

Committee for Risk Assessment

RAC

Annex 2

Background document

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

**Reaction mass of 3-(difluoromethyl)-1-methyl-N-
[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-
1,4-methanonaphthalen-5-yl]pyrazole-4-
carboxamide and 3-(difluoromethyl)-1-methyl-N-
[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-
1,4-methanonaphthalen-5-yl]pyrazole-4-
carboxamide [$\geq 78\%$ *syn* isomers $\leq 15\%$ *anti*
isomers relative content]; isopyrazam**

EC Number: -

CAS Number: 881685-58-1

CLH-O-0000006915-65-01/F

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

Adopted

10 December 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Reaction mass of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide; isopyrazam

EC Number: Not listed

CAS Number: 881685-58-1

Index Number: Not listed

Contact details for dossier submitter: UK Competent Authority
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CONTENTS

1	IDENTITY OF THE SUBSTANCE	1
1.1	NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE.....	1
1.2	COMPOSITION OF THE SUBSTANCE	2
2	PROPOSED HARMONISED CLASSIFICATION AND LABELLING	4
2.1	PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA	4
3	HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING	7
4	JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL.....	8
5	IDENTIFIED USES	8
6	DATA SOURCES.....	8
7	PHYSICOCHEMICAL PROPERTIES.....	9
8	EVALUATION OF PHYSICAL HAZARDS	11
8.1	EXPLOSIVES	11
8.1.1	<i>Short summary and overall relevance of the information provided on explosive properties.....</i>	<i>11</i>
8.1.2	<i>Comparison with the CLP criteria.....</i>	<i>11</i>
8.1.3	<i>Conclusion on classification and labelling for explosive properties</i>	<i>11</i>
8.2	FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES).....	11
8.3	OXIDISING GASES.....	11
8.4	GASES UNDER PRESSURE.....	11
8.5	FLAMMABLE LIQUIDS.....	11
8.6	FLAMMABLE SOLIDS	12
8.6.1	<i>Short summary and overall relevance of the provided information on flammable solids.....</i>	<i>12</i>
8.6.2	<i>Comparison with the CLP criteria.....</i>	<i>12</i>
8.6.3	<i>Conclusion on classification and labelling for flammable solids</i>	<i>12</i>
8.7	SELF-REACTIVE SUBSTANCES.....	12
8.7.1	<i>Short summary and overall relevance of the provided information on self-reactive substances.....</i>	<i>12</i>
8.7.2	<i>Comparison with the CLP criteria.....</i>	<i>12</i>
8.7.3	<i>Conclusion on classification and labelling for self-reactive substances.....</i>	<i>12</i>
8.8	PYROPHORIC LIQUIDS.....	12
8.9	PYROPHORIC SOLIDS	13
8.9.1	<i>Short summary and overall relevance of the provided information on pyrophoric solids.....</i>	<i>13</i>
8.9.2	<i>Comparison with the CLP criteria.....</i>	<i>13</i>
8.9.3	<i>Conclusion on classification and labelling for pyrophoric solids</i>	<i>13</i>
8.10	SELF-HEATING SUBSTANCES.....	13
8.10.1	<i>Short summary and overall relevance of the provided information on self-heating substances</i>	<i>13</i>
8.10.2	<i>Comparison with the CLP criteria</i>	<i>13</i>
8.10.3	<i>Conclusion on classification and labelling for self-heating substances.....</i>	<i>13</i>
8.11	SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES.....	13
8.11.1	<i>Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases.....</i>	<i>13</i>
8.11.2	<i>Comparison with the CLP criteria.....</i>	<i>13</i>
8.11.3	<i>Conclusion on classification and labelling for substances which in contact with water emit flammable gases</i>	<i>14</i>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

8.12	OXIDISING LIQUIDS.....	14
8.13	OXIDISING SOLIDS	14
8.13.1	<i>Short summary and overall relevance of the provided information on oxidising solids</i>	<i>14</i>
8.13.2	<i>Comparison with the CLP criteria</i>	<i>14</i>
8.13.3	<i>Conclusion on classification and labelling for oxidising solids.....</i>	<i>14</i>
8.14	ORGANIC PEROXIDES.....	14
8.15	CORROSIVE TO METALS	15
8.15.1	<i>Short summary and overall relevance of the provided information on the hazard class corrosive to metals</i>	<i>15</i>
8.15.2	<i>Comparison with the CLP criteria.....</i>	<i>15</i>
8.15.3	<i>Conclusion on classification and labelling for corrosive to metals</i>	<i>15</i>
9	TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)	18
9.1	SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S).....	18
10	EVALUATION OF HEALTH HAZARDS.....	20
10.1	ACUTE TOXICITY - ORAL ROUTE	20
10.2	ADDITIONAL INFORMATION ON ACUTE ORAL TOXICITY.....	21
10.2.1	<i>Short summary and overall relevance of the provided information on acute oral toxicity</i>	<i>23</i>
10.2.2	<i>Comparison with the CLP criteria</i>	<i>23</i>
10.2.3	<i>Conclusion on classification and labelling for acute oral toxicity.....</i>	<i>23</i>
10.3	ACUTE TOXICITY - DERMAL ROUTE	24
10.3.1	<i>Short summary and overall relevance of the provided information on acute dermal toxicity.....</i>	<i>24</i>
10.3.2	<i>Comparison with the CLP criteria</i>	<i>24</i>
10.3.3	<i>Conclusion on classification and labelling for acute dermal toxicity</i>	<i>24</i>
10.4	ACUTE TOXICITY - INHALATION ROUTE	25
10.4.1	<i>Short summary and overall relevance of the provided information on acute inhalation toxicity</i>	<i>25</i>
10.4.2	<i>Comparison with the CLP criteria</i>	<i>25</i>
10.4.3	<i>Conclusion on classification and labelling for acute inhalation toxicity</i>	<i>25</i>
10.5	SKIN CORROSION/IRRITATION	31
10.5.1	<i>Short summary and overall relevance of the provided information on skin corrosion/irritation.....</i>	<i>31</i>
10.5.2	<i>Comparison with the CLP criteria</i>	<i>31</i>
10.5.3	<i>Conclusion on classification and labelling for skin corrosion/irritation</i>	<i>31</i>
10.6	SERIOUS EYE DAMAGE/EYE IRRITATION	33
10.6.1	<i>Short summary and overall relevance of the provided information on serious eye damage/eye irritation</i>	<i>33</i>
10.6.2	<i>Comparison with the CLP criteria</i>	<i>33</i>
10.6.3	<i>Conclusion on classification and labelling for serious eye damage/eye irritation</i>	<i>34</i>
10.7	RESPIRATORY SENSITISATION.....	34
10.7.1	<i>Conclusion on classification and labelling for respiratory sensitisation.....</i>	<i>35</i>
10.8	SKIN SENSITISATION	35
10.8.1	<i>Short summary and overall relevance of the provided information on skin sensitisation.....</i>	<i>36</i>
10.8.2	<i>Comparison with the CLP criteria</i>	<i>37</i>
10.8.3	<i>Conclusion on classification and labelling for skin sensitisation</i>	<i>37</i>
10.9	GERM CELL MUTAGENICITY	39
10.9.1	<i>In vitro.....</i>	<i>39</i>
10.9.2	<i>In vivo.....</i>	<i>41</i>
10.9.3	<i>Short summary and overall relevance of the provided information on germ cell mutagenicity.....</i>	<i>42</i>
10.9.4	<i>Comparison with the CLP criteria</i>	<i>42</i>
10.9.5	<i>Conclusion on classification and labelling for germ cell mutagenicity</i>	<i>42</i>
10.10	CARCINOGENICITY	47
10.10.1	<i>Chronic/carcinogenicity study in rats</i>	<i>50</i>
10.10.2	<i>Carcinogenicity study in mice</i>	<i>53</i>
10.10.3	<i>Other information relevant for carcinogenicity</i>	<i>54</i>
10.10.3.1	<i>Liver hepatocellular adenoma.....</i>	<i>54</i>
10.10.3.2	<i>Uterine endometrial adenocarcinoma</i>	<i>62</i>
10.10.4	<i>Short summary and overall relevance of the provided information on carcinogenicity</i>	<i>72</i>
10.10.5	<i>Comparison with the CLP criteria</i>	<i>72</i>
10.10.6	<i>Conclusion on classification and labelling for carcinogenicity.....</i>	<i>72</i>
10.11	REPRODUCTIVE TOXICITY.....	73

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

10.11.1	<i>Adverse effects on sexual function and fertility</i>	95
10.11.2	<i>Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility</i>	97
10.11.3	<i>Comparison with the CLP criteria</i>	100
10.11.4	<i>Adverse effects on development</i>	101
10.10.4.1	<i>Developmental toxicity in rats</i>	104
10.11.5	<i>Short summary and overall relevance of the provided information on adverse effects on development</i> <i>112</i>	
10.11.6	<i>Comparison with the CLP criteria</i>	114
10.11.7	<i>Adverse effects on or via lactation</i>	115
10.11.8	<i>Comparison with the CLP criteria</i>	115
10.11.9	<i>Conclusion on classification and labelling for reproductive toxicity</i>	115
10.12	SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE.....	132
10.12.1	<i>Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure</i>	132
10.12.2	<i>Comparison with the CLP criteria</i>	133
10.12.3	<i>Conclusion on classification and labelling for STOT SE</i>	133
10.13	SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE.....	135
10.13.1	<i>Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure</i>	143
10.13.2	<i>Additional information on repeated-dose toxicity</i>	143
10.13.3	<i>Comparison with the CLP criteria</i>	146
10.13.4	<i>Conclusion on classification and labelling for STOT RE</i>	147
10.14	ASPIRATION HAZARD.....	147
10.14.1	<i>Short summary and overall relevance of the provided information on aspiration hazard</i>	153
10.14.2	<i>Conclusion on classification and labelling for aspiration hazard</i>	154
11	EVALUATION OF ENVIRONMENTAL HAZARDS	155
11.1	RAPID DEGRADABILITY OF ORGANIC SUBSTANCES.....	156
11.1.1	<i>Ready biodegradability</i>	156
11.1.2	<i>BOD₅/COD</i>	157
11.1.3	<i>Hydrolysis</i>	157
11.1.4	<i>Other convincing scientific evidence</i>	157
11.1.4.1	<i>Field investigations and monitoring data (if relevant for C&L)</i>	157
11.1.4.2	<i>Inherent and enhanced ready biodegradability tests</i>	157
11.1.4.3	<i>Water, water-sediment and soil degradation data (including simulation studies)</i>	157
11.1.4.4	<i>Photochemical degradation</i>	161
11.1.4.5	<i>Rapid degradation conclusion</i>	162
11.2	ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION.....	162
11.3	BIOACCUMULATION.....	163
11.3.1	<i>Estimated bioaccumulation</i>	163
11.3.2	<i>Measured partition coefficient and bioaccumulation test data</i>	163
11.3.3	<i>Bioaccumulation conclusion</i>	164
11.4	ACUTE AQUATIC HAZARD.....	164
11.4.1	<i>Acute (short-term) toxicity to fish</i>	167
11.4.2	<i>Acute (short-term) toxicity to aquatic invertebrates</i>	169
11.4.3	<i>Acute (short-term) toxicity to algae or other aquatic plants</i>	171
11.4.4	<i>Acute (short-term) toxicity to other aquatic organisms</i>	172
11.4.5	<i>Summary of acute (short-term) toxicity to aquatic organisms</i>	172
11.5	LONG-TERM AQUATIC HAZARD.....	172
11.5.1	<i>Chronic toxicity to fish</i>	173
11.5.2	<i>Chronic toxicity to aquatic invertebrates</i>	174
11.5.3	<i>Chronic toxicity to algae or other aquatic plants</i>	174
11.5.4	<i>Chronic toxicity to other aquatic organisms</i>	174
11.5.5	<i>Chronic toxicity to aquatic organisms</i>	174
11.6	COMPARISON WITH THE CLP CRITERIA.....	175
11.6.1	<i>Acute aquatic hazard</i>	175
11.6.2	<i>Long-term aquatic hazard (including bioaccumulation potential and degradation)</i>	175
11.7	CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS.....	176
12	EVALUATION OF ADDITIONAL HAZARDS	186

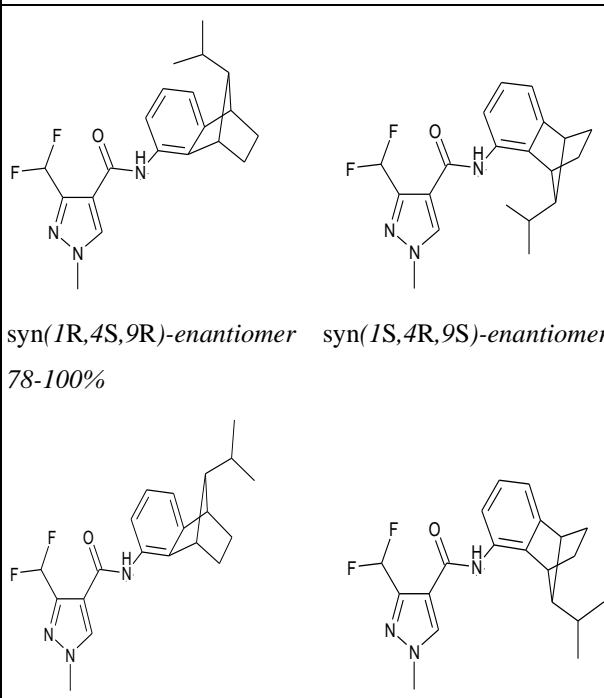
ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

12.1	HAZARDOUS TO THE OZONE LAYER.....	186
12.1.1	<i>Conclusion on classification and labelling for hazardous to the ozone layer.....</i>	<i>186</i>
13	ADDITIONAL LABELLING	187
14	REFERENCES.....	188
15	ANNEXES.....	198

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Reaction mass of 3-(difluoromethyl)-1-methyl-N-[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>RS</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-N-[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>SR</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide [Note, these are referred to as the 'syn' and 'anti'-isomers respectively]
Other names (usual name, trade name, abbreviation)	isopyrazam(*)
ISO common name (if available and appropriate)	isopyrazam(*)
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	881685-58-1 (**)
Other identity code (if available)	CIPAC 963
Molecular formula	C ₂₀ H ₂₃ F ₂ N ₃ O
Structural formula	 <p>syn(1<i>R</i>,4<i>S</i>,9<i>R</i>)-enantiomer syn(1<i>S</i>,4<i>R</i>,9<i>S</i>)-enantiomer 78-100%</p> <p>anti(1<i>R</i>,4<i>S</i>,9<i>S</i>)-enantiomer anti(1<i>S</i>,4<i>R</i>,9<i>R</i>)-enantiomer 0-15%</p>
SMILES notation (if available)	-

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Molecular weight or molecular weight range	359.4 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	See above
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 92% isopyrazam (consisting of 78-100% syn isomer and 0-15% anti isomer)*

* Isopyrazam is the provisional ISO name associated with the substance containing 70-100% syn and 0-30% anti isomers. The specification of the substance placed on the market in the EU and subject to this harmonised classification and labelling proposal contains 78-100% syn and 0-15% anti isomers.

**The CAS number 881685-58-1 is assigned to the substance with the provisional ISO name isopyrazam and is included for consistency with the identification of the active substance in Commission Implementing Regulation (EU) No 1037/2012 of 7 November 2012. It is noted that the CAS number is not specific and covers all isomeric forms.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current Annex VI (CLP)	CLH in Table 3.1	Current classification and self-labelling (CLP)	self-and
3-(difluoromethyl)-1-methyl- <i>N</i> -[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>RS</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (<i>Syn-isomer</i>)	>78 - <100%	Not listed		-#	
3-(difluoromethyl)-1-methyl- <i>N</i> -[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>SR</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (<i>Anti-isomer</i>)	0 – < 15%	Not listed		-#	

The individual isomers are not listed in the C&L Inventory. However, isopyrazam is listed and, at the time of submission, 49 notifiers have classified the substance with Skin Sens. 1; H317, Repr. 2; H361, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. A further 23 notifiers have also applied Carc. 2; H351 in addition to the above classification.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current Annex VI (CLP)	CLH in Table 3.1	Current classification and self-labelling (CLP)	self-and	The impurity contributes to the classification and labelling
None of the identified impurities are relevant for classification.	-	-	-	-	-	-

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None	-	-	-	-	-

Test substances (non-confidential information)

The substance identified by the provisional ISO name 'isopyrazam' (a reaction mass of 3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9RS)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-N-[(1RS,4SR,9SR)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide) is a multi-constituent substance containing two diastereoisomers (epimers) designated syn and anti isomers (referred to in some test reports as SYN534969 and SYN534968 respectively). Both isomers are biologically active and, according to the Commissioning Implementing Regulation (EC) No 1037/2012 approving the active substance 'isopyrazam', the approved specification contains the syn and anti isomers at a percentage ranging from 78-100% syn and 0-15% anti, with an overall minimum purity of 92%.

Most of the toxicology studies have been conducted on batches with an overall purity of 96.4% which consists of 92.8% syn and 7.2% anti (referred to as 93:7 syn:anti in this report). Additional studies are available with batches containing 90:10, 69.7:30.3 (referred to as 70:30 in this report) and 50:50 syn:anti isomers, along with a number of studies on the pure syn and anti isomers themselves. These data are included as supporting information.

The environmental studies have been conducted on batches with varying concentrations of the syn and anti isomers and this is specified in the relevant sections.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	-	Reaction mass of 3-(difluoromethyl)-1-methyl- <i>N</i> -[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>SR</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl- <i>N</i> -[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>SR</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide; isopyrazam	-	88168-58-1*	Repr. 1B Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H360D H317 H400 H410	GHS07 GHS08 GHS09 Dgr	H360D H317 H410		M=10 M=10	
Resulting Annex VI	-	Reaction mass of 3-(difluoromethyl)-1-methyl- <i>N</i> -	-	88168-58-1*	Repr. 1B	H360D	GHS07	H360D	-		-

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

entry if agreed by RAC and COM		[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>RS</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-N-[(1 <i>RS</i> ,4 <i>SR</i> ,9 <i>SR</i>)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide; isopyrazam			Skin Sens 1B	H317	GHS08	H317		M =10	
					Aquatic Acute 1	H400	GHS09			M =10	
					Aquatic Chronic 1	H410	Dgr	H410			

*The CAS number 881685-58-1 is assigned to the substance with the provisional ISO name isopyrazam and is included for consistency with the identification of the active substance in Commission Implementing Regulation (EU) No 1037/2012 of 7 November 2012. It is noted that the CAS number is not specific and covers all isomeric forms.

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data lacking	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

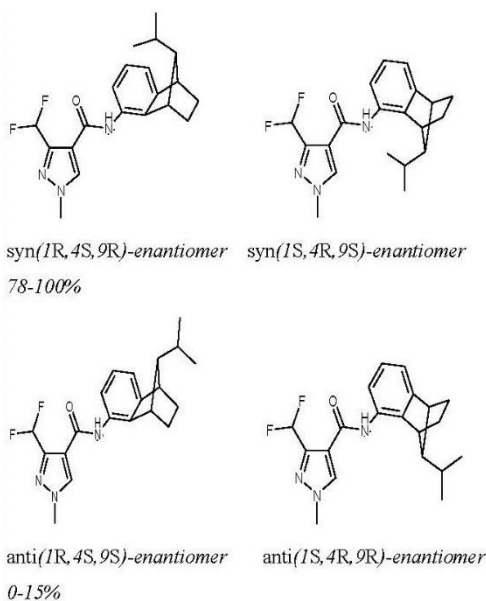
Isopyrazam is an active substance in the scope of Regulation (EC) 1107/2009. It is not listed in Annex VI of CLP and has not been considered for harmonised classification and labelling previously.

At the time of submission, isopyrazam is not registered under REACH.

RAC general comment

Isopyrazam is an active substance in the scope of Regulation (EC) 1107/2009 and is used as a broad-spectrum foliar fungicide. Isopyrazam is not listed in Annex VI of CLP and has not been considered for harmonised classification and labelling previously. Isopyrazam is not registered under REACH.

The substance identified by the provisional ISO name 'isopyrazam' (a reaction mass of 3-(difluoromethyl)-1-methyl-N-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-N-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide [$>78\%$ *syn* isomers $<15\%$ *anti* isomers relative content]) is a multi-constituent substance containing two diastereoisomers (epimers) designated *syn* and *anti* isomers (see figure below). In some test reports they are referred as SYN534969 and SYN534968 respectively. Both isomers are biologically active and, according to the Commissioning Implementing Regulation (EC) No 1037/2012 approving the active substance 'isopyrazam', the approved specification contains the *syn* and *anti* isomers at a percentage ranging from 78-100% *syn* and 0-15% *anti*, with an overall minimum purity of 92%.



Structural formula of isopyrazam

Most of the toxicology studies have been conducted on batches with an overall purity of 96.4% which consists of 92.8% *syn* and 7.2% *anti* (referred to as 93:7 *syn:anti* in this report). Additional studies are available with batches containing 90:10, 69.7:30.3 (referred to as 70:30 in this report) and 50:50 *syn:anti* isomers, along with a number of studies on the pure *syn* and *anti* isomers themselves. These data are included as supporting information. The environmental studies have been conducted on batches with varying concentrations of the *syn* and *anti* isomers and this is specified in the relevant sections.

One MSCA raised an identification issue for the substance as regard the maximal content of the *anti* isomer. The Dossier Submitter (DS) clarified that isopyrazam specifications have been developed to contain a maximum of 15% *anti* isomer.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Isopyrazam is an active substance in the scope of Regulation 1107/2009.

5 IDENTIFIED USES

Isopyrazam is a broad spectrum foliar fungicide.

6 DATA SOURCES

Information submitted for the approval of the pesticide active substance and including;

- Draft Assessment Report (DAR) April 2010 and addenda October 2011.
- EFSA Conclusion for isopyrazam EFSA Journal 2012;10(3):2600

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

§ Study conducted with isopyrazam technical - purity of 95.5% (exact syn:anti ratio not provided but stated as being within the range outlined for the technical material in section 1.)

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Off white solid	Das 2008	Observation Purity 95.5% [§]
Melting/freezing point	Syn: 130.2 °C Anti: 144.5 °C	Geoffroy 2007a & b	OECD 103 99.5% syn 99.6% anti
Boiling point	Syn > 261°C (decomposes) Anti > 274 °C (decomposes)	Geoffroy 2007a & b	OECD 103 99.5% syn 99.6% anti
Relative density	1.332 g/cm ³ at 20 °C	Weissenfeld 2008a	EEC Method A3 95.5% [§]
Vapour pressure	Syn: 2.4 x 10 ⁻⁷ Pa at 20°C 5.6 x 10 ⁻⁷ Pa at 25°C Anti: 2.2 x 10 ⁻⁸ Pa at 20°C 5.7 x 10 ⁻⁸ Pa at 25°C	Geoffroy 2007c	OECD 104 99.5% syn 99.6% anti
Surface tension	63.1 mN/m at 19.8°C (90% saturated solution)	Weissenfeld, 2008K	EEC Method A5 Purity 95.5% [§]
Water solubility	Syn: 0.00105 g/l at 25°C (pH 7) Anti: 0.00055 g/l at 25°C (pH 7)	Weissenfeld 2008b,c,d & e	EC Method A6 99.5% syn 99.6% anti
Partition coefficient n-octanol/water	Syn : Log P _{ow} = 4.1 at 25°C (pH 7.3) Anti : Log P _{ow} = 4.4 at 25°C (pH 7.8)	Weissenfeld 2008g, h, i & j	OECD 107 99.5% syn 99.6% anti
Flash point	Not applicable substance is a solid with melting point of > 130°C	-	-
Flammability	Not highly flammable. In the preliminary study the substance melted on contact with the flame but did not burn.	Jackson, 2008a & b	EEC Method A10 Purity 95.5% [§]

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties	Not explosive within the criteria of this study. The substance did not explode when exposed to heat, mechanical shock or friction.	Jackson, 2008c	EEC Method A14 Purity 95.5% ^s
Self-ignition temperature	No self-ignition up to a temperature of 400 °C. The substance melted during the study.	Jackson, 2008a & b	EEC method A16 Purity 95.5% ^s
Oxidising properties	Not oxidising within the criteria of this study. The burning rate of the test mixture was 1.5mm/s compared to the burring rate of 2.8 mm/s for the reference mixture.	Jackson, 2008d	EEC Method A17 Purity 95.5% ^s
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data. Solubility in other solvents is acetone 314g/l at 25°C dichloromethane 303g/l at 25°C ethylacetate 179g/l at 25°C hexane 1.17g/l at 25°C methanol 119g/l at 25°C octanol 44.1g/l at 25°C toluene 77.1g/l at 25°C	Weissenfeld 2008f	CIPCA MR 157.6 Purity 95.5% ^s
Dissociation constant	Syn and Anti : No dissociation constant was found in the pH range 1 to 12.	Martin, 2007a, 2007b, 2008a & 2008b	OECD 112 99.5% syn 99.6% anti
Viscosity	Not relevant, substance is a solid	-	-

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC Method A14	The substance did not explode when exposed to heat, mechanical shock or friction.	Not explosive within the criteria of this study.	Jackson, 2008c Isopyrazam technical: 95.5%

8.1.1 Short summary and overall relevance of the information provided on explosive properties

In a standard explosivity study (EEC, A14) there was no evidence of shock, friction or thermal sensitivity and isopyrazam was not considered to be explosive within the criteria of this study.

8.1.2 Comparison with the CLP criteria

A substance is considered for classification as explosive where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. There was no evidence of shock, friction or thermal sensitivity when isopyrazam was tested in a standard explosivity study.

The substance was not explosive within the criteria of this study.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – Data conclusive but not sufficient for classification.
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8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable, substance is a solid.

8.3 Oxidising gases

Hazard class not applicable, substance is a solid.

8.4 Gases under pressure

Hazard class not applicable, substance is a solid.

8.5 Flammable liquids

Hazard class not applicable, substance is a solid.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC Method A10 Isopyrazam technical: 95.5%	In the preliminary study the substance melted on contact with the flame but did not burn.	Not highly flammable.	Jackson, 2008a and b

8.6.1 Short summary and overall relevance of the provided information on flammable solids

In the preliminary study, the substance melted on contact with the flame but did not burn. The full study was not conducted. Isopyrazam is not highly flammable.

8.6.2 Comparison with the CLP criteria

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. On attempted ignition, the substance melted but did not burn. Therefore, the criteria for classification as a flammable solid are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – data conclusive but not sufficient for classification.

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No signs of exothermic decomposition were observed in a study conducted in accordance with OECD 113 at temperatures up to 200 °C. In the OECD 103 studies conducted with the syn and anti isomers, exothermic decomposition was noted from 261 °C (with 192 J/g) and 274 °C (with 143 J/g) respectively.

8.7.2 Comparison with the CLP criteria

The available data indicate that the substance does not have to be considered for classification in this hazard class.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified – data conclusive but not sufficient for classification

8.8 Pyrophoric liquids

Hazard class not applicable, substance is a solid.

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No studies are available. However, isopyrazam has been handled in air in the available studies and no incidences of self-ignition have been reported.

8.9.2 Comparison with the CLP criteria

According to Section 2.10.4.1 of Annex I of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of isopyrazam spontaneously igniting when in contact with air.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – Data conclusive but not sufficient for classification.
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8.10 Self-heating substances

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

A study conducted in accordance with EEC A16 is available. In this study, isopyrazam did not self-ignite up to a temperature of 400°C.

8.10.2 Comparison with the CLP criteria

This study is not directly comparable with the CLP criteria. No further data available.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified – data lacking

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No data derived in accordance with the recommended test method in CLP have been provided. However, isopyrazam has been handled in water within many of the studies available in the draft assessment report, and there are no reports of violent reaction and emission of gas.

8.11.2 Comparison with the CLP criteria

According to Section 2.12.4.1 of Annex I of CLP, the classification procedure for this hazard class need not be applied if experience in production or handling shows that the substance does not react with water. Therefore, classification for this class is not applicable for isopyrazam.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – Data conclusive but not sufficient for classification

8.12 Oxidising liquids

Hazard class not applicable, substance is a solid.

8.13 Oxidising solids

Table 10: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC Method A17 Isopyrazam technical: 95.5%	Not oxidising within the criteria of this study.	The burning rate of the test mixture was 1.5mm/s compared to the burring rate of 2.8 mm/s for the reference mixture.	Jackson, 2008d

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

A study conducted according to EEC Method A17, the maximum burring rate of the test substance/cellulose mixture was determined to be 1.5 mm/s compared to 2.8 mm/s for the reference substance (barium nitrate)/cellulose mixture.

8.13.2 Comparison with the CLP criteria

As the maximum burning rate of the test substance/cellulose mixture was less than the maximum burning rate of the reference substance/cellulose mixture, the substance is not considered to be an oxidising substance within the criteria of this study.

Results generated using this method are not directly comparable with the CLP criteria. However, whilst the substance contains oxygen and fluorine, these elements are only bound to carbon. Therefore, in accordance with section 2.13.4.1 of Annex I of CLP the substance is not considered to be an oxidising solid.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified – conclusive but not sufficient for classification.

8.14 Organic peroxides

Not applicable, the substance is not an organic peroxide.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data.

Test C.1 in the UN RTDG Manual of Tests and Criteria is intended to determine the corrosive properties of liquids and solids that may become liquid during transport. Isopyrazam is a solid, with a melting point higher than 55 °C (> 130 °C) and low water solubility (1.05 and 0.55 mg/L for the *syn* and *anti* isomers respectively).

Furthermore, based on experience in manufacture and handling, the substance does not materially damage metallic containers.

8.15.2 Comparison with the CLP criteria

As isopyrazam is a solid with a melting point of 130 °C and a low water solubility, classification as corrosive to metals is not appropriate.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – Data conclusive but not sufficient for classification.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosive

In a standard explosivity study (EEC, A.14) there was no evidence of shock, friction or thermal sensitivity and isopyrazam was not considered to be explosive within the criteria of this study.

Flammable solid

In the preliminary study (EEC, A.10), the substance melted on contact with the flame but did not burn. The full study was not conducted. The criteria for classification as a flammable solid are not met.

Self-reactive substance or mixture

No signs of exothermic decomposition were observed in a study conducted in accordance with OECD TG 113 at temperatures up to 200°C. In the OECD TG 103 studies conducted with the *syn* and *anti* isomers, exothermic decomposition was noted from 261°C (with 192 J/g) and 274°C (with 143 J/g) respectively. The available data indicate that the substance does not warrant classification as self-reactive.

Pyrophoric solid

No specific studies are available. However, the available studies report the use of isopyrazam without any special conditions regarding the exclusion of air or moisture and no incidences of self-ignition have been reported. Hence classification is not warranted.

Self-heating substance or mixture

A study conducted in accordance with EEC A.16 is available. In this study, isopyrazam did not self-ignite up to a temperature of 400 °C. This study is not directly comparable with the CLP criteria and no further data were available.

Substance or mixture which in contact with water emits flammable gas

No data derived in accordance with the test method recommended by CLP have been provided. However, isopyrazam has been handled in water within many of the studies available in the draft assessment report, and there are no reports of violent reaction and emission of gas. Hence classification is not warranted.

Oxidising solid

In a provided study (EEC, A.17), the maximum burning rate of the test substance/cellulose mixture was determined to be 1.5 mm/s compared to 2.8 mm/s for the reference substance (barium nitrate)/cellulose mixture. However, these results are not directly comparable with the CLP criteria. It should be also noted that whilst the substance contains oxygen and fluorine, these elements are only bound to carbon. Weighing these factors, the substance is not considered to be an oxidising solid.

Substance or mixture corrosive to metals

No data available. Test C.1 in the UN RTDG Manual of Tests and Criteria is intended to determine the corrosive properties of liquids and solids that may become liquid during transport. Isopyrazam is a solid, with a melting point higher than 55°C (estimated > 130°C) and with low water solubility (1.05 and 0.55 mg/L for the *syn* and *anti* isomers respectively). Furthermore, based on experience in manufacture and handling, the substance does not materially damage metallic containers. Classification as corrosive to metals is not warranted.

Comments received during consultation

One MSCA pointed out a mistake in referencing a value of solubility of the substance in dichloromethane, this was corrected by the dossier submitter.

Assessment and comparison with the classification criteria**Explosive**

The DS referred to the results of a standard explosivity study indicating no evidence of shock, friction or thermal sensitivity leading to inconclusive data for classification. RAC notes that the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500°C, hence no classification is warranted.

Flammable solid

The presented preliminary study results showed the substance melted on contact with flame but did not burn. As preliminary test according to A.10 (identical to a preliminary test according to UN N.1) was negative, RAC agrees with the DS that the data is conclusive but not sufficient for classification.

Self-reactive substance or mixture

Although no exothermic decomposition occurred in an OECD 113 test with temperatures up to 200°C, it did occur at higher temperatures (*syn*: 261°C; *anti*: 274°C) in an OECD 103 test. Nevertheless, the heat of decomposition was less than 300 J/g, and therefore no classification is warranted based on conclusive information (CLP, Annex I, 2.8.2.1).

Pyrophoric solid

No specific studies are available. However, isopyrazam has been handled in air in the available studies and no incidences of self-ignition have been reported.

Experience in manufacture and handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable based on reports of the available studies). RAC supports the DS proposal for no classification.

Self-heating substance or mixture

As the study conducted in accordance with EEC A.16 is not sufficient to conclude on the classification and no further data is available, RAC proposes not to classify the substance in this hazard class due to inconclusive data.

Substance or mixture which in contact with water emits flammable gas

As most of the studies with isopyrazam are performed in aqueous solutions and there are no reports for emission of gas, RAC support DS proposal for no classification.

Oxidising solid

As the substance contains oxygen and fluorine and these elements are chemically bonded only to carbon, RAC agrees with the DS proposal for no classification.

Substances or mixtures corrosive to metals

RAC agrees with the DS that the available data indicating isopyrazam being a solid with a melting point > 130 °C and having low water solubility, that the substance can be considered not classifiable as corrosive to metals.

Conclusion

Overall, RAC agrees with the rational of the DS and that **no classification with regards to the physical hazards are warranted for isopyrazam.**

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Isopyrazam contains two diastereomers (epimers) designated syn and anti isomers. Both isomers are biologically active and according to the Commissioning Implementing Regulation (EC) No 1037/2012 approving the active substance isopyrazam, the approved specification contains the syn and anti isomers at a percentage ranging from 78:15% to 100:0% syn:anti; with a minimum overall purity of 92%.

The absorption, distribution, excretion and metabolism of isopyrazam were investigated in the rat following oral administration of single (low and high) and multiple (low) doses. In all studies except one, unlabelled isopyrazam (93:7 syn:anti) and the radiolabelled material consisted predominantly of the syn isomer. An additional comparative study (using 70:30 syn:anti), conducted to compare the toxicokinetics of both specifications and to investigate potential differences in absorption and elimination of the syn and anti isomers, used either syn- or anti-labelled isopyrazam. Absorption, distribution, metabolism and excretion were similar in male and female rats.

Absorption

The absorption of isopyrazam has been studied via the oral route of administration; no other routes have been investigated.

The oral absorption of isopyrazam (93:7 syn:anti specification) was estimated following a single low or high gavage dose (1 or 75 mg/kg) to bile-duct cannulated male and female rats. Absorption was estimated to be 63-73% of the administered dose by 48 hours post-dosing (irrespective of sex), with fairly rapid uptake systemically; the majority of absorption occurred within the first 24-hours.

Distribution

Distribution experiments showed that isopyrazam (and/or its metabolites) was rapidly and widely distributed throughout the internal organs after single oral administration. After two hours the majority of the radioactivity was located in the stomach, GI tract, liver and kidneys (lower levels were present in the abdominal fat, brown fat and Harderian gland).

Metabolism

Both the 93:7 and 70:30 syn:anti specifications of isopyrazam were extensively metabolised giving rise to up to 25 metabolite types (including conjugates) with the potential for multiple isomers in most groups. In general, unconjugated metabolites were eliminated in urine and conjugated metabolites in bile. The major routes of biotransformation appeared to be independent of dose level and sex and the metabolite profile did not change following repeated administration. In total, greater than 90% of the administered dose was accounted for by identified metabolites.

Excretion

Following a single oral administration to bile-duct cannulated rats, the major route of elimination was via the bile (48-58% of the administered radioactivity recovered over 48-hours post-dosing) and the majority of the remaining amount of radioactivity was found in the faeces (21-36%); urinary excretion was also apparent to a lesser extent (7-16%). Excretion was fairly rapid and was almost complete by 48-hours post-dose. Although the routes of excretion of isopyrazam after repeated administration for 14 days did not differ from those observed after single administration, excretion was slower after repeated dosing compared to that observed after a single dose. There was no evidence of bioaccumulation potential; however, for the liver and renal fat, steady state was not reached within the 14-day dosing period, so some accumulation potential in these particular tissues over repeated administration cannot be completely ruled out.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Comparison of the toxicokinetics of the 93:7 and 70:30 syn:anti specification materials

There are no major differences in the oral absorption of the 93:7 and the 70:30 syn:anti specifications of isopyrazam, and the metabolite profiles in urine, faeces and bile are broadly similar. Following a single oral administration of the 70:30 syn:anti material to bile-duct cannulated rats, the major route of elimination was via the bile, with means of 37-61% of the administered dose recovered over three days post dose. These figures are broadly similar to those obtained (48-58%) for the 93:7 specification. Hence, there are no major differences in biliary excretion. Furthermore, urinary and faecal excretion of the 70:30 specification was largely similar to the 93:7 syn:anti material.

Overall, the available data show that there are no major differences in the oral absorption, metabolism or excretion of the 93:7 and the 70:30 syn: anti specifications of isopyrazam.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

Acute toxicity has been investigated *in vivo* in rats via the oral, dermal and inhalation routes. The specification of isopyrazam available for the toxicology investigations contains the syn and anti isomers at a ratio of 93:7 and the majority of the acute studies were performed with this specification. However, additional acute oral toxicity studies were available that were conducted with batches containing 70:30 and 50:50 syn:anti and the pure syn and anti isomers. These data have been included as additional information.

10.1 Acute toxicity - oral route

The acute oral toxicity of isopyrazam has been investigated according to the up-and-down procedure (OECD TG 425) using a batch with syn:anti ratio of 93:7. The results are presented below.

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, vehicle, syn:anti ratio	Dose levels, duration of exposure	Results
Up-and-down procedure, gavage OECD 425 GLP Anonymous (2007a)	Rats, HanRcc:WIST, 1 female (limit test) & 5 females (main test)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7 Vehicle: 0.5% carboxymethylcellulose	2500 mg/kg bw (limit test) 2000, 275 & 175 mg/kg bw (main test) Observation period: 15 days	<u>Limit test</u> 1/1 at 2500 mg/kg bw killed in extremis <u>Main test</u> 1/1 at 175, 1/1 at 275 & 3/3 at 2000 mg/kg bw survived LD₅₀ >2000 mg/kg bw

The acute oral toxicity of isopyrazam (batch SMU6AP001; purity 96.4%) was investigated according to the up-and-down procedure as described in OECD TG 425. The batch of isopyrazam used in this study contained the syn and anti isomers at a ratio of 93:7. In a limit test, an initial female received a single gavage dose of 2500 mg/kg bw (it was intended that this animal should be dosed with 5000 mg/kg bw, administered as two separate doses; however this animal was killed *in extremis* and so a further dose was not given). The main test comprised a total of five females at doses of 2000 mg/kg bw (n=3), 275 mg/kg bw (n=1) and 175 mg/kg bw (n=1). An observation period of 15 days followed administration of the test substance and animals were examined for clinical signs and mortality on the day of dosing and twice daily thereafter. A necropsy carried out on all animals revealed no unusual findings and body weights were within the normal ranges. The findings are summarised in the table below.

Table 12: Acute oral toxicity findings for the 93:7 syn:anti isomer specification of isopyrazam

Animals dosed	Dose (mg/kg bw)	Mortality	Clinical observation
1 female	175	Survived	Ruffled fur on day of dosing
1 females	275	Survived	Ruffled fur, hunched posture on day of dosing
3 females	2000	Survived	Hunched posture, poor condition, sedation on day of dosing up to day 4, ruffled fur to day 7
1 female	2500	Killed <i>in extremis</i> 4 h after dosing	Sedation, ruffled fur, hunched posture, poor coordination, ventral recumbency

As all three females survived at 2000 mg/kg bw the acute oral LD₅₀ of the isopyrazam 93:7 syn:anti specification is deemed to be > 2000 mg/kg bw.

10.2 Additional Information on Acute Oral Toxicity

A number of additional studies were conducted using batches with syn:anti ratios of 70:30 and 50:50 and the pure syn and anti isomers. These studies are presented in the table below as additional information.

Table 5: Summary table of additional animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, vehicle, syn:anti ratio	Dose levels, duration of exposure	Results
Up-and-down procedure, gavage OECD 425 GLP Anonymous (2008a)	Rats, HanRcc:WIST, females, 1 female (limit test) & 11 females (main test; across dose groups)	'Isopyrazam' Syn:anti ratio: 70:30 Batch: SMU7DP017 Purity: 90.8% (w/w) Vehicle: 0.5% carboxymethylcellulose	2000 mg/kg bw (limit test) 2000, 550 & 175 mg/kg bw (main test) Observation period: 15 days	<u>Limit test</u> 1 female at 2000 mg/kg bw was killed <i>in extremis</i> <u>Main test</u> 5/7 killed <i>in extremis</i> at 2000 mg/kg bw, 4/4 at 550 & 1/1 at 175 mg/kg bw survived LD₅₀ ≤ 2000 mg/kg bw (95% confidence interval 864-4210 mg/kg).
Up-and-down procedure, gavage OECD 425 GLP Anonymous (2006a)	Rats, HanRcc:WIST, females, 5 females (pure syn), 7 females (pure anti) & 7 females (50:50 syn:anti)	Pure syn epimer (SYN534969) Batch: SMU6BP001 Purity: 99% Pure anti epimer (SYN534968) Batch: SMU6BP001 Purity: 99.5% 'Isopyrazam' Syn:anti ratio:50:50 Batch: SMU6CP014 Purity: 98.2%	2000 mg/kg bw (pure syn) 2000, 550 & 175 mg/kg bw (pure anti) 2000, 550 & 175 mg/kg bw (50:50 syn:anti)	<u>SYN534969 (pure syn)</u> 5/5 survived at 2000 mg/kg bw LD₅₀ >2000 mg/kg bw <u>SYN534968 (pure anti)</u> 1/1 at 2000 mg/kg bw killed <i>in extremis</i> 2 hours post-dose 3/3 at 550 mg/kg bw killed <i>in extremis</i> 1-2 hours post-dose 3/3 survived at 175 mg/kg bw LD₅₀ = 310.2 mg/kg bw (95% confidence interval 175-550 mg/kg bw) <u>Isopyrazam (50:50 syn:anti)</u> 1/1 at 2000 mg/kg bw killed <i>in extremis</i> 2 hours post-dose 3/3 at 550 mg/kg bw killed <i>in extremis</i> 1-2 hours post-dose 3/3 at 175 mg/kg bw survived LD₅₀ = 310.2 mg/kg bw (95% confidence interval 175-550 mg/kg)

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

70:30 syn:anti specification

The acute oral toxicity of a batch containing 70:30 syn:anti isomers was also investigated according to OECD TG 425 (up-and-down procedure). The substance (batch SMU7DP017; purity 90.8%) was administered via gavage to an initial animal in a limit test, at a dose of 2000 mg/kg bw. This animal was killed *in extremis* a few hours post-dose, hence the main test was conducted with a starting dose of 175 mg/kg bw. This animal survived and so a further four females were administered 550 mg/kg bw. The survival of these four animals led to the dosing of an additional six animals at 2000 mg/kg bw; only two of these animals survived and so the investigation was concluded. Body weights and clinical signs were recorded on the day of dosing and then twice daily during the remainder of the 15-day observation period. The findings are summarised in the table below.

