trout (*Oncorhynchus mykiss*) formerly *Salmo gairdneri*	Mortality	Semi-static	96 hours	LC_{50}	27.8 (mm)	[redacted] 1978
Acute toxicity to fish Similar to OECD 203, GLP, purity: 92.4%	Mirror Carp (*Cyprinus carpio*)	Mortality	Semi-static	96 hours	LC_{50}	26 (mm)	[redacted] 1983

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Acute toxicity to fish Similar to OECD 203, GLP, purity: 92.4%	Sheepshead Minnow (*Cyprinidon variegatus*)	Mortality	Semi-static	96 hours	LC_{50}	24.3 (mm)	[redacted] 1985
Prolonged toxicity to fish OECD Guideline 204, GLP, purity: 96.7%	Rainbow Trout (*Oncorhynchus mykiss*) formerly *Salmo gairdneri*	Mortality , weight, length and toxicity symptoms	Flow-through	28 days	NOEC	3.3 (mm)	[redacted], 1990
Daphnia sp Acute Immobilisation US EPA 660/307-5-009, pre-date GLP, purity: 92.46%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>limit of solubility in test media	Hill and Hamer, 1982
Acute toxicity no guideline, GLP, purity:92.4%	Mysid Shrimp (*Americamysis bahia*)	Acute	Semi-static	96 hours	LC_{50}	>9 (mm)	Thompson, 1985a
Acute toxicity ASTM E 724-80, GLP, purity:92.4%	Pacific Oyster larvae (*Crassostrea gigas*)	Acute	Static	48 hours	EC_{50}	>10 (n) Supported by analytical verification	Thompson, 1985b
Daphnia magna Reproduction OECD Guideline 202 modified, GLP, purity: 96.9%	*Daphnia magna*	Survival; reproduction; growth	Semi-static	22 days	NOEC	0.32 (mm)	Stewart *et al*, 1991
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 92.4%	*Pseudo-kirchneriella subcapitata**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>15.2 (mm) 0.98 (mm)	Thompson and Williams, 1984
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 95.1%	*Anabaena flos-aquae*	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>23.23 (mm) 1.8 (mm)	Knauer, 2002
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 95.1%	*Lemna gibba*	Growth	Static	7 days	ErC_{50} NOErC	**0.0283 (n)** **0.002 (n)** Supported by analytical verification	Grade, 2002
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 96.4%	*Lemna gibba*	Growth	Static	7 days	ErC_{50} NOErC	**0.0237 (mm)** **0.00151 (mm)**	Juckeland, 2010a Additional information
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 96.1%	*Lemna minor*	Growth	Static	7 days	ErC_{50} NOErC	2.6 (im) Not determined Supported by analytical verification	Bouwman, 2009a Additional information

Notes:
mm refers to mean measured concentrations
im refers to initial measured concentrations
*formerly *Selenastrum capricornutum*
Bold values indicate most sensitive acute and chronic endpoints

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Four valid acute toxicity to fish studies using paclobutrazol are available. The studies pre-dated standard guidelines but were reviewed under EU Directive 91/414/EEC and considered valid. It was also considered that the study methods agreed in principle with OECD Test Guideline 203. In some cases the studies also pre-dated GLP.

Study 1 ([redacted] 1982)
The semi-static study used Bluegill Sunfish (*Leopmis macrochirus*) the nominal exposure range was 5.6, 10, 18, 24 and 32 mg a.s./l. Exposure solutions were prepared with the aid of the solvent dimethyl sulphoxide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 91.6 to 113% of nominal and results were based on mean measured concentrations. The 96-h LC_{50} was 23.6 mg a.s./l (95% confidence intervals 20.4 to 26.0 mg a.s./l) based on mean measured concentrations.

Study 2 ([redacted] 1978)
Using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 10, 11.5, 13.5, 15.5, 18, 21, 24, 28, 32, 37, 42 and 56 mg a.s./l. Exposure solutions were prepared with the aid of the solvent dimethyl sulphoxide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 79.7 to 111.4% of nominal and results were based on mean measured concentrations. The 96-h LC_{50} was 27.8 mg a.s./l (95% confidence intervals 26.1 to 30 mg a.s./l) based on mean measured concentrations.

