Committee for Risk Assessment

RAC

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

Adopted

8 June 2018

8 June 2018

CLH-O-0000001412-86-213/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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**

The proposal was submitted by **United Kingdom** and received by RAC on **28 March 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 May 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 July 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	603-RST-VW-Y	paclobutrazol (ISO); (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol	-	76738-62-0	Repr. 2 Acute Tox. 4 Acute Tox. 4 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H319 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H302 H319 H410		 M=10 M=10	
RAC opinion	603-RST-VW-Y	paclobutrazol (ISO); (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol	-	76738-62-0	Repr. 2 Acute Tox. 4 Acute Tox. 4 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H319 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H302 H319 H410		inhalation: ATE = 3.13 mg/L (dust and mist) oral: ATE = 490 mg/kg M=10 M=10	
Resulting Annex VI entry if agreed by COM	603-RST-VW-Y	paclobutrazol (ISO); (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol	-	76738-62-0	Repr. 2 Acute Tox. 4 Acute Tox. 4 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H319 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H302 H319 H410		inhalation: ATE = 3.13 mg/L (dust and mist) oral: ATE = 490 mg/kg M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

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.

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.

HUMAN HEALTH HAZARD EVALUATION

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

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Similar to OECD TG 401

Rats, Alderley Park

5 or 10 animals/sex and dose

Purity 97 %</td>
<td>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</td>
<td>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.
<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>
</table>
</td>
<td>Report No CTL/P/748 (1982)</td>
</tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td>
<table>
<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 paclobutrazol was administered by gavage.</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>
<tr><td>640</td><td>4/5</td><td>5/10</td></tr>
<tr><td>800</td><td>5/5</td><td>1/10</td></tr>
<tr><td>1 000</td><td>-</td><td>9/10</td></tr>
<tr><td>1 260</td><td>-</td><td>4/5</td></tr>
<tr><td>2 000</td><td>-</td><td>2/5</td></tr>
<tr><td>2 500</td><td>-</td><td>-</td></tr>
<tr><td>3 200</td><td>-</td><td>-</td></tr>
</table>
LD_{50} in males: 490 mg/kg bw
LD_{50} in females: 1 219 mg/kg bw</td>
<td>Report No CTL/P/748 (1982)</td>
</tr>
<tr>
<td>Similar to OECD TG 401

Guinea Pigs, Dunkin-Hartley</td>
<td>As an aqueous suspension in 0.5 % Lissitan at doses of 320, 400, 500 or 640 mg/kg bw of</td>
<td>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.</td>
<td>Report No CTL/P/748 (1982)</td>
</tr>
</table>

<table>
<tr>
<td>5 animals/sex and dose

Purity 97 %</td>
<td>paclobutrazol by gavage.

A further group of five male Guinea pigs received 800 mg/kg bw.</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>320</td><td>0/5</td><td>0/5</td></tr>
<tr><td>400</td><td>1/5</td><td>0/5</td></tr>
<tr><td>500</td><td>3/5</td><td>3/5</td></tr>
<tr><td>640</td><td>2/5</td><td>5/5</td></tr>
<tr><td>800</td><td>5/5</td><td>-</td></tr>
</table>

LD_{50} in males: 542 mg/kg bw
LD_{50} in females: 400-640 mg/kg bw
</td>
<td></td>
</tr>
<tr>
<td>Similar to OECD TG 401

Rabbits, New Zealand White

5 animals/sex and dose

Purity 97 %</td>
<td>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.</td>
<td>Clinical signs of toxicity were seen at all dose levels within one hour after dosing and included subdued behaviour and unsteady gait.

<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>0/0</td><td>0/0</td></tr>
<tr><td>500</td><td>3/5</td><td>1/5</td></tr>
<tr><td>1000</td><td>1/5</td><td>2/5</td></tr>
<tr><td>2300</td><td>5/5</td><td>5/5</td></tr>
</table>

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
</td>
<td>Report No CTL/P/748 (1982)</td>
</tr>
</table>

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	As an aqueous suspension in propylene glycol at a single concentration of	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)

4 animals/sex and dose Purity 97 %	1 000 mg/kg bw of paclobutrazol under an occlusive dressing.		
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

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 403

Rats, Alderley Park

5/10 animals/sex and concentration

Purity 91.4 %</td><td>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, 1.84, 3.70 and 5.19 mg/L, respectively</td><td>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 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).

Full mortality data recorded in this study
mg/L | Males | Females
0 | 0 | 0
0.54 | 0 | 0
1.84 | 0 | 1
3.70 | 1 | 4
5.19 | 3 | 3

LC_{50} in males = 4.79 mg/L
LC_{50} in females = 3.13 mg/L</td><td>Report No CTL/P/2072 (1988)</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).**

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.**

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 6 females Purity 97 %	500 mg paclobutrazol moistened with 0.5 mL of olive oil Occlusive dressing for 24 hours	<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. 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.	Report No CTL/P/741 (1982)
OECD TG 404 Rabbits, New Zealand White	500 mg paclobutrazol moistened with water	<u>Mean 24-72-hour individual animal scores:</u> Erythema: 0.3, 0.6, 0 Oedema: 0, 0, 0	Syngenta T005847-05 (2006c)

Study	Dose level	Results	Reference
3 animals (2 females and 1 male) Purity 95.7 %	Occlusive dressing for 4 hours		

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.**

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	<u>Mean 24-72-hour individual animal scores:</u> 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	<u>Mean 24-72-hour individual animal scores:</u> 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).**

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.**

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	25 µl of 10, 25 and 65 % paclobutrazol in dimethylformamide during 3	<u>Stimulation index:</u> Paclobutrazol	T005837-05 (2006)