Table 14: Acute oral toxicity findings for the 70:30 syn:anti isomer specification of isopyrazam

Animals dosed	Dose (mg/kg bw)	Mortality	Clinical observation
1 female	175	Survived	Ruffled fur on day of dosing
4 females	550	All survived	Hunched posture, poor coordination, sedation on day of dosing; ruffled fur to day 4
7 females	2000	5 killed <i>in extremis</i> , 1 - 5 hours or 2 days after dosing	Sedation, poor coordination, hunched posture, ruffled fur, cold to touch, ventral or lateral recumbency and convulsions
		2 survived	Poor coordination, hunched posture, sedation on the day of dosing and for up to 3 days, ruffled fur for up to 7 days

Body weights were within normal ranges, but a gross necropsy revealed abnormalities in three of the five animals killed *in extremis* at 2000 mg/kg bw, comprising a distended stomach, liquid contents in the duodenum, a grey material in the stomach/duodenum and an empty jejunum/ileum.

The applicant had proposed an acute oral LD₅₀ of 2000 mg/kg bw (95% confidence interval 864-4210 mg/kg) based on this study; however, as only 2/7 animals survived at this dose the UK considers that the true LD₅₀ is likely to be lower and therefore proposes an LD₅₀ of ≤ 2000 mg/kg bw.

Pure syn and anti and 50:50 specifications

In order to clarify which of the isopyrazam isomers is the more toxic, a third acute oral (gavage) toxicity study was conducted according to the same procedure on SYN534969 (pure syn), SYN534968 (pure anti) and a 50% syn:50% anti specification. Five female rats received a single dose of SYN534969 (pure syn) at 2000 mg/kg bw; all animals survived. One female received a 2000 mg/kg bw dose of SYN534968 (pure anti) and was killed *in extremis* two hours after dosing; an additional three females were killed *in extremis* after receiving 550 mg/kg bw of pure anti and so a final three females were administered doses of 175 mg/kg (all survived). The same pattern was observed for groups administered the 50:50 syn:anti specification. The results are summarised in the table below:

Table 15: Acute oral toxicity findings for the syn and anti isomers

Animals dosed	Dose (mg/kg)	Mortality	Clinical observation
SYN534969 (pure syn)			
5 females	2000	All survived	Hunched posture, slight to moderate poor coordination, ruffled fur on day of dosing and for up to 5 days
SYN534968 (pure anti)			
3 females	175	All survived	Hunched posture, slightly poor coordination, and ruffled fur on the day of dosing and for up to 3 days.
3 females	550	All killed <i>in extremis</i> 1-2 hours after dosing	Hunched posture, sedation, ventral recumbency
1 female	2000	All killed <i>in extremis</i> 2 hours after dosing	Ventral recumbency, marked poor coordination
(50:50 syn:anti)			
3 females	175	All survived	Ventral recumbency on day of dosing. Hunched posture, poor coordination, ruffled fur on day of dosing and for up to 3 days.
3 females	550	All killed <i>in extremis</i> 1-2 hours after dosing	Ventral recumbency, sedation
1 female	2000	All killed <i>in extremis</i> 2 hours after dosing	Ventral recumbency, sedation

Body weights of all animals were within the normal ranges and there were no unusual findings on necropsy. Based on this study the acute oral LD₅₀ of SYN534969 (pure syn) is > 2000 mg/kg bw, whilst the acute oral LD₅₀ of both SYN534968 (pure anti) and the 50:50 syn:anti specification is estimated to be 310.2 mg/kg bw (95% confidence interval 175-550 mg/kg). Hence, it is clear that the anti epimer is more acutely toxic (via the oral route) than the syn epimer.

10.2.1 Short summary and overall relevance of the provided information on acute oral toxicity

The acute oral toxicity study on isopyrazam (with a 93:7 syn:anti ratio) gave an LD₅₀ above the value for classification (i.e., > 2000 mg/kg bw). It is noted that, according to the approved specification, isopyrazam can contain a maximum of 15% of the anti isomer and additional studies on the individual syn and anti isomers indicate that the anti isomer is more acutely toxic than the syn isomer. This is evident in further studies on material containing the syn:anti isomers in a ratio of 70:30 and 50:50, where the LD₅₀ was found to be < 2000 mg/kg bw in each case. No data are available on isopyrazam containing 15% of the anti isomer.

10.2.2 Comparison with the CLP criteria

According to the CLP criteria, classification for acute oral toxicity is warranted if the ATE (LD₅₀) of a substance is ≤ 2000 mg/kg bw. The acute oral toxicity study on isopyrazam (with a 93:7 syn:anti ratio) gave an LD₅₀ above this value. Whilst no data are available on isopyrazam containing 15% of the anti isomer, the ATE is estimated to be > 2000 mg/kg bw, taking account of the ATE for the anti isomer (310.2 mg/kg bw) and the concentration.

Overall, the available data on the substance within the technical specification do not meet the criteria for classification.

10.2.3 Conclusion on classification and labelling for acute oral toxicity

Not classified (conclusive but not sufficient for classification)
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10.3 Acute toxicity - dermal route

Isopyrazam (93:7 syn:anti) was investigated in a reliable, guideline- and GLP-compliant dermal toxicity study, conducted in rats.

Table 16: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, vehicle, syn:anti ratio	Dose levels duration of exposure	Results
Acute dermal toxicity study OECD 402 GLP Anonymous (2007b)	Rats, HanRcc:WIST, males and females, 5/sex	Isopyrazam Batch: SMU6AP001 Purity: 96.4 % (w/w) Syn:anti ratio: 93:7 Vehicle: Moistened with purified water	5000 mg/kg bw Application area: 16 cm ² Duration: 24 hours Observation period: 14 days	No deaths No signs of systemic toxicity or local effects No adverse macroscopic findings

10.3.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In a well conducted acute dermal toxicity study (OECD 402) the 93:7 syn:anti specification of isopyrazam was moistened with purified water and applied to the shorn backs of male and female Wistar rats. The test substance covered an area of at least 10% of the total body surface and was enclosed with a semi-occlusive dressing. After 24 hours, the test substance was removed and the animals were observed for a further 14 days. An initial animal of each sex was treated with 5000 mg/kg bw of the test substance and as no deaths or clinical signs of toxicity were noted after 24 hours, a further 4 animals/sex were treated with 5000 mg/kg bw. All animals survived and there were no signs of systemic toxicity or local reactions. There was no effect on the body weight of the animals and no findings were noted at necropsy. Hence the acute dermal LD₅₀ of the 93:7 syn:anti specification of isopyrazam is >5000 mg/kg bw. The approved specification of isopyrazam for use as an active substance in plant protection products allows for a content of the more acutely toxic (by the oral route) anti isomer at a maximum of 15%, which is greater than the 7% in the specification investigated for dermal toxicity in this study; however, as the LD₅₀ of the specification tested for acute dermal toxicity was > 5000 mg/kg bw/d, a slight increase of the anti isomer would not be expected to result in an LD₅₀ greater than the 2000 mg/kg bw cut-off value for classification in accordance with CLP.

10.3.2 Comparison with the CLP criteria

According to the CLP criteria, a substance is classified for acute dermal toxicity if the LD₅₀ value is ≤ 2000 mg/kg bw. In the available study, isopyrazam (syn:anti ratio 93:7) was found to have an LD₅₀ value of >5000 mg/kg bw. A slight increase in the anti isomer, which is more acutely toxic by the oral route, would not be expected to result in an LD₅₀ greater than 2000 mg/kg bw; hence the dossier submitter concludes that isopyrazam does not meet the criteria for classification.

10.3.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified (conclusive but not sufficient for classification)

10.4 Acute toxicity - inhalation route

One reliable, acute inhalation toxicity study is available, which was conducted in rats in accordance with OECD TG 403 and using the 93:7 syn:anti specification of isopyrazam.

Table 17: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD), syn:anti ratio	Dose levels, duration of exposure	Results
Acute inhalation toxicity study, nose-only exposure OECD 403 GLP Anonymous (2006)	Rats, HsdBr/Han Wistar, males & females, 5/sex	Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7 MMAD: 2.88±3.01 µm (1 hour) & 1.82±1.89 µm (3 hours)	Mean concentration: 5.28±0.08 mg/l air Exposure duration: 4 hours Observation period: 15 days	All animals survived There were no treatment related signs of toxicity or adverse macroscopic findings LC₅₀ = >5.28 mg/l

10.4.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity of isopyrazam (93:7 syn:anti specification) was investigated in a nose-only exposure, inhalation toxicity study. Wistar rats (5/sex) were exposed to a target concentration of 5 mg/l for four hours. The measured mean concentration was 5.28±0.08 mg/l and the mass median aerodynamic diameters (MMAD) of the particles were measured twice during the investigation and on both occasions were within the range of 1 to 4µm; a 15-day observation period (including the day of exposure) followed. All animals survived and gained weight (beginning at day eight and continuing throughout the observation period) and there were no signs of systemic toxicity or unusual macroscopic findings. Therefore the LC₅₀ of isopyrazam (93:7 syn:anti specification) is > 5.28 mg/l. A slight increase in the anti isomer to 15%, as in the approved isopyrazam specification for use as an active substance in plant protection products, would not be expected to have a substantially lower LC₅₀. In the acute oral toxicity studies, the signs of toxicity observed in the animals killed in extremis that had received the specification of isopyrazam containing 70:30 syn:anti isomers were mainly related to the oral route of exposure (gastrointestinal effects comprising a distended stomach, liquid contents in the duodenum, a grey material in the stomach/duodenum and an empty jejunum/ileum). Therefore the acute toxicity of isopyrazam is not likely to be greater when administered via the inhalation route.

10.4.2 Comparison with the CLP criteria

In accordance with the CLP criteria, a substance (dust and mist) is classified for acute inhalation toxicity if it has an LC₅₀ value of ≤ 5 mg/l air. Since in the available study the LC₅₀ value for isopyrazam is > 5.28 mg/l air, the dossier submitter concludes that the substance does not meet the criteria for classification.

10.4.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified (conclusive but not sufficient for classification)

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS assessed a database with three up-down tests (OECD 425) for acute oral toxicity (including pure isomers and three different *syn:anti* ratios), one OECD 402 test for assessing acute dermal toxicity and one OECD 403 test for assessing acute inhalation toxicity. All these studies were performed according to GLP and allowed the DS to propose no classification of isopyrazam for acute toxicity by any of the three relevant routes.

Comments received during consultation

One MSCA considered that it cannot be excluded that the LD₅₀ could be less than 2000 mg/kg bw with a mixture of isopyrazam containing 15% of the *anti* isomer and therefore proposed Acute Tox 4 for oral route. The DS replied that considering the ATE of the pure *anti* isomer the ATE of the substance containing less than 15% could be calculated to be >2000 mg/kg bw.

A second MSCA also demanded Acute Tox 4 for oral route with a 70:30 *syn:anti* isomer ratio. DS agreed with the comment. However, RAC notes that the CLH report is covering a ratio of up to 15% of *anti* isomer.

Assessment and comparison with the classification criteria

Table 1 summarises the acute toxicity studies presented in the CLH report.

Table 1: Summary of animal studies on acute toxicity with isopyrazam.

Study	Dose level	Results	Reference
Up-and-down procedure	Isopyrazam (batch: SMU6AP001)	<u>In all doses and all animals:</u> No unusual findings in necropsies and body weights within normal ranges	Anonymous (2007a)
Gavage	Purity: 96.4%	<u>Limit test:</u>	
OECD 425	93:7 <i>syn:anti</i>	1/1 at 2500 mg/kg bw killed <i>in extremis</i> (sedation, ruffled fur, hunched posture, poor coordination and recumbency)	
GLP	Vehicle: 0.5% carboxymethylcellulose		
HanRcc:WIST rats	2500 mg/kg bw (limit test)	<u>Main test:</u>	
1 female per dose: 175, 275 and 2500 mg/kg bw	2000, 275 and 175 mg/kg bw (main test)	175 mg/kg bw: 1/1 female survived with ruffled fur on day of dosing	
3 females for 2000 mg/kg bw	Observation period: 15 days	275 mg/kg bw: 1/1 female survived with ruffled fur and hunched posture on the day of dosing	
		2000 mg/kg bw: 3/3 females survived with hunched posture, poor condition, sedation on the day of dosing up to day 4 and ruffled fur to day 7	
		Conclusion: LD₅₀ >2000 mg/kg bw	

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Up-and-down procedure	Isopyrazam (batch: SMU7DP017)	<u>In all doses and all animals:</u> Body weights within normal ranges	Anonymous (2008a)
Gavage	Purity: 90.8% (w/w)	<u>Limit test:</u>	
OECD 425	70:30 <i>syn:anti</i>	1/1 female at 2000 mg/kg bw was killed <i>in extremis</i> a few hours after dosing	
GLP	Vehicle: 0.5% carboxymethylcellulose	<u>Main test:</u>	
HanRcc:WIST rats	2000 mg/kg bw (limit test)	175 mg/kg bw: 1/1 female survived with ruffled fur on day of dosing	
175 mg/kg bw: 1 female	2000, 550 and 175 mg/kg bw (main test)	550 mg/kg bw: 4/4 females survived with hunched posture, poor coordination, sedation on day of dosing and ruffled posture to day 4	
550 mg/kg be: 4 females	Observation period: 15 days	2000 mg/kg bw: 5/7 females killed <i>in extremis</i> , 1 female killed 5 hours after dosing and 1 animal killed 2 days after dosing. Clinical signs: Sedation, poor coordination, hunched posture, ruffled fur, cold to touch, ventral or lateral recumbency and convulsions. 3/5 animals had distended stomach, liquid contents in the duodenum, grey material in the stomach/duodenum and an empty jejunum/ileum.	
2000 mg/kg bw: 7 females		2/7 females survived with poor coordination, sedation on the day of dosing and for up to 3 days, ruffled fur for up to 7 days.	
		Conclusion: LD₅₀ ≤ 2000 mg/kg bw (95% confidence interval 864-4210 mg/kg)	
Up-and-down procedure, gavage	Pure <i>syn</i> epimer (SYN534969) (batch SMU6BP001) (purity: 99%)	<u>In all doses and all animals:</u> No unusual findings in necropsies and body weights within normal ranges	Anonymous (2006a)
OECD 425		<u>SYN534969 (pure <i>syn</i>)</u>	
GLP		2000 mg/kg: 5/5 females survived with hunched posture, slight to moderate poor coordination, ruffled fur on day of dosing and for up to 5 days	
Anonymous (2006a)		Conclusion: LD₅₀ >2000 mg/kg bw	
HanRcc:WIST rats		<u>SYN534968 (pure <i>anti</i>)</u>	
<u>Pure <i>syn</i></u> : 5 females for 2000 mg/kg bw	Pure <i>anti</i> epimer (SYN534968) (batch: SMU6BP001) (purity: 99.5%)	2000 mg/kg bw: 1/1 female killed <i>in extremis</i> 2 hours post-dose with ventral recumbency and marked poor coordination	
<u>Pure <i>anti</i></u> : 3 females per dose for 175 and 550 mg/kg be and 1 female for 2000 mg/kg bw		550 mg/kg bw: 3/3 females killed <i>in extremis</i> 1-2 hours post-dose with hunched posture, desation and ventral	
<u>50:50 <i>syn:anti</i></u> : 3 females per dose			

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

for 175 and 550 mg/kg bw and 1 female for 2000 mg/kg bw		recumbency 175 mg/kg bw: 3/3 females survived with hunched posture, slight poor coordination and ruffled fur on day of dosing and for up to 3 days Conclusion: LD₅₀ = 310.2 mg/kg bw (95% confidence interval 175-550 mg/kg bw)	
	Isopyrazam (batch: SMU6CP014) (purity: 98.2%) 50:50 syn:anti ratio: 2000 mg/kg bw (pure syn) 2000, 550 and 175 mg/kg bw (pure anti) 2000, 550 and 175 mg/kg bw (50:50 syn:anti)	<u>Isopyrazam (50:50 syn:anti)</u> 2000 mg/kg bw: 1/1 female killed <i>in extremis</i> 2 hours post-dose with vertebral recumbency and sedation 550 mg/kg bw: 3/3 females killed <i>in extremis</i> 1-2 hours post-dose with vertebral recumbency and sedation 175 mg/kg bw survived: 3/3 females survived with vertebral recumbency on day of dosing, hunched posture, poor coordination, ruffled fur on day of dosing and for up to 3 days Conclusion: LD₅₀ = 310 mg/kg bw (95% confidence interval 175-550 mg/kg)	
Acute dermal toxicity study	Isopyrazam (batch: SMU6AP001)	No deaths No signs of systemic toxicity or local effects	Anonymous (2007b)
OECD 402	Purity: 96.4 % (w/w)		
GLP	93:7 syn:anti Vehicle: Moistened with purified water	No adverse macroscopic findings	
HanRcc:WIST rats		Conclusion: LD ₅₀ > 5000 mg/kg bw	
5 animals/sex	5000 mg/kg bw Application area: 16 cm ² Duration: 24 hours Observation period: 14 days		
Acute inhalation toxicity study	Isopyrazam (batch: SMU6AP001)	All animals survived There were no treatment related signs of toxicity or adverse macroscopic findings	Anonymous (2006)
Nose-only exposure	Purity: 96.4%		
Dust	93:7 syn:anti	Conclusion: LC₅₀ > 5.28 mg/l	
OECD 403	MMAD: 2.88±3.01 µm (1 hour) and		
GLP	1.82±1.89 µm (3 hours)		
HsdBrlHan Wistar rats	Mean concentration: 5.28±0.08 mg/l air		

5 animals/sex

Exposure duration: 4
hours

Observation period: 15
days

The acute oral toxicity study on isopyrazam (with a 93:7 *syn:anti* ratio) gave an LD₅₀ > 2000 mg/kg bw (Table 1). It is noted that, according to the approved product specification, isopyrazam can contain a maximum of 15% of the *anti* isomer and additional studies on the individual *syn* and *anti* isomers indicate that the *anti* isomer is more acutely toxic than the *syn* isomer. This is evident in further studies on material containing the *syn:anti* isomers in a ratio of 70:30 and 50:50, where the LD₅₀ was found to be < 2000 mg/kg bw in each case. No data are available on isopyrazam containing 15% of the *anti* isomer.

The acute dermal LD₅₀ of the 93:7 *syn:anti* specification of isopyrazam is >5000 mg/kg bw (Table 1). The approved specification of isopyrazam for use as an active substance in plant protection products allows for a content of the more acutely toxic (by the oral route) *anti* isomer at a maximum of 15%, which is greater than the 7% in the specification investigated for dermal toxicity in this study. However, as the LD₅₀ of the specification tested for acute dermal toxicity was > 5000 mg/kg bw/d, a slight increase of the *anti* isomer would not be expected to result in an LD₅₀ smaller than the 2000 mg/kg bw cut-off value for classification in accordance with CLP.

The acute inhalation toxicity of isopyrazam dust (93:7 *syn:anti* specification) was investigated in a nose-only exposure, inhalation toxicity study. The LC₅₀ of isopyrazam (93:7 *syn:anti* specification) is > 5.28 mg/l (Table 1). A slight increase in the *anti* isomer to 15%, as in the approved isopyrazam specification for use as an active substance in plant protection products, would not be expected to substantially lower the LC₅₀. In the acute oral toxicity studies, the signs of toxicity observed in the animals killed *in extremis* that had received the specification of isopyrazam containing 70:30 *syn:anti* isomers were mainly related to the oral exposure route (gastrointestinal effects comprising a distended stomach, liquid contents in the duodenum, a grey material in the stomach/duodenum and an empty jejunum/ileum). Therefore, the acute toxicity of isopyrazam is not likely to be greater when administered via the inhalation route compared to the oral route.

Comparison with the criteria

According to the CLP criteria, classification for acute oral toxicity is warranted if the ATE (LD₅₀) of a substance is ≤ 2000 mg/kg bw. The acute oral toxicity study on isopyrazam (with a 93:7 *syn:anti* ratio) gave an LD₅₀ above this value. The database shows that the *anti* isomer is clearly more toxic than the *syn* isomer and RAC notes that no toxicity study is available with a *syn:anti* ratio 85:15; which should be more toxic isomeric ratio compared to 97:3 ratio. RAC notes that considering the available information the LD₅₀ of the 85:15 *syn:anti* ratio could be higher than 2000 mg/kg bw. Nevertheless, some additional uncertainties, such as the ones caused by males not being tested (which could be a much more susceptible sex than females) were noted. Overall, RAC supports the DS proposal for **no classification of isopyrazam for acute oral toxicity** but in this case **based on inconclusive data**.

According to the CLP criteria, a substance is classified for acute dermal toxicity if the LD₅₀ value is ≤ 2000 mg/kg bw. In the available study, isopyrazam (**93:7 *syn:anti***) was found to have an LD₅₀ value of > 5000 mg/kg bw. A slight increase in the *anti* isomer, which is more acutely toxic by the oral route, would not be expected to result in an LD₅₀ lower than 2000

mg/kg bw. Therefore, RAC supports the DS's proposal for **no classification of isopyrazam for acute dermal toxicity.**

In accordance with the CLP criteria, a substance (dust and mist) is classified for acute inhalation toxicity if it has an LC₅₀ value of ≤ 5 mg/l air. In the available study the LC₅₀ value for isopyrazam (93:7 *syn:anti*) is > 5.28 mg/l air, tested in both sexes and without any signs of toxicity. RAC expects the acute inhalation toxicity effects of the *anti* isomer to be similar to the oral exposure route, i.e., a slight increase in the *anti* isomer, which is more acutely toxic by the oral route, would not be expected to result in an LC₅₀ lower than 5 mg/l. RAC supports the DS's proposal for **no classification of isopyrazam for acute inhalation toxicity.**

10.5 Skin corrosion/irritation

One guideline- and GLP-compliant skin irritation study is available, which was conducted in rabbits using the 93:7 syn:anti specification of isopyrazam.

Table 18: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels duration of exposure	Results
Skin irritation study OECD 404 GLP Anonymous (2006b)	Rabbits, New Zealand White, males (n=1) & females (n=2)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7	0.5g moistened with 0.5ml purified water Exposure duration: 4 hours Skin reactions were assessed at 1, 24, 48 & 72 hours post-exposure	Mean scores for each animal (over 24, 48 & 72 hours): Erythema: 0, 0 & 0 Oedema: 0, 0 & 0 Not a skin irritant

10.5.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The skin irritating/corrosive potential of isopyrazam (93:7 syn:anti specification) has been investigated in rabbits. A semi-occlusive application of 0.5g of the test substance (moistened with 0.5 ml water) was applied to the intact shorn left flank of one male and two female New Zealand White rabbits. The test substance was left in contact with the skin for four hours and the area subsequently checked for irritation 1, 24, 48 & 72 hours post-application. There were no signs of irritation at any of these time-points (mean scores for both erythema and oedema were 0). As no signs of irritation were observed for the 93:7 specification of isopyrazam, a slight increase in the content of the anti isomer, which is more acutely toxic by the oral route, would not be expected to increase the skin irritation potential of isopyrazam.

10.5.2 Comparison with the CLP criteria

A substance should be classified for skin irritation category 2 if any of the following criteria are met:

- (1) mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
- (2) inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
- (3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

None of these criteria are met for isopyrazam; the average scores for each animal (over 24, 48 and 72 hours) were 0, 0 and 0 for both erythema and oedema; therefore classification for skin irritancy is not proposed.

10.5.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified (conclusive but not sufficient for classification)
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RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of isopyrazam for skin irritation based on the results of one guideline- and GLP compliant skin irritation study conducted in rabbits using the 93:7 *syn:anti* specification of isopyrazam where no erythema and no oedema was reported.

Comments received during consultation

One MSCA supported the DS's proposal for no classification of isopyrazam for skin irritation/corrosion.

Assessment and comparison with the classification criteria

Table 2 summarises the animal study on skin corrosion/irritation found in the CLH report.

Table 2: Summary of the animal study on skin corrosion/irritation with isopyrazam.

Study	Dose level	Results	Reference
Skin irritation study	Isopyrazam (batch: SMU6AP001)	Skin reactions were assessed at 1, 24, 48 and 72 hours post-exposure	Anonymous (2006b)
OECD 404	Purity: 96.4%	Mean scores for each animal (over 24, 48 and 72 hours): Erythema: 0, 0, 0 Oedema: 0, 0, 0	
GLP	93:7 <i>syn:anti</i>		
New Zealand White rabbits	0.5 g moistened with 0.5 ml purified water		
1 male and 2 females	Exposure duration: 4 hours	Conclusion: Not a skin irritant	

The skin irritating/corrosive potential of isopyrazam (93:7 *syn:anti*) has been investigated in rabbits (Table 2). There were no signs of irritation at any of these time points (mean scores for both erythema and oedema were 0). As no signs of irritation were observed in the 93:7 specification of isopyrazam, a slight increase in the content of the *anti* isomer, which is more acutely toxic by the oral route, would not be expected to increase the skin irritation potential of isopyrazam.

Comparison with the criteria

None of the criteria demanded for triggering classification are met for isopyrazam since the average scores for each animal (over 24, 48 and 72 hours) were 0, 0 and 0 for both erythema and oedema. Therefore, RAC supports the DS's proposal for **no classification of isopyrazam as skin irritant.**

10.6 Serious eye damage/eye irritation

The eye irritating potential of isopyrazam (93:7 syn:anti specification) was investigated in rabbits.

Table 19: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels duration of exposure	Results																			
Eye irritation study OECD 405 GLP Anonymous (2007)	Rabbits, New Zealand White, males (n=1) & females (n=2)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7	0.1g Eye reactions were scored 1, 24, 48 & 72 hours & 7 days after instillation.	There were no deaths or clinical signs of toxicity <table border="1"> <thead> <tr> <th rowspan="2">n</th> <th colspan="4">Mean scores at 24, 48 & 72 h</th> </tr> <tr> <th>Cornea</th> <th>Iris</th> <th colspan="2">Conjunctiva</th> </tr> <tr> <td></td> <td></td> <td></td> <th>Redness</th> <th>Chemosis</th> </tr> </thead> <tbody> <tr> <td>3</td> <td>0, 0, 0</td> <td>0, 0, 0</td> <td>1, 1, 1</td> <td>0.3, 0, 0.3</td> </tr> </tbody> </table> All observed signs had fully reversed by day 7 of treatment Not irritating	n	Mean scores at 24, 48 & 72 h				Cornea	Iris	Conjunctiva					Redness	Chemosis	3	0, 0, 0	0, 0, 0	1, 1, 1	0.3, 0, 0.3
n	Mean scores at 24, 48 & 72 h																						
	Cornea	Iris	Conjunctiva																				
			Redness	Chemosis																			
3	0, 0, 0	0, 0, 0	1, 1, 1	0.3, 0, 0.3																			

10.6.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The potential of isopyrazam to irritate the eyes was investigated in one male and two female New Zealand White rabbits. A 0.1 mg aliquot of the test substance was instilled into the left eye of each animal and scored at 1, 24, 48 and 72 hours (as well as 7 days) post-instillation. The mean scores for both the cornea and the iris were 0, 0 & 0 and the mean scores for conjunctiva redness and erythema were 1, 1, 1 and 0.3, 0, 0.3 respectively. The moderate reddening observed in the conjunctiva was observed in all animals after 1 hour and persisted until 24 hours in two animals. A slight ocular discharge was evident in all animals at the one hour reading; all effects had fully reversed by the end of the observation period (day 7). Such minimal signs of irritation observed in this study would not be expected to increase to any great extent with a slight increase of the content of the anti isomer (i.e. to 15% as in the approved isopyrazam specification).

10.6.2 Comparison with the CLP criteria

A substance is classified as an eye irritant if the following criteria are met in at least 2 of 3 animals:

Corneal opacity ≥ 1 and/or

Iritis ≥ 1 and/or

Conjunctival redness ≥ 2 and or

Conjunctival oedema (chemosis) ≥ 2

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

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

In the available study, the mean eye irritation scores for each animal (24-72 hours) were 0, 0 & 0 for corneal opacity and iritis, whilst the mean scores for conjunctival redness were 1, 1 and 1 and for conjunctival opacity were 0.3, 0 and 0.3. These values are below those that would trigger classification. Furthermore, all effects had fully reversed by day seven of treatment. Therefore isopyrazam does not meet the criteria for classification.

10.6.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified (conclusive but not sufficient for classification)

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritating potential of isopyrazam (93:7 *syn:anti*) was investigated in rabbits. DS proposed no classification of isopyrazam for eye damage/irritation based on the results of one guideline and GLP compliant study where the mean score at 24, 48 and 72 hours were of 0 (cornea), 0 (iris), 1 (conjunctival redness) and 0-0.3 (conjunctival chemosis).

Comments received during consultation

One MSCA supported the DS's proposal for no classification of isopyrazam for serious eye/damage.

Assessment and comparison with the classification criteria

Table 3 summarises the animal study on serious eye damage/eye irritation found in the CLH report.

Table 3: Summary of the animal study on eye damage/irritation with isopyrazam.

Study	Dose level	Results	Reference
Eye irritation study	Isopyrazam (batch: SMU6AP001)	Eye reactions were scored 1, 24, 48 and 72 hours and 7 days after instillation	Anonymous (2007)
OECD 405	Purity: 96.4% (w/w)	There were no deaths or clinical signs of toxicity	
GLP	Syn:anti ratio: 93:7		
New Zealand White rabbits	0.1 g	Mean scores at 24, 48 and 72 h: Cornea: 0, 0, 0	

1 male and 2 females

Iris: 0, 0, 0

Conjunctival redness: 1, 1, 1

Conjunctival chemosis: 0.3, 0, 0.3

All observed signs had fully reversed by day 7 of treatment

The potential of isopyrazam to irritate the eyes was investigated in one male and two female New Zealand White rabbits (Table 3). The mean scores for both the cornea and the iris were 0, 0 and 0 and the mean scores for conjunctiva redness and erythema were 1, 1, 1 and 0.3, 0, 0.3; respectively. The moderate reddening observed in the conjunctiva was observed in all animals after 1 hour and persisted for 24 hours in two animals. A slight ocular discharge was evident in all animals at the 1 hour reading; all effects had fully reversed by the end of the observation period (day 7). Such minimal signs of irritation observed in this study would not be expected to increase to a level that would require classification with a minor increase in the content of the *anti* isomer (i.e. from 7 to 15% as in the approved isopyrazam specification).

Comparison with the criteria

The scores for corneal opacity, iritis, conjunctival redness and conjunctival oedema were below those that would trigger classification. Furthermore, all effects had fully reversed by day seven of treatment. Therefore, RAC supports the DS’s proposal for **no classification of isopyrazam for eye damage/irritation.**

10.7 Respiratory sensitisation

No studies available

10.7.1 Conclusion on classification and labelling for respiratory sensitisation

Not classified (data lacking)

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter’s proposal

The DS proposed no classification of isopyrazam for respiratory sensitisation based on lack of data.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes that: i) there are no data indicating evidence of respiratory tract irritation with isopyrazam; ii) the acute inhalation study showed no evidence of respiratory system impairment; and iii) rabbit dermal and eye irritation studies indicated lack of irritant potential on skin and mucosal membranes. Overall, RAC supports the DS's proposal for **no classification of isopyrazam for respiratory sensitisation.**

10.8 Skin sensitisation

The skin sensitising potential of isopyrazam (93:7 syn:anti specification) has been investigated in a guideline compliant murine local lymph node assay (LLNA).

Table 20: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, batch, purity, vehicle, syn:anti ratio	Concentration levels	Results																				
Mouse Local Lymph Node Assay OECD 429 GLP Anonymous (2016)	Mice, CBA/Ca/Ola/Hsd, females, 4/dose	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Vehicle: dimethyl sulphoxide	10, 25 & 60% (w/v)	8 lymph nodes per concentration were assayed <table border="1"> <thead> <tr> <th>Conc (% w/v)</th> <th>DPM</th> <th>DPM per lymph node</th> <th>SI</th> </tr> </thead> <tbody> <tr> <td>0 (control)</td> <td>3852</td> <td>482</td> <td>-</td> </tr> <tr> <td>10</td> <td>7910</td> <td>989</td> <td>2.1</td> </tr> <tr> <td>25</td> <td>7390</td> <td>924</td> <td>1.9</td> </tr> <tr> <td>60</td> <td>19944</td> <td>2493</td> <td>5.2</td> </tr> </tbody> </table> <p>The positive control gave the expected response Positive EC3 value = > 25%</p>	Conc (% w/v)	DPM	DPM per lymph node	SI	0 (control)	3852	482	-	10	7910	989	2.1	25	7390	924	1.9	60	19944	2493	5.2
Conc (% w/v)	DPM	DPM per lymph node	SI																					
0 (control)	3852	482	-																					
10	7910	989	2.1																					
25	7390	924	1.9																					
60	19944	2493	5.2																					

10.8.1 Short summary and overall relevance of the provided information on skin sensitisation

In a well conducted, guideline- and GLP-compliant LLNA, female CBA mice (4/group) were administered 10, 25 or 60% (w/v) of isopyrazam prepared in the vehicle dimethyl sulphoxide; the vehicle alone served as the negative control whilst hexylcinnamaldehyde provided the positive control. Approximately 25µl of each concentration was applied to the dorsal surface of each ear in accordance with OECD TG 429. Results were expressed as disintegrations per minute (DPM) per lymph node for each group and then divided by the activity of the negative control to give a stimulation index (SI). A positive response was defined as a 3-fold or greater increase relative to the control group. This was the case for the 60% dose-group, for which an S.I of 5.2 was recorded.

10.8.2 Comparison with the CLP criteria

In accordance with the CLP criteria, a substance is classified as a skin sensitiser if data derived from a mouse LLNA conducted in accordance with OECD 429 produces a SI value of ≥ 3 ; this was the case for isopyrazam, for which a concentration of 60% (w/v) resulted in an SI value of 5.2. The CLP criteria include the provision for a substance to be sub-categorised into either 1A or 1B based on its potency, provided that sufficient doses have been tested. An EC3 value (the concentration that results in an SI value of 3) of $\leq 2\%$ would result in a substance being sub-categorised into 1A, and an EC3 value of $> 2\%$ would result in a 1B categorisation. In the study with isopyrazam, doses of 10% and 25% resulted in an SI < 3 whereas, an SI of 5.2 was recorded at 60%. Whilst lower concentrations were not tested and a clear dose response was not determined, it can be concluded that the EC3 value in this study is greater than 25%. Based on these data, classification into subcategory 1A would not be appropriate. As such, it is proposed to classify isopyrazam into subcategory 1B. The batch of isopyrazam tested in this investigation contained 7% of the anti isomer, whilst the isopyrazam specification that has been approved for use as an active substance in plant protection products contains a maximum of 15% of the anti isomer; the results of this skin sensitisation study indicate that an increase of the anti isomer to 15% would not be expected to substantially increase the skin sensitising potential of isopyrazam and that category 1B is appropriate for this specification.

10.8.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens 1B; H317 – May cause an allergic skin reaction

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

DS proposed classification of isopyrazam as Skin Sens. 1B (H317: may cause an allergic skin reaction) based on the results of a one guideline and GLP compliant murine local lymph node assay (LLNA) conducted using the 93:7 *syn:anti* isopyrazam. In this study, a stimulation index of 5.2 was recorded using 60% isopyrazam and an EC₃ $>25\%$ was estimated.

Comments received during consultation

One MSCA supported the DS's proposal for classification of isopyrazam as Skin Sens. 1B (H317).

Assessment and comparison with the classification criteria

Table 4 summarises the result of the murine LLNA found in the CLH report.

Table 4: Summary of the animal studies on skin sensitisation with isopyrazam.

Study	Dose level	Results	Reference
Mouse LLNA	Isopyrazam (batch: SMU6AP001)	8 lymph nodes/concentration	Anonymous (2016)
OECD 429	Purity: 96.4% (w/w)	Conc (% w/v) Stimulation index	
GLP	Syn:anti ratio: 93:7		

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

CBA/Ca/Ola/Hsd mice 4 females/dose	Vehicle: dimethyl sulphoxide	10	2.1	
		25	1.9	
		60	5.2	
10, 25 and 60% (w/v)		The positive control gave the expected response		
EC₃ value > 25%				
<p>In a well conducted, guideline and GLP compliant mouse LLNA a stimulation index ≥ 3 was found in the 60% dose-group (stimulation index of 5.2); while isopyrazam concentrations of 10 and 25% generated stimulation indexes in both cases of around 2 (Table 4).</p> <p>Comparison with the criteria</p> <p>In accordance with the CLP criteria, a substance is classified as a skin sensitiser if data derived from a mouse LLNA conducted in accordance with OECD 429 produces a stimulation index of ≥ 3; as was the case for isopyrazam, for which a concentration of 60% (w/v) resulted in a stimulation index value of 5.2. Thus, the classification of isopyrazam as skin sensitiser is warranted. On the other hand, an EC₃ value (the concentration that results in a stimulation index value of 3) of > 2% would result in a 1B categorisation. In the study with isopyrazam, it can be concluded that the EC₃ value is greater than 25% because this concentration yielded a stimulation index of 1.9. The batch of isopyrazam tested in this investigation contained 7% of the <i>anti</i> isomer, whilst the isopyrazam specification that has been approved for use as an active substance in plant protection products contains a maximum of 15% of the <i>anti</i> isomer. The results of this skin sensitisation study indicate that an increase of the <i>anti</i> isomer to 15% would not be expected to substantially increase the skin sensitising potential of isopyrazam. Overall, RAC supports the DS's proposal for classification of isopyrazam as Skin Sens 1B (H317: may cause an allergic reaction).</p>				

10.9 Germ cell mutagenicity

The genotoxicity of isopyrazam has been investigated in several well conducted, guideline- and GLP-compliant *in vitro* tests and a reliable *in vivo* bone marrow micronucleus test. An unscheduled DNA synthesis (UDS) assay is also available.

10.9.1 In vitro

The gene mutation potential of both the 93:7 and the 70:30 syn:anti specifications of isopyrazam has been investigated *in vitro* in bacteria (Ames tests) and in mammalian cells (mouse lymphoma), whilst the clastogenic potential of both these specifications was investigated *in vitro* in human lymphocytes.

Table 21: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test system (organism, strain)	Test substance, syn:anti ratio	Conc. range	Results		Remarks
				+S9	-S9	
Bacterial Reverse Mutation Assay in <i>S.typhimurium</i> and <i>E. coli</i> Plate incorporation & pre-incubation OECD 471 GLP Callander (2006)	<i>S.typhimurium</i> strains TA1535, TA1537, TA98 & TA100 <i>E.coli</i> strains WP2 (pKM101) & WP2 <i>uvrA</i> (pKM101)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio:93:7	5 – 5000 µg/plate (tested in triplicate)	neg	neg	Precipitation at 500 – 5000 µg/plate Cytotoxicity at 2500 & 5000 µg/plate Appropriate positive & solvent controls gave the expected results
Bacterial Reverse Mutation Assay in <i>S.typhimurium</i> and <i>E. coli</i> Plate incorporation & pre-incubation OECD 471 GLP Sokolowski (2008a)	<i>S.typhimurium</i> strains TA1535, TA1537, TA98 & TA100 <i>E.coli</i> strains WP2 (pKM101) & WP2 <i>uvrA</i> (pKM101)	Isopyrazam Batch: SMU7DP017 Purity: 90.8% (w/w) Syn:anti ratio:70:30	5 – 5000 µg/plate (tested in triplicate)	neg	neg	Precipitation at 333 – 5000 µg/plate No cytotoxicity up to 5000 µg/plate Appropriate positive & solvent controls gave the expected results
<i>In vitro</i> Mammalian Cell Gene Mutation Test (using L5178 TK ^{+/-} mouse lymphoma cells) OECD 476 GLP Clay (2006)	Mouse lymphoma cells, L5178Y TK ^{+/-} locus	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio:93:7	Max 40 µg/ml (+S9) Max 25 µg/ml (-S9)	neg	neg	Cytotoxicity: relative total growth at highest conc was 13% (+S9) & 19% (-S9) Positive controls induced the appropriate increases in mutant frequencies Each conc tested in duplicate (2 independent experiments)

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any	Test system (organism, strain)	Test substance, syn:anti ratio	Conc. range	Results		Remarks
				+S9	-S9	
<i>In vitro</i> Mammalian Cell Gene Mutation Test (using L5178 TK ^{+/-} mouse lymphoma cells OECD 476 Wollny (2008a)	Mouse lymphoma cells, L5178Y TK ^{+/-} locus	Isopyrazam Batch: SMU7DP017 Purity: 90.8% (w/w) Syn:anti ratio:70:30	Max 66 µg/ml (+S9) Max 44 µg/ml (-S9)	neg	neg	Precipitation from 175 µg/l Cytotoxicity: minimum survival levels (compared with controls) at highest conc: 8% (+/-S9) Positive controls induced the appropriate increases in mutant frequencies
<i>In vitro</i> Chromosome Aberration Test in Human Lymphocytes OECD 473 GLP Fox (2006a)	Human Lymphocytes, 2 donors, 1 male & 1 female, pooled	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio:93:7	<u>Assay 1</u> 20 – 40 µg/ml (-S9) & 20 – 50 µg/ml (+S9) 3 h exposure (+/-S9)	neg *	neg	Cytotoxicity: >50% reduction in mitotic activity at the highest conc (conc dependent) *Small increases in the % of aberrant cells in experiment 1 (-S9 at 20 & 30 µg/ml) were within historical control ranges and there were no increases at the highest conc of 40 µg/ml +ve and -ve controls gave the expected results
			<u>Assay 2</u> 10 – 20 µg/ml (-S9) & 20 – 50 µg/ml (+S9) 3h exposure (+S9) & 20 h exposure (-S9)	neg	neg	
<i>In vitro</i> Chromosome Aberration Test in Human Lymphocytes OECD 473 GLP Bohnenberger (2008a)	Human Lymphocytes, 2 donors, 1 male & 1 female, pooled	Isopyrazam Batch: SMU7DP017 Purity: 90.8% (w/w) Syn:anti ratio:70:30	<u>Assay 1</u> 16.9 – 51.7 µg/ml (-S9) & 29.6 – 90.5 µg/ml (+S9) 4h exposure (+/-S9)	neg	neg	Cytotoxicity: >50% conc-dependent reduction in mitotic activity at the highest concentrations +ve and -ve controls gave the expected results
			<u>Assay 2</u> 3 – 16 µg/ml (-S9) & 25 – 75 µg/ml (+S9) 4h exposure (+S9) & 22h exposure (-S9)	neg	neg	

The application of isopyrazam to *Salmonella typhimurium* and *Escherichia coli* strains, up to and including the limit concentration of 5000 µg/plate, did not produce an increase in the number of reversions either with or without metabolic activation. The potential of isopyrazam to induce gene mutations was further investigated *in vitro* at the TK locus of L5178 mouse lymphoma cells and was found to be negative with and

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

without S9 mix, when tested up to appropriate cytotoxic and precipitative concentrations. Furthermore isopyrazam showed no evidence of clastogenic potential *in vitro* in human peripheral blood lymphocytes both in the presence and absence of S9 mix, even when tested up to cytotoxic concentrations.