Study 3 ([redacted] 1983)
Using Mirror Carp (*Cyprinus carpio*) the nominal exposure range was 5.6, 10, 18, 24 and 32mg a.s./l. Exposure solutions were prepared with the aid of the solvent dimethyl sulphoxide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 85.8 to 102.9% of nominal and results were based on mean measured concentrations. The 96-h LC_{50} was 26 mg a.s./l (95% confidence intervals 22.8 to 29.7 mg a.s./l) based on mean measured concentrations.

Study 4 ([redacted] 1985)
Using the marine species Sheepshead Minnow (*Cyprinodon virginica*) the nominal exposure range was 5.6, 10, 18, 24 and 32mg a.s./l. Exposure solutions were prepared with seawater and the aid of the solvent methyl alcohol and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 66.1 to 92.8% of nominal and results were based on mean measured concentrations. The 96-h LC_{50} was 24.3 mg a.s./l (95% confidence intervals 21.9 to 27.2 mg a.s./l) based on mean measured concentrations.

Additional Studies

A toxicity to fish study ([redacted], 1990) is available following OECD Test Guideline 204. The eMS notes the OECD 204 test method is considered a prolonged toxicity to fish test and as such is not considered as a chronic endpoint. In addition, in April 2014, the test guideline was removed by OECD.

The study used Rainbow Trout (*Oncorhynchus mykiss*) with the nominal exposure range 3.2, 5.6, 10, 18 and 32 a.s./l. Exposure solutions were prepared with the aid of the solvent dimethyl formamide and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 88 to 103% of nominal and results were based on mean measured concentrations. The 28-day NOEC was 3.3 mg a.s./l based on mean measured concentrations.

5.4.1.2 Long-term toxicity to fish

No valid available data.

During peer review under EU Directive 91/414/EEC a data gap regarding potential endocrine disrupting properties was identified given paclobutrazol belongs to the triazole chemical family. Following this two studies (Schafers, 2007 and Milburn, 2007) were conducted: a 21-day endocrine disruption screening assay using *Danio rerio* and gonadal fish histopathology. The RMS did not consider the study reliable under EU Directive 91/414/EEC. The subsequent EFSA review concluded that it was unclear if observed effects were due to study design, toxicity or endocrine disruption. On that basis a valid NOEC could not be derived and the data gap remains.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1(Hill and Hamer, 1982)
A static acute toxicity to *Daphnia magna* study using paclobutrazol is available following US EPA guideline 660/307-5-009. The study pre-dated GLP. The nominal exposure range in the main test was 5, 10, 20 and 35 mg a.s./l reflecting the limit of solubility in test media. At the highest exposure treatment undissolved material was present and attached to daphnids.

As 16 out of the 30 daphnia were immobilised in the highest treatment at 48 hours, the study concluded the 48-hour EC_{50} was approximately the level of solubility (mean measured 27.8 mg/l). Given particles were observed attached to daphnids at this concentration the eMS feels it is unclear if immobilisation effects were physical. For the purpose of classification, the eMS considered the 48-h EC_{50} is above the limit of solubility in test media.

Study 2 (Thompson, 1985a)
A semi-static acute toxicity to the marine Mysid Shrimp *Americamysis bahia* is available using paclobutrazol. The study was run to GLP and although a test guideline was not specified, the study was considered valid under Directive 91/414/EEC. The study employed one test concentration – nominally 10 mg a.s./l. prepared with the aid of methanol and a solvent control was included. Based on less than 50% mortality and mean measured concentrations, the 96-h LC_{50} was >9 mg a.s./l.