4 animals/concentration Purity 95.7 %	consecutive days Positive control: 25 % hexylcinnamanic aldehyde	10 % = -0.8 Paclobutrazol 25 % = -0.7 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 %	<u>Induction:</u> Intradermal: 1 % in dimethylformamide/corn oil Topical: 75 % in dimethylformamide (occlusive dressing during 2 days) <u>Challenge:</u> 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. <u>Positive responses:</u> 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.**

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	Report No CTL/P/760 (1983a)

Method	Results	Reference
	Hydropic changes in liver: minimal (4/20), moderate (0/20) 11 % ↑ aminopyrene-N-demethylase activity 50 ppm (3.7 mg/kg bw/d in males and 4.4 mg/kg bw/d in females) Males Hydropic changes in liver: minimal (5/20), moderate (2/20) Females Hydropic changes in liver: minimal (2/20), moderate (0/20) Control Males Hydropic changes in liver: minimal (8/20), moderate (2/20) Females 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)	
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. 1 250 ppm (54 mg/kg bw/d in males and 72 mg/kg bw/d in females) Males ↑ 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 34/50 250 ppm (11 mg/kg bw/d in males and 14 mg/kg bw/d in females) Males Hepatic steatosis 8/50 Females 13 % ↓ body weight gain 50 ppm (2.2 mg/kg bw/d in males and 2.8 mg/kg bw/d in females) 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	There were no differences in mortality rates between treated animals and controls. 750 ppm (81 mg/kg bw/d in males and 89 mg/kg bw/d in	Report No 5014-72/274 / CTL/C/1759A

Method	Results	Reference
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 category 2: 12.5 mg/kg bw/d	females) 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: grade 1: 0/52; grade 2: 3/52; grade 3: 10/52; grade 4: 12/52; grade 5: 12/52. Females ↑ body weight (16 % at terminal sacrifice and 21 % at interim sacrifice) 21 % ↑ body weight gain 125 ppm (14 mg/kg bw/d in males and 16 mg/kg bw/d in females) Males Hepatic hypertrophy/steatosis: grade 1: 4/52; grade 2: 10/52; grade 3: 14/52; grade 4: 6/52; grade 5: 0/52 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)	(1986a)
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	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)	Report No CTL/P/1496 (1987a)

Method	Results	Reference
mg/kg bw/d	 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)	
Reported as "Broadly consistent with OECD TG" one-year Dogs, Beagle 6 animals/sex and dose 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	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 ↑ 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 ↑ 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) 75 mg/kg bw/d Males 13 %↑ Alkaline phosphatase 1.5 ↑ Hepatic aminopyrene-N-demethylase activity ↑ Absolute (24 %) and relative (25 %) liver weights Females 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) 15 mg/kg bw/d Males 1.1 fold ↑ Hepatic aminopyrene-N-demethylase activity LOAEL = 15 mg/kg bw/d (liver changes)	Report No CTL/P/958 (1984)

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.**

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. 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.	Mackay (1990)

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 Bone Marrow micronucleus	Mice, C57/BL 5 animals/sex and group	0, 233 and 373 mg/kg bw in corn oil via gavage Purity: 92 %	Negative.	The positive controls responded as expected. One and five animals were sacrificed in extremis at 233 and 373 mg/kg bw, respectively.	Report No CTL/P/3216 (1991)
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.**

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.**

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.

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 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	<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 hepatocytes (14/30) and inflammatory cell infiltrate (11/30) in females *F1* ↑ Absolute (7 %) and relative (7 %) liver weight in females

Method	Results
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)	 <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 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)	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 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. 100 and 40 mg/kg bw/d No effects observed. Foetuses:

<table>
<tr><th>Method</th><th>Result</th></tr>
<tr><td></td><td>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>

<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)</td><td><u>Dams:</u>

No toxicologically significant changes at any dose level.

<u>Foetal malformations:</u></td></tr>
</table>

<table>
<tr><th>Method</th><th>Result</th></tr>
<tr><td>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>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>
<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>

<u>40 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>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>

<u>10 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>
</table>
</td></tr>
</table>

<table>
<tr><th>Method</th><th>Result</th></tr>
<tr><td></td><td>
<table>
<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>
<u>2.5 mg/kg bw/d</u>

No toxicologically significant changes.</td></tr>
<tr><td>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)</td><td><u>Maternal toxicity:</u>

No treatment related adverse effects observed.

<u>Foetuses:</u>

No treatment-related adverse effects observed.</td></tr>
<tr><td>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/1460 (1986)</td><td><u>Maternal toxicity:</u>

No treatment-related adverse effects observed.

<u>Foetuses:</u>

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</td><td>8*/5</td><td>2/2</td></tr>
</table></td></tr>
</table>

Method	Result
	ossified | | | 5th sternebrae partially ossified | 47*/13 | 30/9 HC = 19-58 % *Statistically different from control at p<0.05 HC= Historical control 25 mg/kg bw/d Alterations in ossification (number of foetuses affected/number of litters affected) | Exposed | Control 5th sternebrae partially ossified | 55*/15 | 30/9 HC = 19-58 % *Statistically different from control at p<0.05 HC= Historical control

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 Report No 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).**

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.

ENVIRONMENTAL HAZARD EVALUATION

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, pre-date GLP, purity: 97 %	*Oncorhynchus mykiss*	Mortality	Semi-static	96 hours	LC_{50}	27.8 (mm)
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)

Guideline / GLP status	Species	Endpoint	Exposure		Results	
			Design	Duration	Endpoint	Toxicity (mg a.s./L)
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 Test OECD TG 221, GLP, purity: 95.1 %**	***Lemna gibba***	**Growth**	**Semi-static**	**7 days**	**ErC_{50}** **NOE_rC**	**0.0283 (n)** **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 < $L(E)C_{50}$ ≤ 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**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).