10.9.2 In vivo

The potential of isopyrazam to induce chromosomal damage in rats has been investigated *in vivo* in a bone marrow micronucleus test. An unscheduled DNA synthesis assay is also available. Only the 93:7 syn:anti specification of isopyrazam has been investigated *in vivo*.

Table 22: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance, batch, purity, syn:anti ratio	Dose levels	Results
<i>In vivo</i> Rat bone marrow micronucleus test OECD 474 GLP Anonymous (2006b)	Rats, HsdRCCHan:WIST, 5 males	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7	2000 mg/kg bw, oral (limit dose) Bone marrow sampled at 24 & 48 hours	Negative 2000 erythrocytes examined No clinical signs of toxicity noted
<i>In vivo</i> Rat Liver Unscheduled DNA Synthesis Assay OECD 486 GLP Anonymous (2006c)	Rats, HsdRCCHan:WIST, 3 males	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7	2000 mg/kg bw, oral (limit dose) Hepatocytes sampled at 2 and 16 hours	Negative No clinical signs of toxicity noted No histopathological changes to the liver Positive control induced marked increase in UDS

Isopyrazam was investigated for its ability to induce micronucleated immature erythrocytes in the bone marrow of male HsdRCCHan:WIST rats. A single oral dose of 2000 mg/kg bw (limit dose) was administered to five rats, before bone marrow was sampled at 24 and 48 hours; 2000 immature erythrocytes/animal were examined for the presence of micronuclei. Cyclophosphamide (20 mg/kg bw) provided the positive control whilst carboxymethylcellulose (CMC) served as the vehicle (negative) control.

No clinical signs of toxicity were noted and no unusual findings were found at necropsy.

There were no increases in the incidences of micronucleated immature erythrocytes (when compared with vehicle control values) at either sampling time; a biologically meaningful increase in micronuclei was induced by the positive control, thus demonstrating the sensitivity of the assay. Exposure of the bone marrow to the test substance was demonstrated by a reduction in the mean % of immature erythrocytes in the treated groups compared with the vehicle control groups. Under the conditions of this study the 93:7 syn:anti specification of isopyrazam was not clastogenic *in vivo* in rats.

Isopyrazam did not induce unscheduled DNA synthesis in the livers of rats in an *in vivo* UDS study. A single oral limit dose of 2000 mg/kg bw administered via gavage to 3 male HsdRCCHan:WIS rats did not result in any changes to the mean net nuclear grain counts or the % of DNA repair in hepatocytes sampled at 2 and 16 hours post-dose. The positive control (N-nitrosodimethylamine) induced marked increases in UDS demonstrating the sensitivity of the assay.

10.9.3 Short summary and overall relevance of the provided information on germ cell mutagenicity

Isopyrazam has been tested for potential genotoxic properties in a standard battery of good quality *in vitro* and *in vivo* assays. Both the 93:7 and the 70:30 *syn:anti* isomer specifications were tested *in vitro* whilst only the 93:7 specification was investigated *in vivo*.

There was no evidence that either specification of isopyrazam was mutagenic or clastogenic in six well conducted, reliable *in vitro* tests.

The genotoxicity of isopyrazam was tested *in vivo* in a bone marrow micronucleus test conducted in rats. The test did not reveal an increase in the frequency of micronucleated polychromatic erythrocytes following administration of a limit dose of 2000 mg/kg bw. Furthermore, the administration of a 2000 mg/kg bw limit dose of isopyrazam did not induce unscheduled DNA synthesis (UDS) in the livers of rats.

There is no evidence from the available data set that isopyrazam is a somatic cell mutagen. There is therefore no reason to believe that isopyrazam would have the potential to induce mutations in germ cells.

10.9.4 Comparison with the CLP criteria

In accordance with the CLP criteria, isopyrazam did not demonstrate any genotoxic potential in six *in vitro* and two *in vivo*, guideline- and GLP-compliant studies and therefore the criteria for classification are not met.

10.9.5 Conclusion on classification and labelling for germ cell mutagenicity

Not classified (conclusive but not sufficient for classification)

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The gene mutation potential of both the 93:7 and the 70:30 *syn:anti* ratios of isopyrazam has been investigated *in vitro* in bacteria (Ames tests) and in mammalian cells (mouse lymphoma). The clastogenic potential of both specifications was investigated *in vitro* in human lymphocytes. The potential of the 93:7 *syn:anti* specification of isopyrazam to induce chromosomal damage in rats has been investigated *in vivo* in a bone marrow micronucleus test and in an unscheduled DNA synthesis assay. All *in vivo* and *in vitro* tests yielded negative results hence the DS proposed no classification of isopyrazam for germ cell mutagenicity.

Comments received during consultation

One MSCA supported the DS’s proposal for no classification of isopyrazam for germ cell mutagenicity.

Assessment and comparison with the classification criteria

Tables 8 and 9 summarise the *in vitro* and *in vivo* mutagenicity studies with isopyrazam; respectively.

Table 8: Summary of mutagenicity/genotoxicity in vitro studies with isopyrazam.

Method	Tested concentrations	Results	Reference
Bacterial reverse mutation assay	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w)	Precipitation at 500-5000 µg/plate Cytotoxicity at 2500 and 5000 µg/plate Appropriate positive and solvent controls gave the expected results Tested in triplicate	Callander (2006)
S. typhimurium strains TA1535, TA1537, TA98 and TA100	93:7 syn:anti 5-5000 µg/plate	Results: +S9: Negative - S9: Negative	
E.coli strains WP2 (pKM101) and WP2 uvrA (pKM101)			
OECD 471			
GLP			
Bacterial reverse mutation assay	Isopyrazam (batch: SMU7DP017) Purity: 90.8% (w/w)	Precipitation at 333-5000 µg/plate No cytotoxicity up to 5000 µg/plate Appropriate positive and solvent controls gave the expected results Tested in triplicate	Sokolowski (2008a)
S. typhimurium strains TA1535, TA1537, TA98 and TA100	70:30 syn:anti 5-5000 µg/plate	Results: +S9: Negative - S9: Negative	
E. coli strains WP2 (pKM101) and WP2 uvrA			

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

(pKM101)			
OECD 471			
GLP			
<i>In vitro</i> mammalian cell gene mutation test	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w)	Cytotoxicity: relative total growth at highest concentration was 13% (+S9) and 19% (-S9) Positive controls induced the appropriate increases in mutant frequencies	Clay (2006)
Mouse lymphoma cells, L5178Y TK+/- locus	93:7 syn:anti Max 40 µg/ml (+S9) Max 25 µg/ml (-S9)	Each concentration tested in duplicate (2 independent experiments)	
OECD 476		Results: +S9: Negative - S9: Negative	
<i>In vitro</i> mammalian cell gene mutation test	Isopyrazam (batch: SMU7DP017) Purity: 90.8% (w/w)	Precipitation from 175 µg/l Cytotoxicity: minimum survival levels (compared with controls) at highest concentration: 8% (+/-S9)	Wollny (2008a)
Mouse lymphoma cells, L5178Y TK+/- locus	70:30 syn:anti Max: 66 µg/ml (+S9) Max: 44 µg/ml (-S9)	Positive controls induced the appropriate increases in mutant frequencies	
OECD 476		Results: +S9: Negative - S9: Negative	
<i>In vitro</i> chromosome aberration test	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w)	Cytotoxicity: >50% reduction in mitotic activity at the highest concentration (concentration dependent) Small increases in the % of aberrant cells in experiment 1 (-S9 at 20 and 30 µg/ml) were within historical control ranges and there were no increases at the highest concentration of 40 µg/ml	Fox (2006a)
Human lymphocytes: 2 donors (1 male and 1 female, pooled)	93:7 syn:anti <u>Assay 1:</u> 20-40 µg/ml (-S9) and 20-50 µg/ml (+S9)	Positive and negative controls gave the expected results	
OECD 473	3 h exposure (+/-S9)	Results assay 1: +S9: Negative -S9: Negative	
GLP	<u>Assay 2:</u> 10-20 µg/ml (-S9) and 20-50 µg/ml (+S9) 3 h exposure (+/-S9)	Results assay 2: +S9: Negative -S9: Negative	
<i>In vitro</i> chromosome aberration test	Isopyrazam (batch: SMU7DP017) Purity: 90.8% (w/w)	Cytotoxicity: >50% concentration-dependent reduction in mitotic activity at the highest concentrations Positive and negative controls gave the	Bohnenberger (2008a)

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Human lymphocytes: 2 donors (1 male and 1 female, pooled)	70:30 <i>syn:anti</i> <u>Assay 1:</u> 16.9-51.7 µg/ml (-S9) and 29.6-90.5 µg/ml (+S9)	expected results Results assay 1: +S9: Negative - S9: Negative
OECD 473	4h exposure (+/- S9)	Results assay 2:
GLP	<u>Assay 2:</u> 3-16 µg/ml (-S9) and 25-75 µg/ml (+S9) 4h exposure (+S9) and 22h exposure (-S9)	+S9: Negative -S9: Negative

The application of isopyrazam to *Salmonella typhimurium* and *Escherichia coli* strains, up to and including the limit concentration of 5000 µg/plate, did not produce an increase in the number of reversions either with or without metabolic activation. The potential of isopyrazam to induce gene mutations was further investigated *in vitro* at the TK locus of L5178 mouse lymphoma cells and was found to be negative both with and without S9 mix, when tested up to appropriate cytotoxic and precipitative concentrations. Furthermore, isopyrazam showed no evidence of clastogenic potential *in vitro* in human peripheral blood lymphocytes both in the presence and absence of S9 mix, even when tested up to cytotoxic concentrations.

Table 9: Summary of mutagenicity/genotoxicity *in vivo* studies with isopyrazam.

Method	Tested concentrations	Results	Reference
<i>In vivo</i> rat bone marrow micronucleus test	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w)	2000 erythrocytes examined No clinical signs of toxicity noted	Anonymous (2006b)
OECD 474	93:7 <i>syn:anti</i>	Negative	
GLP	2000 mg/kg bw, oral (limit dose)		
HsdRCCHan:WIST rats	Bone marrow sampled at 24 and 48 hours		
5 males			
<i>In vivo</i> rat Liver unscheduled DNA Synthesis Assay	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w)	Hepatocytes sampled at 2 and 16 hours No clinical signs of toxicity noted	Anonymous (2006c)
OECD 486	93:7 <i>syn:anti</i>		
GLP	2000 mg/kg bw, oral (limit dose)	No histopathological changes to the liver	
HsdRCCHan:WIST rats	Hepatocytes sampled at 2 and 16 hours	Positive control induced marked increase in unscheduled DNA synthesis	
3 males			
Negative			

Isopyrazam (93:7 *syn:anti*) was investigated for its ability to induce micronucleated immature erythrocytes in the bone marrow of male HsdRCCHan:WIST rats. There were no increases in the incidences of micronucleated immature erythrocytes (when compared with

vehicle control values) at either sampling time; a biologically meaningful increase in micronuclei was induced by the positive control, thus demonstrating the sensitivity of the assay. Exposure of the bone marrow to the test substance was demonstrated by a reduction in the mean percentage of immature erythrocytes in the treated groups compared with the vehicle control groups. Under the conditions of this study, isopyrazam was not clastogenic *in vitro* in rats.

Isopyrazam (93:7 *syn:anti*) did not induce unscheduled DNA synthesis in the livers of rats in an *in vivo* unscheduled DNA synthesis study. A single oral limit dose of 2000 mg/kg bw did not result in any changes to the mean net nuclear grain counts or the % of DNA repair in hepatocytes sampled at 2 and 16 hours post-dose. The positive control (N-nitrosodimethylamine) induced marked increases in unscheduled DNA synthesis demonstrating the sensitivity of the assay.

Comparison with the criteria

There is no evidence from the available data set that isopyrazam is a somatic cell mutagen. Moreover, there is no reason to believe that isopyrazam would have the potential to induce mutations in germ cells. Therefore, RAC supports the DS's proposal for **no classification of isopyrazam for germ cell mutagenicity.**

10.10 Carcinogenicity

The carcinogenic potential and chronic toxicity of isopyrazam have been thoroughly investigated in a standard set of studies in rats and mice; additional studies have been conducted to investigate the mode of action (MoA) and human relevance (see section 10.10.3).

Table 23: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any,	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	Results (% change from controls)
Rats				
Two-year chronic toxicity and carcinogenicity study (with histological extension), dietary OECD 453 GLP Anonymous (2008a) & Anonymous (2009)	Rats, HsdRccHn: WIST, M & F 12/sex/dose (1-yr) 52/sex/dose (2-yr)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7	0, 100, 500 & 3000 ppm Equivalent to: Males: 0, 5.5, 28 & 173 mg/kg bw/d Females: 0, 7, 35 & 233 mg/kg bw/d Chronic phase: 52 weeks Carc' phase: 104 weeks	There were no treatment-related deaths. There were no treatment-related effects on survival. <u>Chronic phase (12 months)</u> <u>3000 ppm</u> ↑ incidence of scabs (M & F) & subcutaneous masses (M) ↓ mean body weight (BW): -10.3%** (M) & -18.8%** (F) ↓ haemoglobin (Hb) (-3.4%**), ↑ Platelet count (14%*) in F ↑ gamma glutamyl transferase (GGT) in M (12.9%**), ↓ alkaline phosphatase (ALP) in M (-28.9%** & F (-41.7%**), ↓ aspartate aminotransferase (AST) in F (-34.8%**) ↑ liver weight in M (17%** & F (14.4%**) Hepatocellular hypertrophy in 12/12** M (5 minimal, 7 slight) & 12/12** F (slight), hepatocyte pigmentation in 6/12* M (5 minimal, 1 slight) & 10/12* F (minimal), minimal hepatocyte vacuolation in 11/12** M & 8/12** F <u>500 ppm</u> ↓ mean BW in F (-3.5%*) ↑ GGT in M (24.8%**) Hepatocellular hypertrophy in 4/12 M (minimal) & 9/12** F (minimal), hepatocyte vacuolation in 10/12 M** (9 minimal, 1 slight) & 8/12** F (minimal) <u>100 ppm</u> No adverse effects <u>Carcinogenicity phase (24 months)</u> <u>Non-neoplastic findings</u> <u>3000 ppm</u> ↓ mean BW: -13%** (M) & -27%** (F) ↓ Hb (-5.4%**), ↓ haematocrit (-4.4%**), ↓ red blood cell count (RBC) (-5.5%**), ↑ platelet count (10.8%*) in

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any,	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	Results (% change from controls)																																												
				<p>F</p> <p>↑ GGT in M (49.9%**) & F (42.8%**), ↓ ALP in M (-33.7%**) & F (-30.7%*), ↓ AST in F (-21%*)</p> <p>↑ liver weight in M (17.5%**) & F (26.4%**)</p> <p>Pale spots on liver & liver masses in F</p> <p>Centrilobular hepatocellular hypertrophy in M (49/52**) & F (50/52**), hepatocyte vacuolation in M (39/52**), centrilobular hepatocellular pigmentation in M (32/52**) & F (46/52**), minimal bile duct hyperplasia in M (12/52**), minimal bile duct fibrosis in M (12/52**), eosinophilic foci in M (32/52**) & F (29/52**)</p> <p>Brown pigment in kidney tubules in F, sinus erythrocytosis in lymph nodes in M</p> <p><u>500 ppm</u></p> <p>↓ mean BW in F -11%**</p> <p>↑ GGT in M (-24.8%**), ↓ ALP in M (-17.1%**), ↓ AST in F (-22.2%*)</p> <p>↑ relative liver weight in F (12%**) and M (5%*)</p> <p>Centrilobular hepatocellular hypertrophy in M (45/52**) & F (49/52**), hepatocyte vacuolation in M (32/52**) & F (18/52**), centrilobular hepatocellular pigmentation in F (49/52**), minimal bile duct hyperplasia in M (19/52**), minimal bile duct fibrosis in M (10/52**), eosinophilic foci in M (23/52**) & F (26/52**)</p> <p>Brown pigment in kidney tubules in F</p> <p><u>100 ppm</u></p> <p>↓ mean BW in F (-5.3%*)</p> <p><u>Neoplastic findings</u></p> <p><u>Thyroid</u></p> <table border="1"> <thead> <tr> <th rowspan="2">Parameter</th> <th colspan="4">Dietary conc. of isopyrazam (ppm)</th> </tr> <tr> <th>0</th> <th>100</th> <th>500</th> <th>3000</th> </tr> </thead> <tbody> <tr> <td>Organs exam.</td> <td>52</td> <td>52</td> <td>52</td> <td>52</td> </tr> <tr> <td colspan="5">Follicular cell tumours- Males</td> </tr> <tr> <td>Adenoma</td> <td>1</td> <td>4</td> <td>2</td> <td>6</td> </tr> <tr> <td>Carcinoma</td> <td>0</td> <td>0</td> <td>5</td> <td>0</td> </tr> <tr> <td colspan="5">Follicular cell tumours - Females</td> </tr> <tr> <td>Adenoma</td> <td>5</td> <td>1</td> <td>3</td> <td>3</td> </tr> <tr> <td>Carcinoma</td> <td>0</td> <td>1</td> <td>0</td> <td>0</td> </tr> </tbody> </table> <p>Laboratory Historical Control Data[§]: Follicular cell adenoma (males) 1.6 – 9.6%, 3 studies 2007 - 2009</p> <p><u>Liver</u></p>	Parameter	Dietary conc. of isopyrazam (ppm)				0	100	500	3000	Organs exam.	52	52	52	52	Follicular cell tumours- Males					Adenoma	1	4	2	6	Carcinoma	0	0	5	0	Follicular cell tumours - Females					Adenoma	5	1	3	3	Carcinoma	0	1	0	0
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Mice				
80-week carcinogenicity, dietary OECD 451 GLP Anonymous (2008b)	Mice, C57BL/10J _f CD-1, M & F, 50/sex/group	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7	0, 70, 500 & 3500 ppm Equivalent to: Males: 0, 8, 56 & 433 mg/kg bw/d Females: 0, 10, 75 & 554 mg/kg bw/d 80-weeks	There were no treatment-related deaths <u>Non-neoplastic findings</u> <u>3500 ppm</u> ↓ BW in M (-23%**) and F (-11%**) ↑ liver weights in M (41%**) & F (38%**) Eye discharge in 26/46 M Hepatocellular hypertrophy in 42/50 M & 47/50 F <u>500 ppm</u> ↑ liver weights in M (14%**) & F (7%**) Hepatocellular hypertrophy in 5/50 M & 13/50 F, eosinophilic droplets in gall bladder epithelium in 25/50 F, inflammation in the nasolacrimal ducts in 38/50 M <u>70 ppm</u> No adverse effects <u>Neoplastic findings</u> There were no treatment related effects on the incidence, appearance, onset or character of tumours at any dose

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

§Laboratory historical control data are taken from 3 studies conducted in the same strain of rat between 2007-2009. HCD are limited to 3-studies as the laboratory changed the strain of rat used for long-term repeat dose toxicity studies in 2005-2006 from the Alderley Park rat to the Han Wistar. The available HCD are considered relevant to the current study.

10.10.1 Chronic/carcinogenicity study in rats

In a two-year combined chronic and carcinogenicity study in rats, isopyrazam with a syn:anti ratio of 93:7 was administered for either 52 (12/sex/group) or 104 (52/sex/group) weeks at dietary concentrations of 0, 100, 500 and 3000 ppm. These doses equated to mean intakes of 0, 5.5, 28 & 173 mg/kg bw/d in males and 0, 7, 35 & 233 mg/kg bw/d in females.

Non-neoplastic findings

There were no treatment-related effects on survival. In males at 52 weeks, survival was 100% in all dose groups, whilst in females at 52 weeks survival was reported to be 100%, 97%, 97% and 98% in the 0, 100, 500 and 3000 ppm dose-groups respectively. At 104 weeks, survival was 71%, 73%, 81% and 71% in males and 75%, 68%, 60% and 64% in females in the 0, 100, 500 and 3000 ppm groups. Treatment-related clinical signs at the high-dose comprised scabbing in both sexes accompanied by subcutaneous masses in males. Body-weight gain was lower in both sexes at 3000ppm, such that by the end of the study overall body weights were 13% (males) and 27% (females) lower than controls, and in females overall body-weight gain

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

was approximately 40% lower than controls. Food consumption at this dose was lower than controls for the first 13 weeks only, suggesting that food utilisation efficiency was reduced in these animals. Blood was collected for haematological analysis from 13/animals/sex at weeks 14, 27, 53 and 79 and concurrently from a further 13 animals/sex for clinical-chemistry analysis. There were treatment-related effects on some haematological parameters in females: at all time-points haemoglobin, haematocrit and red blood cell counts were reduced, whilst platelet counts were increased in comparison with controls. With regard to clinical chemistry changes, GGT activity was increased from week 53 in males and females at 3000ppm and also at 500ppm in males. Triglycerides were reduced at 500ppm and 3000ppm from week 53 in females and at 3000ppm (throughout the study) in males. Increased cholesterol was noted in both sexes at the top dose at most time points; plasma bilirubin was reduced in males at certain time-points. At 3000ppm alanine transaminase activity (ALT) activity was increased in males whilst alkaline phosphatase (ALP) activity was generally lower in males and females.

A standard range of organs from all animals at the 52 and 104 week scheduled kills were weighed and subjected to a gross necropsy. Treatment-related organ weight increases were confined to the liver (see table below).

Table 24: Selected organ weight after 52- or 104-weeks' exposure to isopyrazam

Organ/week	Dietary Concentration of Isopyrazam (ppm)							
	Males				Females			
	0	100	500	3000	0	100	500	3000
Liver, 53	15.1	14.3	15.2	17.7**	9.0	8.9	9.6	10.3**
Liver, 105	16.6	16.5	17.5*	19.5**	10.6	11.2	11.9**	13.4**

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Pale spots and liver masses were noted amongst 3000 ppm females at the 104 week kill and there was a dose-related increase in hepatocellular changes at the 104 week kill, as summarised in the table below.

Table 25: Incidence of selected microscopic non-neoplastic findings in the liver after 104 weeks exposure to isopyrazam (decedents)

Finding	Dietary Concentration of Isopyrazam (ppm)							
	Males				Females			
	0	100	500	3000	0	100	500	3000
Total number examined (decedents)	52 (15)	52 (14)	52 (11)	52 (12)	52 (14)	52 (17)	52 (22)	52 (19)
Hypertrophy, hepatocellular, centrilobular (decedents)	0 (0)	12 (0)**	45 (6)**	49 (11)**	0 (0)	22 (5)**	49 (19)**	50 (17)**
Vacuolation, hepatocellular, centrilobular (decedents)	3 (2)	7 (1)	32 (6)**	39 (6)**	1 (1)	0 (0)	18 (4)**	3 (0)
Pigment, hepatocellular, centrilobular (decedents)	0 (0)	0 (0)	1 (0)	32 (6)**	3 (0)	34 (12)**	49 (19)**	46 (16)**
Hyperplasia, bile ducts (decedents)	3 (1)	11 (2)*	19 (2)**	12 (1)**	15 (4)	12 (5)	17 (9)	23 (6)
Fibrosis, bile ducts (decedents)	1 (1)	5 (1)	10 (0)**	12 (1)**	6 (1)	6 (2)	12 (5)	11 (3)
Altered hepatocytes, eosinophilic (decedents)	7 (1)	10 (2)	23 (3)**	32 (3)**	9 (2)	15 (3)	26 (11)**	29 (7)**

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Further microscopic findings were noted in the kidney tubules (brown pigment) at a higher incidence than controls (18/52) at 500ppm (30/52*) and 3000ppm (42/52**) at the terminal kill. The only other necropsy finding was an increase in the incidence of sinus erythrocytosis in the mesenteric lymph nodes of high dose males at the terminal kill (14/52** compared with 3/52 in controls).

Neoplastic findings

There were no treatment-related neoplastic findings at the interim kill (52 weeks). In the main study (104 weeks) there was an increase in the incidence of uterine adenocarcinomas and liver hepatocellular adenomas in females at 3000ppm (233 mg/kg bw/d). In males there was an increase in the incidence of thyroid follicular cell adenoma at the high-dose. Neoplastic findings at the 104 week kill are summarised below.

Table 26: Incidence of selected microscopic neoplastic findings at 52 & 104 weeks

Finding	Dietary Concentration of Isopyrazam (ppm)			
	0	100	500	3000
Total number of animals examined (includes 12 at interim kill, and 52 up to 104 weeks)	64	64	64	64
Males: thyroid follicular cell adenoma				
Terminal sacrifice and intercurrent deaths (n/52)	1	4	2	6
Incidence at interim kill (n/12)	0	0	0	1
Females: thyroid follicular cell adenoma				
Terminal sacrifice and intercurrent deaths (n/52)	5	1	3	3
Incidence at interim kill (n/12)	0	0	0	2
Females: hepatocellular adenoma				
Terminal sacrifice and intercurrent deaths (n/52)	0	1	1	11
Incidence at interim kill (n/12)	0	0	0	0
Females: uterine endometrial adenocarcinoma				
Terminal sacrifice and intercurrent deaths (n/52)	1	2	3	15
Incidence at interim kill (n/12)	0	0	0	0

Thyroid follicular cell adenoma

The incidence of thyroid follicular cell adenomas in males only at 3000 ppm was 11.5% which is statistically significantly higher than that of the concurrent controls; however, there was no evidence of a dose response (total incidence was 1/52, 4/52, 2/52 and 6/52 at 0, 100, 500 and 3000 ppm respectively). Furthermore, no increase in carcinomas was noted. Limited historical data was available for this strain of rat from the concurrent laboratory in the appropriate time period, owing to a change in the species used for carcinogenicity studies. The HCD available provided a range of 1.6% to 9.6% based on just three previous carcinogenicity studies (conducted between 2007-2009). Further historical control data was available from the Registry of Toxicology Animal (RITA) database and was based on 104 studies conducted on Wistar rats between 1983 and 2004; although the studies from this database were not conducted in the concurrent laboratory and span a time period greater than ±5 years of the current study, it is considered that these data provide supporting evidence of the propensity of this rat strain to develop this type of tumour (i.e., up to an incidence of 28%). Therefore, the increase in thyroid follicular cell adenomas observed in males of the high-dose group is only marginally outside the values for the laboratory's historical control data and falls well within the historical control values of the RITA database for this strain of rat. Therefore the dossier submitter

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

concludes that this is a spurious finding and not related to treatment with isopyrazam. This conclusion is supported by the absence of a clear dose-response relationship in tumour incidence.

Hepatocellular adenoma

The incidence of hepatocellular adenomas was increased at 3000ppm in females (11/52 compared with 0/52 in the control group). A non-genotoxic MoA for isopyrazam-induced liver hepatocellular adenomas has been proposed, for which supporting information is available (see section 10.10.3).

Uterine endometrial adenocarcinoma

The incidence of uterine endometrial carcinoma in females at 3000ppm was 28.8% (total incidence 15/52); the incidence in the control animals was just 1/64 at terminal kill. Only limited laboratory historical control data is available from the appropriate time period, which provides a substantially lower upper incidence for this carcinoma in Wistar rats (up to 7.8% based on 3 studies, conducted over a two-year period covering 2007-2009). Further historical control data is available from the RITA database on 32 studies conducted on the Wistar rat between 1997 and 2005. This reports incidences of up to 28%, with an average incidence of 6.7%. Although the RITA historical control data is not strictly relevant as it was not conducted in the concurrent laboratory and covers a period of time broader than ± 5 years of the current study, it does provide supporting evidence of the propensity of this strain of rat to spontaneously develop this type of tumour up to an incidence of 28%. A non-genotoxic MoA has been proposed for this tumour (supporting data can be found in section 10.10.3).

Overall the dossier submitter concludes that the increases in the incidence of hepatocellular adenoma and uterine carcinoma, both in female rats, are treatment related. The genotoxicity profile for this substance is negative (see section 10.8), suggesting a non-genotoxic MoA. Therefore, additional studies have been undertaken in order to investigate potential non-genotoxic modes of action and their relevance to humans. These are summarised in section 10.10.3.

10.10.2 Carcinogenicity study in mice

Isopyrazam (93:7 syn:anti) was administered to male and female mice for 80-weeks at doses of 0, 70, 500 & 3500 ppm, which equated to mean intakes of 0, 8, 56 & 433 mg/kg bw/d in males and 0, 10, 75 & 554 mg/kg bw/d in females.

Non-neoplastic findings

There was no effect on the survival rate of the mice and the only treatment-related sign of toxicity was a discharge from the eyes of the high-dose males. Body weights throughout the study were lower than controls for both sexes at 3500ppm and by the end of the study were 23% and 11% lower in males and females respectively. There was no effect on food consumption but food utilisation efficiency was reduced for both sexes at 3500ppm and additionally for females at 500ppm. There was no effect on haematology parameters. Relative liver weights were increased in both sexes at 500 and 3500ppm when compared with controls, whilst relative kidney (females) and spleen (both sexes) weights were reduced at 3500ppm.

Table 27: Intergroup comparison of mean organ weights (g)

Organ	Dietary Concentration of Isopyrazam (ppm)							
	Males				Females			
	0	70	500	3500	0	70	500	3500
Liver – adjusted ^a	1.97	1.98	2.24** (13.7%)	2.78** (41%)	1.30	1.30	1.39** (6.9%)	1.79** (37.7%)
Kidney - adjusted	0.50	0.49	0.50	0.52	0.38	0.38	0.38	0.35** (-7.9%)
Spleen – adjusted ^b	0.100	0.100	0.098	0.083* (-17%)	0.106	0.115	0.102	0.086* (-18.9%)

Adjusted = weight adjusted for final bodyweight ^aexcludes animals with selected microscopic findings in the liver

^bexcludes animals with malignant lymphoma/extramedullary haematopoiesis

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

The increases in liver weights were accompanied by non-neoplastic findings of hepatocellular hypertrophy in both sexes, with a predominantly mid-zonal distribution in males and periportal distribution in females. There were no microscopic findings that could account for the decreases in the relative weights of the kidneys and spleen and so these were unlikely to be related to treatment with isopyrazam. Other microscopic findings included eosinophilic droplets in the gall bladder epithelium and inflammation/exudate in the nasolacrimal duct, which was accompanied by macroscopic findings and was likely to be related to the discharge observed during the clinical examination.

Table 28: Intergroup comparison of selected microscopic non-neoplastic findings

Finding	Dietary Concentration of Isopyrazam (ppm)							
	Males				Females			
	0	70	500	3500	0	70	500	3500
Number of animals examined	50	50	50	50	50	50	50	50
Liver – hepatocellular hypertrophy – mid-zonal	0	0	5	40	0	0	0	0
hepatocellular hypertrophy – periportal	0	0	0	2	0	0	13	47
Gall bladder – eosinophilic droplets in the epithelium	14	7	12	15	14	11	15	25
Nasal cavity Nasolacrimal ducts – inflammation/exudate	6	9	13	38	5	4	4	6

Neoplastic findings

Exposure to isopyrazam had no effect on the incidence, appearance or onset of tumours in male or female mice.

10.10.3 Other information relevant for carcinogenicity

Data is available to assess the proposed MoA and the human relevance of the liver hepatocellular adenomas and uterine endometrial adenocarcinomas.

10.10.3.1 Liver hepatocellular adenoma

Limited evidence was available to support a possible carcinogenic response in rat livers; an increase in hepatocellular adenomas was observed in female rats at the high-dose of 3000 ppm (11/52 female rats at the high-dose presented with hepatocellular adenomas compared with 0/52 in the control group and 1/52 in the mid- and low-dose groups). There was no increase in the incidence of carcinomas and the observed increase in adenomas was not seen in male rats or in mice of either sex. In female rats the increase in hepatocellular adenomas only occurred at the high-dose in the presence of systemic toxicity which was characterised by an 18.8% reduction in mean body weight.

There are various possible mechanistic explanations for this apparent weak carcinogenic response in high-dose female rats, including genotoxicity, PPAR α receptor activation, AhR receptor activation, CAR/PXR receptor activation, oestrogenic stimulation, Statins, cytotoxicity, immunosuppression, porphyria and increased apoptosis.

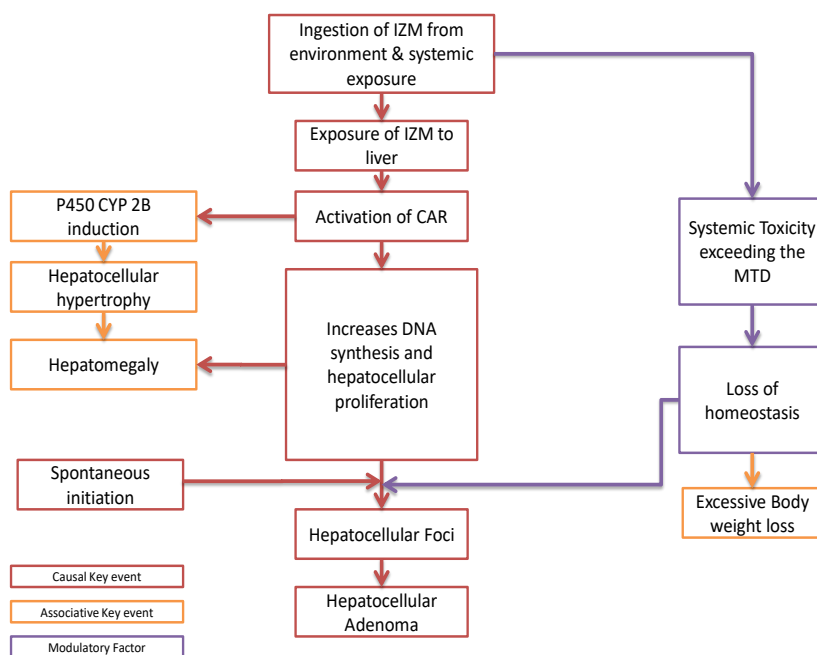
Proposed Mode of Action

A document has been provided by the applicant, outlining the MoA and human health relevance assessment of the increased incidence of liver tumours in female Han Wistar rats, dosed with isopyrazam (see Annex II). This document outlines a phenobarbital-like MoA which involves the activation of the constitutive androgen receptor (CAR) as the most likely cause. This mechanism is generally accepted to be implausible in humans on a qualitative basis (Elcombe *et al*, 2014).

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

The proposed MoA for the development of liver tumours in female rats is summarised below:

IZM = isopyrazam



After exposure of the liver to sufficiently high free concentrations of isopyrazam from the systemic circulation, activation of the constitutive androstane receptor (CAR, NR1I1) results in an increased expression of its target genes' messenger RNA (mRNA), including those that regulate xenobiotic metabolism, DNA proliferation and the cell cycle; consequently, it is proposed that isopyrazam activated CAR results in increased DNA synthesis, increased proliferation and ultimately hepatocellular adenoma.

The dose-response of CAR-promoted key events and tumours is likely to be modulated by any perturbation of normal homeostasis (i.e. alterations to normal physiology), including doses that produce systemic toxicity exceeding the maximum tolerated dose (MTD), such as that demonstrated by marked decreases in bodyweight and body-weight gain in females, throughout the entire two-year rat carcinogenicity study.

Summary of supporting data for the key events of proposed Mode of Action

The CAR activation step of the MoA also induces a variety of xenobiotic metabolism genes (e.g. P450 CYP 2B) which results in smooth endoplasmic reticulum proliferation, leading to hepatocyte hypertrophy and hepatomegaly (when combined with mitogenesis). These events associated with CAR activation serve as measurable markers for this key event in the proposed MoA. Hence CAR activation can be demonstrated by the measurement of these associated markers. For example, increased P450 CYP2B activity (indirectly measured by PROD and BROD activity), hepatocellular hypertrophy and increased liver weight along with increased DNA synthesis and cell proliferation all provide supportive evidence for the proposed MoA. *In vitro* and *in vivo* mechanistic data to support the proposed MoA and to exclude human relevance are available and are summarised below.

In-vitro studies with rat hepatocytes**Table 29: In vitro study demonstrating enzyme induction, cytotoxicity and cell proliferation in Wistar rat hepatocytes**

Test substance, test system	Conc/dose, replicates, duration of exposure	Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Positive controls: Phenobarbital (PB) & epidermal growth factor (EGF) Vehicle control: DMSO Cultured hepatocytes from female Han Wistar Rats (same strain as the carc. study) Non GLP compliance (abides to GLP principles)	Isopyrazam: 1 – 100 μ M Phenobarbital: 10 – 1000 μ M 96-hours' exposure <u>CYP2B activity</u> 3 replicates <u>Proliferation</u> 5 replicates, 1500 hepatocytes/conc <u>ATP depletion</u> 6 replicates	Cytotoxicity assay	Cytotoxicity of isopyrazam demonstrated by ATP depletion from 65 μ M (effects observed at non-cytotoxic concentrations)	Ellcombe (2011)
		Enzyme Induction	Isopyrazam: ↑↑ PROD (6.6- & 4.1-fold increase at 10 & 30 μ M) ↑ BROD (1.4- to 2.2-fold increase at 3 – 30 μ M) Phenobarbital: ↑↑ PROD (4.0- to 5.8-fold increase at 100 & 1000 μ M) ↑ BROD (2- to 2.6-fold increase at 100-1000 μ M)	
		Replicative DNA Synthesis	Isopyrazam: ↑ hepatocyte proliferation (at all concentrations, maximum 2.6 times control at 3 μ M) Phenobarbital: ↑ hepatocyte proliferation (2.4-fold at 1000 μ M)	

In cultured rat hepatocytes isolated by *in situ* perfusion, treatment with isopyrazam at concentrations of 10 and 30 μ M resulted in a 6.6- and 4.1-fold increase in PROD activity; for comparison purposes, phenobarbital at concentrations of 100 and 1000 μ M induced a 4.0- and 5.8-fold increase in activity. There was a 1.4- to 2.2-fold increase in BROD activity after treatment of the hepatocytes with 3 – 30 μ M isopyrazam, corresponding to a 2- to 2.6-fold increase with phenobarbital. Significant increases in DNA synthesis were observed in the rat hepatocytes at all concentrations tested; up to a maximum of 2.6 times control at 3 μ M (the increase with phenobarbital was 2.4-fold at 1000 μ M).

In-vitro studies with human hepatocytes**Table 30: In vitro study demonstrating enzyme induction and lack of proliferation in human hepatocytes**

Test substance, test system	Conc/dose, replicates, duration of exposure	Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Positive controls: PB and EGF Vehicle control: DMSO Cryopreserved, cultured human hepatocytes; one female donor; viability > 70% Non GLP compliance (abides to GLP principles)	Isopyrazam: 1 – 100 μ M Phenobarbital: 10 – 1000 μ M 96-hours' exposure 3 replicates 5 replicates, 1500 hepatocytes/conc 6 replicates	Cytotoxicity assay	Cytotoxicity of isopyrazam demonstrated by ATP depletion from 65 μ M (effects observed at non-cytotoxic concentrations)	Ellcombe (2011a)
		Enzyme Induction	Isopyrazam: ↑ PROD (max. 1.7-fold at 30 μ M) ↑↑ BROD (2.9-, 3.8-, 4.2- & 4.9-fold at 1, 3, 10 & 30 μ M) Phenobarbital: ↑ PROD (1.8-fold at 1000 μ M) ↑↑ BROD (6.3-fold at 1000 μ M) Hepatocyte proliferation absent	
		Replicative DNA Synthesis	Isopyrazam and Phenobarbital: Hepatocyte proliferation absent EGF: Induced proliferation	

When the same concentrations of isopyrazam were applied to human hepatocytes under the same conditions, PROD activity was increased to a magnitude of 1.7-fold at 30 μ M whilst BROD activity was increased at 1, 3, 10 and 30 μ M to an extent of 2.9-, 3.8-, 4.2- and 4.9-fold control levels respectively (for phenobarbital, PROD and BROD activity was increased at 1000 μ M to 1.8- and 6.3-fold respectively). Neither isopyrazam nor phenobarbital produced a proliferative response in human hepatocytes. The capacity of the human hepatocytes to produce a proliferative response was confirmed by the addition of EGF which produced such a response.

In vivo studies in rats**Table 31: In vivo study measuring enzyme induction and cell proliferation in rats**

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Aim, results/remarks
Repeated dose mode-of-action study in rats Non GLP compliance (abides to GLP principles) Anonymous (2011)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Rats, Han Wistar [CrL:WI(Han)] (same strain as carc. Study), female, 10/dose Peroxisomal & microsomal subcellular fractions	0, 500 & 3000 ppm Equivalent to: 0, 58 & 327 mg/kg bw/d 3-, 7- & 14-days' exposure <u>Measurements:</u> Peroxisome proliferation (palmitoyl co-enzyme A metabolism) Total CYP (reduced-CO binding spectra of CYP) CYP1A (ethoxyresorufin-O deethylase (EROD)) CYP2B (PROD) CYP3A ([14C]-testosterone 6 β -hydroxylation) CYP4A ([14C]-lauric acid 12-hydroxylation) Cell proliferation (uptake of BrdU into the nucleus of S-phase cells)	General toxicity
			There were no treatment-related deaths or clinical signs of toxicity 327 mg/kg bw/d <u>3-days</u> ↓ BW gain (-45%*), ↓ FC, ↑ liver weights (16%**), ↑ mitosis <u>7-days</u> ↓ BW gain (-25%**) ↑ liver weights (15%**), centrilobular hypertrophy (5/10 minimal) <u>14-days</u> ↑ liver weights (24%**), centrilobular hepatocellular hypertrophy (5/10 minimal, 5/10 mild) 58 mg/kg bw/d <u>3-days</u> ↑ liver weights (20%**) <u>7-days</u> ↑ liver weights (6%**) <u>14-days</u> ↑ liver weights (10%**) Enzyme induction There were no treatment related effects on peroxisome proliferation, CYP3A or CYP4A 327 mg/kg bw/d <u>3-days</u> Total P450: ↑ 1.6-fold CYP2B (PROD): ↑ 346-fold CYP1A (EROD): ↑ 2.1-fold <u>7-days</u> Total P450: ↑ 1.5-fold CYP2B (PROD): ↑ 162-fold CYP1A (EROD): ↑ 2.5-fold

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Aim, results/remarks
			<p><u>14-days</u></p> <p>Total P450: ↑ 1.6-fold CYP2B (PROD):↑ 340-fold CYP1A (EROD): ↑ 2.7-fold</p> <p>58 mg/kg bw/d</p> <p><u>3-days</u></p> <p>Total P450: ↑ 1.8-fold CYP2B (PROD):↑ 303-fold CYP1A (EROD): ↑ 3.3-fold</p> <p><u>7-days</u></p> <p>Total P450: ↑ 1.5-fold CYP2B (PROD):↑ 110-fold CYP1A (EROD): ↑ 2.5-fold</p> <p><u>14-days</u></p> <p>Total P450: ↑ 1.5-fold CYP2B (PROD):↑ 209-fold CYP1A (EROD): ↑ 2.4-fold</p> <p>Proliferation</p> <p>↑ liver cell proliferation after 3-days: 3000 ppm group mean s-phase labelling index (%) was increased 2-fold after 3-days and returned to control levels at days 7 and 14</p> <p>There was no evidence of cell proliferation at 500 ppm at any time-point</p>

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Liver enzyme activity and cell proliferation were also measured *in vivo* in rat microsomes, after 3-, 7- and 14-days' exposure. A non-carcinogenic (500ppm/58 mg/kg bw/d) and a carcinogenic dose (3000ppm/327 mg/kg bw/d) were selected to reflect those of the main two-year carcinogenicity study in rats. Both doses resulted in significant increases in total microsomal CYP content comprising an increase in EROD activity (a marker of CYP1A) and PROD activity (a marker of CYP2B). The increase in PROD activity (up to several hundred-fold of control activity levels) was much greater than the increase in EROD activity which was up to ~3-fold control activity (a large increase in EROD (CYP1A) would indicate activation of AhR rather than CAR) Activation of PPAR α was discounted by the lack of a significant increase in lauric acid 12-hydroxylation (CYP4A) in the microsomes and peroxisomal palmitoyl Co-A oxidase activity in the peroxisomes at either 500 or 3000 ppm. No increase in testosterone 6 β -hydroxylation (a marker of CYP3A activity) was noted at any concentration. Liver cell proliferation was assessed by measuring the uptake of BrdU into the nuclei of cells undergoing S-phase; a two-fold increase was observed at 3000ppm after three days of treatment and had returned to control levels thereafter. No increase in proliferation occurred at 500ppm.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Overall, these studies demonstrate that isopyrazam activates CAR in both rat and human female hepatocytes, and that this activation results in proliferation in rat but not human cells.