Study 3 (Thompson, 1985b)
A static acute toxicity to marine Eastern Oyster (*Crassostrea virginica*) larvae is available using paclobutrazol. The study was run to GLP and followed ASTM E 724-80. The nominal exposure range was 1, 1.8, 3.2, 5.6 and 10 mg a.s./l. Exposure solutions were prepared with the aid of the solvent methanol and a solvent control was included. Mean measured concentrations were 99 to 130% of nominal. No treatment related larval mortality or abnormalities were observed in the higher treatments. Therefore the 48-hour EC_{50} was considered >10 mg a.s./l based on nominal concentrations. While measured concentrations were not within 20% of nominal concentrations, the quoted endpoint is considered conservative and acceptable given measured concentrations were generally in excess of nominal concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A semi-static chronic toxicity to *Daphnia magna* study (Stewart et al, 1991) using paclobutrazol is available following GLP and modified OECD Test Guideline 202. The 22 day study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.32, 0.56, 1.0, 1.8, 3.2 and 5.6 mg a.s./l. Exposure solutions were prepared with the aid of the solvent methanol at 0.1 ml/l and a solvent control was included. Measured concentrations were 100 to 106% of nominal and results were based on mean measured values: 0.32, 0.58, 1.0, 1.9, 3.2 and 5.6 mg a.s./l. Validity criteria were met and the test is considered reliable. The most sensitive endpoint was length. The study 22-d NOEC was 0.32 mg a.s./l based on mean measured concentrations.

5.4.3 Algae and aquatic plants

Algae:

Study 1 (Thompson, 1984)
A static algal growth inhibition test using *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available following GLP and OECD Test Guideline 201. The nominal exposure range was 1, 1.8, 3.2, 5.6, 10 and 18 mg a.s./l. Exposure solutions were prepared with the aid of the solvent methanol –a test substance concentration above 8 mg/l could not be achieved without exceeding 0.1 ml/l so the highest two exposure concentrations were prepared with 0.225 ml/l methanol. Two solvent controls were included with results pooled for statistical comparison. Measured concentrations were 85 to 104% of nominal.

At the highest treatment 21% growth inhibition was observed. On this basis, the 96-h E_rC_{50} was considered >15.2 mg a.s./l based on mean measured concentrations. The 72-hour NOE_rC was 0.98 mg a.s./l based on mean measured concentrations.

Study 2 (Knauer, 2002)
A static algal growth inhibition test using *Anabaena flos-aquaea* is available following GLP and OECD Test Guideline 201. The nominal exposure range was 0.94, 1.88, 3.75, 7.5, 15 and 30 mg a.s./l. Measured concentrations were 77 to 117% of nominal. As 24% inhibition of growth was observed at the highest exposure concentration, the 96-h E_rC_{50} was >23.23 mg a.s./l based on mean measured concentrations. The 72-hour NOE_rC was 1.8 mg a.s./l based on mean measured concentrations.

Aquatic plants:

Three study are available using *Lemna* spp. The first study was reviewed under Directive 91/414/EEC and considered valid. The other two studies were conducted for data matching under Directive 91/414/EEC. They are considered reliable for the purpose of classification.

Study 1 (Grade, 2002)
A semi-static 7-day toxicity to *Lemna gibba* study using paclobutrazol is available following GLP and OECD Test Guideline 221. The nominal exposure range was 0.002, 0.004, 0.008, 0.016, 0.032, 0.064 and 0.128 mg a.s./l. Measured concentrations were 83 to 94% of nominal and results were based on nominal values. Validity criteria were met and the test is considered reliable. The study 7-d E_rC_{50} was 0.0283 mg a.s./l based on nominal concentrations. The 7-d NOE_rC was 0.002 mg a.s./l based on nominal concentrations.

Study 2 (Juckeland, 2010a)
A static 7-day toxicity to *Lemna gibba* study using paclobutrazol is available following GLP and OECD Test Guideline 221. The nominal exposure range was 0.002, 0.005, 0.0157, 0.0441, 0.1234 mg a.s./l. Measured concentrations were <80% of nominal and results were based on geometric mean measured concentrations: 0.00151, 0.00482, 0.0129, 0.0441, 0.909 mg a.s./l. Validity criteria were met and the test is considered reliable. The study 7-d E_rC_{50} was 0.0237 mg a.s./l based on frond number. The 7-d NOE_rC was 0.00151 mg a.s./l based on frond number.

Study 3 (Bouwman, 2009a)
A semi-static 7-day toxicity to *Lemna minor* study using paclobutrazol is available following GLP and OECD Test Guideline 221. The study used dilutions of a 100 mg/l stock solution which were filtered using 0.45µm. The measured exposure range was <LOQ of 0.1, 0.16, 0.93, 5.0 5.9 and 29 mg/l based on initial measured concentrations. Measured concentrations in expired media were 97-102% of initial measured concentrations and results were based on initial measured concentrations. Validity criteria were met and the test is considered reliable. The study 7-d E_rC_{50} was 2.6 mg/l based on frond numbers. Study NOECs were not determined although given the exposure range, a potential NOEC would not be below the other available NOECs for *Lemna* spp..

5.4.4 Other aquatic organisms (including sediment)

No available data.

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

Paclobutrazol is considered hydrolytically stable. Under experimental conditions paclobutrazol undergoes minimal photodegradation. The actual degree of photodegradation in the aquatic environment depends on local conditions and seasons. Therefore, in reality the potential for aquatic photolysis is likely to be limited.

In ready biodegradation studies minimal (<5%) degradation was observed and paclobutrazol is considered not readily biodegradable.

In an aerobic water-sediment study paclobutrazol was observed to dissipate slowly from the water column to sediment in two systems. Limited transformation to degradants was also observed. Estimated whole system DT_{50} values for paclobutrazol were $DT_{50\ total\ system}$ 167 to 1,378 days. Minimal mineralisation was observed <0.7 to 7.4% AR CO_2 after 84 days. Subsequent data re-analysis using FOCUS determined a $DT_{50\ total\ system}$ of 193 days.

Overall, the degradation information does not provide sufficient data to show paclobutrazol is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, paclobutrazol is considered non-rapidly degradable for the purpose of classification and labelling.

The experimental log K_{ow} for paclobutrazol is 3.11 at 23 ^{o}C (no pH dependence).

An experimental whole fish BCF was 44 l/kg based on ^{14}C-residues considered as paclobutrazol equivalents.

Overall, the log K_{ow} is below the CLP log K_{ow} trigger value of ≥ 4 and the whole fish BCF for parent paclobutrazol (or TRR) is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

Identified degradants are relatively less toxic than the parent substance (see Annex II) and are not considered further for classification of paclobutrazol.

Aquatic acute toxicity data on paclobutrazol are available for fish, invertebrates, algae and aquatic plants. Aquatic plants are the most acutely sensitive trophic group. Data are available for two *Lemna* species with *Lemna gibba* exhibiting the most acute sensitivity. The two *Lemna gibba* E_rC_{50} values of 0.0283 and 0.0237 mg/l are in the range 0.01 to 0.1 mg/l. On this basis paclobutrazol should be classified as Aquatic Acute 1 with an acute M-factor of 10.

At present there are no valid chronic toxicity data on fish, although further relevant data may be available in the future. Based on current data, fish are the least sensitive species in acute studies. Adopting the surrogate approach using available acute data would not result in a more stringent classification than the chronic classification proposal below. This is partially supported by the NOEC from a prolonged fish toxicity study (to OECD TG 204)

Adequate chronic toxicity data on paclobutrazol are available for invertebrates, algae and aquatic plants. Data are available for two *Lemna* species with *Lemna gibba* exhibiting the most chronic sensitivity. The two *Lemna gibba* NOE_rC values of 0.002 and 0.00151 mg/l are in the range 0.001 to 0.01 mg/l. Given this and because the substance is also considered non-rapidly degradable, paclobutrazol should be classified as Aquatic Chronic 1 with a chronic M-factor of 10.

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

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 10

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Paclobutrazol is a plant growth regulator of the triazole group, which is mainly taken up by roots. It also has some fungicidal activity. It retards vegetative growth by suppressing gibberellin production resulting in the reduction of cell expansion. It is a racemic mixture with two enantiomers. Fate and ecotoxicity testing did not consider individual enantiomers. Consequently, endpoints are based on the sum of the two enantiomers, which were tested in similar ratios to that occurring in the marketed technical material. The DS proposed to classify paclobutrazol as Aquatic Acute 1; H400 and as Aquatic Chronic 1; H410 with an M-factor of 10 for both acute and chronic classification. The classification is based on the substance being non-rapidly degradable, having no potential to bioaccumulate and being very toxic to aquatic organisms. The two *Lemna gibba* E_rC_{50} values of 0.0283 and 0.0237 mg/L are in the range 0.01 to 0.1 mg/L. The two *Lemna gibba* NOE_rC values of 0.002 and 0.00151 mg/L are in the range of 0.001 to 0.01 mg/L.