Other supporting evidence

In addition to the specific studies investigating MoA, liver enzyme investigations were also carried out as part of the 28-day repeated-dose toxicity studies in rats (see section 10.12). P450 isoenzyme (7-ethoxyresorufin (EROD) and 7-pentoxoresorufin O-depentyldase (PROD)) activity was measured. Increases in total P450 activity (particularly of PROD) were noted after treatment with isopyrazam.

In the *in vivo* mechanistic study, the two-year carcinogenicity study and several repeated-dose toxicity studies, CAR activation was evidenced by findings of dose-related increases in the incidences of hepatocellular hypertrophy and increases in liver weights. Similar findings were observed in mice but did not result in tumour formation in this species. The formation of hepatocellular foci (a key event in the proposed mode-of-action) was noted in both sexes in the 2-year carcinogenicity study in rats; in this study eosinophilic foci were noted in 7/52, 10/52, 23/52 & 32/52 males (without tumour formation) and 9/52, 15/52, 26/52 & 29/52 females (with tumour formation) in the control-, low-, mid- and high-dose groups respectively. The fact that hepatocellular foci formation also occurred in males in the absence of tumour development, means that this key event has not been sufficiently demonstrated; however, a measurable increase in foci formation at carcinogenic doses is not always observed, and is dependent on several factors, including the specific properties of the test substance itself.

Relevance to humans

The proposed non-genotoxic mode of action, which involves stimulation via the CAR receptor leading to increased proliferation, has been shown to be of little relevance to humans (Elcombe *et al* 2014). Furthermore, it has been demonstrated that whilst isopyrazam-mediated CAR activation occurred to a similar extent in both rat and human hepatocytes, only in rats did this lead to increased DNA synthesis and proliferation. The human hepatocytes used in this study originated from only one female donor and were therefore not broadly representative of the human population; however, positive control agents have previously shown consistency across human hepatocytes with regards to a lack of known CAR activators and so a single human donor is considered acceptable (Peffer *et al*, 2018). Therefore it is considered that the relevance to humans of isopyrazam-mediated CAR activation has been adequately excluded.

Biological plausibility and the consideration of other possible Modes of Action

The key events in the proposed isopyrazam mediated CAR activation MoA are similar to those already established for phenobarbital and other related compounds. When considering the plausibility of a MoA, other possible MoA should be excluded. Overall, nine other possible mechanisms for the formation of liver tumours have been described in the literature. The following table outlines these and considers their plausibility with regard to isopyrazam.

Table 32: Consideration of alternative MoA

Alternative MOA	Reason for Exclusion
Genotoxicity	Isopyrazam was negative in six <i>in vitro</i> investigations of genetic toxicity (bacterial mutation - Callander 2006 and Sokolowski 2008; <i>in vitro</i> cytogenetics- Fox 2006a and Bohnenberger 2008; Mammalian cell gene mutation Clay 2006 and Wollny 2008) and two <i>in vivo</i> tests to investigate genetic toxicity (rodent micronucleus - Fox 2006b and rodent liver UDS - Fox 2006c).
PPAR α receptor activation	Isopyrazam did not increase peroxisomal palmitoyl Co-A oxidase or P450 Cyp4a activities in liver peroxisome and microsome preparations respectively (Murchison 2010)
AhR receptor activation	Isopyrazam did not produce a large increase in P450 Cyp1a EROD activity in liver microsomes (Milburn 2007a and Murchison 2010)
Estrogenic stimulation	Isopyrazam was not estrogenic in an Oestrogen Receptor transactivation assay <i>in vitro</i> (Toole 2011) or uterotrophic <i>in vivo</i> in the ovariectomised rat (Kuhl 2011) (see section 10.9.3.1)
Statins	Isopyrazam was not designed to inhibit HMG-CoA reductase, nor is there any evidence to suggest that cholesterol levels were increased in rats (Milburn 2007a,c, 2008)
Cytotoxicity	Isopyrazam did not produce elevations in markers of hepatocyte damage nor was there any evidence of cytotoxicity or regenerative proliferation (Milburn 2007a,b,c,d, 2008, and Shearer 2008)
Immunosuppression	Isopyrazam did not produce any signs of infection, cytotoxicity and regenerative proliferation in micropathology (Milburn 2007a,b,c,d, 2008, and Shearer 2008)
Porphyria	Isopyrazam did not produce elevations in hepatocyte damage and regenerative proliferation in micropathology or clinical chemistry (Milburn 2007a,b,c,d, 2008, and Shearer 2008)
Increased apoptosis	Isopyrazam did not increase rates of apoptosis and regenerative proliferation as determined by micropathology in multiple studies (Milburn 2007a,b,c,d, 2008, and Shearer 2008)

Conclusion

Overall, it has been experimentally demonstrated that the liver induction profile of isopyrazam is consistent with CAR/PXR activation; The induction of CYP2B (PROD & BROD) and CYP3A (BROD) have been demonstrated *in vitro* and the induction of PROD has been demonstrated *in vivo* in a specific enzyme induction study along with several other investigations. Increased BrdU labelling has been demonstrated in rat (both *in vitro* and *in vivo*) but not human hepatocytes. Furthermore, in all studies an increase in liver weights has been accompanied by hepatocellular hypertrophy, which is indicative of this MoA. The dossier submitter therefore concludes that owing to qualitative differences, the hepatocellular adenomas found in rats as a result of CAR activation by isopyrazam, are of little relevance to humans; hence it would not be appropriate to classify isopyrazam on the basis of these liver tumours.

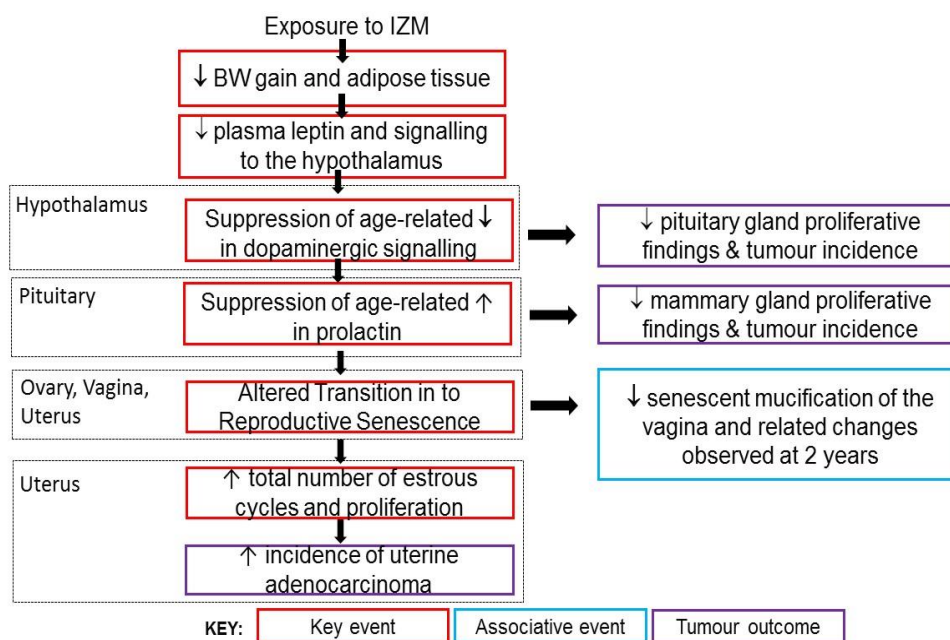
10.10.3.2 Uterine endometrial adenocarcinoma

The incidence of uterine endometrial carcinoma was increased in females at 3000ppm compared to the concurrent control and the limited laboratory historical control data. There are several possible mechanisms by which the observed increase in uterine tumours could have been mediated, including genotoxicity, oestrogenicity, effects on the dopamine transporter, effects on oestrogen metabolism in the uterus and a delay into reproductive senescence. The latter has been proposed as the most likely mode of action and all other potential MoA have been adequately excluded (refer to table 39). The shift in tumour profile observed in high-dose rats (increased uterine tumours and decreased mammary and pituitary tumours) and the proposed mechanism is a recognised biological phenomenon which is rat specific (Harleman et al 2012). Furthermore, this mode of action in the induction of uterine tumours has been repeatedly shown to occur as a result of dietary restriction in rats (Roe et al 1995, Tucker 1979).

Proposed Mode-of-Action

The proposed MoA for the observed shift in the incidence of uterine endometrial adenocarcinomas in the female Han Wistar rat is summarised below:

IZM = isopyrazam



In normal ageing rats (from approximately 12-months) blood levels of prolactin are elevated after the usual dopamine-mediated inhibition of its release is diminished (dopaminergic neurones are lost in the hypothalamus of ageing rats via a process of senescence). Elevated levels of prolactin stimulate continued progesterone synthesis; thus the normal state of the ageing female rat is high levels of prolactin and progesterone and low levels of oestrogen, follicle stimulating hormone (FSH) and luteinising hormone. The maintenance of this low oestrogen: progesterone ratio protects against endometrial cancer, owing to a decreased oestrogenic proliferative drive on the uterus; a coincident increase in mammary and pituitary tumours also occurs in the normal ageing rat as a consequence of senescence.

In the isopyrazam treated rats (high-dose group) of the two-year carcinogenicity study, along with the increase in the incidence in uterine endometrial adenocarcinomas there was a corresponding decrease in the incidence of mammary gland fibroadenomas and pituitary adenomas. A large sustained decrease in body-weight gain (up to 30-40%) was noted in females at the top-dose (the only dose at which alterations in the incidence of these tumours were observed), which continued throughout the duration of the study. It is proposed that the decrease in body-weight gain was a consequence of a loss of adipose tissue, which has the potential to cause a delay in the normal age-related loss of tuberoinfundibular dopaminergic (TIDA) neurons

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

of the hypothalamus. As the treated rats age, the retention of a greater number of functional TIDA neurons would result in the continued production of dopamine, thus suppressing prolactin release from the anterior pituitary (via activation of the dopamine-2 (D2) receptor). In isopyrazam treated rats, this delay in the physiological age-related increase in prolactin affects the timing of progression into reproductive senescence, subsequently exposing the uterus to a higher oestrogen: progesterone ratio. The result of this continued exposure to higher oestrogen/lower progesterone over time would lead to a pro-proliferative oestrogenic stimulation of the uterine endometrial cells, the increased promotion of spontaneous tumours and ultimately an increase in the observed incidence of uterine endometrial adenocarcinomas. Maintenance of higher dopamine levels also blocks proliferative changes in the pituitary and would account for the lower incidence of pituitary adenoma, whilst the lower prolactin released by the pituitary would explain the observed decline in the incidence of mammary fibroadenomas in the high-dose treated groups.

The applicant has provided a document outlining the proposed MoA and human relevance of the uterine endometrial adenocarcinomas observed in female rats (see Annex III). The key data are summarised below.

Summary of supporting data for proposed mode-of-action

To evaluate the key events of the proposed MoA, an 18-month investigative toxicity study has been conducted in which female Han Wistar rats were administered isopyrazam for 13, 26, 52, 66 and 80 weeks, the doses reflecting those of the 2-year carcinogenicity study (0, 500 and 3000ppm equating to 0, 28 & 194 mg/kg bw/d).

Table 33: Summary table of 18-month study in the rat investigating the MoA and human relevance of uterine endometrial adenocarcinomas induced by isopyrazam

Type of study/data	Test substance, test system	Methods	Aim, results/remarks
18-month investigative study in the female Han Wistar rat GLP Anonymous (2018)	Isopyrazam (purity 96.4%) Rats, Han Wistar [CrL:WI(Han)], female, 145/group	Isopyrazam: 0, 500, 3000 ppm Equivalent to 0, 28 & 194 mg/kg bw/d 13, 26, 52, 66 and 80 weeks' exposure	Aim: To support the proposed MoA for the development of uterine tumours. There were no treatment-related deaths or clinical signs of toxicity <u>Control</u> Age related ↑ ↑ in prolactin and leptin <u>3000ppm (isopyrazam)</u> ↓ BW gain (-31.7%**), ↓ final BW (-20.6%**), ↓ absolute fat pad weight (max -59% wk. 66), ↓ FC (last 4-months) ↓ oestrous cycle duration (from wk. 29) compared with controls ↓ prolactin, ↓ leptin (until week 80) corresponds to ↑ in dopamine (26.3% at wk. 26) and dihydroxyphenylacetic acid (29.4% at wk. 52 & 30% at wk. 80) ↑ liver weight <u>500ppm (isopyrazam)</u> ↓ BW gain (-8.9%; last 6-m only) ↓ final BW (-5.4%) ↑ prolactin (age-related), ↑ leptin (age-related; week 52 compared with day 28) ↑ liver weight

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Relevant information can also be derived from the previously described two-year rat carcinogenicity study (table 23), the retained tissues of which have been further investigated to explore the signalling pathways of the MoA. The information gathered from this investigation.

Table 34 Investigation of retained tissue from the rat two-year chronic toxicity and carcinogenicity study (refer to table 23 for study details)

Type of study/data	Test substance, test system	Methods	Aim, results/remarks
Isopyrazam – Evaluation of pituitary prolactin and hypothalamic tyrosine hydroxylase by immunohistochemistry and <i>in situ</i> hybridisation Anonymous (2015b)	Formalin fixed paraffin embedded (FFPE) tissues from the 2-year rat carcinogenicity study	0, 500 & 3000 ppm <u>RNA Scope <i>in situ</i> hybridisation (ISH)</u> To quantify mRNA of tyrosine hydroxylase (TH) – enzyme in dopamine synthesis-measured in tuberoinfundibular dopaminergic (TIDA) neurons of the hypothalamus <u>Immunohistochemistry (IHC)</u> To quantify prolactin (PRL) protein expression in the anterior pituitary (PRL IHC) & to quantify dopaminergic neurons in the hypothalamus (TH IHC) <u>Quantitative image analysis</u> To measure PRL protein expression in the anterior pituitary	Aim: To explore the signalling pathways of the proposed MoA using tissues from the 2-year rat carcinogenicity study Higher levels of TH mRNA and protein at 3000ppm compared with controls No differences in prolactin (PRL) expression in the anterior pituitary between control, 500 and 3000ppm treated groups, suggesting that the production of prolactin is normal and just its release (dopamine mediated) is affected. Isopyrazam is associated with delayed senescence of dopaminergic neurons in the TIDA region of the hypothalamus.
Isopyrazam – evaluation of hypothalamic tyrosine hydroxylase in control female Wistar rats at 3, 12 or 24 months by immunohistochemistry and <i>in situ</i> hybridisation Anonymous (2015a)	Formalin fixed paraffin embedded (FFPE) tissues from the control animals of the 90-day rat study and the 2-year rat carcinogenicity study	<u>RNA Scope <i>in situ</i> hybridisation (ISH)</u> To quantify tyrosine hydroxylase (TH) mRNA in the arcuate nucleus (ARC) and median eminence (ME) areas of the TIDA <u>Immunohistochemistry (IHC)</u> To detect TH protein in the ARC and ME areas of the hypothalamus	Aim: To determine the normal changes over time in the number of dopaminergic neurons TH proteins were lower for the 2-year animals compared with the 1-year animals; TH proteins were higher in the 1-year animals compared with the 90-day animals TH mRNA was lower in the 2-year animals than in the 1-year animals; no statistically significant differences between the 1-year and 90-day animals mRNA and protein expression of TH decreases progressively with age (from 1- to 2-years) in control rats

Key event one: body-weight gain deficit and loss of adipose tissue

In the 18-month investigative toxicity study, the first key event in the MoA hypothesis was demonstrated by a reduction in body-weight gain and final body weight when compared with controls (-31.7%** and -20.6%** respectively) in the 3000ppm isopyrazam treated group, along with a particularly marked reduction in absolute fat- pad weights (-59%** in week 66); the decrease in body-weight gain and final body weight for the 500ppm isopyrazam treated group was less than 10% when compared with controls. In the 2-year rat carcinogenicity study there was a large deficit in body-weight gain throughout the study in the 3000 ppm treated group, to a magnitude of 40% lower than controls by the end of the study. Although there were some small effects on food consumption in the initial weeks of the two-year carcinogenicity study, food consumption in females treated with 3000 ppm (232.8 mg/kg bw/d) was broadly similar to controls; similarly, in the 18-month investigative study food consumption, although erratic, was generally similar to controls throughout the treatment period. Hence, in both studies, a marked reduction in food utilisation efficiency was indicated. An increase in the incidence of uterine tumours (with a concomitant reduction in the incidence of mammary and pituitary tumours) as a consequence of body-weight loss has been previously demonstrated in rats after dietary restriction (particularly in the Wistar rat). In fact, as a consequence of reductions in body-weight gain, because of calorific restriction, both Sprague Dawley and Wistar rats showed reductions in mammary and pituitary tumours, but only in Wistar rats was there an increase in uterine tumours under such conditions (Harleman *et al* 2012).

Key event two: decrease in plasma leptin and signalling to the hypothalamus

In accordance with the hypothesised MoA, body weight/adipose tissue losses should lead to a reduction in plasma leptin and signalling to the hypothalamus. Circulating leptin levels were measured in the 18-month investigative study using a rat leptin enzyme-linked immunoassay (EIA) kit. The expected age-related increases in plasma leptin were evident in the control and the 500ppm isopyrazam treated animals from week 52 onwards (statistically significant compared with the day-28 values). In animals of the 3000ppm group this increase was delayed until week 80 of treatment (at all ages the mean leptin values were numerically smaller than control values with statistical significance being reached at weeks 52, 66 and 80). Although the expected age-related increases in plasma leptin occurred in animals treated with 500ppm isopyrazam, plasma leptin values were still statistically significantly lower than controls at certain time-points in these animals (although to a lesser magnitude and duration than those observed in the 3000ppm animals). Adiponectin (hormone involved in fatty acid breakdown) levels between control and treated groups showed no discernible differences.

Key event three: Suppression of age-related decrease in dopaminergic signalling

The proposed MoA postulates that treatment with 3000ppm isopyrazam preserves dopaminergic signalling in the TIDA neurons of the hypothalamus as the rats age in comparison with the physiological reduction in signalling which occurs in ageing control animals. The TIDA neurons' cell bodies are situated in the arcuate nucleus (ARC) region of the hypothalamus with their axons extending into the median eminence (ME) region. Dopamine is synthesised in the TIDA neurons and released from the ME to reach the anterior pituitary. In the 18-month investigative study, the median eminence (ME) of the hypothalamus was isolated, extracted and analysed to determine the concentrations of dopamine (DA) and its metabolite 3, 4 dihydrophenylacetic acid (DOPAC). During each statistically significant event in the 3000ppm isopyrazam treated group, an elevation of mean DA or DOPAC was observed. In this group DOPAC levels were higher than controls at week 52 (29.4%*), week 66 (15.9%) and week 80 (30%**); corresponding to the lower leptin values observed at these time-points; DOPAC and DA values were also higher than controls at week 26 (31% and 26.3%* respectively). DOPAC and DA values for the 500ppm group showed much smaller differences from the controls and none were of statistical significance. Dopamine turnover was not affected in control and treated groups and the DA/DOPAC ratio was similar across all groups.

The age-related decrease in the number and activity of dopaminergic neurons in normal ageing rats has been investigated via measurement of tyrosine hydroxylase (TH), which is the rate limiting step in the synthesis of dopamine. The relative abundance of TH in control rats sacrificed at 90-days, 1-year and 2-years (using preserved tissues from the 90-day repeated-dose toxicity study and the 2-year carcinogenicity study) was assessed by *in-situ* hybridisation (ISH) which selectively stained mRNA for TH, and by

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

immunohistochemistry (IHC) which selectively stained for TH protein. TH mRNA levels at 2-years were statistically significantly lower than those at 90-days and TH protein was statistically significantly lower at 2-years than at 1-year in these control rats, suggesting that tyrosine hydroxylase and therefore dopamine synthesis reduces over time in normal ageing female rats. When tyrosine hydroxylase in the tissues of isopyrazam treated animals was analysed using the same methods (500 & 3000ppm treated groups from the 2-year carcinogenicity study), there was a statistically significant higher level of TH mRNA in the ARC and the combined ARC and ME regions when compared with controls as well as statistically significant higher levels of TH protein in the ARC (3000ppm) and the combined ARC and ME regions (500 & 3000 ppm) with evidence of a dose-response. Hence it has been demonstrated that there is a higher capacity for dopamine production in the TIDA neurons of rats treated with 3000ppm isopyrazam compared with controls, after 2-years of treatment.

Key event four: suppression of age-related increase in prolactin

Plasma prolactin (PRL) levels were measured in the 18-month study by radioimmunoassay (RIA) in samples taken after 4, 13, 26, 52, 66 and 80 weeks. It was demonstrated that an age-related increase in plasma prolactin observed in the control and 500ppm isopyrazam treated animals was delayed by treatment with 3000ppm isopyrazam after 66- and 80-weeks' treatment (statistical significance was reached at week 66). With the delay in the rise in prolactin levels there was a concomitant delay in the onset of reproductive senescence; this is discussed in further detail below.

Key events five and six: altered transition to reproductive senescence, increased total number of oestrus cycles and proliferation

The 18-month investigative study monitored the pattern and onset of reproductive senescence in female Han Wistar rats as well as the effect of isopyrazam treatment. This was facilitated by the daily measurement of oestrus cycling status over alternate 2-week intervals (i.e. two weeks with daily measurements alternated with two weeks without). An oestrus cycle is expected to measure between 4 and 5 days in normal sexually mature rats. The four stages that could potentially be observed via vaginal lavage are proestrus (P) during which ovulation occurs, oestrus (E), metestrus (M) and diestrus (D). The assessment of oestrus cycling took account of the duration of each cycle and the number/frequency of cycles in a given period, along with the number of days that an animal is in an oestrogenic state (E or P). Only complete cycles were included (total number of returns to M or D from E or P from the beginning to the end of the sampling period). All animals showed mean cycle lengths of approximately four days for the first 25 days of treatment and then all showed a tendency for fewer animals with complete cycles and longer mean cycle durations at week 29. From weeks 29-43 animals treated with 3000ppm isopyrazam had a greater number of complete oestrus cycles compared with the controls and during the last three sampling intervals (weeks 68-69, 73-74 and 77-79) there was a tendency for 3000ppm isopyrazam treated groups to have shorter mean cycling durations than the other groups (at weeks 77-79 high-dose animals had a mean cycle duration of 6.1 days compared with 7.7 and 7.8 days in the control and 500ppm groups respectively). The control group showed the expected physiological response of females entering reproductive senescence with an initial increase in the percentage of days in an oestrogenic state (between sampling weeks 3-4 and 42-43) followed by a slow decline. In contrast, in 3000 ppm animals, the percentage of days in an oestrogenic state continued to increase up to weeks 55-57 before showing a slow decline. In the 3000ppm group there was a lower incidence of "reproductive cycle alteration" a term which is assigned when oestrous cycling cannot be determined owing to the onset of reproductive senescence. Relevant findings to the evaluation of oestrus cycling are summarised in the table below:

Table 35: Summary of findings from the evaluation of oestrus cyclicity

Parameter	0 ppm (control)	500 ppm isopyrazam	3000 ppm isopyrazam
Oestrus cycle length weeks 0-51 (days)	3.9-4.8	4.0-4.9	4.0-4.7
Oestrus cycle length weeks 52-80 (days)	5.5-7.7	5.3-7.8	4.7-6.1
Animals with complete cycles – weeks 0-25	86.4% - 100%	82.4% - 99.3%	89.6% - 100%
Animals with complete cycles – weeks 29-57	50.5% - 100%	44.2% - 81%	48.6% - 93.3%
Animals with complete cycles – weeks 60-80	37.5% - 44.8%	26.7% - 62.1%	24.2% - 62.5%
Peak oestrogenic state (% E + % P)	59.8%	63.5%	67.3%
Timing of peak oestrogenic state	42-43 weeks	50-51 weeks	55-57 weeks

Histopathological findings demonstrating a difference across groups in the progression of animals into reproductive senescence were also reported. In the 500 and 3000 ppm isopyrazam treated groups these comprised a lower incidence of vaginal/cervical mucification at week 80 (an associative event) and a lower incidence of atrophy in the uterus/vagina; however in both these cases a dose-response was not evident. There was a lower incidence of lobuloalveolar hyperplasia of the mammary gland in the 500 ppm (week 52) and 3000 ppm (week 66) when compared with controls but no changes were noted at week 80. There were no consistent differences in the incidence of hyperplasia or proliferative lesions in the pituitary or the uterus/cervix.

Tumour outcome

The pattern of alterations in the incidence of uterine, mammary and pituitary tumours in the 2-year rat carcinogenicity study supports the proposed MoA hypothesis. Uterine tumours were increased, whilst mammary and pituitary tumours were decreased at the top-dose of 3000ppm (232.8 mg/kg bw/d).

Table 36: Incidence of Uterine Endometrial Tumours, Pituitary Adenoma and Mammary Fibroadenoma in the Combined Chronic Toxicity and Carcinogenicity Study with Isopyrazam

Histopathology findings	2-year carcinogenicity study			
	0 ppm	100 ppm (6.9 mg/kg/day)	500 ppm (34.9 mg/kg/day)	3000 ppm (232.8 mg/kg/day)
Uterus (n)	(64)	(64)	(64)	(64)
ADENOMA [B]	1	0	1	0
ADENOCARCINOMA [M]	1	2	3	15**
Mammary Gland (n)	(63)	(64)	(63)	(64)
FIBROADENOMA [B]	14	16	9	4*
Pituitary Gland (n)	(64)	(64)	(64)	(64)
ADENOMA [B]	33	34	28	24

^aData from 64 animals per group. [B] = benign; [M] = malignant. *, ** statistically significant by pairwise Fisher's Exact Test (p<0.05, 0.01)

Table 37: Historical Control Data for uterine tumours in Wistar Rats

	Historic Control Data - Range	
Uterine tumours:	Lab (CTL)	RITA
Adenomas	0-1.6%	0-6%
Adenocarcinomas	1.9-7.8%	0-28%

Lab (Syngenta Central Toxicology Laboratory; CTL) data refers to 3 studies conducted at CTL from 2007-2009 . RITA (Registry of Industrial Toxicology Animal Data) data refers to 32 studies conducted in the Wistar rat from 1997-2009

The proposed MoA and the resulting pattern of alterations in uterine, mammary and pituitary tumours are well described in the literature (Harleman *et al* 2012). The Han Wistar rat is reported as being particularly susceptible to these changes following significant reductions in body weights; this is demonstrated in the 2-year carcinogenicity study in which the observed shift in tumour profile was related to the magnitude of the effect on body-weight gain.

Relevance to humans

There are fundamental differences between humans and rats with regard to both hormonal control of the hypothalamic-pituitary-gonad (HPG) axis and the process of transitioning into reproductive senescence. Furthermore the human reproductive (menstrual) cycle has markedly different control mechanisms compared with the 4-5 day cycle of rats (see table below).

Table 38: Reproductive cycle regulation in rats and humans

	Rat	Human
Prolactin surge	Occurs in proestrus	No significant changes during cycle
Luteal phase length	Short (1-day)	Long (14- to 16-days)
Role of prolactin in Luteal phase	Luteotrophic which rescues new CLs and ↑ progesterone synthesis	None; mediated by LH (initially) & chorionic gonadotropin (in pregnancy)
	Luteolytic which recruits macrophages to degrade prior CLs – prolactin surge in proestrus	None

The prolactin surge that occurs in rats during proestrus is not observed in humans. In rats the normal luteal phase is only one day and the new corpora lutea will regress; however maintenance of a higher prolactin level (i.e. on mating) rescues the corpora lutea and stimulates it to produce further prolactin. In contrast, human corpora lutea are maintained by luteinising hormone (LH) during a menstrual cycle and also by chorionic gonadotropin from the placenta (in pregnancy). In rats, luteolysis is under the partial control of prolactin whilst luteotrophic actions of prolactin do not occur in humans. With regard to reproductive senescence processes, in Wistar rats the onset of senescence is driven by a progressive decrease in the activity of dopaminergic (TIDA) neurons in the hypothalamus and a consequential loss of dopamine-mediated inhibition of prolactin. Prolactin levels are thus elevated resulting in a luteotrophic effect on the corpora lutea, leading to elevated progesterone and lower oestrogen in the blood. This regulation does not occur in humans; in contrast menopause and reproductive senescence in humans are driven by an eventual depletion (with age) of the limited number of primordial follicles in the ovaries and are associated with a marked decrease in circulating oestrogen and progesterone. The state of persistent oestrus is unique to rats with no equivalent state in humans.

Exclusion of other possible MoA

To support the proposed Mode of Action, other alternatives have been evaluated in the context of the existing data and excluded. Data supporting the exclusion of alternative MoA is summarised in the table below.

Table 39: Data supporting the exclusion of alternative MoA

Type of study/data	Test substance, test system	Methods	Aim, results/remarks
Oestrogenicity (<i>in vitro</i>)			
Stably transfected human oestrogen receptor- α transcriptional activation, <i>in vitro</i> Non-GLP compliance (abides to GLP principles) Toole (2011)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 hER α -HeLa-9903 cell line	10 ⁻¹² – 10 ⁻⁵ M 24-hours' exposure	Aim: To exclude an estrogenic effect as an alternative MoA Isopyrazam was not an agonist of the human oestrogen receptor when tested up to cytotoxic concentrations Positive control substances (17 α -estradiol and 17 β -estradiol) gave the expected response Isopyrazam shows no estrogenic potential <i>in vitro</i> under the conditions of this study
Oestrogenicity (<i>in vivo</i>)			
Uterotrophic assay in ovariectomised rats, gavage OECD 404 Non-GLP compliance (abides to GLP principles) Anonymous (2011)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Vehicle: 0.5% CMC Rats, , Han Wistar [CrL:WI(Han)], female, 6/group	300 mg/kg bw/d 3-days' exposure	Aim: To exclude an estrogenic effect as a potential alternative MoA ↓ BW, ↓ FC No effect on relative uterus weight when compared with control Positive control substance (17 α -ethynylestradiol) gave the expected result Isopyrazam shows no estrogenic potential <i>in vivo</i> under the conditions of this study
Dopamine agonist/effects on the dopamine transporter (<i>in vitro</i>)			
Isopyrazam – effects on dopamine transport <i>in vitro</i> Robinson (2013)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Rat striatal synaptosomes and radio ligand [³ H]-dopamine	3, 10, 30 & 100 μ M (triplicates) Incubation time: 15 mins Positive control: GBR-12909	Aim: To evaluate the ability of isopyrazam to inhibit [³ H]-dopamine uptake into the synaptosomes (mediated by dopamine transporter proteins) ≥50% inhibition indicates activity Mean % inhibitions of [³ H]-dopamine uptake were - 10.9%, -15.91%, 20.27% and 100.33% at concentrations of 3, 10, 30 & 100 μ M Isopyrazam is an inhibitor of dopamine transport <i>in vitro</i> at a concentration of 100 μM

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Type of study/data	Test substance, test system	Methods	Aim, results/remarks
Isopyrazam – <i>in vitro</i> dopamine transporter binding assay Jolas (2015)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Human recombinant dopamine transporter (from CHO cells expressing human DAT1 gene)	3, 10, 30 & 100 µM (triplicates) in 1% DMSO Positive control: Non titrated BTCP 0.1nM – 3 µM	Aim: To determine the potential of isopyrazam to bind the dopamine transporter <i>in vitro</i> Binding was assessed by displacement of the known binder of the dopamine transporter [³ H] – benzothiophenylcyclohexylpiperidine Greater than 50% inhibition of control specific binding was observed at concentrations ≥ 30 µM Isopyrazam binds to the dopamine transporter <i>in vitro</i> at a concentration of ≥ 30 µM
Isopyrazam and CSCD459488 – effects on dopamine transport and dopamine D2 receptor ligand binding <i>in vitro</i> Weismann (2012)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 CSCD459488 Synaptosomes and radio ligand [³ H]-dopamine	10 µM (triplicate)	Aim: To evaluate the effects of isopyrazam and its metabolite CSCD459488 on dopamine transport and dopamine D2 receptor ligand binding <i>in vitro</i> The binding of a single concentration of labelled ligand in the presence of a concentration of unlabelled test item (competes with labelled ligand for receptor binding). ≥ 50% inhibition indicates activity <u>Isopyrazam</u> Mean % of radiolabelled ligand binding was 27% and 6% for dopamine transport and dopamine D2 receptor assays <u>CSCD459488</u> Mean % of radiolabelled ligand binding was 1% and 14% for dopamine transport and dopamine D2 receptor assays Neither test substance was an inhibitor of dopamine transport or dopamine D2 receptor <i>in vitro</i>
Dopamine agonist/effects on the dopamine transporter (<i>in vivo</i>)			
Isopyrazam – a study to assess the effects on the 17β-Estradiol induced prolactin surge in Han Wistar rats Anonymous (2015)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Han Wistar rats, female, ovariectomised, n=12 treated group/n=13 control	0 & 300 mg/kg bw Vehicle/control: 0.5 ml carboxymethylcellulose (CMC) Gavage, 5 ml/kg bw 3-days' exposure, E2 administered on third day to induce prolactin surge Plasma PRL levels measured by radioimmunoassay	Aim: To test the hypothesis that isopyrazam inhibits the dopamine transporter <i>in vivo</i> (inhibitors of the dopamine transporter will suppress a 17β-Estradiol (E2) mediated prolactin surge). Isopyrazam did not suppress E2 mediated prolactin surge in ovariectomised Han Wistar rats Isopyrazam is not an inhibitor of the dopamine transporter <i>in vivo</i>

Genotoxicity

The genotoxic potential of isopyrazam has been thoroughly investigated in a standard battery of well-conducted *in vitro* and *in vivo* tests; conclusive negatives in all of these tests allow the exclusion of this potential MoA.

Oestrogenicity

One possible mode of action in the induction of uterine tumours is the binding to/activation of the oestrogen receptors (ER α and ER β) by either the parent compound or its metabolites; it was demonstrated that, when tested up to cytotoxic concentrations, isopyrazam was not an agonist of the human oestrogen receptor *in vitro* (Toole, 2011). This was confirmed *in vivo* when no effect on uterine weight was observed in ovariectomised Han Wistar rats after administration of isopyrazam, despite reductions in mean body weights and food consumption throughout the study (Anonymous, 2011). Therefore this MoA can be excluded.

Dopamine agonist/effects on dopamine transport

Compounds acting as dopamine agonists (such as bromocriptine) can decrease prolactin levels in rats and thus lead to increases in uterine tumours and corresponding decreases in mammary and pituitary tumours. The effect of isopyrazam on dopamine transport and dopamine D2 receptor binding has been measured *in vitro* in rat striatal synaptosomes. The ability of isopyrazam to inhibit the binding of a radiolabelled ligand to the D2 receptor was measured, with > 50% inhibition indicating activity. Isopyrazam was inactive in this assay as inhibition was found to be 27% and 6% for dopamine transport and ligand binding respectively. Two further *in vitro* assays, one investigating binding to the dopamine transporter and the other investigating effects on dopamine transport were conducted. In the first, isopyrazam was found to bind to the dopamine transporter *in vitro*, with greater than 50% inhibition at ≥ 30 μg . In the second assay a 100 μM concentration of isopyrazam inhibited the uptake of radio ligand [^3H]-dopamine into the synaptosomes by 100.33%. As an inhibition of uptake of $\geq 50\%$ is considered a positive result, isopyrazam is a dopamine inhibitor under the conditions of this study. This could potentially be owing to an indirect effect on the synaptosomal integrity and not a direct inhibiting effect and so a definitive *in vivo* study was conducted. Isopyrazam was tested for its potential to inhibit the dopamine transporter by way of facilitating an inhibition of the E2 mediated prolactin surge in Han Wistar rats. In this assay isopyrazam and its metabolite CSCD459488 were shown not to suppress this prolactin surge and hence did not inhibit the dopamine transporter *in vivo*. It has been adequately demonstrated that isopyrazam is not a dopamine agonist and does not affect dopamine transport, thus allowing the exclusion of this potential MoA.

Prolactin secreting tumours in the anterior pituitary

According to the literature, the majority of rat pituitary hyperplasias and adenomas are functional prolactin-producing tumours (Kovacs *et al.* 1977). The formalin fixed paraffin embedded (FFPE) tissues from the 2-year rat study have been examined to evaluate prolactin protein expression using immunohistochemistry (IHC). No significant differences in the expression of prolactin protein were observed between the control and treated groups (in control animals 22/30 anterior pituitary adenomas were prolactin positive compared with 20/25 from the high dose group). Therefore the ability of these tumours to secrete prolactin is unlikely to be the cause of the uterine tumours and can therefore be excluded.

Oestrogen metabolism in the uterus

Modulation of oestrogen metabolism in the uterus could potentially lead to higher net oestrogenic stimulation of the uterus and ultimately uterine adenocarcinomas. An example is 17 β -estradiol which when hydroxylated to 4-hydroxyestradiol has a stronger carcinogenic potential via oxidative stress and DNA damage on the uterus, kidney and pituitary. Cytochrome P450s (predominantly CYP1B1) are responsible for the hydroxylation of 17- β -estradiol.

Isopyrazam increases metabolic capacity in the liver via CAR activation and induction of cytochrome P450 enzymes (see section 10.9.3.1). It was demonstrated *in vitro* that isopyrazam elevated levels of CYP2B and CYP3A activity (indirectly measured by associated markers); *in vivo* it was shown that isopyrazam increased total hepatic microsomal CYP content. The administration of isopyrazam in the diet for 14-days (500 and 3000ppm) resulted in a significant increase in the metabolism of 17- β -estradiol to 2- and 4- estradiol in liver microsomes, however no changes in uterine CYP1B1 were observed. This suggests that the increase in

oestrogen metabolism is a result of CYP2B/3A microsomal enzyme induction, secondary to CAR activation and that peripheral induction of estradiol hydroxylation does not occur in isopyrazam treated rats. Furthermore the observed shift in tumour profile is inconsistent with this MoA. Contrary to the reduction in mammary and pituitary tumours observed after isopyrazam exposure, such a MoA would be expected to cause an increase in tumours in the oestrogen sensitive mammary and pituitary tissues. Therefore this MoA can be excluded.

Conclusion

Overall the proposed MoA for the observed increase in the incidence of uterine tumours is plausible. This MoA is not relevant to humans and so therefore classification for carcinogenicity on the basis of the increased incidence of uterine tumours in rats is not recommended.

10.10.4 Short summary and overall relevance of the provided information on carcinogenicity

The carcinogenic potential of isopyrazam has been investigated in two life-time feeding studies, one in rats and one in mice. Toxicity was evident at the high-dose (mice) and at the mid- and high-dose (rats), characterised by reduced body-weight gain, lower overall body weights and reduced food consumption. In mice, isopyrazam had no effect on the appearance, onset or incidence of tumours. In female rats there was a treatment-related increase in the incidence of uterine endometrial adenocarcinomas and liver hepatocellular adenomas. With regard to the liver adenomas, a plausible MoA has been established involving CAR activation, the human relevance of which has been experimentally excluded. Other possible modes of action for the induction of liver tumours were excluded.

The increase in the incidence of uterine adenocarcinomas occurred at doses that induced toxicity far above the maximum tolerated dose (MTD), demonstrated by reductions in overall body weights of approximately 40%. The increase in uterine tumours was accompanied by a decrease in mammary and pituitary tumours, a tumour profile which is characteristic of the proposed MoA. The large reductions in body weights (mainly adipose tissue) in the high-dose group were likely to have ultimately resulted in delaying the progression of affected animals into reproductive senescence; it is presumed that this then led to an increase of oestrogen over progesterone and an increased proliferative drive on the uterus. An 18-month mechanistic study in Wistar rats has been conducted and (along with other *in vitro* and *in vivo* mechanistic data) adequately demonstrates the stages of the proposed MoA. The human relevance of this MoA has been excluded because the onset of reproductive senescence in humans is driven by the gradual depletion of primordial follicles in the ovaries, leading to an overall reduction in progesterone and oestrogen; the age-related loss of dopamine-mediated prolactin inhibition does not occur in humans. Furthermore, other potential modes of action have been excluded.

Overall, there is adequate and convincing evidence to indicate that alterations in the incidence of tumours observed in female rats after administration of isopyrazam resulted from modes of action that are not relevant to humans. On the basis of the available evidence, therefore, the dossier submitter concludes that isopyrazam is not carcinogenic to humans.

10.10.5 Comparison with the CLP criteria

Specific studies have been conducted which have established the MoA for both the liver and uterine tumours in rats. Furthermore it has been demonstrated that both these modes of action are not of relevance to humans. Therefore it would not be appropriate to classify isopyrazam for carcinogenicity.

10.10.6 Conclusion on classification and labelling for carcinogenicity

Not classified (conclusive but not sufficient for classification)
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RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS presented two carcinogenicity studies in the CLH report, one in mice and one in rats. The study in mice showed no neoplastic findings; while the study in rats showed neoplastic findings in thyroid, liver, uterus, mammary gland and thyroid gland. Only uterine and hepatocellular tumours were considered as treatment-related. However, for both types of tumours, the CLH report presents specific studies conducted for establishing modes of action not relevant for humans. In conclusion, the DS proposed no classification of isopyrazam for carcinogenicity.

Comments received during consultation

One company/manufacturee agreed with the DS's proposal for no classification of isopyrazam for carcinogenicity and submitted attachments with additional information. This same company/manufacturee released a second similar comment providing a summary of the submitted documents in the text. DS thanked and replied that this additional information should be considered by RAC. RAC notes that the position paper entitled "*Isopyrazam-Human Relevance Framework Assessment of Liver Tumour Induction in Female Rats*" was prepared with the aim to assess a hypothesised mode of action for isopyrazam-induced liver tumours using the framework developed by the International Programme on Chemical Safety (IPCS) and the International Life Science Institute (ILSI). RAC has taken this paper into consideration in its assessment but notes that is quite similar to Annex 2 of the CLH report.

One MSCA raised concerns about the relevance of liver tumours for humans considering that the key events relationships of the constitutive androstane receptor (CAR) mediated mode of action have not been established by a weight of evidence analysis. Moreover, this MSCA also raised the question that alternative modes of actions such as sustained aryl hydrocarbon receptor (AhR) activation leading to rodent liver tumours; inhibition of inducible nitric oxide synthase (iNOS), hepatotoxicity, and regenerative proliferation leading to liver tumours; or mutagenic mode-of-action leading to hepatocellular carcinoma have not been ruled out. Overall, this MSCA considered that classification as Carc. 2 is more appropriate than the no classification proposed by the DS.