Degradation

No hydrolysis was observed in a study following OECD TG 111.

There were three studies available on aqueous photolysis. No degradation was observed in the study following SETAC Guidelines. In a GLP study following Japanese Agchem Test Guideline 12 calculated half-lives at 24-25 °C were 77 days and 38 days in Test 1 and Test 2, respectively. Minimal mineralisation was observed in both tests. Various degradants, all less than 10 % were observed in Test 1. In Test 2, where approximately three times more light photons reached the test solutions, 1,2,4-triazole was the most significant degradant (14.4 % of applied radioactivity (AR)). In the third study (GLP) performed according to the OECD TG 316 the calculated half-lives at room temperature were > 64, > 72 and > 82 days for the latitudes 30°N, 40°N and 50°N, respectively.

There were two GLP biodegradation studies available following OECD TG 301F. The studies were run at ~ 15.7 mg/L and ~ 100 mg/L paclobutrazol, respectively. Negligible degradation (< 5 %, 4 %) was observed over 28 days.

Two GLP water-sediment studies were available using radiolabelled paclobutrazol. In the first study performed according to SETAC Guidelines (GLP), the total system half-life was from 167 to 1 378 days. Paclobutrazol was observed to dissipate slowly from the water

column to sediment. Mineralisation after 84 days was from < 0.7 to 7.4 %. Subsequent analysis of the data using FOCUS and single first-order kinetics calculated a half-life to the whole system of 193 days. The second study, involving flooding fresh paddy soil, followed Japanese Agchem Test Guidelines (2-5-1). The total system half-life was 639 days. Mineralisation after 120 days was < 0.1 %.

Available information on the toxicity of identified degradation products are presented in the CLH report, in Annex II. The lowest E_rC_{50} was 0.616 mg/L for *Lemna sp*. and the NOE_rC from the same study was 0.024 mg/L.

The DS concluded that the degradation information does not provide sufficient data to show that paclobutrazol is ultimately degraded within 28 days (corresponding to half-life < 16 days) or transformed to non-classifiable products. Consequently, paclobutrazol was considered non-rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

An experimental aquatic BCF study is available. The study used ^{14}C-triazole paclobutrazol. Bluegill sunfish (*Lepomis macrochirus*) was tested in a flowthrough system at nominal concentration of 0.5 mg/L for 14 days followed by a 14-d depuration period. The highest BCF value was 44 L/kg on day 10. Data were not available for lipid normalisation. While the study has limitations, the DS was of the opinion that it is sufficient to indicate that BCF is below the CLP trigger value of ≥ 500. In a Shake flask method test the partition coefficient n-octanol/water (Log K_{ow}) was 3.11 at 23 °C, with no evidence of pH dependence, which is below the CLP Log K_{ow} trigger value of ≥ 4.

Toxicity

The measured water solubility in distilled water was 22.9 mg/L following the shake flask method. Paclobutrazol was not anticipated to dissociate. The available valid information on the aquatic toxicity of paclobutrazol is presented in the table below. Studies were reviewed under EU Directive 91/414/EEC and were considered valid and suitable for use in hazard classification. Two additional reliable studies on the toxicity of paclobutrazol to *Lemna spp*. were available and included below. Further details are presented for studies conducted on the active substance paclobutrazol but not for its degradants as these are less toxic and not considered further for classification of paclobutrazol.

Table: Summary of relevant information on aquatic toxicity for paclobutrazol

Guideline / GLP status	Species	Endpoint	Exposure		Results	
			Design	Duration	Endpoint	Toxicity (mg a.s./L)
Acute toxicity to fish Similar to OECD TG 203, GLP, purity: 92.4 %	*Lepomis macrochirus*	Mortality	Semi-static	96 hours	LC_{50}	23.6 (mm)
Acute toxicity to fish Similar to OECD TG 203,	*Oncorhynchus mykiss*	Mortality	Semi-static	96 hours	LC_{50}	27.8 (mm)

pre-date GLP, purity: 97 %						
Acute toxicity to fish Similar to OECD TG 203, GLP, purity: 92.4 %	*Cyprinus carpio*	Mortality	Semi-static	96 hours	LC_{50}	26 (mm)
Acute toxicity to fish Similar to OECD TG 203, GLP, purity: 92.4 %	*Cyprinidon variegatus*	Mortality	Semi-static	96 hours	LC_{50}	24.3 (mm)
Prolonged (* toxicity to fish OECD TG 204, GLP, purity: 96.7 %	*Oncorhynchus mykiss*	Mortality, weight, length and toxicity symptoms	Flow-through	28 days	NOEC	3.3 (mm)
Daphnia sp Acute Immobilisation US EPA 660/307-5-009, pre-date GLP, purity: 92.46 %	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	> limit of solubility in test media
Acute toxicity no guideline, GLP, purity:92.4 %	*Americamysis bahia*	Acute	Semi-static	96 hours	LC_{50}	> 9 (mm)
Acute toxicity ASTM E 724-80, GLP, purity:92.4%	C*rassostrea gigas* Pacific Oyster larvae	Acute	Static	48 hours	EC_{50}	> 10 (n) Supported by analytical verification
Daphnia magna Reproduction OECD TG 202 modified, GLP, purity: 96.9 %	*Daphnia magna*	Survival; reproduction; growth	Semi-static	22 days	NOEC	0.32 (mm)
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 92.4 %	*Pseudo-kirchneriella subcapitata*(**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	> 15.2 (mm) 0.98 (mm)
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 95.1 %	*Anabaena flos-aquae*	Cell multiplication inhibition	Static	72 hours	E_rC_{50} NOE_rC	> 23.23 (mm) 1.8 (mm)
Lemna sp. Growth Inhibition	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50}	0.0283 (n)

Test OECD TG 221, GLP, purity: 95.1 %					NOE_rC	0.002 (n) Supported by analytical verification
Lemna sp. Growth Inhibition Test OECD TG 221, GLP, purity: 96.4 %	*Lemna gibba*	Growth	Static	7 days	E_rC_{50} NOE_rC	0.0237 (mm) 0.00151 (mm)
Lemna sp. Growth Inhibition Test OECD TG 221, GLP, purity: 96.1 %	*Lemna minor*	Growth	Semi-static	7 days	E_rC_{50} NOE_rC	2.6 (im) Not determined Supported by analytical verification

Notes:
mm refers to mean measured concentrations
im refers to initial measured concentrations
(* not used for short-term or long-term classification in this case

(** formerly *Selenastrum capricornutum*
Bold values indicate most sensitive acute and chronic endpoints

Short-term (acute)

Fish

Four acute fish studies were available. The lowest LC_{50} value was 23.6 mg/L for *Lepomis macrochirus*.

Invertebrates

There were altogether three studies available on *Daphnia magna*, *Americamysis bahia* and Eastern Oyster (*Crassostrea virginica*) larvae. In the *Daphnia* test it was concluded that the 48-h EC_{50} was approximately the limit of solubility (mean measured 27.8 mg/L). Because there were particles attached to daphnids at this concentration, it was unclear if the effects were physical. Therefore, the 48-h EC_{50} was considered to be above the limit of solubility in test media. The *Americamysis bahia* test only employed one test concentration – nominal 10 mg/L. Based on less than 50 % mortality and mean measured concentrations, the 96-h LC_{50} was >9 mg/L. In the *Crassostrea virginica* study, no treatment related larval mortality or abnormalities were observed in the higher treatments. Therefore, the 48-h EC_{50} was considered >10 mg/L based on nominal concentrations. Mean measured concentrations were 99 to 130 % of nominal.

Algae and aquatic plants

There were altogether two algae studies available. In the *Pseudokirchneriella subcapitata* study, 21% growth inhibition was observed at the highest treatment of 18 mg/L (nominal). Measured concentrations were 85 to 104 % of nominal. The 96-h E_rC_{50} was considered > 15.2 mg/L based on mean measured concentrations. In the *Anabaena flos-aquaea* test, 24% inhibition of growth was observed at the highest concentration of 30 mg/L (nominal).

The 96-h ErC_{50} was >23.23 mg/L based on mean measured concentrations. Measured concentrations were 77 to 117 % of nominal.