A second MSCA also proposed classification of isopyrazam as Carc. 2 in line with the argumentation for classification of sedaxane, as sedaxane and isopyrazam are structural analogues and both induce liver and uterine tumours. Moreover, this MSCA also demanded a careful analysis of isopyrazam carcinogenic effects in the light of the succinate dehydrogenase inhibition. The DS replied that a robust assessment of the data on isopyrazam had not been presented at the time of assessment of sedaxane by RAC and now the full data on isopyrazam should be given due consideration. The classification of isopyrazam cannot be automatically extrapolated from the sedaxane classification because the database is not identical and because isopyrazam contains some mechanistic studies not previously considered for the case of sedaxane; for example, the mechanistic 18-month study in rats. RAC supports the DS's view that the data should be given full consideration, however, notes that isopyrazam mechanistic information including the 18-months study and evaluation of hypothalamic

tyrosine hydroxylase in samples from 13-week dietary and 2-year carcinogenicity studies, was indeed submitted also for sedaxane assessment. The same Mode of action for uterine carcinoma was claimed for both substances. The 18-month investigative isopyrazam study was submitted as new data, with a detailed weight of evidence document describing the Mode of action and human non-relevance of uterine tumours. At that time RAC carefully looked at all information provided, including the isopyrazam data, to understand the proposed Mode of action hypothesis.

The DS also replied that there is no convincing evidence that succinate dehydrogenase inhibitors induce liver or uterine tumours since a genetic defect of succinate dehydrogenase gene in humans lead to encephalopathies and cardiomyopathies in addition to the fact that isopyrazam is extensively metabolised, and therefore it is unlikely that it is able to induce a sustained inhibition over time.

A third MSCA also raised concerns about the capability of succinate dehydrogenase inhibitors to induce uncontrolled proliferation of cells causing cancer. This same MSCA also raised concerns about the uncertainties that remains regarding the mode of action of isopyrazam for induction of liver carcinomas. The concerns being based on the lack of data from *in vitro* CAR/PXR assay, gene expression, CAR-knock out animals and the fact that hepatocytes from a single donor were used.

Finally, the MSCA highlighted uncertainties also regarding the mode of action of isopyrazam for induction of uterine tumours. These uncertainties were addressed towards key events 3, 5 and 6. In conclusion, the MSCA considered that the classification of isopyrazam as Carc. 2 is warranted. The DS replied in similar terms to those stated above for the second MSCA.

A fourth MSCA expressed concerns on all the justifications provided by the DS questioning whether the induction of CYP could be caused as an adaptative response of the liver, the target organ of isopyrazam, the mode of action behind these carcinogenic effects And whether all relevant uncertainties have been clarified?

Assessment and comparison with the classification criteria

The US EPA classifies isopyrazam as "*Likely to be Carcinogenic to Humans*" based on increased incidence of uterine endometrial adenocarcinomas and liver hepatocellular adenomas in female rats and increased incidence of thyroid follicular cell adenomas and/or carcinomas in male rats.

According to the EFSA opinion 2012 the long-term exposure to isopyrazam produces liver hepatocellular adenomas and uterine endometrial adenocarcinomas in rats and therefore proposed the classification as a carcinogen category 3, R40 "*limited evidence of a carcinogenic effect*".

Carcinogenicity study in mice (Anonymous 2008b)

Isopyrazam (93:7 *syn:anti*) was administered to male and female mice for 80-weeks at doses of 0, 70, 500 and 3500 ppm, which equals to mean intakes of 0, 8, 56 and 433 mg/kg bw/day in males and 0, 10, 75 and 554 mg/kg bw/day in females. There was no effect on the survival rate of the mice and the only treatment-related sign of toxicity was a discharge from the eyes of the high dose males. Table 6 summarises the non-neoplastic findings of this study. Exposure to isopyrazam had no effect on the incidence, appearance or onset of tumours in male or female mice.

Two-year dietary toxicity and carcinogenicity study in rats and histological extension (Anonymous 2008a and Anonymous 2009)

In a two-year combined chronic and carcinogenicity study in rats, isopyrazam 93:7 *syn:anti* was administered for either 52 (12/sex/group) or 104 (52/sex/group) weeks at dietary concentrations of 0, 100, 500 and 3000 ppm. These doses equal mean intakes of 0, 5.5, 28 and 173 mg/kg bw/day in males and 0, 7, 35 and 233 mg/kg bw/day in females. Table 5 summarises the non-neoplastic findings of this study.

Neoplastic findings

There were no treatment-related neoplastic findings at the interim kill (52 weeks). In the main study (104 weeks), there was a dose-dependent increase in the incidence of uterine adenocarcinomas (low-to high dose groups) and an increase in liver hepatocellular adenomas in females at 3000 ppm (233 mg/kg bw/day). In males, there was an increase in the incidence of thyroid follicular cell adenoma at the high dose.

Thyroid follicular cell adenoma

The incidences of thyroid cell tumours are shown in the Table 10. The incidence of thyroid follicular cell adenomas in males at 3000 ppm was 12%; which is statistically significantly higher than that of the concurrent controls. However, there was no evidence of a dose response (total incidence was 1/52, 4/52, 2/52 and 6/52 at 0, 100, 500 and 3000 ppm respectively). Furthermore, no increase in carcinomas was noted. The historical control data (HCD) available provided a range of 1.6% to 9.6% based on just three previous carcinogenicity studies (conducted between 2007-2009).

Table 10: Thyroid follicular cell tumours in the 2-year carcinogenicity study in rats.

Parameter	Dietary isopyrazam (ppm)				HCD
	0	100	500	3000	
Number of organs examined	52	52	52	52	
Males					
Adenoma	1	4	2	6 (12%)*	Performing facility: 1.6- 9.6% (3 studies 2007- 2009)
Carcinoma	0	0	5	0	
Females					
Adenoma	5	1	3	3	
Carcinoma	0	1	0	0	

*Statistically significant increase, p not available.

RAC does not consider thyroid follicular cell adenomas in males relevant for classification purposes based mainly on the lack of dose-response and supported by the fact that the increase of incidence is only marginally outside the values for the laboratory's historical control data.

Hepatocellular adenoma

The incidences of liver tumours are shown in the Table 11. The incidence of hepatocellular adenomas was increased at 3000 ppm in females (11/52 (21%) compared with 0/52 in the control group) and a few adenomas were reported for the high dose males. Statistical assessment has not been provided in the CLH report, however, the incidence in females is clearly above the HCD of the performing facility. There are also single incidences of hepatocellular carcinoma in the high dose males and females, while the HCD incidence for the

carcinoma is zero. A non-genotoxic mode of action for isopyrazam-induced liver hepatocellular adenomas has been proposed (see sections below).

Table 11: Hepatocellular tumours in the 2-year carcinogenicity study in rats. Statistical assessment was not provided.

Parameter	Dietary isopyrazam (ppm)				HCD
	0	100	500	3000	
Number of organs examined	52	52	52	52	
Males					
Adenoma	1	0	0	3	
Carcinoma	0	0	0	1	
Females					
Adenoma	0	1	1	11 (21%)	Performing facility: 0-1.9% (3 studies 2007-2009)
Carcinoma	0	0	0	1	0% (3 studies 2007-2009)

Uterine endometrial adenocarcinoma

The incidence of uterine tumours is shown in Table 12. The incidences of uterine endometrial carcinoma in females were zero for all groups at the interim sacrifice (12 animals/group), while at terminal sacrifice of the 52 animals a dose-dependent increase with 1/52, 2/52, 3/52, 15/52 for control, low, mid and high dose group, respectively, is reported, the high dose of 3000 ppm shows an incidence of 29%); the incidence in the control animals was just 1/52 at terminal kill. Statistical assessment has not been provided in the CLH report. HCD of the performing laboratory from the appropriate time period provides a substantially lower upper incidence for this carcinoma in Wistar rats (range 1.9-7.8%, no mean available) and there is no indication that the concurrent control group is not reliable and thus considered the most relevant control for the assessment for the findings. A non-genotoxic mode of action has been proposed for this tumour (see sections below).

Table 12: Uterine tumours in the 2-year carcinogenicity study in rats. Statistical assessment was not provided.

Parameter	Dietary isopyrazam (ppm)				HCD
	0	100	500	3000	
Number of organs examined	52	52	52	52	
Adenoma	1	0	1	0	
Carcinoma	1	2	3	15 (29%)	Performing facility: 1.9-7.8% (3 studies 2007-2009)

Other neoplastic incidences

The Table 13 provides a summary of neoplastic findings in mammary and pituitary glands. In both organs, the incidences of both adenomas and carcinomas was not statistically different from the incidences of controls. No tumour induction and thus no concern is identified for these two glandular tissues. RAC notes that tumour incidences were reduced at the mid and high doses. The observation that mammary and pituitary gland tumour incidences were concomitantly reduced with the increased uterine tumour incidences, is a similar finding as for the structural analogue sedaxane, and thus it may be treatment-related. The reduction of mammary and pituitary gland tumour incidence is considered by the applicant a relevant and treatment related tumour outcome in the Mode of action hypothesis for uterine adenocarcinomas induced by isopyrazam.

Table 13: Mammary and pituitary tumours in the 2-year carcinogenicity study in rats.

Parameter	Dietary isopyrazam (ppm)				HCD
	0	100	500	3000	
Number of organs examined	52	52	52	52	
Mammary tumours - Females					
Adenoma	14	16	9	4	Not available
Carcinoma	2	7	5	0	Not available
Pituitary tumours - Females					
Adenoma	32	33	28	24	Not available
Carcinoma	0	1	0	0	Not available

Conclusion:

Overall, RAC concludes that the increases in the incidence of hepatocellular adenoma and carcinomas in males and females and the uterine carcinoma with a dose-response relationship are treatment related. The genotoxicity profile for this substance is negative (see section about germ cell mutagenicity), suggesting a non-genotoxic mode of action.

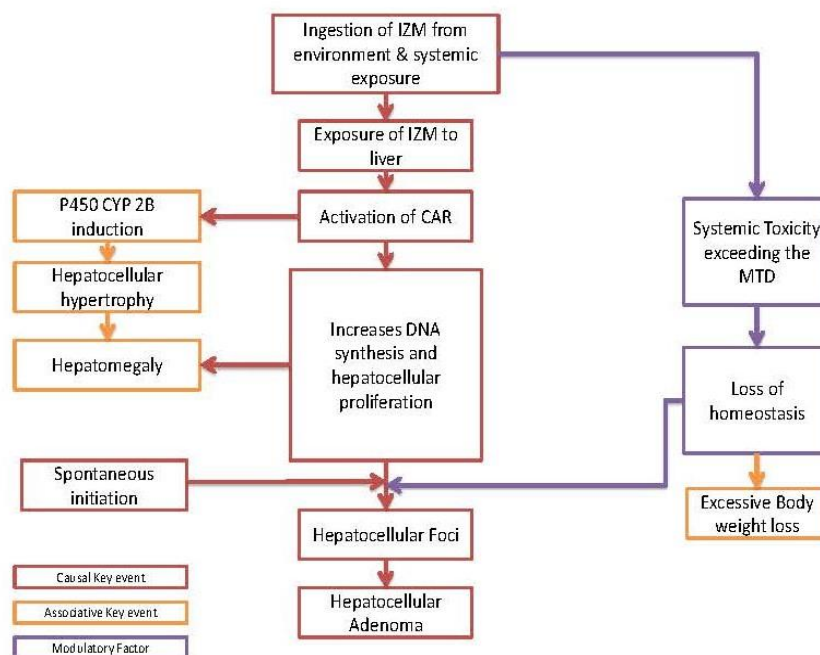
PROPOSED MODE OF ACTION FOR LIVER HEPATOCELLULAR ADENOMAS

There are various possible mechanistic explanations for this weak carcinogenic response in high dose female rats, including genotoxicity, peroxisome proliferator activated receptor alpha (PPAR α) receptor activation, AhR receptor activation, CAR/PXR receptor activation, oestrogenic stimulation, statins, cytotoxicity, immunosuppression, porphyria and increased apoptosis.

Annex 2 to the CLH report contains a document provided by the applicant describing the mode of action and the human health relevance assessment of the increased incidence of liver tumours in female Han Wistar rats dosed with isopyrazam. This document outlines a phenobarbital-like mode of action that involves the activation of the CAR as the most likely cause. This mechanism is generally accepted to be implausible in humans on a qualitative basis (Elcombe et. al, 2014).

The proposed Mode of action for the development of liver tumours in female rats is summarised below:

IZM = isopyrazam



After exposure of the liver to sufficiently high free concentrations of isopyrazam from the systemic circulation, activation of the CAR, NR1I1 results in an increased expression of its target genes' messenger RNA (mRNA), including those that regulate xenobiotic metabolism, DNA proliferation and the cell cycle. Consequently, it is proposed that isopyrazam activated CAR results in increased DNA synthesis, increased proliferation and ultimately hepatocellular adenoma.

Mechanistic studies: CAR activation

The CAR activation step of the mode of action also induces a variety of xenobiotic metabolism genes (e.g. P450 CYP 2B) which results in smooth endoplasmic reticulum proliferation, leading to hepatocyte hypertrophy and hepatomegaly (when combined with mitogenesis). These events associated with CAR activation serve as measurable markers for this key event in the proposed mode of action. Hence, CAR activation can be demonstrated by the measurement of these associated markers. Increased P450 Cyp2b activity (indirectly measured by activity of pentoxyresorufin O-dealkylase (PROD) and benzyloxyresorufin O-dealkylation (BROD)), hepatocellular hypertrophy and increased liver weight along with increased DNA synthesis and cell proliferation all provide supportive evidence for the proposed mode of action.

Table 14 summarises *in vitro* experiments with human and rat hepatocytes. RAC notes that isopyrazam and phenobarbital caused increases in the expression of both PROD and BROD in both rat hepatocytes and human hepatocytes (Table 14). However, isopyrazam and phenobarbital triggered cell proliferation in rat hepatocytes but not in human hepatocytes. Data contained in Table 14 suggests that the response of human hepatocytes to isopyrazam is different to the response of rat hepatocytes to this substance because, despite the activation of CAR receptor, human hepatocytes do not undergo cell proliferation. RAC notes that, despite the fact that no proliferation was noted in human hepatocytes in three different donors at three different isopyrazam concentrations (1, 10 and 30 μM), the hepatocytes coming from all these three human donors are able to proliferate when they are exposed to the positive control. RAC also notes that use of *in vitro* hepatocytes from only female rats

leads to some uncertainties on the Mode of action.

Table 14: *In vitro* study for assessing enzyme induction, cytotoxicity and cell proliferation in Wistar rat and human hepatocytes

Test substance, test system, Reference	Conc/dose, replicates, duration of exposure	Type of data	Relevant information about the study (as applicable) (values are compared to controls)																																										
Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w) 93:7 <i>syn:anti</i> Positive controls: Phenobarbital (PB) and epidermal growth factor (EGF) Vehicle control: DMSO Cultured hepatocytes from female Han Wistar Rats (same strain as the carc. study) Cryopreserved, cultured human hepatocytes; viability > 65% Non GLP compliance (abides to GLP principles) Ellcombe (2011) and Ellcombe (2011a)	Isopyrazam: 1-100 µM PB: 10-1000 µM 96-hours exposure <u>CYP2B activity:</u> 3 replicates <u>Proliferation:</u> 5 replicates, 1500 hepatocytes/conc <u>ATP depletion:</u> 6 replicates	Cytotoxicity assay	Both rats and humans: Cytotoxicity of isopyrazam demonstrated by ATP depletion from 65 µM (effects observed at non-cytotoxic concentrations)																																										
		Enzyme Induction CYP2B activity measured by PROD CYP2B/CYP3A activity measured by BROD	Isopyrazam <table border="1"> <thead> <tr> <th></th> <th>Isopyrazam [µM]</th> <th>HUMAN</th> <th>RAT</th> </tr> </thead> <tbody> <tr> <td rowspan="2">PROD</td> <td>10</td> <td>-</td> <td>↑ 4.1</td> </tr> <tr> <td>30</td> <td>↑ 1.7</td> <td>↑ 6.6</td> </tr> <tr> <td rowspan="4">BROD</td> <td>1</td> <td>↑ 2.9</td> <td>-</td> </tr> <tr> <td>3</td> <td>↑ 3.8</td> <td>↑ 1.4</td> </tr> <tr> <td>10</td> <td>↑ 4.2</td> <td>↑ 2.2</td> </tr> <tr> <td>30</td> <td>↑ 4.9</td> <td>-</td> </tr> </tbody> </table> Phenobarbital <table border="1"> <thead> <tr> <th></th> <th>Phenobarbital [µM]</th> <th>HUMAN</th> <th>RAT</th> </tr> </thead> <tbody> <tr> <td rowspan="2">PROD</td> <td>100</td> <td>-</td> <td>↑ 4.0</td> </tr> <tr> <td>1000</td> <td>↑ 1.8</td> <td>↑ 5.8</td> </tr> <tr> <td rowspan="2">BROD</td> <td>100</td> <td>↑ 2.0</td> <td>-</td> </tr> <tr> <td>1000</td> <td>↑ 2.6</td> <td>↑ 6.3</td> </tr> </tbody> </table>		Isopyrazam [µM]	HUMAN	RAT	PROD	10	-	↑ 4.1	30	↑ 1.7	↑ 6.6	BROD	1	↑ 2.9	-	3	↑ 3.8	↑ 1.4	10	↑ 4.2	↑ 2.2	30	↑ 4.9	-		Phenobarbital [µM]	HUMAN	RAT	PROD	100	-	↑ 4.0	1000	↑ 1.8	↑ 5.8	BROD	100	↑ 2.0	-	1000	↑ 2.6	↑ 6.3
			Isopyrazam [µM]	HUMAN	RAT																																								
PROD	10	-	↑ 4.1																																										
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Replicative DNA synthesis	Hepatocyte proliferation <table border="1"> <thead> <tr> <th></th> <th>HUMAN</th> <th>RAT</th> </tr> </thead> <tbody> <tr> <td>Isopyrazam</td> <td>Absent</td> <td>Yes</td> </tr> <tr> <td>PB</td> <td>Absent</td> <td>Yes</td> </tr> <tr> <td>EGF</td> <td>Yes</td> <td>-</td> </tr> </tbody> </table>		HUMAN	RAT	Isopyrazam	Absent	Yes	PB	Absent	Yes	EGF	Yes	-																																
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Mechanistic information: *In vivo* enzyme induction and cell proliferation

The CLH report contains an *in vivo* study in rats devoted to determine enzyme induction and cell proliferation in rats. This study is summarised in Table 15. Two doses (one carcinogenic and the other not; 327 mg/kg bw/day and 58 mg/kg bw/day, respectively) resulted in significant increases in total microsomal CYP content comprising an increase in ethoxyresorufin O-deethylase (EROD) activity (a marker of CYP1A) and PROD activity (a marker of CYP2B). The increase in PROD activity (up to several hundred-fold of control

activity levels) was much greater than the increase in EROD activity that was up to ~3-fold control activity. A large increase in EROD (CYP1A) would indicate activation of AhR rather than CAR. Activation of PPAR α was discounted by the lack of a significant increase in lauric acid 12-hydroxylation (CYP4A) in the microsomes and peroxisomal palmitoyl CoA oxidase activity in the peroxisomes. No increase in testosterone 6 β -hydroxylation (a marker of CYP3A activity) was noted. A two-fold increase of liver cell proliferation was observed at the carcinogenic dose but not at the non-carcinogenic dose after three days of treatment and had returned to control levels thereafter. Overall, RAC notes that this study demonstrated that isopyrazam *in vivo* activates CAR in rat hepatocytes, suggesting the triggering of cell proliferation. RAC also notes that, although weaker, AhR activation is indicated and thus a role of AhR in cell cycle regulation and hepatocyte proliferation cannot be fully ruled out.

Table 15: *In vivo* study measuring enzyme induction and cell proliferation in rats.

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Aim, results/remarks																																																																																	
Repeated dose mode-of-action study in rats Non GLP compliance (abides to GLP principles) Anonymous (2011)	Isopyrazam (batch: SMU6AP001) Purity: 96.4% (w/w) 93:7 syn:anti Han Wistar [CrL:WI(Han)] rats (same strain as carc. study), 10 females/dose Peroxisomal and microsomal subcellular fractions	0, 58 and 327 mg/kg bw/d 3-, 7- and 14-days exposure <u>Measurements:</u> Peroxisome proliferation (palmitoyl co-enzyme A metabolism) Total CYP (reduced-CO binding spectra of CYP) CYP1A (ethoxyresorufin-O deethylase (EROD)) CYP2B (PROD) CYP3A ([¹⁴ C]-testosterone 6 β -hydroxylation) CYP4A ([¹⁴ C]-lauric acid 12-hydroxylation) Cell proliferation (uptake of BrdU into the nucleus of S-phase cells)	General toxicity (in all cases statistically different from control for p<0.01) <table border="1"> <thead> <tr> <th></th> <th colspan="2">mg/kg bw/day</th> </tr> <tr> <th></th> <th>58</th> <th>327</th> </tr> </thead> <tbody> <tr> <td colspan="3"><i>Day 3</i></td> </tr> <tr> <td>↓ Body weight gain</td> <td>-</td> <td>45%</td> </tr> <tr> <td>↑ Liver weights</td> <td>20%</td> <td>16%</td> </tr> <tr> <td>↑ mitosis</td> <td>-</td> <td>YES</td> </tr> <tr> <td colspan="3"><i>Day 7</i></td> </tr> <tr> <td>↓ Body weight gain</td> <td>-</td> <td>25%</td> </tr> <tr> <td>↑ Liver weights</td> <td>6%</td> <td>15%</td> </tr> <tr> <td>Centrilobular hypertrophy</td> <td>-</td> <td>5 (minimal)</td> </tr> <tr> <td colspan="3"><i>14-days</i></td> </tr> <tr> <td>↑ Liver weights</td> <td>10%</td> <td>24%</td> </tr> <tr> <td>Centrilobular hypertrophy</td> <td>-</td> <td>5 minimal + 5 mild</td> </tr> </tbody> </table> Enzyme induction There were no treatment related effects on peroxisome proliferation, CYP3A or CYP4A <table border="1"> <thead> <tr> <th></th> <th colspan="2">mg/kg bw/day</th> </tr> <tr> <th></th> <th>58</th> <th>327</th> </tr> </thead> <tbody> <tr> <td colspan="3"><i>Day 3</i></td> </tr> <tr> <td>Total P450</td> <td>↑ 1.8-fold</td> <td>↑ 1.6-fold</td> </tr> <tr> <td>CYP2B (PROD)</td> <td>↑ 303-fold</td> <td>↑ 346-fold</td> </tr> <tr> <td>CYP1A (EROD)</td> <td>↑ 3.3-fold</td> <td>↑ 2.1-fold</td> </tr> <tr> <td colspan="3"><i>Day 7</i></td> </tr> <tr> <td>Total P450</td> <td>↑ 1.5-fold</td> <td>↑ 1.5-fold</td> </tr> <tr> <td>CYP2B (PROD)</td> <td>↑ 110-fold</td> <td>↑ 162-fold</td> </tr> <tr> <td>CYP1A (EROD)</td> <td>↑ 2.5-fold</td> <td>↑ 2.5-fold</td> </tr> <tr> <td colspan="3"><i>14-days</i></td> </tr> <tr> <td>Total P450</td> <td>↑ 1.5-fold</td> <td>↑ 1.6-fold</td> </tr> <tr> <td>CYP2B (PROD)</td> <td>↑ 209-fold</td> <td>↑ 340-fold</td> </tr> <tr> <td>CYP1A (EROD)</td> <td>↑ 2.4-fold</td> <td>↑ 2.7-fold</td> </tr> </tbody> </table>		mg/kg bw/day			58	327	<i>Day 3</i>			↓ Body weight gain	-	45%	↑ Liver weights	20%	16%	↑ mitosis	-	YES	<i>Day 7</i>			↓ Body weight gain	-	25%	↑ Liver weights	6%	15%	Centrilobular hypertrophy	-	5 (minimal)	<i>14-days</i>			↑ Liver weights	10%	24%	Centrilobular hypertrophy	-	5 minimal + 5 mild		mg/kg bw/day			58	327	<i>Day 3</i>			Total P450	↑ 1.8-fold	↑ 1.6-fold	CYP2B (PROD)	↑ 303-fold	↑ 346-fold	CYP1A (EROD)	↑ 3.3-fold	↑ 2.1-fold	<i>Day 7</i>			Total P450	↑ 1.5-fold	↑ 1.5-fold	CYP2B (PROD)	↑ 110-fold	↑ 162-fold	CYP1A (EROD)	↑ 2.5-fold	↑ 2.5-fold	<i>14-days</i>			Total P450	↑ 1.5-fold	↑ 1.6-fold	CYP2B (PROD)	↑ 209-fold	↑ 340-fold	CYP1A (EROD)	↑ 2.4-fold	↑ 2.7-fold
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			<p>Proliferation</p> <p>↑ liver cell proliferation after 3-days: 327 mg/kg bw/day group mean S-phase labelling index (%) was increased 2-fold after 3-days and returned to control levels at days 7 and 14</p> <p>There was no evidence of cell proliferation at 58 mg/kg bw/day at any time-point</p>
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Integration of mechanistic studies

RAC in the past discussed minimum requirements to demonstrate that a CAR Mode of action is plausible. Table 16 summarises the data made available in the case of hepatoadenomas and carcinoma induced by isopyrazam. Human hepatocytes have shown that CAR is also activated, although presumably at a lower level than in rats, but the key event of cellular proliferation that triggers downstream hypertrophy, altered foci and carcinomas does not take place (Table 15). Importantly, *in vivo* CAR knock-out studies demonstrating lack of enzyme expression, replicative DNA synthesis and liver tumour promotion have not been conducted, which leaves uncertainties in the Mode of action hypothesis.

Table 16: Isopyrazam datasets to establish a CAR-mediated mode of action for rodent liver tumors

EVENT	RATS	HUMAN
CAR activation based on <i>in vitro</i> and <i>in vivo</i> enzyme expression studies: Increased CYP2b, Cyp3a	YES (Table 10)	YES (Table 10)
Hepatocellular proliferation	YES (Table 14, Table 15)	NO (Table 14) -
Hepatocellular hypertrophy	YES (Table 5)	Not tested
Altered foci	YES (Table 5)	Not tested
Adenomas or carcinomas	YES (see discussion above)	Not tested
CAR activation <i>in vivo</i> transgenic CAR knock-out (KO).	Not tested	n.a.

Other possible modes of action

Overall, nine other possible mechanisms for the formation of liver tumours have been described in the literature. Table 17 outlines these and considers their plausibility with regard to isopyrazam.

Table 17: Consideration of alternative modes of action.

Alternative mode of action	Reason for exclusion
Genotoxicity	Isopyrazam was negative in six <i>in vitro</i> investigations of genetic toxicity (see Table 8) and two <i>in vivo</i> tests to investigate genetic toxicity (see Table 9).
PPARα receptor activation	Isopyrazam did not increase peroxisomal palmitoyl CoA oxidase or P450 Cyp4a activities in liver peroxisome and microsome preparations respectively (see Table 11)
AhR receptor activation	Isopyrazam did not produce a large increase in P450 Cyp1a EROD activity in liver microsomes (see Table 11)

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Estrogenic stimulation	Isopyrazam was not estrogenic in an Oestrogen Receptor transactivation assay <i>in vitro</i> (Toole 2011)* or uterotrophic <i>in vivo</i> in the ovariectomised rat (Kuhl 2011)*
Statins	Isopyrazam was not designed to inhibit HMG-CoA reductase, nor is there any evidence to suggest that cholesterol levels were increased in rats (Milburn 2007a,c, 2008)*
Cytotoxicity	Isopyrazam did not produce elevations in markers of hepatocyte damage nor was there any evidence of cytotoxicity or regenerative proliferation (Milburn 2007a,b,c,d, 2008, and Shearer 2008)*
Immunosuppression	Isopyrazam did not produce any signs of infection, cytotoxicity and regenerative proliferation in micropathology (Milburn 2007a,b,c,d, 2008, and Shearer 2008)*
Porphyria	Isopyrazam did not produce elevations in hepatocyte damage and regenerative proliferation in micropathology or clinical chemistry (Milburn 2007a,b,c,d, 2008, and Shearer 2008)*
Increased apoptosis	Isopyrazam did not increase rates of apoptosis and regenerative proliferation as determined by micropathology in multiple studies (Milburn 2007a,b,c,d, 2008, and Shearer 2008)*
*Only summaries available to RAC and therefore the robustness of these studies could not be assessed	

Remaining uncertainties to the proposed mode of action

The applicant provided a number of mechanistic studies for demonstrating that rat hepatocellular adenoma and carcinomas develop through a phenobarbital-like CAR-mediated mechanism that is widely accepted as not being relevant for humans and that these tumours would not warrant classification for carcinogenicity.

During the Public Consultation, the following uncertainties were raised:

1. *Lack of data with CAR-knock out animals*

RAC notes this weakness and agrees that it is widely accepted that the indirect measurement of CAR activation through determinations of either mRNA codifying for Cyp2b or EROD activity is a surrogate of CAR activation. However, convincingly demonstrating the role of CAR in cell proliferation and tumour promotion would need further support with CAR-knock out animals and its absence leaves significant uncertainties in the hypothesis that CAR has been responsible for liver tumour formation by isopyrazam.

2. *Data with human hepatocytes have been generated with a single donor*

It is noted that for isopyrazam the human hepatocytes from one particular donor were receptive to proliferation after exposure to epidermal growth factor. Therefore, the lack of response after exposure to isopyrazam casts doubt on the intrinsic incapability of hepatocytes to proliferate. Testing material from only one donor, even if responding to a positive control, leaves uncertainties with regards to biological donor-to-donor variability. In the PC further data have been submitted on 2 donors and RAC considers that this reduced the uncertainties.

The AOP 41 (Sustained AhR Activation leading to Rodent Liver Tumours) has not been fully ruled out

In this respect, RAC notes that, despite the lack of interaction between isopyrazam and the AhR has not been directly demonstrated, no large activation of Cyp1a was noted; which is a requirement for disproving AhR activation (Peffer *et al.*, 2018). Therefore, RAC notes that the activation of AhR is very unlikely because in this case a large increase in EROD activity would have to have been detected.

3. *The AOP 32 (Inhibition of iNOS, hepatotoxicity, and regenerative proliferation leading to liver tumours) has not been verified*

This AOP has been developed for the only thiamethoxam metabolite that is able to inhibit nitric oxide synthases. Isopyrazam and thiamethoxam have very different molecular structures and therefore it is not to be expected that isopyrazam or its metabolites could inhibit nitric oxide synthases. Overall, RAC notes this uncertainty as of very low concern.

4. *The AOP 46 (AFB1: Mutagenic Mode-of-Action leading to Hepatocellular Carcinoma) has not been fully verified.*

Aflatoxin B1, after metabolic activation, forms a pro-mutagenic DNA adduct that causes DNA mutations. Isopyrazam was negative in six *in vitro* investigations of genetic toxicity (see Table 8) and two *in vivo* tests to investigate genetic toxicity (see Table 9). Therefore, this AOP is not considered by RAC as a concern.

5. *Isopyrazam is a succinate dehydrogenase inhibitor*

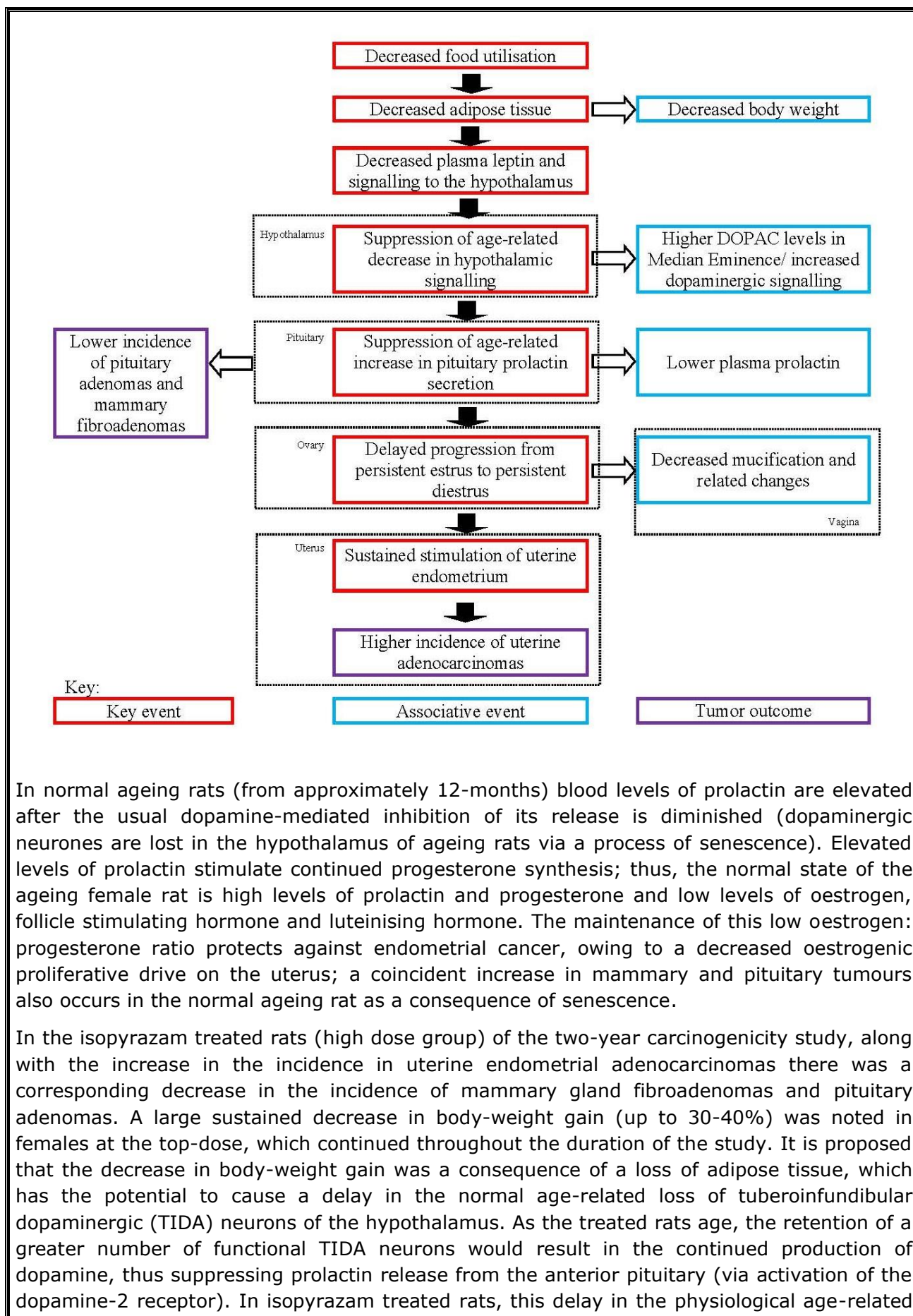
A concern with succinate dehydrogenase inhibitors has been raised since it is noted that humans with mutations in the succinate dehydrogenase gene can suffer uncontrolled proliferation of cells causing cancer. The tumour formation seems to be a consequence of a long-term accumulation of succinate. In this respect, RAC notes that, according to toxicokinetic information provided in the DAR, the mean terminal elimination half-life is 4.6 hours and bioaccumulation of isopyrazam has not been detected. Thus, it is unlikely that isopyrazam exposure could cause succinate accumulation comparable to patients with a genetic disease in which succinate dehydrogenase is not active.

Conclusion on hepatocarcinogenicity

RAC notes that the liver carcinogenicity induced by isopyrazam in rats is consistent with mode of action based on the CAR activation (Table 16) that is not relevant to humans. However, RAC notes that other possible mechanisms of action have not been sufficiently ruled out and therefore the relevance of the isopyrazam-induced hepatocarcinogenicity for humans has to be considered.

PROPOSED MODE OF ACTION FOR UTERINE ENDOMETRIAL ADENOCARCINOMAS

The incidence of uterine endometrial carcinoma was dose-dependently increased in females, at 3000 ppm, with 29%, exceeding the concurrent control and the available limited laboratory HCD. A delay into reproductive senescence has been proposed by the applicant and DS as the most likely mode of action. The hypothesis is based on the shift in tumour profile observed in high dose rats (increased uterine tumours and decreased mammary and pituitary tumours). The proposed mechanism is described as a biological phenomenon specific for rats, in particular Wistar rats (Harleman *et al.* 2012). The proposed mode of action for the observed shift in the incidence of uterine endometrial adenocarcinomas in the female Han Wistar rat is summarised in the Figure below and was described as follows:



In normal ageing rats (from approximately 12-months) blood levels of prolactin are elevated after the usual dopamine-mediated inhibition of its release is diminished (dopaminergic neurones are lost in the hypothalamus of ageing rats via a process of senescence). Elevated levels of prolactin stimulate continued progesterone synthesis; thus, the normal state of the ageing female rat is high levels of prolactin and progesterone and low levels of oestrogen, follicle stimulating hormone and luteinising hormone. The maintenance of this low oestrogen: progesterone ratio protects against endometrial cancer, owing to a decreased oestrogenic proliferative drive on the uterus; a coincident increase in mammary and pituitary tumours also occurs in the normal ageing rat as a consequence of senescence.

In the isopyrazam treated rats (high dose group) of the two-year carcinogenicity study, along with the increase in the incidence in uterine endometrial adenocarcinomas there was a corresponding decrease in the incidence of mammary gland fibroadenomas and pituitary adenomas. A large sustained decrease in body-weight gain (up to 30-40%) was noted in females at the top-dose, which continued throughout the duration of the study. It is proposed that the decrease in body-weight gain was a consequence of a loss of adipose tissue, which has the potential to cause a delay in the normal age-related loss of tuberoinfundibular dopaminergic (TIDA) neurones of the hypothalamus. As the treated rats age, the retention of a greater number of functional TIDA neurones would result in the continued production of dopamine, thus suppressing prolactin release from the anterior pituitary (via activation of the dopamine-2 receptor). In isopyrazam treated rats, this delay in the physiological age-related

increase in prolactin affects the timing of progression into reproductive senescence, subsequently exposing the uterus to a higher oestrogen:progesterone ratio. The result of this continued exposure to higher oestrogen/lower progesterone over time would lead to a proliferative oestrogenic stimulation of the uterine endometrial cells, an increased promotion of spontaneous tumours and ultimately an increase in the observed incidence of uterine endometrial adenocarcinomas. Maintenance of higher dopamine levels also blocks proliferative changes in the pituitary and would account for the lower incidence of pituitary adenoma, whilst the lower prolactin released by the pituitary would explain the observed decline in the incidence of mammary fibroadenomas in the high dose treated groups.

Annex 3 to the CLH report outlines the proposed mode of action and human relevance of the uterine endometrial adenocarcinomas observed in female rats. The key data are summarised below.

Study 1: 18-month investigative study in female rats (Anonymous 2018)

To evaluate the key events of the proposed mode of action, a 18-month investigative toxicity study has been conducted in which female Han Wistar rats were administered isopyrazam for 13, 26, 52, 66 and 80 weeks, the doses reflecting those of the 2-year carcinogenicity study (0, 500 and 3000 ppm equating to 0, 28 and 194 mg/kg bw/day). There were no treatment-related death or clinical signs of toxicity and the results of this study are summarised in Table 18 (for details see CLH report and its Annex):

Table 18: Summary of 18-month study in the rat investigating the mode of action and human relevance of uterine endometrial adenocarcinomas induced by isopyrazam

Control	500 ppm	3000 ppm
↑↑ in prolactin and leptin (age related)	↓ Body weight gain (-9%; last 6-m only) ↓ Final body weight (-5%) ↑ Prolactin (age-related) ↑ Leptin (age-related; week 52) ↑ Liver weight	↓ Body weight gain (-32%**) ↓ Final body weight (-21%**) ↓ Absolute fat pad weight (max -59% week 66) ↓ Oestrous cycle duration (from week 29) ↓ Prolactin (sign. at wk 80 compared to control) ↓ Leptin (wk 52-66-80) (age-related increase delayed) - No concomitant alteration in adiponectin (↑) Dopamine (DA) (26% at wk. 26 only) ↑ Dihydroxyphenylacetic acid (DOPAC: 29% at wk 52 and 30% at wk 80) – no clear time course - No change in tyrosine hydroxylase expression - No change in DA/DOPAC turnover ↑ Liver weight

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

In the 18-months mechanistic study, body weight gain and adipose fat tissues were significantly reduced at the high dose. Leptin concentration was altered (age-related increase was delayed), but not adiponectin. The extent of the leptin reduction however was consistent with the reduction in adipose tissue (about 60% at week 66). According to the Annex to CLH report, there were no differences between control and treated groups for dopamine concentrations or DOPAC/dopamine ratio (indicative of dopamine turnover). The biological significance of DA increase at week 26 is considered questionable by RAC. The DA/DOPAC ratio was similar across all groups. DOPAC levels tended to be greater than control, but only at week 52 and 80 significant difference was achieved, thus some evidence is available for isopyrazam effects on dopaminergic system. RAC notes that no alteration of TH mRNA or

protein expression was detected.

Age-related increase in prolactin was reduced in the high dose group at week 66 and 80, only at 80 weeks levels were significantly different compared to the control group.

Study 2: Evaluation of pituitary prolactin and hypothalamic tyrosine hydroxylase by immunohistochemistry and in situ hybridisation (Anonymous 2015b, tissues from 2-year study)

Pituitary glands from the 2-year rat carcinogenicity study were assessed to explore signalling pathways of the mode of action. Dose dependent higher levels of tyrosine hydroxylase (TH) mRNA, at 500 and 3000 ppm, compared with controls, were reported in the arcuate nucleus alone and in the arcuate nucleus and median eminence. The corresponding protein expression was only slightly increased in the arcuate nucleus alone at 3000 ppm and, for the arcuate nucleus and median eminence, the protein expression was higher at 500 and 3000 ppm, however, with inverted dose-response. No differences in prolactin between control and the two treated groups were noted; which should suggest that the production of prolactin is normal and just its release (dopamine mediated) is affected. It was concluded that isopyrazam is associated with delayed senescence of dopaminergic neurons in the TIDA region of the hypothalamus. RAC notes that no DA/DOPAC data are available for the 2-year carcinogenicity study.

Study 3: Evaluation of hypothalamic tyrosine hydroxylase in control female Wistar rats at 3, 12 or 24 months (Anonymous 2015a, tissues from 90-day and 2-year studies)

Arcuate nucleus and median eminence in the hypothalamus from control animals of 90-day and 2-year rat studies were assessed for quantification of both mRNA and protein tyrosine hydroxylase. Tyrosine hydroxylase proteins were lower for the 2-year animals compared with the 1-year animals; and were also higher in the 1-year animals compared with the 90-day animals. Messenger RNA coding for tyrosine hydroxylase was lower in the 2-year animals than in the 1-year animals and no statistically significant differences between the 1-year and 90-day animals. In conclusion, mRNA and protein expression of tyrosine hydroxylase decreases progressively with age (from 1- to 2-years) in control rats.