There were three *Lemna sp*. studies available. Two with *Lemna gibba* and one with *Lemna minor*. All tests were GLP and were performed according to the OECD TG 221. The test design was static or semi-static and the test duration was 7 days. The effects endpoint investigated was growth. In the first semi-static study, measured concentrations were 83-94 % of nominal and the results were based on nominal values. The 7-d E_rC_{50} was 0.0283 mg/L. In the second static study, measured concentrations were < 80 % of nominal and results were based on geometric mean concentrations. The 7-d E_rC_{50} was 0.0237 mg/L. In a third semi-static study, measured concentrations in expired media were 97-102 % of initial measured concentrations and the 7-d ErC_{50} was 2.6 mg/L, based on frond numbers.

Long-term (chronic)

Fish

There was no valid data available. There was an OECD TG 204 prolonged study with *Oncorhynchys mykiss*. The 28-d NOEC was 3.3 mg/L based on mean measured concentrations which were 88 to 103 % of nominal. The study is not, however, a chronic study and was not used for classification.

Invertebrates

A semi-static chronic *Daphnia magna* study was available. The 22-d study assessed survival, reproduction, length and weight. Measured concentrations were 100 to 106 % of nominal. The most sensitive endpoint was length. The NOEC was 0.32 mg/L based on mean measured concentrations.

Algae and aquatic plants

There were altogether two algae studies available. In the *Pseudokirchneriella subcapitata* study 21 % growth inhibition was observed at the highest treatment, 18 mg/L nominal. Measured concentrations were 85 to 104 % of nominal. The 72-h NOEC was 0.98 mg/L based on mean measured concentrations. In the *Anabaena flos-aquaea* test 24 % inhibition of growth was observed at the highest concentration, 30 mg/L nominal. The 72-h NOE_rC was 1.8 mg/L based on mean measured concentrations. Measured concentrations were 77 to 117 % of nominal.

There were three *Lemna sp*. studies available. Two with *Lemna gibba* and one with *Lemna minor*. All tests were GLP and were performed according to the OECD TG 221. Test design was static or semi-static and test duration was 7 days. The endpoint investigated was growth. In the first semi-static study measured concentrations were 83-94 % of nominal and results were based on nominal values. The 7-d NOErC was 0.002 mg/L. In the second static study measured concentrations were < 80 % of nominal and results were based on geometric mean concentrations. The 7-d NOErC was 0.00151 mg/L. In a third semi-static study no NOEC was determined.

Comments received during public consultation

Three MSCAs supported the proposed classification as Aquatic Acute 1; H400, M=10 and Aquatic Chronic 1; H410, M=10. One MSCA clarified certain points related to degradation data and the Virginia water system with very slow degradation, in particular.

Assessment and comparison with the classification criteria

Paclobutrazol is not rapidly degradable. No hydrolysis was observed (OECD TG 111). In two ready biodegradation studies (OECD TG 301F) negligible degradation was observed over 28 days. In two water-sediment studies, mineralisation was negligible and the total system half-lives were ≥ 167 days. At least one of the degradation products is classifiable for environmental hazards.

Paclobutrazol has no potential to bioaccumulate. An experimental BCF for fish was 44 L/kg. As this is below the CLP cut-off criterion ≥ 500, Paclobutrazol is considered to have a low potential to bioaccumulate.

There are short-term (acute) data available on fish, invertebrates, algae and *Lemna*. The lowest values are from two different *Lemna gibba* tests: E_rC_{50}s of 0.0283 mg/L and 0.0237 mg/L. The lowest acute ErC_{50} of 0.0237 mg/L fills the Category Aquatic Acute 1 criteria ≤ 1 mg/L. The value is in the range $0.01 < L(E)C_{50} \leq 0.1$, giving an M-factor of 10.

There is no long-term (chronic) data on fish. Using the surrogate system (as in Figure 4.1.1 of the CLP Regulation), when adequate chronic toxicity data are available for the other trophic levels, there is a need to assess both the criteria given in Table 4.1.0(b)(i) or 4.1.0(b)(ii) of the CLP Regulation (depending on information on rapid degradation) and the criteria given in Table 4.1.0(b)(iii). The classification would, subsequently, be according to the most stringent outcome.

In the current case, the substance is not rapidly degradable. Therefore, assessing the criteria of Table 4.1.0(b)(i), would lead to classification as Aquatic Chronic 1, based on the lowest chronic NOErC of 0.00151 mg/L.