Key event one: body-weight gain deficit and loss of adipose tissue

In the 18-month investigative toxicity study, the postulated initial key event in the mode of action hypothesis was demonstrated (Table 18, Annex 3 to the CLH report) by a reduction in body-weight gain and final body weight when compared with controls (-32%**[†], p<0.01 and -21%**[†], p<0.01, respectively). In the 3000 ppm isopyrazam treated group in this same study a particularly marked reduction in absolute fat pad weight was also noted (-59%**[†] in week 66) (Table 18). In the 2-year rat carcinogenicity study (Table 5, Annex 3 to the CLH report), there was a large deficit in body-weight gain throughout the study in the 3000 ppm treated group, to a magnitude of 40% lower than controls by the end of the study. Hence, in both studies, a marked reduction in food utilisation efficiency was indicated because no significant differences in food consumption were noted. An increase in the incidence of uterine tumours (with a concomitant reduction in the incidence of mammary and pituitary tumours) as a consequence of body-weight loss has been previously demonstrated in rats after dietary restriction (particularly in the Wistar rat). In fact, as a consequence of reductions in body-weight gain, because of calorific restriction, both Sprague Dawley and Wistar rats showed reductions in mammary and pituitary tumours, but only in Wistar rats there was an increase

in uterine tumours under such conditions (Harleman *et al.* 2012). RAC notes the decrease in body weight gain and decrease in adipose tissue, however questions that the decreased body weight gain is considered to be a specific initiating event for the isopyrazam induced uterine tumours. Such tumours were not systematically observed as a result of exposure to substances at high doses even if significant reductions in body weight gain were observed. Could the marked decrease in body weight gain have masked a more pronounced tumourigenic effect? It is also noted that in the 2-year study the pituitary and mammary gland tumours are already reduced at 500 ppm, and the uterine tumour incidences are also slightly increased at this dose. A causal relationship for body weight is considered questionable by RAC.

Key event two: decrease in plasma leptin and signalling to the hypothalamus

In accordance with the hypothesised mode of action, body weight/adipose tissue losses should lead to a reduction in plasma leptin and signalling to the hypothalamus. Circulating leptin levels were measured in the 18-month investigative study (Table 18, Annex 3 to the CLH report). The expected age-related increases in plasma leptin were evident in the control and the 500 ppm isopyrazam treated animals from week 52 onwards (statistically significant compared with the day-28 values). In animals of the 3000 ppm group this increase was delayed until week 80 of treatment (at all ages the mean leptin values were numerically smaller than control values with statistical significance being reached at weeks 52, 66 and 80). RAC acknowledges that leptin was indeed decreased at week 52-80 weeks, at levels consistent with the decrease in adipose tissue (about 60% at week 66), however, no concomitant changes in adiponectin were reported (but would be expected). Importantly also, no causality has been demonstrated for leptin and its signalling and downstream events of the postulated Mode of action.

Key event three: Suppression of age-related decrease in dopaminergic signalling

The proposed mode of action postulates that treatment with 3000 ppm isopyrazam preserves dopaminergic signalling in the TIDA neurons of the hypothalamus in comparison with the physiological reduction in signalling which occurs in control animals of the same age. The TIDA neurons' cell bodies are situated in the arcuate nucleus region of the hypothalamus with their axons extending into the median eminence region. Dopamine is synthesised in the TIDA neurons and released from the median eminence to reach the anterior pituitary.

The age-related decrease in the number and activity of dopaminergic neurons in normal ageing rats has been investigated via measurement of tyrosine hydroxylase (TH); which is the rate limiting step in the synthesis of dopamine. TH mRNA levels at 2-years were statistically significantly lower than those at 90-days and tyrosine hydroxylase protein was statistically significantly lower at 2-years than at 1-year in these control rats, suggesting that tyrosine hydroxylase and therefore dopamine synthesis reduces over time in normal ageing female rats (investigative study 3, Annex 3 to the CLH report).

In the 18-month investigative study, the median eminence of the hypothalamus was isolated to determine the concentrations of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). An elevation of mean DOPAC was observed in the 3000 ppm isopyrazam treated group (Table 18, Annex 3 to the CLH report). In this group, DOPAC levels were higher than controls at week 52 (29%* $p < 0.05$), week 66 (16%) and week 80 (30%** $p < 0.01$); corresponding to the lower leptin values observed at these time-points; DOPAC and dopamine values were also higher than controls at week 26 (31% and 26%* respectively). Dopamine/DOPAC ratio was similar across all groups. RAC takes note of these effects and the

time courses observed in the 18-months study: The mean dopamine (DA) concentrations in the median eminence of the hypothalamus were only statistically significantly higher at week 26, and were not affected later, thus biological significance of this isolated finding at week 26 is questionable. DOPAC showed some alterations without clear time course at week 52 and 80. There were no treatment-related alterations in tyrosine hydroxylase (enzyme in dopamine synthesis) staining in the arcuate nucleus nor in the median eminence. Dopamine turnover was not affected in control and treated groups and the DA/DOPAC ratio was similar across all groups. Evidence for clear effects on dopamine synthesis, concentrations and dopamine/DOPAC turnover, thus functional activity of TIDA neurons seem rather weak.

There was a statistically significantly higher level of TH mRNA in the arcuate nucleus region and the combined arcuate nucleus region and median eminence regions than controls in the 2-year carcinogenicity study (investigative study 2, Annex 3 to the CLH report). Hence, it has been concluded that there is a higher capacity for dopamine production in the TIDA neurons of rats treated with 3000 ppm isopyrazam compared with controls, after 2-years of treatment. However, no DA/DOPAC data are available for the 2-year study and RAC takes note of the inconsistencies in TH mRNA and protein expression in the 2-years study (as described above study 2, 2015b). The 18-month and 2-year study results are not in line as there were no treatment-related alterations in TH in the arcuate nucleus nor in the median eminence in the 18-months study.

Despite the uncertainties related to inconsistencies in the results itself, RAC notes that these experiments have research study character and do not follow validated methodologies. They also do not include positive controls or guideline values for evaluation hampering result interpretation.

Key event four: suppression of age-related increase in prolactin

Plasma prolactin levels were measured in the 18-month study in samples taken after 4, 13, 26, 52, 66 and 80 weeks (investigative study 1, Annex 3 to the CLH report). It was demonstrated that an age-related increase in plasma prolactin observed in the control and 500 ppm isopyrazam treated animals was delayed by treatment with 3000 ppm isopyrazam after 66- and 80-weeks' treatment (statistical significance was reached only at week 80). With the delay in the rise in prolactin levels there was a concomitant delay in the onset of reproductive senescence, postulated by the applicant. RAC notes that no prolactin levels are available for the 2-year carcinogenicity study, however mammary gland and pituitary tumours were reduced for mid and high dose. The dose response of the mammary gland tumours in the 2-year study (↓ at 500 and 3000 ppm) is not in line with the dose response for prolactin in the 18-months study (↑ at 500 ppm, ↓ at 3000 ppm).

Key events five and six: altered transition to reproductive senescence, increased total number of oestrus cycles and proliferation

The 18-months investigative study monitored the pattern and onset of reproductive senescence in female Han Wistar rats as well as the effect of isopyrazam treatment (investigative study 1, Annex 3 to the CLH report). An oestrus cycle is expected to measure between 4 and 5 days in normal sexually mature rats. The four stages that could potentially be observed via vaginal lavage are proestrus (P) during which ovulation occurs, oestrus (E), metestrus (M) and diestrus (D).

All animals showed mean cycle lengths of approximately four days for the first 25 days of treatment and then all showed a tendency for fewer animals with complete cycles and longer mean cycle durations at week 29. Animals treated with 3000 ppm isopyrazam had a greater

number of complete oestrus cycles compared with the controls (Table 19). There was a tendency for 3000 ppm isopyrazam treated groups to have shorter mean cycling durations than the other groups (at weeks 77-79 high dose animals had a mean cycle duration of 6.1 days compared with 7.7 and 7.8 days in the control and 500 ppm groups respectively (Table 19)). The control group showed the expected physiological response of females entering reproductive senescence with an initial increase in the percentage of days in an oestrogenic state (between sampling weeks 3-4 and 42-43) (Table 19) followed by a slow decline. In contrast, in 3000 ppm animals, the percentage of days in an oestrogenic state continued to increase up to weeks 55-57 (Table 19) before showing a slow decline. In the 3000 ppm group there was a lower incidence of "reproductive cycle alteration", a term which is assigned when oestrous cycling cannot be determined owing to the onset of reproductive senescence. RAC acknowledges that the data may indicate that isopyrazam has the potential to delay reproductive senescence, probably at 500 and 3000 ppm. However, RAC notes that the GLP statement of the study report mentions that the systems used for calculation and tabulation of estrous cycle data were not validated. The estrogenic state unopposed by progesterone, compared to control group, was postulated, however estrogen:progesterone levels that would lead to a sustained stimulation of the uterine endometrium have not been demonstrated. The demonstration of an alteration of estrus cycle-sensitive estrogen:progesterone ratios would have substantiated such hypothesis. In particular, it is also noted by RAC that across all time intervals, there were no definitive adverse test substance-related histologic changes, and no apparent test substance related effects on proliferative lesions in the uterus, cervix or vagina, which would be indicative of sustained stimulation of the uterine endometrium due to elevated estrogenic/progesterone levels. Thus, the last key event leading to the adverse outcome endometrial adenocarcinoma/hyperplasia, has not been demonstrated.

Table 19: Summary of findings from the evaluation of oestrus cyclicity.

Parameter	0 ppm (control)	500 ppm isopyrazam	3000 ppm isopyrazam
Oestrus cycle length weeks 0-51 (days)	3.9-4.8	4.0-4.9	4.0-4.7
Oestrus cycle length weeks 52-80 (days)	5.5-7.7	5.3-7.8	4.7-6.1
Animals with complete cycles-weeks 0-25	86.4% - 100%	82.4% - 99.3%	89.6% - 100%
Animals with complete cycles-weeks 29-57	50.5% - 100%	44.2% - 81%	48.6% - 93.3%
Animals with complete cycles-weeks 60-80	37.5% - 44.8%	26.7% - 62.1%	24.2% - 62.5%
Peak oestrogenic state (% E + % P)	59.8%	63.5%	67.3%
Timing of peak oestrogenic state	42-43 weeks	50-51 weeks	55-57 weeks

Tumour outcome

The pattern of alterations in the incidence of uterine, mammary and pituitary tumours in the 2-year rat carcinogenicity study supports the proposed mode of action hypothesis. Uterine tumours (Table 12) were increased, whilst mammary and pituitary tumours were decreased at the top-dose of 3000 ppm (Table 13). The proposed mode of action and the resulting pattern of alterations in uterine, mammary and pituitary tumours are well described in the literature (Harleman *et al.* 2012). The Han Wistar rat is reported as being particularly

susceptible to these changes following significant reductions in body weights; this is demonstrated in the 2-year carcinogenicity study in which the observed shift in tumour profile was related to the magnitude of the effect on body-weight gain.

RAC notes that based on the tumour pattern the hypothesis is plausible, but also notes inconsistencies in the dose-response and time course of the initiating event, key events and the adverse outcome. An example is the change in tumour pattern at mid dose, while body weights and adipose tissue remain fairly unchanged at 500 ppm (as commented above).

Relevance to humans

The CLH report elaborates on the human relevance of the postulated Mode of action. There are fundamental differences between humans and rats with regard to both hormonal control of the hypothalamic-pituitary-gonad axis and the process of transitioning into reproductive senescence. Furthermore, the human reproductive (menstrual) cycle has markedly different control mechanisms compared with the 4-5 day cycle of rats.

The prolactin surge that occurs in rats during proestrus is not observed in humans. In rats, the normal luteal phase is only one day and the new corpora lutea will regress; however, maintenance of a higher prolactin level (i.e. on mating) rescues the corpora lutea and stimulates it to produce further prolactin. In contrast, human corpora lutea are maintained by luteinising hormone during a menstrual cycle and by chorionic gonadotropin from the placenta (in pregnancy). In rats, luteolysis is under the partial control of prolactin whilst luteotrophic actions of prolactin do not occur in humans.

With regard to reproductive senescence processes, in Wistar rats the onset of senescence is driven by a progressive decrease in the activity of TIDA neurons in the hypothalamus and a consequential loss of dopamine-mediated inhibition of prolactin. Prolactin levels are thus elevated resulting in a luteotrophic effect on the corpora lutea, leading to elevated progesterone and lower oestrogen in the blood. This regulation does not occur in humans; in contrast, menopause and reproductive senescence in humans are driven by an eventual depletion (with age) of the limited number of primordial follicles in the ovaries and are associated with a marked decrease in circulating oestrogen and progesterone. The state of persistent oestrus is unique to rats with no equivalent state in humans.

RAC notes as regards to the uterine tumours in general, these tumours are malignant and endometrial cancer is considered highly relevant for women; both in humans and in rodents this type of cancer is an oestrogen sensitive lesion.

Exclusion of other possible mode of action

To support the proposed mode of action, other alternatives have been evaluated in the context of the existing data. Data supporting the exclusion of alternative mode of action is summarised in the Table 20 (see Annex 3 to CLH report for details). In addition, RAC complemented the assessment available in the CLH report:

Table 20: Data supporting the exclusion of alternative mode of action.

Alternative mode of action	Reason for exclusion
Genotoxicity	Isopyrazam was negative in six <i>in vitro</i> investigations of genetic toxicity (see Table 8) and two <i>in vivo</i> tests to investigate genetic toxicity (see Table 9).

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Direct oestrogenicity	<p>Isopyrazam shows no estrogenic potential <i>in vitro</i> in an assay with stably transfected human oestrogen receptor-α transcriptional activation*</p> <p>Isopyrazam shows no estrogenic potential <i>in vivo</i> in an uterotrophic assay in ovariectomised rats*</p>
Dopamine agonist/effects on the dopamine transporter	<p>Isopyrazam 10 μM (or its metabolite CSCD459488) were not inhibitors of dopamine transport (27% ligand binding) or of dopamine D2 receptor (6% ligand binding) <i>in vitro</i>* based on competition ligand binding ($\geq 50\%$ inhibition indicates activity). RAC notes uncertainties in the results interpretation as only the result of one single concentration has been presented and no log competition curve of ligand binding, which is usually set up in such type of studies. This is considered important, as the dopamine receptor is a neuro-endocrine receptor and as such operates rather in the nM range. RAC acknowledges further experiments linked to the DA transporter:</p> <p>Isopyrazam is an inhibitor of dopamine transport (uptake into rat striatal synaptosomes) <i>in vitro</i> at a concentration of 100 μM*</p> <p>Isopyrazam inhibits control ligand binding to the dopamine transporter protein <i>in vitro</i> at a concentration of ≥ 30 μM*</p> <p>Isopyrazam is not an inhibitor of the dopamine transporter <i>in vivo</i> assessed based on suppression of a 17β-Estradiol (E2) mediated prolactin surge*</p>
Prolactin secreting tumours in the anterior pituitary	<p>According to the literature, the majority of rat pituitary hyperplasias and adenomas are functional prolactin-producing tumours (Kovacs <i>et al.</i> 1977*). No significant differences in the expression of prolactin protein were observed between the control and treated groups in the 2-year rat study. Therefore, the ability of these tumours to secrete prolactin is unlikely to be the cause of the uterine tumours.</p>
Oestrogen metabolism in the uterus	<p>Modulation of oestrogen metabolism in the uterus could potentially lead to higher net oestrogenic stimulation of the uterus and ultimately uterine adenocarcinomas. An example is 17β-estradiol which when hydroxylated to 4-hydroxyestradiol has a stronger carcinogenic potential on the uterus, kidney and pituitary. Cytochrome P450s (predominantly CYP1B1) are responsible for the hydroxylation of 17-β-estradiol. It was demonstrated <i>in vitro</i> that isopyrazam elevated levels of CYP2B and CYP3A activity (indirectly measured by associated markers) (Tables 14 and 15); <i>in vivo</i> it was shown that isopyrazam increased total hepatic microsomal CYP content (Table 15). The administration of isopyrazam in the diet for 14-days (500 and 3000 ppm) resulted in a significant increase in the metabolism of 17-β-estradiol to 2- and 4- estradiol in liver microsomes, however no changes in uterine CYP1B1 were observed. This suggests that the increase in oestrogen metabolism is a result of CYP2B/3A microsomal enzyme induction, secondary to CAR activation and that peripheral induction of estradiol hydroxylation does not occur in isopyrazam treated rats. Furthermore, the observed shift in tumour profile is inconsistent with this mode of action. Contrary to the reduction in mammary and pituitary tumours observed after isopyrazam exposure (Table 13), such a mode of action would be expected to cause an increase in tumours in the oestrogen sensitive mammary and pituitary tissues. Therefore, the modulation of oestrogen metabolism in the uterus is unlikely to be the cause of the uterine tumours.</p>
Increase in oestrogen:progesterone ratio	<p>Indirect senescent but also chemically-induced imbalance in sex steroid hormones in the ovary leads to decrease of both oestrogen and progesterone and a status similar to a high oestrogen status may manifests by persistent oestrous in vaginal cytology, an</p>

	atrophic ovary with cystic atretic follicles, lack/few corpora lutea, cornification of the cervix/vagina mucosa, and/or squamous metaplasia of endometrial epithelial cells. Atypical precancerous hyperplasia may be increased (Yoshida <i>et al.</i> , 2015; Cruz <i>et al.</i> , 2017). For clarification, no oestrous-cycle-sensitive hormone measurements on oestrogen:progesterone ratio are available. RAC considers that the data available do not allow a firm conclusion on the role of this Mode of action.
Increased <i>in situ</i> aromatase	According to Yoshida <i>et al.</i> (2015), this is a mechanism with no evidence so far in rodents, but human relevance has been demonstrated with increased protein and mRNA expression for aromatase (and related <i>in situ</i> oestrogen production) in epithelial stromal cells in endometrial carcinomas (Watanabe <i>et al.</i> 1995). Aromatase is a key factor for mammary carcinogenesis. Based on the tumour profile of isopyrazam (tumour shift with decrease in mammary tumours) such pathway is unlikely.
Cytotoxicity / regenerative cell proliferation	Chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced. For isopyrazam no persistent inflammation/ cytotoxicity and regenerative hyperplasia is evident in the repeated dose and carcinogenicity studies. Carcinogenesis due to excessive toxicity and necrosis that would trigger regenerative proliferation is unlikely as there is no histopathological hint for this mechanism. RAC concludes that the data do not support a cytotoxicity-mediated Mode of action.
*Only summaries available to RAC and therefore the robustness of these studies could not be assessed.	
Uncertainties in the proposed mode of action	
<p>The applicant has provided a number of mechanistic studies for demonstrating that rat uterine tumours are developed through a mode of action based on a delay into the reproductive senescence induced by isopyrazam that is considered non relevant for humans and that would not qualify these tumours for warranting classification for carcinogenicity. Overall, RAC notes the following uncertainties:</p> <ul style="list-style-type: none"> • <i>AOP and previous general considerations of RAC about this particular mode of action</i> <p>RAC provided an opinion on about the CLH of sedaxane in 2019. This substance is another 4-carboxamide derivate causing uterine tumours similar to those found in the case of isopyrazam. For sedaxane the mode of action proposed for isopyrazam-induced uterine tumours was also assessed. Some of the concerns raised by RAC were:</p> <p><i>It is not considered very plausible, as not all body weight reductions lead to increased tumour formation in carcinogenicity studies, not all chemically induced alterations in reproductive senescence lead to uterine tumour formation, and not all Wistar rat feed restriction studies show an increase in uterine adenocarcinoma. However, it is acknowledged that some studies have shown an association of diet / feeding status with uterine tumour incidences (Tucker <i>et al.</i>, 1979; Roe <i>et al.</i>, 1995), but the diet composition itself rather than caloric restriction could play a role. Also, an association of caloric/feeding status with (decrease) in mammary gland tumours is known. In the view of RAC it is true that reproduction and oestrous cycling is sensitive to feeding and weight loss (McShane and Wise, 1996; Frisch <i>et al.</i>, 1975; Tropp <i>et al.</i>, 2001). However, not all studies on caloric restriction that show a delay in reproductive senescence result in induction of uterine carcinoma (Keenan <i>et al.</i>, 1995). Then, in the literature it is not well demonstrated whether activity of TIDA neurons changes during</i></p>	

different feeding states and inconsistent results have been reported. Recent findings in transgenic mice suggested that short-term fasting attenuated TIDA neuron activity and increased serum prolactin levels (Kubota et al., 2018).

Moreover, other points highlighted by RAC were:

Based on literature references, the applicant postulated the mode of action involving sustained dopaminergic activity and prolactin-dependent alteration of reproductive senescence with an estrogenic state leading to sustained cell proliferation, the adverse outcome, typically observed with higher frequency in Wistar rats, an increase incidence in uterine adenocarcinoma and concomitant decrease in mammary gland and pituitary tumours. This pathway is discussed by Harleman et al. (2012). The authors point out that such a tumour shift is seen in dietary restriction studies in Wistar rats, but less frequent in SD rats. The prolactin-mediated effect in rats on increased oestradiol:progesterone ratio and resulting uterine tumour formation is considered as not relevant for humans, since prolactin is not luteotrophic in humans. Yoshida et al. (2015) concluded that for such a mode of action extrapolation to humans, more clear evidence is needed.

RAC notes that an AOP in Wistar rats is not so far robustly developed enough with its molecular initiating and key events, and thus, at this stage, the relevance for humans is still uncertain. A putative AOP is under development by U.S. EPA ("*Increased dopaminergic activity leading to endometrial adenocarcinoma (in Wistar rats)*", with reference to Harleman et al. 2012 (AOP 112: MIE: *increased dopaminergic activity*, → KE: *Decrease Prolactin* → KE: *Increase Estrogen receptor activity* → KE: *Decrease Progesterone from corpus luteum* → KE: *Increase Hyperplasia (glandular epithelial cells of endometrium* → AO: *Increase Endometrial adenocarcinoma*). When considering this AOP in its preliminary stage, not all relevant key events were investigated or demonstrated in the case of isopyrazam (such as estrogen receptor activity, also in relation to decreased progesterone, endometrial hyperplasia). On the contrary other key events have been postulated and investigated but no causal relationship has been proven (such as bodyweight, adipose tissue and leptin decrease). Although RAC acknowledges the efforts of the applicant, the Committee is of the view that the postulated Mode of action remains, to a considerable extend, hypothetical.

- *Experimental methodologies to investigate the AOP and evaluation of results*

When it comes to the experimental tools and methodologies to investigate postulated key events, RAC notes further uncertainties. To robustly demonstrate an AOP, methods should preferably have been reviewed previously and agreed by a recognized authority. Other methods should be well established in the published literature and described regarding their fitness for purpose, direct or indirect (i.e. a surrogate) relation to a key event relevant to the final adverse effect in question, assay repeatability and reproducibility. In the case of isopyrazam, some mechanistic studies have a largely research study character. The interpretation of results is hampered because methodologies are not reviewed/validated, and, for some postulated key events, it is unclear to RAC what should be measured/detected. For other key events it seems that other methods should have been used, such as the measurement of E2/P ratio to proof decreased progesterone. In addition, no studies are included that would provide evidence for causality.

Looking at the results itself, as indicated in the previous text section, RAC notes a range of inconsistencies in dose-responses, time courses, and unclear biological significance of the alterations detected or not detected.

To summarise, during the ECHA Consultation and/or Committee consultation, the following uncertainties were raised:

- *Key event one: body-weight gain deficit and loss of adipose tissue*

RAC notes that a decrease in adipose tissue is a very broad key event, and causal relationship remains difficult to establish. Decreased body weight gain is considered not to be a specific initiating event for uterine tumours, and on the other hand, it needs to be considered whether marked decrease in body weight gain could have masked a more pronounced tumourigenic effect.

- *Key event two: decrease in plasma leptin and signalling to the hypothalamus*

RAC notes that no changes in adiponectin were detected where an increase would have been expected.

- *Key event three: Suppression of age-related decrease in dopaminergic signalling and dopamine turnover*

RAC considers dopaminergic signalling not robustly demonstrated due to inconsistencies in response, time course and dose response of DOPAC, DA and TH. DA level was fairly unchanged, no treatment-related alterations were observed in TH in the arcuate nucleus nor in the median eminence in the 18-months study. Further, DA/DOPAC ratio remained similar across all groups, while DOPAC tended to be greater for the high dose group at weeks 26-80, but significant only at week 52 and 80 with an unclear time course. In contrast, in samples from the 2-year study, TH mRNA and protein levels were increased, but mRNA and protein levels were inconsistently altered for 500 and 3000 ppm dose groups.

- *Key event four: suppression of age-related increase in prolactin*

RAC notes that decrease in prolactin started at 66 weeks. However, there was no DOPAC change at 66 weeks which would have been expected, and further, no data are available on prolactin for the actual 2-years carcinogenicity study.

- *Key events five and six: altered transition to reproductive senescence, increased total number of oestrus cycles and proliferation*

The 18-month isopyrazam study seems to suggest that isopyrazam can delay the time of reproductive senescence onset. It is, however, noteworthy that in the GLP statement of the study report, it is mentioned that the systems used for calculation and tabulation of estrous cycle data were not validated. An alteration of (cycle-sensitive) oestrogen:progesterone ratios should have been demonstrated. There were no definitive adverse test substance-related histologic changes, and no apparent test substance-related effects on proliferative lesions in the uterus, cervix or vagina, indicative of sustained stimulation of the uterine endometrium due to elevated estrogenic/progesterone levels.

Uncertainties are also related to alternative Mode of action.

- *Isopyrazam is a succinate dehydrogenase inhibitor*

See RAC consideration about this issue above in the section about uncertainties for mode of action of liver hepatocarcinomas.

- *Other Mode of action*

RAC considers that the available data on the Mode of action of uterine adenocarcinoma are insufficient to support the -postulated Mode of action by the applicant. A prediction of an

alternative Mode of action is not possible and thus the Mode of action remains undefined (see decision tree in Yoshida *et al.*, 2015).

Conclusion on uterine adenocarcinomas

RAC notes that, despite the mechanistic studies provided in the CLH report and its Annex 3, a number of questions about the plausibility of the proposed mode of action and its relevance for humans still remain unanswered. Therefore, based on the information available in the CLH report, RAC considers that the increased incidence of uterine adenocarcinoma with a dose response relationship is potentially a treatment related effect and relevant for classification as human relevance cannot be excluded.

Comparison with the criteria

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Since there are no human data it cannot be concluded that isopyrazam has known carcinogenic potential for humans; therefore Category 1A is not applicable.

Category 1B is for substances where sufficient evidence of carcinogenic potential for humans exist. For that, increases incidences of malignant neoplasms or an appropriate combination of benign and malignant neoplasm must be seen in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP, can also provide sufficient evidence. In the case of isopyrazam, the data package contains one study showing increment of malignant (uterine) lesions and hepatocellular adenomas and carcinomas in a single species and therefore the conditions for category 1B are not met.

Category 2 is reserved for substances with evidence of carcinogenicity not sufficiently convincing to place the substance in Category 1A or 1B. It can be set in this category if the evidence of carcinogenicity is restricted to a single experiment, as is the case of isopyrazam.

Overall, based on a weight-of-evidence approach, **RAC proposes classification of isopyrazam as Carc. 2; H351 (suspected of causing cancer).**

10.11 Reproductive toxicity

The reproductive toxicity of isopyrazam has been investigated in a two-generation study in rats and in several developmental toxicity studies in rats and rabbits.

10.11.1 Adverse effects on sexual function and fertility

A two-generation study in rats is available to assess the effect of isopyrazam (93:7 syn:anti specification) on sexual function and fertility.

Table 40: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
Multigeneration study Rats, HsdRCCHan:WIST, males & females, 26/sex/group OECD 416 GLP Anonymous 2008b	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 0, 100, 500 & 3000ppm	There were no treatment related deaths or clinical signs of toxicity
		Parental toxicity
		<u>F₀ generation</u>
		<u>3000ppm</u>
		↓ BW in M (-11%**) & F (-8%**), ↓ FC, ↑ liver weights in M (18%**) & F (29%**), ↓ ovary weight (-13%**), ↓ uterus + cervix weight (-27%**), hepatocellular hypertrophy
		<u>500ppm</u>
		↓ BW in F (-2%*), ↓ FC, ↑ liver weights in F (8%*), hepatocellular hypertrophy
		<u>100ppm</u>
		No adverse effects
		<u>F₁ generation</u>
<u>3000ppm</u>		
↓ BW in M (-5%**) & F (-6%**), ↓ FC, ↑ liver weights in M (27%**) & F (31%**), ↓ ovary weight (-27%**), ↓ uterus + cervix weight (-39%**), hepatocellular hypertrophy		
<u>500ppm</u>		
↓ BW in F (-8%**), ↓ FC, ↑ liver weights in M (10%**) & F (15%**), hepatocellular hypertrophy		
<u>100ppm</u>		
↑ liver weights in F (10%*)		
Fertility		
<u>F₀ generation</u>		
No adverse effects		
<u>F₁ generation</u>		
No adverse effects		
Offspring toxicity		
<u>F₁ generation</u>		
<u>3000 ppm</u>		
↓ total litter size (-13%*), ↓ BW-gain during lactation, ↓ BW at weaning in M (-12%**) & F (-10%**), ↑ liver weights in M & F (-26%**), ↑ thymus weights in F (15%*)		
<u>500 ppm</u>		

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		<p>↑ liver weights in M (9%**) & F (6%*), ↑ thymus weights in F (5%*)</p> <p><u>100 ppm</u></p> <p>↑ thymus weights in F (4%*)</p> <p><u>F2 generation</u></p> <p><u>3000 ppm</u></p> <p>↓ total litter size (-12%*), ↓ BW-gain during lactation, ↓ BW at weaning in M (-15%**) & F (-16%**), ↑ liver weights in M (23%**) & F (32%**)</p> <p><u>500 ppm</u></p> <p>↑ liver weights in M (9%*) & F (14%**)</p> <p><u>100 ppm</u></p> <p>No adverse effects</p>

10.11.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a guideline-compliant, two-generation reproductive toxicity study, isopyrazam (93:7 syn: anti specification) was administered continuously in the diet of Han Wistar rats (26/group). Dietary concentrations of 0, 100, 500 and 3000 ppm were adjusted to obtain the following mean target doses:

Table 41: Mean doses received (mg/kg bw/d)

Dietary Concentration of Isopyrazam (ppm)	100	500	3000
F ₀ parental males, pre mating	8	41	250
F ₀ parental females, pre mating	9	47	277
F ₀ parental females, gestation	7	37	217
F ₀ parental females, lactation	25	119	700
F ₁ parental males, pre mating	9	48	288
F ₁ parental females, pre mating	10	50	301
F ₁ parental females, gestation	8	41	239
F ₁ parental females, lactation	24	129	774

After 10-weeks' exposure, F₀ animals were mated to produce the F₁ generation. After weaning, the F₁ parental animals were selected and similarly mated following 10-weeks' post weaning exposure to produce the F₂ generation (with non-selected pups being killed at weaning); the study was concluded when the F₂ generation pups were approximately 4 weeks old.

Parental toxicity

Table 42: Fertility and reproductive performance in isopyrazam treated F₀ and F₁ generations

Observation	Dietary Concentration of Isopyrazam (ppm)			
	0	100	500	3000

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

F₀ parents, F₁ offspring				
Mean precoital interval (days)	3.2	2.5	2.7	3.1
% females mating	100	100	100	100
% matings resulting in a live litter	96	92	96	100
Mean gestation length (days)	22.1	22.1	22.1	22.1
Number of viable litters	26	24	25	26
F₁ parents, F₂ offspring				
% mean precoital interval (days)	2.4	2.6	3.8*	2.9
% females mating	96	100	100	100
% matings resulting in a live litter (%)	92	100	92	100
Mean gestation length (days)	22.1	22.1	22.2	22.1
Number of viable litters	24	26	24	26

Toxicity was evident in both the F₀ and the F₁ parental generations. At 3000ppm body weights were lower than controls throughout the entire study. By the end of the premating period, body weights in males and females of the F₀ generation were 11% and 8% lower than controls, whilst those of the F₁ generation were 5% and 6% lower. Body weights of females at 500 ppm were also lower than controls by the end of pre-mating period (2% and 8% lower in the F₀ and F₁ generations respectively).

Food consumption was reduced in a dose-related manner in both parental generations at 500 and 3000 ppm, with food utilisation efficiency being reduced in the 3000 ppm F₀ parents. Liver weights were increased in all treated animals of both parental generations. Relative liver weights for the F₀ generation were 18% and 29% greater than controls in males and females respectively at 3000 ppm, and 8% greater in females at 500 ppm. For the F₁ generation the increases were 27% and 31% at the top-dose and 10% and 15% at the mid-dose in males and females respectively; liver weights were also increased in females of the F₁ generation at 100 ppm (10%). Accompanying histopathological signs were present in both parental generations at 500 and 3000 ppm and comprised a dose-related increase in the incidence of hepatocellular hypertrophy (diffuse at the high-dose and centrilobular at the mid-dose) with fine cytoplasmic vacuolation apparent in the hypertrophic hepatocytes. Ovary and uterus weights were increased at the top-dose in both generations; however in the absence of microscopic findings or functional effects on fertility these findings were unlikely to be treatment related.

Fertility

Isopyrazam exposure had no adverse effects on fertility or reproductive performance in either generation. Oestrous cycling and sperm parameters were unaffected by treatment. It is noted that there was a decrease in the number of implantation sites in the F₀ and F₁ mothers. The relevance of these findings is discussed below.

Offspring toxicity

Table 43: Key litter data

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Observation	Dietary Concentration of Isopyrazam (ppm)			
	0	100	500	3000
F₀ parents, F₁ offspring				
No. of implantation sites	12.3	12.7	11.7	10.7*
Historical control values ^a	11.6, 10.3			
Mean litter size at birth (inc dead pups)	11.5	12.3	11.2	10.0*
Historical control values ^a	11.0, 9.6			
% pups born alive	94.4	98.5	99.1	98.4
Mean litter size day 29 (weaning)	10.8	10.3	10.2	9.5
% pups born alive surviving to weaning	99.0	97.9	96.5	96.7
Mean pup weight:				
Males day 1	5.8	5.4*	5.7	5.5
Males day 29 (weaning)	75.8	77.3	74.7	66.9**
Females day 1	5.5	5.1*	5.5	5.3
Females day 29 (weaning)	71.3	72.2	69.7	64.1**
F₁ parents, F₂ offspring				
No. of implantation sites	12.8	11.9	12.3	11.4*
Historical control values ^a	11.3, 11.5			
Mean litter size at birth (inc dead pups)	12.5	11.2	11.6	11.0*
Historical control values ^a	10.1, 10.9			
% pups born alive	94.0	97.2	97.9	100.0*
Mean litter size day 29 (weaning)	10.0	10.1	10.6	10.2
% pups born alive surviving to weaning	95.6	97.6	94.8	93.2
Mean pup weight:				
Males day 1	5.5	5.8*	5.5	5.4
Males day 29 (weaning)	76.3	76.1	76.0	64.5**
Females day 1	5.2	5.4*	5.2	5.1
Females day 29 (weaning)	73.2	71.2	70.2	61.3**

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

^aHistorical control values, from two previous studies conducted at Central Toxicology Laboratory (2007-2008)

At the top-dose of 3000 ppm, the total litter size was less than controls for both the F₁ (-13%*) and F₂ (-12%*) generations; the number of implantation sites in the F₀ and F₁ mothers were also lower than controls (13% and 11% respectively). However, these values were within the range of the laboratory's historical control data (based on two studies, conducted with the same strain of rat within 2-years of the current study) and so are most likely to reflect normal background variation. There was no effect on pup appearance, pup survival or sex ratio. Although pup body-weight at birth was not affected by treatment, body-weight gain during lactation was reduced at the top-dose in both generations, such that at weaning body weights were approximately 11% and 16% lower than controls for the F₁ and F₂ generations respectively.

Similarly to the parental generations, liver weights of the F₁ and F₂ offspring killed at weaning were increased by 23-32% (3000ppm) and 6-14% (500ppm). Thymus weights were increased in the F₁ but not in the F₂ females; the lack of consistency across generations suggests that this is a chance finding. In F₁ males at the top-dose, the age of preputial separation was 2.3 days later than controls, whilst in females of this group, vaginal opening occurred 2 days later than controls; however, these observations are likely to be a secondary consequence of the reduced body-weight gain and the subsequent lower post-weaning body-weights that were observed in this dose-group.

10.11.3 Comparison with the CLP criteria

According to the CLP criteria, adverse effects on sexual function and fertility include those that interfere with the reproductive system, onset of puberty, gamete production/transport, reproductive cycle, sexual behaviour, fertility, parturition, pregnancy outcome, reproductive senescence or any other function that is dependent on the reproductive system. All of these have been sufficiently investigated in a guideline-compliant two-generation study, conducted in accordance with OECD TG 416. Exposure to isopyrazam showed no indication of adverse effects on sexual function or fertility according to these criteria. Therefore classification is not proposed.

10.11.4 Adverse effects on development

The potential of isopyrazam to adversely affect development has been assessed in rats in one study using the 93:7 syn:anti specification of isopyrazam and in another using the 70:30 specification. In rabbits, three dose-range finding studies and one main study are available to assess the developmental toxicity of isopyrazam (all conducted using the 93:7 syn:anti specification).

Information is also available from the rat multigeneration study (refer to the section on offspring toxicity in section 10.11.2.).

Table 44: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
Developmental toxicity in rats		
Prenatal developmental toxicity study (gavage) OECD 414 GLP Rats, HsdRccHan:WIST, female, 24/group Anonymous (2007)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 0, 20, 75 & 250 mg/kg bw/d Dosing from GD5-21	<u>Maternal toxicity</u> <u>250 mg/kg bw/d</u> 1 female killed <i>in extremis</i> on day 21 (poor physical condition/body-weight loss) ↓ BW-gain (-23% by day 22), ↓ BW (-7%** on day 22), adjusted for gravid uterine weight ↓ BW 4% on day 22) ↓ FC** (-43% on days 5-8, -22.5% on days 17-20) <u>75 mg/kg bw/d</u> No treatment-related effects <u>20 mg/kg bw/d</u> No treatment-related effects <u>Developmental toxicity</u> <u>250 mg/kg bw/d</u> ↑ post-implantation loss, ↓ mean foetal BW, ↑ incidence of incomplete ossification: cervical centra (5.6 – 35.7%**), sternum (12.4%**), caudal arches (4 – 8%), hind-paw bones (17%**), and fore-paw bones (8%**) <u>75 & 20 mg/kg bw/d</u> No effects
Prenatal developmental toxicity study (gavage) OECD 414 GLP Rats, HsdRccHan:WIST, female, 24/group Anonymous (2008a)	Isopyrazam Batch: SMU7DP017 Purity: 90.8% (w/w) Syn:anti ratio: 70:30 0, 20, 75 & 200 mg/kg bw/d Dosing from GD 4-20	<u>Maternal toxicity</u> There were no treatment-related deaths <u>200 mg/kg bw/d</u> Ventral recumbency and sedation in 24/24 dams at start of dosing ↓ BW-gain (-48%**), ↓ BW (-17%** on day 21), when corrected for gravid uterine weight BW-loss** (-5.7g compared with a gain of 24.2g in control), ↓ FC** <u>75 mg/kg bw/d</u> ↓ BW-gain (-13%*), ↓ BW (-5%* on day 22), when corrected for gravid uterine weight ↓BW-gain (-41%**), ↓ FC** <u>20 mg/kg bw/d</u>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		<p>No treatment-related adverse effects</p> <p><u>Developmental toxicity</u></p> <p>External malformations and skeletal malformations and variations were unaffected by treatment.</p> <p><u>200 mg/kg bw/d</u></p> <p>↓ mean foetal BW in M & F (18%**)</p> <p>↑ foetuses with visceral variations 76/151 (50%) compared with 52/150 (35%) in controls (overall and specific incidences were within historical control ranges)</p> <p>↑ incidence of non-ossification in several vertebral centra and hind limb phalanges</p> <p><u>75 mg/kg bw/d</u></p> <p>↓ mean foetal BW in M & F (6%**)</p> <p>↑ incidence of non-ossification in one vertebral centrum</p> <p><u>20 mg/kg bw/d</u></p> <p>No effects</p>
Developmental toxicity in rabbits		
<p>Dose-range finding prenatal developmental toxicity study (gavage)</p> <p>GLP</p> <p>Rabbits, Himalayan, females, 10/dose</p> <p>Anonymous (2008b)</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4% (w/w)</p> <p>Syn:anti ratio: 93:7</p> <p>Vehicle: 0.5% w/v aqueous carboxymethylcellulose (CMC)</p> <p>0, 100, 200 & 400 mg/kg bw/d</p> <p>Dosing from GD 4-27</p>	<p><u>Maternal toxicity</u></p> <p>There were no deaths or treatment-related clinical signs of toxicity at any dose</p> <p>There was no effect on body weight or food consumption at any dose</p> <p>There were no macroscopic or necropsy findings at any dose</p> <p><u>Developmental toxicity</u></p> <p>There was no effect on litter size or pre- and post-implantation loss</p> <p><u>400 mg/kg bw/d</u></p> <p>7 (6)* foetuses with visceral malformations (cardiovascular), 2(1) foetuses with slightly reduced eye size</p> <p><u>200 & 100 mg/kg bw/d</u></p> <p>No treatment-related adverse effects</p>
<p>Dose-range finding prenatal developmental toxicity study (gavage)</p> <p>GLP</p> <p>Rabbits, Himalayan, females, 5/dose</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4% (w/w)</p> <p>Syn:anti ratio: 93:7</p> <p>Vehicle: 0.5% w/v CMC</p> <p>0, 600, 800 & 1000</p>	<p><u>Maternal toxicity</u></p> <p>There were no deaths or clinical signs of toxicity at any dose</p> <p>There was no effect on body weight or food consumption at any dose</p> <p>There were no macroscopic or necropsy findings at any dose</p> <p><u>Developmental toxicity</u></p> <p>There was no effect on litter size or pre- and post-implantation loss</p> <p><u>1000 mg/kg bw/d</u></p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
Anonymous (2008c)	mg/kg bw/d Dosing from GD4-27	<p>5(3)* fetuses with ‘small eye’(malformation), 10(3)** fetuses with ‘slightly small eye’ (variation), 17(4) fetuses with intraocular abnormalities consistent with microphthalmia</p> <p><u>800 mg/kg bw/d</u></p> <p>5(2)** fetuses with ‘slightly small eye’, 9(3) fetuses with intraocular abnormalities consistent with microphthalmia</p> <p><u>600 mg/kg bw/d</u></p> <p>2 (litter incidence not provided) fetuses with ‘small eye’, 9(3)** fetuses with ‘slightly small eye’, 15(4) fetuses with intraocular abnormalities consistent with microphthalmia</p>
<p>Dose-range finding prenatal developmental toxicity study (gavage) OECD 414 GLP Rabbits, New Zealand White, females, 10/dose Anonymous (2008a)</p>	<p>Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Vehicle: 0.5% w/v CMC 0, 400, 700 & 1000 mg/kg bw/d Dosing from GD7-28</p>	<p><u>Maternal toxicity</u></p> <p><u>1000 mg/kg bw/d</u> One female killed <i>in extremis</i> on day 21 ↓ BW-gain (53%*) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation</p> <p><u>700 mg/kg bw/d</u> One female aborted on day 25 ↓ BW-gain (63%**) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation</p> <p><u>400 mg/kg bw/d</u> One female killed <i>in extremis</i> on day 23, one female aborted on day 25 ↓ BW-gain (41%) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation</p> <p><u>Developmental toxicity</u></p> <p>There was no effect on litter size or pre-implantation loss, post implantation loss was ↑ in all treated groups but without statistical significance</p> <p><u>1000 mg/kg bw/d</u> ↓ mean male foetal weight (14%*) ↑ incidence of fetuses with eye malformations 5(2) compared with 1, 0 & 0 in the control, 700 & 400 mg/kg bw/d groups (incidence of 7% compared with 0-0.9% from historical control data)</p> <p><u>700 & 400 mg/kg bw/d</u> No treatment-related adverse effects</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
<p>Prenatal developmental toxicity study (gavage)</p> <p>OECD 414</p> <p>GLP</p> <p>Rabbits, New Zealand White, females, 25/dose</p> <p>Anonymous (2008b)</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4% (w/w)</p> <p>Syn:anti ratio: 93:7</p> <p>Vehicle: 0.5% w/v CMC</p> <p>0, 30, 150 & 500 mg/kg bw/d</p> <p>Dosing from GD7-28</p>	<p><u>Maternal toxicity</u></p> <p><u>500 mg/kg bw/d</u></p> <p>One female found dead on day 24</p> <p>↓ faeces production, ↓ FC (20%** days 7-13), ↑ absolute liver weight (36%), hepatocellular hypertrophy, centrilobular hepatocellular vacuolation</p> <p><u>150 mg/kg bw/d</u></p> <p>↑ absolute liver weight (13%), hepatocellular hypertrophy, centrilobular hepatocellular vacuolation</p> <p><u>30 mg/kg bw/d</u></p> <p>No treatment-related adverse effects</p> <p><u>Developmental toxicity</u></p> <p>There was no effect on litter size or pre- and post-implantation loss</p> <p><u>500 mg/kg bw/d</u></p> <p>↓ male foetal body weight (8%*)</p> <p>1 foetus with microphthalmia</p> <p><u>50 and 30 mg/kg bw/d</u></p> <p>No treatment-related adverse effects</p>

10.10.4.1 Developmental toxicity in rats

Two prenatal developmental toxicity studies conducted in rats are available; one study was performed with a batch of isopyrazam which contained the syn:anti isomers at a ratio of 93:7 and the other with a batch containing 70:30 syn:anti.

Syn:anti ratio 93:7

In the first study, isopyrazam (syn:anti ratio 93:7) was administered via gavage to groups of 24 mated rats at doses of 0, 20, 75 and 250 mg/kg bw/day, during days 5 to 21 of gestation; day of mating (detection of sperm detected by vaginal smear) was presumed to be gestation day one. Some maternal toxicity was evident at 250 mg/kg bw/d, characterised by lower food consumption throughout the study to a magnitude of 43% lower than controls on days 5-8 and 22.5% lower on days 17-20. Maternal body-weight gain was lower than controls throughout dosing (23% lower by day 22), which culminated in mean body weights that were 7% lower than controls on day 22 (4% lower when adjusted for gravid uterine weight). Body-weight losses and poor clinical condition led to the premature death of one female from this dose-group.

The percentage of pre-implantation losses (8.1% litter mean) was statistically significantly higher than those of the controls; however it should be noted that pre-implantation losses for the concurrent control (1.9%) was notably lower than the historical control value based on five other studies conducted by the laboratory in

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

2006 (range 2.7-13% mean 7.5%) and the control animals of a subsequent developmental toxicity study in rats also had a much higher loss of 4.8%. Furthermore, pre-implantation losses in a developmental toxicity study cannot be confidently attributed to treatment, as dosing commenced on GD 5 (likely after implantation occurred). Hence the dossier submitter concludes that the increases in pre-implantation losses observed in the 250 mg/kg bw/d dose-group are not a consequence of treatment with isopyrazam.

A higher percentage of early intra-uterine deaths at 250 mg/kg bw/d (22/273 compared with 3/306 in controls) meant that the value for overall post-implantation losses was statistically significantly higher than concurrent controls for this dose-group. Consequently the number of live foetuses in this dose group was also lower than controls. However, it is again noted that the post-implantation losses in the concurrent control group (1.6%) were considerably lower than the historical control range of 3.2-9.7% (mean 5.5%) based on five other studies conducted in 2006. The early interuterine deaths were just outside of the available HCD (range 1.1-7.8% mean 4.38%).

Table 45: Caesarean section data

Observation	Dose (mg/kg/day)			
	0	20	75	250
No. of pregnant dams alive on day 22	23	24	23	22
Mean no. corpora lutea/dam	13.6	13.7	13.0	13.6
Mean no of implantations/dam	13.3	12.5	12.0*	12.4
Mean no. live foetuses/dam	13.1	12.0	11.6*	11.3**
% pre-implantation loss (litter mean)	1.9	8.6**	7.7*	8.1**
% post-implantation loss (litter mean)	1.6	4.2	3.8	8.4**
Mean male foetal weight (g)	4.86	5.04	4.94	4.59*
Mean female foetal weight (g)	4.64	4.74	4.75	4.30**

A statistically significant lower number of live foetuses in the 75 mg/kg bw/d occurred in the absence of a specific effect on post-implantation loss and so therefore is likely to be a consequence of the observed pre-implantation losses in this dose-group and thus not related to treatment with isopyrazam.

The overall numbers of foetuses with malformations or variations was not influenced by treatment (see table below).

Table 46: Overall numbers (%) of foetuses with malformations or variations

Observation	Dose (mg/kg/day)			
	0	20	75	250
Number of foetuses examined	301	288	266	249
External/visceral malformations	1 (0.4%)	3 (1%)	0 (0%)	2/249 (0.8%)
External/visceral variations	58 (20%)	41 (13%)	53 (21%)	40/249 (16%)
Skeletal malformations	0 (0%)	2 (0.6%)	0 (0%)	2/249 (0.7%)
Skeletal variations	286 (95%)	273 (95%)	247 (93%)	232/249 (93%)

However there were differences from controls in the percentage of treated foetuses with the following specific skeletal variations:

Table 47: Selected specific skeletal variations (number (%)) of affected foetuses)

Description	Dose (mg/kg/day)			
	0	20	75	250
No. of foetuses examined	301	288	266	249
Cervical centra: Centrum 2 not ossified	53 (17.6%)	50 (17.4%)	60 (22.6%)	89** (35.7%)
Centrum 3 not ossified	8 (2.7%)	9 (3.1%)	22** (8.3%)	33** (13.3%)
Centrum 4 not ossified	4 (1.3%)	6 (2.1%)	10 (3.8%)	23** (9.2%)
Centrum 5 not ossified	1 (0.3%)	2 (0.7%)	9** (3.4%)	18** (7%)
Centrum 6 not ossified	2 (0.7%)	2 (0.7%)	5 (1.9%)	14** (5.6%)
Odontoid not ossified	28 (9.3%)	39 (13.5%)	43* (16.2%)	62** (24.9%)
Ventral arch bipartite ossification	85 (28.2%)	64 (22.2%)	62 (23.3%)	38** (15.3%)
Ventral arch fragmented ossification	33 (11.0%)	22 (7.6%)	19 (7.1%)	14* (5.6%)
Sternum: Xiphoid cartilage incomplete	15 (5.0%)	23 (8.0%)	25* (9.4%)	31** (12.4%)
Caudal arches: Arch 2 incompletely ossified	1 (0.3%)	3 (1.0%)	4 (1.5%)	20** (8.0%)
Arch 2 not ossified	1 (0.3%)	2 (0.7%)	2 (0.8%)	10** (4.0%)
Fore paws: Reduced ossification manus	4 (1.3%)	19** (6.6%)	10 (3.8%)	20** (8.0%)
Hind paws: Calcaneum ossified	123 (40.9%)	111 (38.5%)	101 (38.0%)	44** (17.7%)
Reduced ossification pes	27 (9.0%)	29 (10.1%)	26 (9.8%)	43** (17.3%)

Treatment-related specific skeletal variations at 250 mg/kg bw/d were observed as retardations of ossification of some vertebral centra and arches, as well as several hind and fore-paw bones, an increase in the number of foetuses with an incomplete xiphoid cartilage of the sternum was also observed at this dose. Some similar effects were also seen at 75 mg/kg bw/d; however these were only marginally different from controls and are likely to reflect biological variation and are thus not a result of treatment with isopyrazam.

Syn:anti ratio 70:30

In a second prenatal developmental toxicity study, isopyrazam (syn:anti ratio 70:30) was administered via gavage to groups of 24 presumed-pregnant rats. Doses of 0, 20, 75 and 200 mg/kg bw/d were given from gestation days 4 to 20. There were no treatment-related deaths but ventral recumbency and sedation was noted in all dams of the high-dose group for the first few days of dosing. Throughout dosing, maternal body-weight gain and food consumption were reduced at 200 and 75 mg/kg bw/d, such that by the end of the study a body-weight loss of -5.7g in the high-dose group was apparent (compared with a gain of 34.2g in the controls), whilst in the mid-dose group, body-weight gain was 41% lower than that of the controls. Isopyrazam had no effect on litter size or on pre- or post-implantation loss; however mean foetal body-weights were reduced in the 75 mg/kg bw/d (-6%) and 200 mg/kg bw/d (-18%) dose groups (see table below).

Table 48: Caesarean section data

Observation	Dose (mg/kg/day)			
	0	20	75	200
No. of pregnant dams alive on day 21	23	23	24	24
Mean no. corpora lutea/dam	14.4	14.8	14.4	14.2
Mean no of implantations/dam	13.7	13.9	13.9	13.2
Mean no. live foetuses/dam	12.4	13.1	13.1	12.2
% pre-implantation loss (litter mean)	4.8	6.2	3.5	7.0
% post-implantation loss (litter mean)	9.2	5.9	5.4	7.9
Mean male foetal weight (g)	5.0	4.8	4.7*	4.1**
Mean female foetal weight (g)	4.7	4.6	4.4*	3.9**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

The overall percentage of foetuses with malformations or skeletal variations (excluding ossification) was not influenced by treatment with isopyrazam.

Table 49: Overall numbers (%) of foetuses with malformations or variants

Observation	Dose (mg/kg/day)			
	0	20	75	200
External examination: number foetuses examined	286	301	315	291
External malformations	0	0	0	0
Visceral examination: number foetuses examined	150	156	163	151
visceral malformations	0	1 (0.6%)	3 (1.8%)	1 (0.7%)
visceral variations	52 (35%)	55 (35%)	63 (39%)	76 (50%)*
Skeletal examination: number foetuses examined	136	145	152	141
skeletal malformations	1 (0.7%)	0 (0%)	2 (1.3%)	0 (0%)
skeletal variations (excluding ossification stage and presence of supernumerary ribs)	23 (17%)	31 (21%)	38 (25%)	34 (24%)

The overall incidence of foetuses with visceral variations was statistically significantly greater than controls at the high-dose; however, the incidence of each specific visceral variation fell within the range of the historical-control data (based on six previous studies conducted over 2006-2007, in the same strain of rat and in the same laboratory) and hence was not a consequence of treatment with isopyrazam. Slight retardation of ossification was again observed at the high-dose of 200 mg/kg bw/d (see below).

Table 50: Selected skeletal examination findings

Description	Dose (mg/kg/day)			
	0	20	75	200
No. of foetuses examined	136	145	152	141
Cervical centra: Centrum 1 not ossified	7 (5%)	13 (9%)	12 (8%)	17 (12%)**
Centrum 2 not ossified	3 (2%)	7 (5%)	9 (6%)	19 (13%)**
Centrum 3 not ossified	3 (2%)	6 (4%)	10 (7%)*	13 (9%)**
Hind limb, digit: 2 proximal phalanx, left not ossified	3 (2%)	5 (3%)	5 (3%)	10 (7%)*
3 proximal phalanx left not ossified	3 (2%)	5 (3%)	3 (2%)	10 (7%)*
4 proximal phalanx left not ossified	3 (2%)	5 (3%)	3 (2%)	10 (7%)*
5 proximal phalanx left not ossified	10 (7%)	12 (8%)	14 (9%)	21 (15%)**
5 proximal phalanx right not ossified	10 (7%)	10 (7%)	11 (7)	18 (13%)*

At 200 mg/kg bw/d the incidence of non-ossification of certain vertebral centra and hind limb phalanges was increased, whilst at 75 mg/kg bw/d the incidence of non-ossification of the centrum of one vertebra (the 3rd cervical) was increased. This retardation of ossification is presumed to be related to treatment with isopyrazam.

10.10.4 Developmental toxicity in rabbits

Developmental toxicity has been investigated in rabbits in three dose-range finding studies (two in the Himalayan strain of rabbit and one in the New Zealand White); a main prenatal developmental toxicity study has also been conducted in the New Zealand White rabbit.

Dose-range finding study: Anonymous (2008b)

In the first dose-range finding study, gavage administration of isopyrazam at doses of 0, 100, 200 and 400 mg/kg bw/d isopyrazam (over days 4 to 27 of gestation) caused no observable signs of maternal toxicity in 10/group female Himalayan rabbits. There were no deaths or treatment-related clinical signs of toxicity, body weight and food consumption was not affected and there were no treatment-related macroscopic or necropsy findings.

There was no effect on litter size, pre- or post-implantation losses or foetal body-weights. Foetuses were individually weighed and examined for gross external abnormalities; the heads were separated from half of the foetuses and serially sectioned for internal evaluation (eyes, brain, nasal passages and tongue). At the high-dose, the incidence of foetuses with visceral malformations (specifically relating to the cardiovascular system) was greater than controls with seven foetuses from six litters being malformed in this way; however, as these findings were not repeated in several other developmental toxicity studies in rabbits (including those at higher doses) this is not likely to be a treatment-related effect. There was no effect on the number of foetuses with skeletal and/or head malformations/variations (see table below).

Table 51: Summary of overall numbers of fetuses with malformations or variations

Observation	Dose (mg/kg/day)			
	0	100	200	400
Number fetuses examined- external, visceral and skeletal examinations (litters)	43 (8)	80 (10)	60 (10)	57 (9)
No. with external and visceral malformations (litters)	0	2 (2)	1 (1)	7* (6)
No. with external and visceral variations (litters)	26 (8)	56 (10)	39 (10)	41 (9)
No. with skeletal malformations (litters)	0	0	1 (1)	2 (1)
No. with skeletal variations (litters)	9 (4)	13 (7)	9 (6)	19 (7)
Number fetuses examined- sectioned head (litters)	21 (8)	39 (10)	30 (10)	29 (9)
No. with malformations (litters)	0	0	0	1 (1)
No. with variations (litters)	1 (1)	0	0	2 (2)

* Statistically significant difference from control, $p < 0.05$

Two fetuses at 400 mg/kg bw/d presented with eyes of slightly reduced size (approximately 75% of normal size). One of these fetuses also presented with eye retinal folds and narrow choanal. Only limited evidence of a relationship with treatment has been demonstrated in this specific case; however, as eye abnormalities have been observed in subsequent studies in rabbits, a treatment-related effect cannot be ruled out.

Dose-range finding study: Anonymous (2008c)

In a second dose-range finding study, female Himalayan rabbits (5/group) were administered isopyrazam via gavage at dose levels of 0, 600, 800 and 1000 mg/kg bw/d over gestation days 4 to 27. Again, no maternal toxicity was evident at these doses. There were no treatment-related deaths or clinical signs of toxicity. There were no macroscopic or necropsy findings and isopyrazam had no effect on body weight or food consumption.

With regard to developmental toxicity there was no effect on pre- or post-implantation loss or litter size. The following morphological differences comprising 'small eyes' (microphthalmia) or 'slightly small eyes' were noted in isopyrazam treated groups; however, there was no clear dose-response relationship.

Table 52: Summary of fetuses (litters) with malformations, variants and eye malformations or variations

Observation	Dose (mg/kg/day)			
	0	600	800	1000
No. fetuses examined macroscopically- external, visceral and skeletal examinations (no. of litters)	33 (5)	27 (5)	23 (5)	32 (5)
No. fetuses with any external and visceral malformation (litters)	1 (1)	4 (2)	0	6 (4)
No. fetuses with eye malformation: small, ~50-75% of normal (litters)	0	2 (1)	0	5* (3)
No. of fetuses with any external and visceral finding (litters)	20 (5)	21 (5)	16 (5)	29 (5)
No. fetuses with eye variant: slightly small, ~75-<100% of normal (litters)	0	9** (3)	5** (2)	10** (3)

The laboratory has defined 'small eyes' as an abnormality (50% to 75% of normal size) and 'slightly small eyes' as a variant (75% to <100% of normal size). According to the laboratory historical control data for this strain of rabbit (based on 5 studies comprising 679 fetuses conducted in 2006-2007) only one animal has presented with microphthalmia ('small eyes') and the variant 'slightly small eyes' has never been reported for this laboratory. Microscopic examination of foetal head sections revealed intraocular abnormalities in at least those fetuses that presented with 'small' or 'very small eyes' comprising retinal dysplasia, choroidal dysplasia and/or posterior fibre disarray (see below).

Table 53: Summary of foetuses (litters) with intraocular abnormalities

Observation	Dose (mg/kg/day)			
	0	600	800	1000
No. foetuses subjected to microscopic examination of eye (litters)	13 (2)	24 (4)	16 (4)	27 (4)
No. foetuses with microscopic intraocular abnormalities consistent with microphthalmia (litters)	1 (1)	15 (4)	9 (3)	17 (4)

These findings are consistent with the presence of microphthalmia and thus confirm the occurrence of eye abnormalities in the treated groups.

Dose-range finding study: Anonymous (2008a)

A third dose-range finding study has been conducted in which doses of 0, 400, 700 and 1000 mg/kg bw/d were administered via gavage to female New Zealand White rabbits (10/group), over gestation days 7 to 28. There was evidence of maternal toxicity in all dose-groups comprising reduced food consumption, body weights and faeces production. This resulted in two females being killed *in extremis* on days 23 (400 mg/kg bw/d) and 21 (1000 mg/kg bw/d) and also led to two females aborting on day 25 at 400 and 700 mg/kg bw/d. Initial body-weight losses in all treated groups, up to a magnitude of -97g in the high-dose group, resulted in overall body weight-gains that were 63% and 53% lower than controls in the 700 and 1000 mg/kg bw/d treated groups respectively. Relative liver weights were increased in treated groups by a magnitude of 39%-77% in comparison with controls.

There was no effect on litter size or pre-implantation loss; however, post implantation losses were higher than controls in all treated groups (albeit without statistical significance) and at 1000 mg/kg bw/d mean foetal body-weights were lower than controls.

Table 54: Caesarean section data

Observation	Dose (mg/kg/day)			
	0	400	700	1000
No. of pregnant dams alive on day 29	10	8	8	9
Mean no. corpora lutea/dam	10.1	9.9	9.6	10.7
Mean no of implantations/dam	9.6	8.5	9.1	9.8
Mean no. live foetuses/dam	9.5	7.8	8.3	8.4
% pre-implantation loss (litter mean)	4.7	14.2	5.3	8.5
% post-implantation loss (litter mean)	1.1	6.9	8.5	12.8
Mean male foetal weight (g)	42.2	42.2	39.3	36.1*
Mean female foetal weight (g)	40.0	39.6	40.0	36.6

Similar to the previous dose-range finding studies that were conducted in Himalayan rabbits, there was an increase in the incidence of foetuses with microphthalmia at 1000 mg/kg bw/d (see below).

Table 55: Summary of foetuses with malformations, variants and eye malformations

Observation	Dose (mg/kg/day)			
	0	400	700	1000
Number foetuses examined- external and visceral and skeletal examinations (litters)	95	62	66	76
Total number of foetuses with any external and visceral malformations (litters)	1 (1)	1 (1)	0	5 (2)
Number of foetuses with eye malformations:	1 (1)	0	0	5 (2)

The incidence of rabbits presenting with eye variations and malformations at 1000 mg/kg bw/d (5/76 animals) was substantially greater than the range of the historical control data for this strain of rabbit of 0-0.9% (total incidence of 4/6125 foetuses from 33 studies). In three of these five cases, microphthalmia was

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

associated with a haemorrhagic ring around the eye (one foetus), reddened eyes (two foetuses) and/or dark red areas of the eye (one foetus). Microphthalmia has been observed in other developmental toxicity studies in rabbits, therefore the incidences seen in this study are likely to be related to treatment with isopyrazam.

Main study

The main prenatal developmental toxicity study was conducted in New Zealand White rabbits. Gavage doses of 0, 30, 150 and 500 mg/kg bw/d isopyrazam were administered to 25/group mated females from day 7 to day 28 of gestation. One female at 500 mg/kg bw/d was found dead on day 24 following body-weight losses and reduced food consumption. Food consumption was reduced for this group overall with an associated reduction in faeces production, although maternal body weights were generally not affected by treatment. Liver weights were increased at 150 and 500 mg/kg bw/d (13% and 36% respectively) and associated findings of minimal to mild centrilobular hepatocellular hypertrophy/vacuolation were evident.

Isopyrazam treatment had no effect on litter size or on pre- or post-implantation losses. Mean foetal body-weights were lower than controls at 500 mg/kg/day with statistical significance being reached for males only (see table below).

Table 56: Caesarean section data

Observation	Dose (mg/kg/day)			
	0	30	150	500
No. of pregnant dams alive on day 29	23	24	23	23
Mean no. corpora lutea/dam	9.0	9.9	9.7	9.9
Mean no of implantations/dam	8.3	9.3	8.9	9.4*
Mean no. live foetuses/dam	7.9	8.8	8.6	8.8
% pre-implantation loss (litter mean)	8.0	5.6	7	5.1
% post-implantation loss (litter mean)	4.6	4.8	3.4	6.2
Mean male foetal weight (g)	42.2	40.6	40.1	39.0*
Mean female foetal weight (g)	41.6	38.7	39.4	38.4

Treatment with isopyrazam had no influence on the overall incidence of foetal malformations or variations, as shown in the table below.

Table 57: Summary of overall numbers of foetuses with malformations or variations

Observation	Dose (mg/kg/day)			
	0	30	150	500
Number foetuses examined- external, visceral and skeletal examinations (litters)	182 (23)	212 (24)	197 (23)	202 (23)
Number of foetuses with external, visceral or skeletal malformations (litters)	2 (2)	6 (6)	5 (5)	7 (6)
Mean % of foetuses with external, visceral or skeletal variations	72	76	77	71

Of the seven foetuses presenting with malformations at 500 mg/kg/day, only one had microphthalmia. Although this incidence did not exceed the laboratory historical control range (0-0.9% from 33 studies, overall control incidence 4/6125 foetuses), similar eye malformations have been reported in other isopyrazam rabbit developmental toxicity studies (at higher doses), and so a relationship with treatment cannot be unequivocally excluded.

10.11.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of isopyrazam has been investigated in rats and rabbits. Information is also available from the rat multigeneration study.

In the rat multigeneration study, a decrease in the number of implantation sites and a decrease in total litter size was observed in both generations at the top dose of 3000ppm. However, these findings were within the range of the historical control data provided by the laboratory and are considered to reflect background variation. As such they are not considered further in the assessment of developmental toxicity.

Two prenatal developmental toxicity studies are available in rats. In the first, after administration of the 93:7 syn:anti specification of isopyrazam, some maternal toxicity was evident at the high-dose of 250 mg/kg bw/d, characterised by reduced food consumption and lower body-weight gains. Body weights were lower than controls throughout the study, but at no point was the difference more than 10% lower than the controls. At the end of the study (day 22) body weights in the high-dose group were 7% lower than controls (4% when adjusted for gravid uterine weight). However, it is noted that the body-weight losses and poor clinical condition led to the premature death of one female from this dose-group. At this dose some developmental toxicity was apparent, characterised by a statistically significant increase in post-implantation loss due to a statistically significantly higher percentage of early intra-uterine deaths. As a consequence, the number of live foetuses was lower in this group in comparison with the controls and mean foetal weight was also significantly lower. It is noted that the post implantation losses in the concurrent controls was much lower than the laboratory HCD and that the incidence in the high dose group was just within this range (range of 3.2-9.7% mean of 5.5%). Slight retardation of ossification was also observed but these changes are considered to be transient and do not necessarily represent an adverse effect.

In a second prenatal developmental toxicity study, in which the 70:30 syn:anti specification of isopyrazam was administered to rats, maternal toxicity was noted at 75 and 200 mg/kg bw/d (lower body-weight gain, reduced food consumption and reduced faeces production). At these doses, foetal body-weights were lower than controls and a slight retardation of ossification was evident. Developmental toxicity was observed in rats (reduced foetal weight and slight retardation of ossification) at dose levels also eliciting maternal toxicity. These types of developmental effects are commonly seen in rats in conjunction with maternal toxicity and are therefore likely to be secondary consequences at these doses and not a specific developmental effect arising as a result of treatment with isopyrazam. There were no quantitative or qualitative differences in the rat, with regard to the developmental toxicity of the 93:7 and the 70:30 syn:anti specifications of isopyrazam.

Developmental toxicity was investigated in Himalayan rabbits in two dose-range finding studies and in New Zealand White rabbits in a dose-range finding study and a main prenatal developmental toxicity study. In the studies conducted in Himalayan rabbits no maternal toxicity was evident up the top doses tested (400 mg/kg bw/d in the first study and 1000 mg/kg bw/d in the second study). In New Zealand White rabbits maternal toxicity was evident in the dose-range finding study across all treated groups (400, 700 and 1000 mg/kg bw/d) characterised by reductions in food consumption, body-weight gain and faeces production which led to two females being killed *in extremis* and two females aborting. In the main prenatal developmental toxicity study in the New Zealand White, doses of 30, 150 and 500 mg/kg bw/d resulted in the death of one female at the top-dose; maternal toxicity at this dose presented as reduced food consumption and faeces production, but there was no resulting effect on body-weights.

Isopyrazam caused developmental toxicity in rabbits, manifested as eye abnormalities to a varying degree across all four studies. Incidences of microphthalmia ('small eyes') or 'slightly small eyes' were evident in two preliminary studies (one in the Himalayan rabbit and one in the New Zealand White rabbit) and were also occasional features of the other rabbit studies (although a link with treatment was not unequivocally demonstrated in these latter cases). No abnormalities/variations of this nature were detected in the rat studies; however, neither used such high doses as were used in rabbits.

Table 58: Summary of eye malformations/variatio ns in rabbits

Study	Strain	Dose-levels where eye effects seen	Maternal toxicity	No animals examined	Incidence of microphthalmia foetus (litter)	Incidence of 'slightly small eyes' foetus (litter)	Historical control data
Dose-range finding prenatal developmental toxicity study	Himalayan Rabbit	400 mg/kg bw/d	None	57 (9)	0	2(1)	1/679
Dose-range finding prenatal developmental toxicity study	Himalayan rabbit	1000 mg/kg bw/d	None	32(5)	5(3)*	10(3)**	1/679
		800 mg/kg bw/d		23(5)	0	5(2)**	
		600 mg/kg bw/d		27(5)	2(1)	9(3)**	
Dose-range finding prenatal developmental toxicity study	New Zealand White rabbit	1000 mg/kg bw/d	400 mg/kg bw/d: ↓ BW-gain (63%) 1000 mg/kg bw/d: ↓ BW-gain (53%)	76	5(2)		4/6125
Prenatal developmental toxicity study	New Zealand White rabbit	500 mg/kg bw/d	↓ food consumption/faeces production No effect on body weight	202 (23)	1	0	4/6125

At the top-dose of 1000 mg/kg bw/d in the second dose-range finding study (Himalayan rabbit), 5 (3) foetuses (litter) presented with 'slightly small eyes' whilst 10 (3) at 1000 mg/kg bw/d and 5 (2) litters at 800 mg/kg bw/d presented with the malformation microphthalmia (small eyes). According to the laboratory historical control data, only one incidence of microphthalmia has been seen in the Himalayan rabbit and an incidence of 'slightly small eyes' has never been recorded. In this particular study, microscopic examination of the foetal head sections revealed intraocular abnormalities that are consistent with microphthalmia. In the previous range-finding study in the Himalayan rabbit, only two foetuses had presented with 'eyes of slightly reduced size' and none with 'eyes of reduced size'; however the doses were much lower in this study (top-dose of 400 mg/kg bw/d) and in the context of findings from other studies at higher doses, a relationship to treatment with isopyrazam cannot be excluded. No maternal toxicity was observed at any dose in these preliminary studies conducted in the Himalayan rabbit.

In New Zealand White rabbits, the incidence of microphthalmia observed in the dose-range finding study at the top-dose of 1000 mg/kg bw/d (5/76 from 2 litters) was substantially above the laboratory historical data for this strain (4/6125 foetuses from 33 studies); maternal toxicity in this study was evident from 400 mg/kg bw/d, which is well below the dose at which the eye malformations were seen (1000 mg/kg bw/d). In the main study at the top-dose of 500 mg/kg bw/d only 1/202 foetuses presented with microphthalmia, which was within the range of the laboratory historical control data; however when taken in consideration with the findings from previous studies a relationship to treatment with isopyrazam cannot be ruled out. In this study the incidences of microphthalmia were only observed at the high-dose of 500 mg/kg bw/d, a dose at which

mild maternal toxicity was evident; at this dose food consumption was reduced but to an extent not sufficient to have an effect on body-weight.

In conclusion isopyrazam caused developmental effects in rabbits in the form of eye abnormalities (microphthalmia) in two range-finding studies in the Himalayan and New Zealand White strains; sporadic eye effects in another range-finding study and in a main prenatal developmental toxicity study were also likely to be related to treatment with isopyrazam.

10.11.6 Comparison with the CLP criteria

According to the CLP criteria, developmental toxicity includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during pre-natal development, or postnatally, to the time of sexual maturation. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth and functional deficiency.

Classification for developmental toxicity is appropriate when these effects are observed in the absence of other toxic effects, or if the adverse effect on development is considered not to be a secondary non-specific consequence of the other toxic effects. The CLP criteria recommend that classification of a substance into category 1 for developmental toxicity (1A or 1B) is appropriate when that substance is known to have produced an adverse effect on development in humans, or there is strong evidence available from animal studies. Category 2 is more appropriate when there is some uncertainty about the relevance of the findings to humans.

No human data are available. However, there is evidence from animal studies of an adverse effect on development in both rats (post-implantation losses) and rabbits (microphthalmia).

The post implantation losses (resulting from early interuterine deaths) seen in the first rat developmental toxicity study were not observed in the presence of marked maternal toxicity; at the end of the study the body weights of the high-dose dams were just 7% lower than controls (4% when adjusted for gravid uterine weight) and never exceeded 10% at any time during the study. However, it is noted that the body-weight losses and poor clinical condition led to the premature death of one female from this dose-group. It is also noted that the incidence was just within the range of the laboratory HCD and that the losses observed in the concurrent control group were considerably low. Further, there were no effects on post-implantation loss (or early interuterine death) in a second study in rats at a slightly reduced top dose of 200 mg/kg bw/day. Overall, these findings are not considered to support classification for developmental toxicity.

Microphthalmia was observed in rabbits of both the Himalayan and the New Zealand White strains and was not always accompanied by excessive maternal toxicity.

Two preliminary studies were conducted in this Himalayan rabbits and the relevant historical control data reported microphthalmia at an incidence rate of 1/679. In the first study, two foetuses (from one litter) at 400 mg/kg bw/d and one control animal, presented with the variation 'eyes of slightly reduced size' whilst no cases of microphthalmia ('eyes of reduced size') were reported. No maternal toxicity was evident in this study up to and including the high-dose of 400 mg/kg bw/d.

In the second preliminary study in the Himalayan rabbit, 'eyes of slightly reduced size' were reported in 10(3), 5(2) and 9(3) foetuses (litter) in the 1000, 800 and 600 mg/kg bw/d dose-groups. In addition, 5(3) and 2(1) foetuses (litter) at 1000 and 600 mg/kg bw/d presented with the eye malformation microphthalmia ('eyes of reduced size'). No signs of maternal toxicity were evident in this study up to and including the dose at which the eye effects became apparent.

One preliminary and one main study have been conducted in the New Zealand White strain of rabbit; according to the historical control data for this strain, microphthalmia has been reported in 4/6125 foetuses. In the preliminary study, 76 animals were examined and microphthalmia was noted in five foetuses (from two litters). All of these cases occurred at the top-dose of 1000 mg/kg bw/d, a dose level which also induced severe maternal toxicity. Such maternal toxicity became evident from 400 mg/kg bw/d and was characterised by mortality, abortions, lower body weight and body-weight gain, reduced food consumption and lower faeces production. Body-weight losses throughout the study at the top-dose of 1000 mg/kg bw/d (up to -97g

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

at weeks 10-13) resulted in overall body-weight gains that were 53% lower than controls by the end of the treatment period. Large reductions in body-weight gain were also seen at the mid-dose of 700 mg/kg bw/d (63% lower than controls by the end of the study). Hence the microphthalmia observed in the New Zealand White rabbits in this preliminary study occurred only in the presence of marked maternal toxicity.

A high-dose of 500 mg/kg bw/d was selected for the main study. There were no signs of maternal toxicity at this dose and 1/202 rabbits presented with microphthalmia. This was within the range of the historical control data for this strain; however, as similar effects were seen across all developmental studies in rabbits, a relationship with treatment cannot be excluded.

Conclusion

In conclusion, there were incidences of microphthalmia or 'slightly small eyes' that were outside the historical control data and could reasonably be considered to be related to treatment with isopyrazam. Such effects only occurred in the presence of marked maternal toxicity in the New Zealand White rabbit (dose-range finding study) whereas in the Himalayan strain of rabbit, no maternal toxicity accompanied the finding. However, it is noted that the studies in the Himalayan rabbit were preliminary studies only and there is a question about the reporting of the finding by the test laboratory (i.e., the test report refers to the finding as 'eyes of slightly reduced size' and 'eyes of reduced size' rather than microphthalmia). That said, the overall conclusion of the test report (which included a microscopic examination of the foetal head sections and consideration of the intraocular abnormalities) was that the findings were consistent with microphthalmia.

Given the uncertainties, classification in Category 2 could be considered. However, as the same effect on the eyes has been observed in multiple studies and is consistent across two different strains of rabbit, it cannot be confidently dismissed as a secondary effect of maternal toxicity. Therefore, it is the opinion of the dossier submitter that classification in Category 1B would be the more appropriate classification for isopyrazam.

10.11.7 Adverse effects on or via lactation

Information relevant to any potential adverse effects on or via lactation after the administration of isopyrazam can be derived from the two-generation study in rats (see section 10.10.1).

At the top-dose of 3000 ppm (equivalent to 774 mg/kg bw/d), pup body-weights were lower than controls by 11% and 6% in the F₁ and F₂ generations respectively during lactation. Marked maternal toxicity was evident at this dose throughout the study, characterised by reduced food consumption, reduced body-weight gains and lower overall mean body weights. By the end of lactation, the magnitude of the body weight reductions (in comparison with controls) were 6.6% and 8% for the F₀ and F₁ dams respectively, whilst food consumption was 18% and 12% lower than controls. Consequently, the dossier submitter concludes that the observed effects on pups during lactation were most probably secondary to maternal toxicity.

10.11.8 Comparison with the CLP criteria

According to the CLP criteria, classification for effects on or via lactation are assigned if there is human evidence that indicates a hazard to babies during lactation and/or clear evidence from animals that a substance causes adverse effects in offspring because of transfer in the milk or adverse effects on the quality of the milk and/or ADME studies indicate that the substance is present in the milk at potentially toxic concentrations. No human evidence is available and there is no toxicokinetic information to indicate that toxic concentrations of isopyrazam or its metabolites may be present in milk. In a two-generation reproductive toxicity study signs of toxicity in the offspring during lactation (reductions in body weight gain) are attributable to maternal toxicity. Therefore, the criteria for classification for effects on or via lactation are not met.

10.11.9 Conclusion on classification and labelling for reproductive toxicity

Repr. 1B; H360D – May damage the unborn child
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RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of isopyrazam has been investigated in a two-generation study in rats and in several developmental toxicity studies in rats and rabbits. The DS proposed no classification for sexual function and fertility and adverse effects on or via lactation based on the lack of effects in the 2-generation study. On the contrary, DS proposed classification of isopyrazam as Repr. 1B (H360D) based on the microphthalmia or "slight small eyes" consistently found in four developmental toxicity studies in rabbits.

Comments received during consultation

One company/major manufacturer disagreed with the proposal of classification released by the DS. This company submitted a position paper entitled "*Isopyrazam-Technical Position on the Classification of Isopyrazam for Developmental Toxicity in Rabbits*" with detailed comments. The position paper highlights the following points:

- There is no evidence that isopyrazam causes developmental toxicity in the rat and the only finding of relevance to classification is the observation of microphthalmia and small eyes at high dose levels of isopyrazam (≥ 600 mg/kg/day) in the rabbit.
- Rabbit studies on isopyrazam were performed by two separate contract research organizations (CRO). The isopyrazam rabbit prenatal developmental toxicity studies were some of the first externally managed studies conducted for Syngenta. Due to differences in ways of working, there were uncertainties in the procedures in place for minimising bias and reporting. Consequently, Syngenta changed CRO during isopyrazam development, which caused a need to change the rabbit strain. Preliminary and definitive prenatal developmental toxicity studies were conducted at WIL Research Laboratories (Ashland, US) in New Zealand White rabbits (WIL 639020 and 639021).
- Two preliminary studies in Himalayan rabbit reported foetal findings of eyes smaller than normal, but not sufficiently small to be described as microphthalmia. Small eyes had not previously been described by RCC CRO company and were not in the laboratory's glossary of foetal effects. In the preliminary study at WIL Research Laboratories in New Zealand Whites, a higher incidence of microphthalmia was noted at 1000 mg/kg/day, which was considered related to administration of isopyrazam. However, the maternal toxicity at this dose level was excessive (severe weight loss and abortion necessitating termination). Increased liver weight and centrilobular hepatocyte hypertrophy was noted in New Zealand White rabbits from 150 mg/kg/day.
- In the definitive regulatory rabbit study, there were no occurrences of malformations outside of the laboratories historical control data range.
- Genetics of albinism predisposes laboratory animals to microphthalmia. Albinism of laboratory strains has been associated with a predisposition to microphthalmia due to the expression patterns of genes, which differ from wild type (pigmented) animals.
- Microphthalmia and anophthalmia is common in laboratory rabbits. Microphthalmia / anophthalmia is the second most common major malformation in New Zealand White

rabbits, with a maximum incidence of 1.23% per litter ($0.07 \pm 0.2\%$, mean \pm SD) from 2007 to 2017. Demonstrating a propensity for this abnormality, Charles River (Ashland, USA) reported 20 cases in 17 litters over the same time period. The minor and variant findings reported in Himalayan rabbits were not considered in their foetal glossary, consequently, there was no historical control data available to contextualise the findings. There are significant differences in the calling criteria for microphthalmia and "small eye".

- Higher incidence of microphthalmia associated with poor clinical condition of the dams. The induction of microphthalmia has been associated with the clinical condition of the dam. Consequently, the interpretation of microphthalmia in rabbits is not clear-cut, and it is vital to consider both the species inherent propensity for the malformation and confounders such as maternal toxicity.
- The microphthalmia / small eyes observed in rabbits administered high dose levels of isopyrazam are not considered a specific or sensitive indicator of developmental toxicity. The increases in microphthalmia / small eyes are considered secondary to enhancement of an underlying physiological process predisposing the animals to this malformation. The litter incidence of microphthalmia in isopyrazam treated groups was only a single incidence higher than the concurrent controls, and, in light of the excessive toxicity observed at this dose level, the microphthalmia and small eyes were considered a secondary consequence of maternal toxicity.

Overall, the company/manufacturer considered that the evidence is not sufficiently clear for Category 1B 'Presumed human reproductive toxicant' (H360D) and considered Category 2 'suspected of damaging the unborn child' (H361d) the only suitable remaining category. DS thanked the comments, commented that they should be taken into consideration by RAC but remained in the opinion that Repr 1B (H360D) is appropriate.

One MSCA supported Repr. 1B H360D but requested more information for substantiating the lack of relevance of the delay in vaginal opening and preputial separation. This MSCA also questioned the validity of the HCD (only 2 studies were available) for the number of implantation sites and mean litter size and demanded more weight for the concurrent control group. The DS thanked the comment, provided the requested information for delayed preputial separation and vaginal opening (also shown below in this opinion) and highlighted the fact that the available HCD is relevant in terms of the timing and supports the conclusion that the findings reflect normal background variation. The DS considered that the HCD should not be disregarded and, on the opposite, should be considered in a weight of evidence approach together with other evidences.

A second MSCA also supported Repr. 1B (H360D) but proposed also H360f based on the fact that total litter size was significantly decreased for both the F1 ($-13\%*$ $p < 0.05$) and F2 ($-12\%*$ $p < 0.05$) generations, in comparison with the controls; the number of implantation sites in the F0 and F1 mothers were also significantly decreased (12.3 v.s. $10.7*$ $p < 0.05$ and 12.8 v.s. $11.4*$ $p < 0.05$, in controls and F0 then F1 mothers, respectively) compared to controls and the low reliability of HCD since only 2 studies were available in HCD. DS thanked the comments and replied the same considerations stated above as regard the suitability of the HCD.

Assessment and comparison with the classification criteria**Sexual function and fertility**

Table 21 summarises the 2-generation study in rats.

Table 21: Summary of animal studies on adverse effects on sexual function and fertility with isopyrazam.

Method	Results	Reference
Multigeneration study		Anonymous 2008b
HsdRCChan:WIST rats		
OECD 416		
GLP		
Isopyrazam (batch: SMU6AP001)		
Purity: 96.4% (w/w)		
93:7 syn:anti 0, 100, 500 and 3000ppm		
	mg/kg bw/day	
	100 ppm 500 ppm 3000 ppm	
	M F M F M F	
F0 pre mating	8 9 41 47 250 277	
F0 gestation	- 7 - 37 - 217	
F0 lactation	- 25 - 119 - 700	
F1 pre mating	9 10 48 50 288 301	
F1 gestation	- 8 - 41 - 239	
F1 lactation	- 24 - 129 - 774	

There were no treatment related deaths or clinical signs of toxicity

Parental toxicity (all effects were statistically significant)

3000 ppm

	F0		F1	
	males	females	males	females
↓ Body weight (end pre mating period)	11%	8%	5%	6%
↑ Liver weights	18%	29%	27%	31%
↓ Ovary weight	-	13%		27%
↓ uterus + cervix weight	-	27%		39%
Hepatocellular hypertrophy	Minimal or slight		Minimal or slight	

500ppm

	F0		F1	
	males	females	males	females
↓ Body weight (end pre mating period)	-	2%	-	8%
↑ Liver weights	-	8%	10%	15%
Hepatocellular hypertrophy	Minimal or slight		Minimal or slight	

100ppm

	F0		F1	
	males	females	males	females
↑ Liver weights	-	-	-	10%

Fertility

F0 generation: No adverse effects

F1 generation: No adverse effects

Offspring toxicity (all effects were statistically significant)

3000 ppm

	F1	F2
Mean litter size at birth (inc dead pups)	10.0 vs 11.5 g (HCD ^a : 11.0/9.6)	11.0 vs 12.5 g (HCD ^a : 10.1/10.9)
Implantation sites	10.7 vs 12.3 (HCD ^a : 11.6/10.3)	11.4 vs 12.8 (HCD ^a : 11.3/11.5)
↓ Mean pup weight males day 29 (weaning)	12%	15%
↓ Mean pup weight females day 29 (weaning)	10%	16%
↑ Liver weights in males	26%	23%
↑ Liver weights in females	26%	32%
↑ thymus weights in females	15%	-
^a Historical control values, from two previous studies conducted at Central Toxicology Laboratory (2007-2008)		

Parental toxicity

Toxicity was evident in both the F0 and the F1 parental generations (Table 21). At 3000 ppm body weights were lower than controls throughout the entire study. By the end of the pre-mating period, body weights in males and females of the F0 generation were 11% and 8% lower than controls, whilst those of the F1 generation were 5% and 6% lower. Body weights of females at 500 ppm were also lower than controls by the end of pre-mating period (2% and 8% lower in the F0 and F1 generations respectively).