Furthermore, assessing the criteria given in Table 4.1.0(b)(iii), would lead to classification as Aquatic Chronic 3, based on the acute fish data. Based on this overall comparison with the CLP criteria, the proposed classification for long-term hazards would be Aquatic Chronic 1 (most stringent outcome) and with a corresponding M-factor of 10.

5.7 Hazardous to the ozone layer

A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

The low volatility of paclobutrazol precludes an ozone-layer-depleting potential.

5.7.1 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified – conclusive but not sufficient for classification.

6 OTHER INFORMATION

No other relevant informaiton**.**

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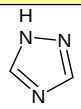
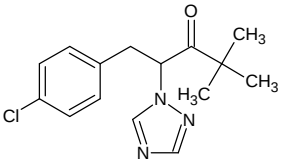
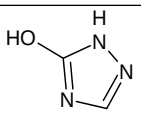
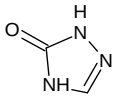
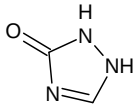
7 ANNEXES

Annex I - Environmental degradant information: code, chemical name and structure.

Annex II - Aquatic toxicity data for paclobutrazol degradants.

ANNEX I – Environmental degradant information: code, chemical name and structure.

	Report name, Structure **IUPAC name** **CAS name**
1,2,4,-triazole **CGA 71019** Soil and aquatic degradant	 1*H*-1,2,4-triazole
Ketone **CGA 149907** Soil and aquatic degradant	 (2*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pentan-3-one
Hydroxy triazole **NOA457654** Aquatic degradant	   1*H*-1,2,4-triazol-5-ol 2,4-dihydro-3*H*-1,2,4-triazol-3-one 1,2-dihydro-3*H*-1,2,4-triazol-3-one

ANNEX II – Aquatic toxicity data for paclobutrazol degradants.

Table 1: Summary of relevant information on aquatic toxicity for paclobutrazol degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
1,2,4-Triazole (CGA 71019)							
Acute toxicity to fish OECD Guideline 203, GLP, purity 91.9%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>498 (mm)	[redacted]1983
Toxicity to fish OECD Guideline 213, GLP, purity 99.9%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Semi-static	28 days	NOEC	>100 (n) Supported by analytical verification	[redacted] 2002
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 100.8%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>100 (n) Supported by analytical verification	[redacted]1995
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours 96 hours	ErC_{50} NOErC ErC_{50} NOErC	>31 (mm) 3.1 (mm) >31 (mm) 6.8 (mm)	Palmer, Kendall and Krueger, 2001
Ketone (CGA 149907)							
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 99%	*Lemna gibba*	Growth	Static	7 days	ErC_{50} (frond number) NOErC (frond number)	1.37 (n) 0.31 (n) Supported by analytical verification	Swarbrick, 2003
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 97.8%	*Lemna minor*	Growth	Semi-static	7 days	ErC_{50} (frond number) NOErC (frond number)	1.5 (im) Not determined Supported by analytical verification	Bouwman, 2009b Additional data matching information
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 98.2%	*Lemna gibba*	Growth	Static	7 days	ErC_{50}(frond number) NOErC (frond number)	0.616 (mm) 0.024 (mm)	Juckeland, 2010b Additional data matching information
Hydroxy triazole (NOA 457654)							
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 99%	*Lemna gibba*	Growth	Static	7 days	ErC_{50} (frond number) NOErC (frond number)	>100 (n) 10 (n) Supported by analytical verification	Grade and Wydra, 2007

Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 99.7%	*Lemna minor*	Growth	Static	7 days	ErC_{50} (frond number) NOErC (frond number)	59 (n) 25 (n) Supported by analytical verification	Vryenhoef and Mullee, 2010 Additional data matching information
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 99.5%	*Lemna gibba*	Growth	Static	7 days	ErC_{50}(frond number) NOErC (frond number)	314.8 (n) 1.0 (n) Supported by analytical verification	Juckeland, 2011 Additional data matching information

Notes:
mm refers to mean measured concentrations
n refers to nominal concentrations
im refers to initial measured concentrations
*formerly *Selenastrum capricornutum*