Liver weights were increased in all treated animals of both parental generations (Table 21). Relative liver weights for the F0 generation were 18% and 29% greater than controls in males and females respectively at 3000 ppm, and 8% greater in females at 500 ppm. For the F1 generation the increases were 27% and 31% at the top-dose and 10% and 15% at the mid dose in males and females respectively; liver weights were also increased in females of the F1 generation at 100 ppm (10%). Accompanying histopathological signs were present in both parental generations at 500 and 3000 ppm and comprised a dose-related increase in the

incidence of hepatocellular hypertrophy (diffuse at the high dose and centrilobular at the mid dose) with fine cytoplasmic vacuolation apparent in the hypertrophic hepatocytes. RAC notes that these hepatic effects were already noted in the repeated dose toxicity studies and were considered as adaptive. Ovary and uterus weights were increased at the top-dose in both generations. However, in the absence of microscopic findings or functional effects on fertility RAC notes that these findings were unlikely to be treatment related.

Fertility

Isopyrazam exposure had no adverse effects on fertility or reproductive performance in either generation. Oestrous cycling and sperm parameters were unaffected by treatment.

Offspring toxicity

At the top-dose of 3000 ppm, the total litter size was smaller than controls for both the F1 (-13%* p<0.05) and F2 (-12%* p<0.05) generations; the number of implantation sites in the F0 and F1 mothers were also lower than controls (13%* and 11%* respectively) (Table 21). However, these values were within the range of the laboratory's historical control data (based on two studies conducted with the same strain of rat within 2-years of the current study) and so are most likely to reflect normal background variation. There was no effect on pup appearance, pup survival or sex ratio. Although pup body-weight at birth was not affected by treatment, body-weight gain during lactation was reduced at the top-dose in both generations, such that at weaning body weights were approximately 11% and 16% lower than controls for the F1 and F2 generations respectively.

Similarly, to the parental generations, liver weights of the F1 and F2 offspring killed at weaning were increased by 23-32% (3000 ppm) and 6-14% (500 ppm). Thymus weights were increased in the F1 but not in the F2 females; the lack of consistency across generations suggests that this is a chance finding.

In F1 males at the top-dose, the age of preputial separation was 2.3 days later than controls, whilst in females of this group, vaginal opening occurred 2 days later than controls; however, these observations are likely to be a secondary consequence of the reduced body-weight gain and the subsequent lower post-weaning body-weights that were observed in this dose-group. Table 22 shows further information relating to the preputial separation and vaginal opening.

Table 22. Relationships between days of preputial separation and vaginal opening with mean body weight in F1 generation.

	Dietary concentration			
	0	100	500	3000
No animals	26	26	26	26
Day of preputial separation (mean)	45.0	45.1	45.8	47.3**
SD	1.7	1.6	2.5	2.4
Mean body Weight at landmark (g)	171.9	170.4	171.0	159.2**
SD	15.7	14.5	19.5	17.6
Day of vaginal opening (mean)	36.2	36.9	36.6	38.2**
SD	1.2	1.8	1.8	2.0
Mean body Weight at landmark (g)	105.3	104.5	102.4	103.7
SD	7.8	11.2	8.4	13.8

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Development

The potential of isopyrazam to adversely affect development has been assessed in rats in one study using the 93:7 *syn:anti* isopyrazam and in another using the 70:30 ratio. In rabbits, three dose-range finding studies and one main study are available to assess the developmental toxicity of isopyrazam (all conducted using the 93:7 *syn:anti*).

Developmental toxicity study in rats with preparation *syn:anti* ratio 93:7 (Anonymous 2007)

Isopyrazam (93:7 *syn:anti*, purity 96.4%) was administered via gavage to groups of 24 mated HsdRccHan:WIST rats at doses of 0, 20, 75 and 250 mg/kg bw/day, during days 5 to 21 of gestation; day of mating (detection of sperm detected by vaginal smear) was presumed to be gestation day one. Some maternal toxicity was evident at 250 mg/kg bw/d. It was characterised by lower food consumption throughout the study to a magnitude of 43% lower than controls on days 5-8 and 22.5% lower on days 17-20. Maternal body-weight gain was lower than controls throughout dosing (23% lower by day 22), which culminated in mean body weights that were 7% lower than controls on day 22 (4% lower when adjusted for gravid uterine weight). Body-weight loss and poor clinical condition led to the premature death of one female from this dose-group.

Table 23 summarises the caesarean section data. The percentages of pre-implantation losses were statistically significantly higher than those of the controls for all doses. However, it should be noted that pre-implantation losses for the concurrent control (1.9%) was notably lower than the historical control value based on five other studies conducted by the laboratory in 2006 (range 2.7-13% mean 7.5%) and the control animals of a subsequent developmental toxicity study in rats also had a much higher loss of 4.8%. DS concludes that the increases in pre-implantation losses observed in the 250 mg/kg bw/day dose-group are not a consequence of treatment with isopyrazam, especially considering that dosing commenced on gestation day 5, likely after implantation occurred.

A higher percentage of early intra-uterine deaths at 250 mg/kg bw/day (8% (22/273) compared with 1% (3/306) in controls) meant that the value for overall post-implantation losses was statistically significantly higher than concurrent controls for this dose-group (Table 23). Consequently, the number of live foetuses in this dose group was also lower than controls. However, it is again noted that the post-implantation losses in the concurrent control group (1.6%) were considerably lower than the historical control range of 3.2-9.7% (mean 5.5%) based on five other studies conducted in 2006.

A statistically significant lower number of live foetuses in the 75 mg/kg bw/day occurred in the absence of a specific effect on post-implantation loss, therefore it is likely to be a consequence of the observed pre-implantation losses in this dose-group and thus not related to treatment with isopyrazam.

Table 23: Caesarean section data in the developmental toxicity study in rats with preparation 93:7 *syn:anti* (Anonymous 2007)

Observation	Dose (mg/kg/day)			
	0	20	75	250
No. of pregnant dams alive on day 22	23	24	23	22
Mean no. corpora lutea/dam	13.6	13.7	13.0	13.6
Mean no of implantations/dam	13.3	12.5	12.0*	12.4
Mean no. live foetuses/dam	13.1	12.0	11.6*	11.3**
% pre-implantation loss (litter mean)	1.9	8.6**	7.7*	8.1**
% post-implantation loss (litter mean)	1.6	4.2	3.8	8.4**
Mean male foetal weight (g)	4.86	5.04	4.94	4.59**
Mean female foetal weight (g)	4.64	4.74	4.75	4.30**

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)
 ** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

The overall numbers of fetuses with malformations or variations was not influenced by treatment. However, there were differences from controls in the percentage of treated fetuses with the following specific skeletal variations shown in Table 24. Treatment-related specific skeletal variations at 250 mg/kg bw/day were observed as retardations of ossification of some vertebral centra and arches, as well as in several hind and fore-paw bones. An increase in the number of fetuses with an incomplete xiphoid cartilage of the sternum was also observed at this dose. Some similar effects were also seen at 75 mg/kg bw/d; however, these were only marginally different from controls and are likely to reflect biological variation and are thus not a result of treatment with isopyrazam.

Table 24: Selected specific skeletal variations (number (%) of affected fetuses) in the developmental toxicity study in rats with preparation 93:7 *syn:anti*(Anonymous 2007).

Description	Dose (mg/kg/day)			
	0	20	75	250
No. of fetuses examined	301	288	266	249
Cervical centra:				
Centrum 2 not ossified	53 (17.6%)	50 (17.4%)	60 (22.6%)	89** (35.7%)
Centrum 3 not ossified	8 (2.7%)	9 (3.1%)	22** (8.3%)	33** (13.3%)
Centrum 4 not ossified	4 (1.3%)	6 (2.1%)	10 (3.8%)	23** (9.2%)
Centrum 5 not ossified	1 (0.3%)	2 (0.7%)	9** (3.4%)	18** (7%)
Centrum 6 not ossified	2 (0.7%)	2 (0.7%)	5 (1.9%)	14** (5.6%)
Odontoid not ossified	28 (9.3%)	39 (13.5%)	43* (16.2%)	62** (24.9%)
Ventral arch bipartite ossification	85 (28.2%)	64 (22.2%)	62 (23.3%)	38** (15.3%)
Ventral arch fragmented ossification	33 (11.0%)	22 (7.6%)	19 (7.1%)	14* (5.6%)
Sternum:				
Xiphoid cartilage incomplete	15 (5.0%)	23 (8.0%)	25* (9.4%)	31** (12.4%)
Caudal arches:				
Arch 2 incompletely ossified	1 (0.3%)	3 (1.0%)	4 (1.5%)	20** (8.0%)
Arch 2 not ossified	1 (0.3%)	2 (0.7%)	2 (0.8%)	10** (4.0%)
Fore paws:				
Reduced ossification manus	4 (1.3%)	19** (6.6%)	10 (3.8%)	20** (8.0%)
Hind paws:				
Calcaneum ossified	123 (40.9%)	111 (38.5%)	101 (38.0%)	44** (17.7%)
Reduced ossification pes	27 (9.0%)	29 (10.1%)	26 (9.8%)	43** (17.3%)

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Developmental toxicity study in rats with preparation 70:30 *syn:anti* ratio (Anonymous 2008a)

In a second prenatal developmental toxicity study, isopyrazam (70:30 *syn:anti*, purity 90.8%) was administered via gavage to groups of 24 presumed-pregnant HsdRccHan:WIST rats. Doses of 0, 20, 75 and 200 mg/kg bw/day were given from gestation days 4 to 20. There were no treatment-related deaths but ventral recumbency and sedation was noted in all dams of the high dose group for the first few days of dosing. Throughout dosing, maternal body-weight gain and food consumption were reduced at 200 and 75 mg/kg bw/d, such that by the end of the study a corrected for gravid uterus body-weight loss of -5.7 g in the high dose group was apparent (compared with a gain of 34.2 g in the controls). In the

mid dose group, the maternal, corrected for gravid uterus, body-weight gain was 41% lower than that of the controls.

Isopyrazam had no effect on litter size or on pre- or post-implantation loss; however, mean foetal body-weights were reduced in the 75 mg/kg bw/day (-6%) and 200 mg/kg bw/day (-18%) dose groups (Table 25).

Table 25: Caesarean section data in the Developmental toxicity in rats with preparation 70:30 *syn:anti* (Anonymous 2008a).

Observation	Dose (mg/kg/day)			
	0	20	75	200
No. of pregnant dams alive on day 21	23	23	24	24
Mean no. corpora lutea/dam	14.4	14.8	14.4	14.2
Mean no of implantations/dam	13.7	13.9	13.9	13.2
Mean no. live foetuses/dam	12.4	13.1	13.1	12.2
% pre-implantation loss (litter mean)	4.8	6.2	3.5	7.0
% post-implantation loss (litter mean)	9.2	5.9	5.4	7.9
Mean male foetal weight (g)	5.0	4.8	4.7*	4.1**
Mean female foetal weight (g)	4.7	4.6	4.4*	3.9**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

The overall percentage of foetuses with malformations or skeletal variations (excluding ossification) was not influenced by treatment with isopyrazam. The overall incidence of foetuses with visceral variations (50%) was statistically significantly greater than controls (35%) at the high dose. However, the incidence of each specific visceral variation fell within the range of the historical-control data (based on six previous studies conducted over 2006-2007, in the same strain of rat and in the same laboratory) and hence was not a consequence of treatment with isopyrazam.

Slight retardation of ossification was again observed at the high dose of 200 mg/kg bw/day (Table 26). At 200 mg/kg bw/day the incidence of non-ossification of certain vertebral centra and hind limb phalanges was increased, whilst at 75 mg/kg bw/day the incidence of non-ossification of the centrum of one vertebra (the 3rd cervical) was increased. This retardation of ossification is presumed to be related to treatment with isopyrazam.

Table 26: Selected skeletal examination findings in the developmental toxicity in rats with preparation 70:30 *syn:anti* (Anonymous 2008a).

Description	Dose (mg/kg/day)			
	0	20	75	200
No. of foetuses examined	136	145	152	141
Cervical centra:				
Centrum 1 not ossified	7 (5%)	13 (9%)	12 (8%)	17 (12%)**
Centrum 2 not ossified	3 (2%)	7 (5%)	9 (6%)	19 (13%)**
Centrum 3 not ossified	3 (2%)	6 (4%)	10 (7)*	13 (9%)**
Hind limb, digit:				
2 proximal phalanx, left not ossified	3 (2%)	5 (3%)	5 (3%)	10 (7%)*

3 proximal phalanx left not ossified	3 (2%)	5 (3%)	3 (2%)	10 (7%)*
4 proximal phalanx left not ossified	3(2%)	5 (3%)	3 (2%)	10 (7%)*
5 proximal phalanx left not ossified	10 (7%)	12 (8%)	14 (9%)	21 (15%)**
5 proximal phalanx right not ossified	10 (7%)	10 (7%)	11 (7)	18 (13%)*

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

First dose-range finding developmental toxicity study in rabbits (Anonymous 2008)

In the first dose-range finding study, gavage administration of isopyrazam (93:7 *syn:anti*, purity 96.4%) at doses of 0, 100, 200 and 400 mg/kg bw/day isopyrazam (over days 4 to 27 of gestation) caused no observable signs of maternal toxicity in 10/group female Himalayan rabbits. There were no deaths or treatment-related clinical signs of toxicity, body weight and food consumption were not affected and there were no treatment-related macroscopic or necropsy findings.

There was no effect on litter size, pre- or post-implantation losses or foetal body-weights. Foetuses were individually weighed and examined for gross external abnormalities; the heads were separated from half of the foetuses and serially sectioned for internal evaluation (eyes, brain, nasal passages and tongue). At the high dose, the incidence of foetuses with visceral malformations (specifically relating to the cardiovascular system) was greater than controls with altogether seven foetuses from six litters being malformed in this way; however, as these findings were not repeated in several other developmental toxicity studies in rabbits (including those at higher doses) this is not likely to be a treatment-related effect. There was no effect on the number of foetuses with skeletal and/or head malformations/variations.

Two foetuses at 400 mg/kg bw/day presented with eyes of slightly reduced size (approximately 75% of normal size). One of these foetuses also presented with eye retinal folds and narrow choanal. Only limited evidence of a relationship with treatment has been demonstrated in this specific case; however, as eye abnormalities have been observed in subsequent studies in rabbits, a treatment-related effect cannot be ruled out.

Second dose-range finding developmental toxicity study in rabbits (Anonymous 2008c)

In a second dose-range finding study, female Himalayan rabbits (5/group) were administered isopyrazam (93:7 *syn:anti*, purity 96.4%) via gavage at dose levels of 0, 600, 800 and 1000 mg/kg bw/day over gestation days 4 to 27. Again, no maternal toxicity was evident at these doses. There were no treatment-related deaths or clinical signs of toxicity. There were no macroscopic or necropsy findings and isopyrazam had no effect on body weight or food consumption.

With regard to developmental toxicity there was no effect on pre- or post-implantation loss or litter size. The following morphological differences comprising 'small eyes' (microphthalmia) or 'slightly small eyes' were noted in isopyrazam treated groups (Table 27); however, there was no clear dose-response relationship.

Table 27: Summary of fetuses (litters) with malformations, variants and eye malformations or variations in the second dose-range finding developmental toxicity study in rabbits (Anonymous 2008c).

Observation	Dose (mg/kg/day)			
	0	600	800	1000
No. fetuses examined macroscopically- external, visceral and skeletal examinations (no. of litters)	33 (5)	27 (5)	23 (5)	32 (5)
No. fetuses with any external and visceral malformation (litters)	1 (1)	4 (2)	0	6 (4)
No. fetuses with eye malformation: small, ~50-75% of normal (litters)	0	2 (1)	0	5* (3)
No. of fetuses with any external and visceral finding (litters)	20 (5)	21 (5)	16 (5)	29 (5)
No. fetuses with eye variant: slightly small, ~75- <100% of normal (litters)	0	9** (3)	5** (2)	10** (3)

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

The laboratory has defined 'small eyes' as an abnormality (50% to 75% of normal size) and 'slightly small eyes' as a variant (75% to <100% of normal size). According to the laboratory HCD for this strain of rabbit (based on 5 studies comprising 679 fetuses conducted in 2006-2007) only one animal presented microphthalmia ('small eyes') and the variant 'slightly small eyes' has never been reported for this laboratory. Microscopic examination of foetal head sections revealed intraocular abnormalities in at least those fetuses that presented with 'small' or 'very small eyes' comprising retinal dysplasia, choroidal dysplasia and/or posterior fibre disarray. These findings are consistent with the presence of microphthalmia and thus confirm the occurrence of eye abnormalities in the treated groups.

Third dose-range finding developmental toxicity study in rabbits (Anonymous 2008a)

A third dose-range finding study has been conducted in which doses of 0, 400, 700 and 1000 mg/kg bw/day of isopyrazam (93:7 *syn:anti*, purity 96.4%) were administered via gavage to female New Zealand White rabbits (10/group) over gestation days 7 to 28. There was evidence of maternal toxicity in all dose-groups comprising reduced food consumption, body weights and faeces production. This resulted in two females being killed *in extremis* on days 23 (400 mg/kg bw/d) and 21 (1000 mg/kg bw/d) and led to two females aborting on day 25 at 400 and 700 mg/kg bw/day. Initial body-weight losses in all treated groups, up to a magnitude of -97 g in the high dose group, resulted in overall body weight-gains (not corrected with gravid uterus weight) that were 63% and 53% lower than controls in the 700 and 1000 mg/kg bw/day treated groups, respectively. Relative liver weights were increased in treated groups by a magnitude of 39-77% in comparison with controls.

There was no effect on litter size or pre-implantation loss; however, post implantation losses were higher than in controls in all treated groups (albeit without statistical significance) and at 1000 mg/kg bw/day mean foetal body-weights were 14% lower than controls.

Similarly to the previous dose-range finding studies that were conducted in Himalayan rabbits, there was an increase in the incidence of fetuses with microphthalmia at 1000 mg/kg bw/day. The incidence of rabbits presenting eye variations and malformations at 1000 mg/kg bw/day (7%, 5/76 animals) was substantially greater than the range of the HCD for this strain of rabbit of 0-0.9% (total incidence of 4/6125 fetuses from 33 studies). In three of these five cases, microphthalmia was associated with a haemorrhagic ring around the eye (one foetus), reddened eyes (two fetuses) and/or dark red areas of the eye (one foetus). Microphthalmia has been observed in other developmental toxicity studies in rabbits, therefore the incidences seen in this study are likely to be related to treatment with isopyrazam.

Prenatal developmental toxicity study in rabbits (Anonymous 2008b)

The main prenatal developmental toxicity study was conducted in New Zealand White rabbits. Gavage doses of 0, 30, 150 and 500 mg/kg bw/day isopyrazam (93:7 *syn:anti*, purity 96.4%) were administered to 25/group mated females from day 7 to day 28 of gestation. One female at 500 mg/kg bw/day was found dead on day 24 following body-weight losses and reduced food consumption. Food consumption was reduced for this group overall with an associated reduction in faeces production, although maternal body weights were generally not affected by treatment. Liver weights were increased at 150 and 500 mg/kg bw/day (13% and 36% respectively) and associated findings of minimal to mild centrilobular hepatocellular hypertrophy/vacuolation were evident.

Isopyrazam treatment had no effect on litter size or on pre- or post-implantation losses. Mean foetal body-weights were 8% lower than controls at 500 mg/kg/day with statistical significance being reached for males only. Treatment with isopyrazam had no influence on the overall incidence of foetal malformations or variations.

Of the seven fetuses presenting malformations at 500 mg/kg/day, only one had microphthalmia. Although this incidence did not exceed the laboratory HCD range (0-0.9% from 33 studies, overall control incidence 4/6125 fetuses), similar eye malformations have been reported in other isopyrazam rabbit developmental toxicity studies (at higher doses) therefore a relationship with treatment cannot be unequivocally excluded.

Adverse effects on or via lactation

Information relevant to any potential adverse effects on or via lactation after the administration of isopyrazam can be derived from the two-generation study in rats. In this study, it was concluded that no effects on pups during lactation were attributable to isopyrazam administration.

COMPARISON WITH THE CRITERIA

Sexual function and fertility

The 2-generation reproduction study in rats showed slight lower mean litter size at birth and implantation sites in both F1 and F2 (Table 21). However, these values were within the HCD. RAC notes that the HCD package is formed by only two studies. However, these studies are relevant in terms of timing and performing facility. Overall, considering the small magnitude in the reductions of the mean litter size at birth and implantation sites and the HCD in a weight of evidence approach, RAC considers these effects as non-treatment related and therefore not able to support a classification.

The 2-generation reproduction study in rats reported a delay of 2.3 days in preputial separation and a delay of 2.0 days in the vaginal opening of the F1 animals. Table 22 shows that the mean body weight of males at the moment of preputial separation was 7% lower than controls (statistically significant); while the mean body weight of females at the moment of vaginal opening was 2% lower than controls (non-statistically significant). Overall, considering the weight reductions and that the effects appear only in F1 and not in F2, RAC considers vaginal opening and preputial separation delays as incidental and not robust enough for warranting a classification.

According to the CLP criteria, adverse effects on sexual function and fertility include those that interfere with the reproductive system, onset of puberty, gamete production/transport, reproductive cycle, sexual behaviour, fertility, parturition, pregnancy outcome, reproductive senescence or any other function that is dependent on the reproductive system. All of these have been sufficiently investigated in a guideline-compliant two-generation study, conducted in accordance with OECD TG 416. Exposure to isopyrazam showed no indication of adverse effects on sexual function or fertility according to these criteria. Therefore, RAC supports the DS's proposal for **no classification of isopyrazam for adverse effects on sexual function and fertility.**

Development

Alterations in post-implantation loss and reductions in male and female foetal weight were noted in both developmental toxicity studies in rats (Tables 23 and 25). However, RAC notes that these effects were reported at top doses where severe maternal toxicity (ventral recumbency and sedation for the first days of dosing, poor physical condition warranting sacrifice by day 21 and body weight loss by the end of the study) was concurrent. It is also noted that the incidence of post-implantation loss was just within the range of the laboratory HCD and that the losses observed in the concurrent control group were considerably low. Further, there were no effects on post-implantation loss (or early intrauterine death) in a second study in rats at a slightly reduced top dose of 200 mg/kg bw/day. Overall, these findings are not considered by RAC to support classification for developmental toxicity.

The developmental toxicity studies in rats also showed delayed ossification (cervical

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

centra, sternum, caudal arches, fore paws, hind paws) (Tables 24 and 26) at top maternally toxic doses. However, RAC notes that these effects also appear, mainly in the study with 93:7 *syn:anti*) at the non-maternally toxic dose of 75 mg/kg bw/day (Table 24). RAC notes that this delay in ossification can be used for supporting a classification for developmental toxicity at least as Repro 2.

Microphthalmia was observed in rabbits of both the Himalayan and the New Zealand White strains and was not always accompanied by excessive maternal toxicity and even in some cases appeared without any maternal toxicity. Table 28 summarises these malformations.

Table 28: Summary of eye malformations/variations in rabbits.

Study	Strain	Dose levels	Dose - levels where eye effects seen	Maternal toxicity	No animals examined (No of litters)	Incidence of microphthalmia foetus (litter)	Incidence of 'slightly small eyes' foetus (litter)	HCD
Dose-range finding prenatal developmental toxicity study	Himalayan rabbit	0, 100, 200 and 400 mg/kg bw/day	400 mg/kg bw/day	None	57 (9)	0	2(1)	1/679
Dose-range finding prenatal developmental toxicity study	Himalayan rabbit	0, 600, 800 and 1000 mg/kg bw/day	1000 mg/kg bw/day	None	32 (5)	5 (3)*	10 (3)*	1/679
			800 mg/kg bw/day		23 (5)	0	5 (2)**	
			600 mg/kg bw/day		27 (5)	2 (1)	9 (3)**	
Dose-range finding prenatal developmental toxicity study	New Zealand White rabbit	0, 400, 700 and 1000 mg/kg bw/day	1000 mg/kg bw/day	↓ body weight-gain (53%)	76	5(2)		4/6125
Prenatal developmental toxicity	New Zealand White rabbit	0, 30, 150 and	500 mg/kg bw/d	↓ food consumption/faeces production	202 (23)	1	0	4/6125

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

study		500 mg/k g bw/d ay	ay	No effect on body weight				
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* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Two preliminary studies were conducted in this Himalayan rabbits and the relevant historical control data reported microphthalmia at an incidence rate of 1/679. In the first study, two fetuses (from one litter) at 400 mg/kg bw/day and one control animal, presented with the variation 'eyes of slightly reduced size' whilst no cases of microphthalmia ('eyes of reduced size') were reported. No maternal toxicity was evident in this study up to and including the high dose of 400 mg/kg bw/day.

In the second preliminary study in the Himalayan rabbit, 'eyes of slightly reduced size' were reported in 10(3), 5(2) and 9(3) fetuses (litter) in the 1000, 800 and 600 mg/kg bw/day dose-groups. In addition, 5(3) and 2(1) fetuses (litter) at 1000 and 600 mg/kg bw/day presented with the eye malformation microphthalmia ('eyes of reduced size'). No signs of maternal toxicity were evident in this study up to and including the dose at which the eye effects became apparent.

One preliminary and one main study have been conducted in the New Zealand White strain of rabbit; according to the historical control data for this strain, microphthalmia has been reported in 4/6125 fetuses. In the preliminary study, 76 animals were examined and microphthalmia was noted in five fetuses (from two litters). All these cases occurred at the top-dose of 1000 mg/kg bw/day, a dose level that also induced severe maternal toxicity. Such maternal toxicity became evident from 400 mg/kg bw/day and was characterised by mortality, abortions, lower body weight and body-weight gain, reduced food consumption and lower faeces production. Body-weight losses throughout the study at the top-dose of 1000 mg/kg bw/day (up to -97 at weeks 10-13) resulted in overall body-weight gains that were 53% lower than controls by the end of the treatment period. Large reductions in body-weight gain were also seen at the mid dose of 700 mg/kg bw/day (63% lower than controls by the end of the study). Hence, the microphthalmia observed in the New Zealand White rabbits in this preliminary study occurred only in the presence of marked maternal toxicity.

A high dose of 500 mg/kg bw/day was selected for the main study. There were no signs of maternal toxicity at this dose and 1/202 rabbits presented microphthalmia. This was within the range of the HCD for this strain; however, as similar effects were seen across all developmental studies in rabbits, a relationship with treatment cannot be excluded.

In conclusion, RAC notes that there were incidences of microphthalmia or 'slightly small eyes' that were outside the historical control data and could reasonably be considered to be related to treatment with isopyrazam. Such effects only occurred in the presence of marked maternal toxicity in the New Zealand White rabbit (dose-

range finding study) whereas in the Himalayan strain of rabbit, no maternal toxicity accompanied the finding. However, it is noted that the studies in the Himalayan rabbit were preliminary studies only and there is a question about the reporting of the finding by the test laboratory (i.e., the test report refers to the finding as 'eyes of slightly reduced size' and 'eyes of reduced size' rather than microphthalmia). That said, the overall conclusion of the test report (which included a microscopic examination of the foetal head sections and consideration of the intraocular abnormalities) was that the findings were consistent with microphthalmia.

Overall, as the same effect on the eyes has been observed in multiple studies and is consistent across two different strains of rabbit, it cannot be confidently dismissed as a secondary effect of maternal toxicity. The effects were supported by other eye effects observed, such as eye retinal folds, retinal dysplasia, choroidal dysplasia, posterior fibre disarray, haemorrhagic ring around the eye, reddened eyes and dark red areas of the eye, supporting that the eye is a target organ. Therefore, RAC, based mainly on these effects on eyes and supported by the delayed ossification in the studies in rats, supports the DS's proposal **for classification of isopyrazam as Repr 1B (H360D: May damage the unborn child).**

Adverse effects on or via lactation

According to the CLP criteria, classification for effects on or via lactation are assigned can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No human evidence is available and there is no toxicokinetic information to indicate that toxic concentrations of isopyrazam or its metabolites may be present in milk. In a 2-generation reproductive toxicity study signs of toxicity in the offspring during lactation (reductions in body weight gain) are not attributable to isopyrazam. Therefore, the criteria for classification for effects on or via lactation are not met and RAC supports the DS's proposal for **no classification of isopyrazam for effects on or via lactation.**

Concentration limits

According to ECHA guidance, when the evidence does not indicate which of the ED₁₀ (pups or litter) is more appropriate both should be derived and the lower should be used.

Table 28 demonstrated that ED₁₀ for pup incidence must be higher than 400 mg/kg bw/day.

The first dose-range finding prenatal developmental toxicity study in Himalayan rabbits show that the incidence for litters is 11% (1/9) at 400 mg/kg bw/day with no effects at 200 mg/kg bw/day; while the second study in this species show an ED₁₀ for

litters lower than 600 mg/kg bw/day. The database with New Zealand white rabbit shows no effects at 30, 150 and 400 mg/kg bw/day, with effects starting at 500 mg/kg bw/day. Overall, the ED₁₀ for litter incidence is below 400 mg/kg bw/day but above 4 mg/kg bw/day; which suggests that concentration limits for isopyrazam should be set based on the medium potency group. However, the ED₁₀ for litter incidence is very close to 400 mg/kg bw/day, and all the other levels are above, which points the weight of evidence towards low potency group.

Furthermore, when considering modifying factors:

- Severity: Microphthalmia should be considered as severe malformation. For these severe malformations a concentration limit lower than the GCL should be set when ED₁₀ are close to the lower border of the group. However, RAC notes that, although incidence for litters is lower than 400 mg/kg bw/day the whole database suggests that such ED₁₀ is clearly higher above the lower limit of 4 mg/mg bw/day.
- Data availability: RAC notes no issues of concern as regard as this modifying factor.
- Shallow dose-response curve: RAC notes no issues of concern as regard as this modifying factor.
- Mode of action: RAC notes the lack of information on differences in sensitivities between rabbit and human for this effect.
- Toxicokinetics: Considering comprehensive knowledge of all involved toxicokinetic factors RAC notes no information on toxicokinetics suggesting significant differences between rabbits and humans.
- Bio-accumulation: According to CLH dossier, there was no evidence of bioaccumulation potential; however, for the liver and renal fat, steady state was not reached within the 14-day dosing period, so some accumulation potential in these particular tissues over repeated administration cannot be completely ruled out.

Overall, based on the ED₁₀ for both litter and foetus incidences and considering modifying factors **RAC considers that isopyrazam should be assigned to the low potency group and proposes the adoption of the SCL (3%).**

10.12 Specific target organ toxicity-single exposure

Information to inform on the specific target organ toxicity of isopyrazam after single exposure is available and is summarised in the acute toxicity section of this report (10.1 to 10.4). An acute neurotoxicity study is also available and is summarised below.

Table 59: Summary of acute neurotoxicity study with isopyrazam

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels duration of exposure	Results
Acute (gavage) neurotoxicity OECD 424 GLP Rats, Wistar, 10/sex/group Anonymous (2009a)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 Vehicle: 0.5% aqueous CMC	0, 30, 250 & 2000 mg/kg bw Single gavage doses Observation period: 16 days	There were no deaths <u>2000 mg/kg bw</u> ↓ activity & weak appearance in M & F (1h post-dose) ↓ BW-gain in F (33%; first week), ↓ FC (first week) ↓ rearing (day 1) ↓ locomotor activity (total distance, total centre time & number of rears) on day 1 <u>250 mg/kg bw</u> ↓ activity, weak appearance & swaying gait in M & F (1h post-dose) ↓ BW-gain in F (30%; first week) ↓ rearing (day 1) ↓ locomotor activity (total distance, total centre time & number of rears) on day 1 <u>30 mg/kg bw</u> No treatment-related effects

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

A single gavage dose of isopyrazam (93 syn: 7 anti) in 0.5% aqueous CMC, was administered to 10/sex/group Wistar rats at dose levels of 0, 30, 250 and 2000 mg/kg bw. Body-weight and food consumption were measured throughout the study whilst cage side observations were made prior to the study, one hour after dose administration and once daily thereafter for a 16-day observation period. Detailed clinical observations were made prior to the study and subsequently on days 1 (included in FOB assessments), 8 and 15; FOB evaluations (comprising landing foot splay, sensory perception and muscle weakness) were performed prior to the study, two hours post-dose and then on days 8 and 15, with locomotor activity being assessed for 30 minutes following each FOB examination. At the end of the observation period the brains of 5/sex/group rats were weighed and selected nervous system tissues of these animals were subject to microscopic examination.

All animals survived to the end of the observation period. Body-weight gain in females was 30% and 33% lower than controls in the 250 and 2000 mg/kg bw dose groups during the first week of dosing and food consumption in the first week was lower than controls for females at 2000 mg/kg bw. There was no effect on body-weight or food consumption in males at any dose. Cage side observations noted at one-hour post-dose

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

comprised decreased activity (250 & 2000 mg/kg bw; males & females), weak appearance (250 & 2000 mg/kg bw/d; females) and swaying gait (250 mg/kg bw; females). Reduced activity, along with reduced rearing activity was also noted during the FOB assessments on day one only (250 & 2000 mg/kg bw; males & females); however, the incidence was low and no dose-response was evident. During the day-one locomotor activity assessment, reduced total distance, total centre time and number of rears was reported in females at the mid- and high-doses. There was no effect on brain weight and no microscopic or macroscopic findings in tissues that would suggest a specific effect on the nervous system.

Several acute oral toxicity studies are available. In these studies, at doses of up to 2500 mg/kg, the observed clinical signs (ruffled fur, hunched posture, sedation, poor co-ordination and ventral recumbency) are signs commonly associated with general toxicity in rats and are therefore not indicative of a toxic effect on any specific target organ. In an acute dermal toxicity study there were no clinical signs of toxicity up the top-dose tested of 5000 mg/kg bw. An acute inhalation toxicity study was conducted in which rats were exposed nose-only to 5.28 mg/l isopyrazam; the only clinical signs noted were those associated with restraint, and animals had fully recovered by day three. There was no indication of any direct toxic effect on the respiratory system.

From the information available there is no indication that isopyrazam exerts a toxic effect on any specific organ after single exposure.

10.12.2 Comparison with the CLP criteria

Classification as either STOT-SE1 or 2 is applicable to substances that have produced non-lethal toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following a single exposure.

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

No human data are available but a battery of acute toxicity tests (including a specific acute neurotoxicity study) has demonstrated that no specific target organ toxicity occurs after a single exposure to isopyrazam. Therefore, the criteria for classification for STOT-SE 1 or 2 are not met.

No indications of an effect on the respiratory tract were observed in the acute inhalation toxicity study and there were no specific findings indicative of a narcotic effect in any of the studies. Consequently, the criteria for classification for STOT-SE 3 are not met.

10.12.3 Conclusion on classification and labelling for STOT SE

Not classified (conclusive but not sufficient for classification)

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification of isopyrazam based on the absence of specific effects reported in the acute toxicity tests (see Table 1) and the absence of neurotoxicity in one

acute neurotoxicity study using doses up to 2000 mg/kg bw.

Comments received during consultation

One MSCA supported the DS's proposal for no classification of isopyrazam for STOT SE.

Assessment and comparison with the classification criteria

RAC notes the absence of organ-specific effects in the acute toxicity studies via oral, dermal and inhalation routes (Table 1). In these studies, at doses up to 2500 mg/kg bw, the observed clinical signs (ruffled fur, hunched posture, poor coordination, sedation and ventral recumbency) are commonly associated with general toxicity and not indicative of toxic effects on any specific target organ.

The CLH report presents also an acute neurotoxicity study (Anonymous 2009a) performed in rats conducted according to OECD 424 Guideline and observing GLP. On this study, 10 rats/sex/group were treated with a single dose of 30, 250 or 2000 mg/kg bw of isopyrazam (purity 96.4%, 93:7 *syn:anti*) in 0.5% w/v caroxymethylcellulose in water. Animals were further observed for 16 days. All animals survived until the end of the observation period. Body weight gain in females was 30% and 33% lower than controls in the 250 and 2000 mg/kg bw dose groups, respectively, during the first week of dosing. Observations noted at one-hour post-dose comprised decreased activity (250 and 2000 mg/kg bw; males and females), weak appearance (250 and 2000 mg/kg bw/d; females) and swaying gait (250 mg/kg bw; females). Reduced activity, along with reduced rearing activity was also noted during the Functional Observational Battery (FOB) assessments on day one only (250 and 2000 mg/kg bw; males and females); however, the incidence was low and no dose-response was evident. There was no effect on brain weight and no microscopic or macroscopic findings in tissues that would suggest a specific effect on the nervous system.

Comparison with the criteria

None of the single dose animal studies contained in the CLH report provided evidence of organ specific toxicity; which does not support classification as STOT SE 1 or 2. Moreover, no respiratory tract irritation was found in the studies; hence not supporting classification as STOT SE 3 H335. According to CLP classification criteria, narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex and ataxia, and are usually readily reversible on cessation of exposure with no permanent damage or changes. Poor coordination and sedation (lethargy) were observed in acute toxicity studies at doses below and above LD50 value. Decreased activity and swaying gait (may be considered as lethargy and poor coordination/ataxia) were detected one-hour post exposure in mid and high dose group during the acute neurotoxicity study. These effects may be indications of transient CNS depression. However, they may also be considered as indications of general or secondary toxicity. In the absence of any indications of potential neurotoxicity-based effects observed in other studies, or on the structure or mechanism/mode of action of the substance, and considering that the effects were observed still at substantially high doses, RAC does not consider that the criteria for classification regarding narcotic effects are met. Therefore, RAC supports the DS's proposal for **no classification of isopyrazam as STOT SE.**

10.13 Specific target organ toxicity-repeated exposure

Information is available to inform on the specific target organ toxicity of isopyrazam upon repeated oral exposure from several repeated-dose toxicity studies, conducted in rats (28- and 90-day), mice (90-day) and dogs (90-day & 1-year). The majority of the studies used a specification of isopyrazam comprising 93:7 syn:anti isomers; further 90-day studies in rats and dogs have been conducted using a 70:30 syn:anti specification, whilst several additional 28-day rat studies used an 89:11 or 50:50 syn:anti specification along with the pure syn and anti isomers.

Table 60: Summary table of animal studies on STOT RE

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
Rats					
<p>28-day oral (dietary)</p> <p>OECD 407</p> <p>Deviations: no FOB, only liver & uterus examined</p> <p>GLP</p> <p>Anonymous (2007b)</p>	<p>Rats, HsdRccHan: WIST, males & females, 5/sex/group</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4%</p> <p>Syn:anti ratio: 93:7</p>	<p>0, 300, 4000 & 8000 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 29, 392 & 793 mg/kg bw/d</p> <p>Females: 0, 28, 390 & 720 mg/kg bw/d</p>	<p>Cat 1 = 30</p> <p>Cat 2 = 300</p>	<p>There were no deaths or clinical signs of toxicity</p> <p><u>793/720 mg/kg bw/d</u></p> <p>↓ terminal BW in M (-14%**) & F (-18%**)</p> <p>↓ neutrophils (-30-50%) & eosinophils in F (-30%)</p> <p>↑ urea (34%**), cholesterol (34%**), GGT (45%**) & phosphorous (44%**) in F, ↓ triglycerides (-53%**) & creatinine (-9%*) in M</p> <p>↑ relative liver weights in M (31%**) & F (28%**)</p> <p>Minimal/slight hepatocellular hypertrophy in all animals</p> <p><u>392/390 mg/kg bw/d</u></p> <p>↓ terminal BW in M (-12%) & F (-10%**)</p> <p>↑ urea (20%**), cholesterol (33%**) & phosphorous (24%**) in F, ↓ triglycerides in M (54%**)</p> <p>↑ relative liver weights in M (24%**) & F (22%**)</p> <p>Minimal/slight hepatocellular hypertrophy in all animals</p> <p><u>29/28 mg/kg bw/d</u></p> <p>No adverse effects</p>
<p>28-day oral (dietary)</p> <p>OECD 407</p> <p>Deviations: conc, stability & homogeneity of test material not determined</p> <p>GLP</p> <p>Anonymous (2007a)</p>	<p>Rats, HsdRccHan: WIST, males & females, 5/sex/group</p>	<p>Isopyrazam</p> <p>Batch: TE-5854/7</p> <p>Purity: 100%</p> <p>Syn:anti ratio: 89:11</p>	<p>0, 100, 500 & 2000 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 9, 46 & 175 mg/kg bw/d</p> <p>Females: 0, 10, 48 & 191 mg/kg bw/d</p>	<p>Cat 1 = 30</p> <p>Cat 2 = 300</p>	<p>There were no deaths or clinical signs of toxicity</p> <p><u>175/191 mg/kg bw/d</u></p> <p>↓ final BW (-7%**) & FC (wk. 4; -24%) in F</p> <p>↓ lymphocyte counts in M (-31%); ↑ creatinine (19.8%*) & creatinine kinase (56%*) and ↓ triglycerides (-39.4*) in M</p> <p>↑ relative liver weights in M (13%**) and F (14%**)</p> <p>↑ P450 activity in M (200%**) & F (56%*)</p> <p>Hepatocellular hypertrophy in all M & F</p> <p><u>46/48 mg/kg bw/d</u></p> <p>↑ liver weights in F (9%*)</p> <p>Hepatocellular hypertrophy in 1/5 M</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
					9/10 mg/kg bw/d No adverse effects
<p>28-day oral (dietary) OECD 407 Deviations: No FOB GLP Anonymous (2007c)</p>	<p>Rats, HsdRccHan: WIST, males & females, 5/sex/group (main study) & 6/sex/group (satellite phase)</p>	<p><u>SYN534969</u> Batch: SMU6BP001 Purity: 99% Pure Syn <u>SYN534968</u> Batch: SMU6BP001 Purity: 99.5% Pure anti <u>'Isopyrazam'</u> Batch: SMU6CP014 Purity: 98.2% Syn:anti ratio:50:50</p>	<p>0, 500, 2000 & 5000 ppm Equivalent to: <u>Pure syn</u> Males: 0, 47, 178.9 & 449.4 mg/kg bw/d Females: 0, 46.8, 181.7 & 458.9 mg/kg bw/d <u>Pure anti</u> Males: 0, 43.8, 170 & 406.5 mg/kg bw/d Females: 0, 44.4, 182.6 & 372.3 mg/kg bw/d <u>50% syn:50% anti</u> Males: 0, 44.7, 181.1 & 456 mg/kg bw/d Females: 0, 44.6, 197.8 & 371.9 mg kg bw/d</p>	<p>Cat 1 = 30 Cat 2 = 300</p>	<p>There were no deaths There was a dose-dependent increase in P450 activity for all test substances Hepatocellular hypertrophy (minimal to moderate) was observed for all test substances at all dose levels in M and at the mid- and high-doses in F Pure syn <u>449.4/458.9 mg/kg bw/d</u> ↓ BW (-5%; week 1) in M & F ↓ platelets (-12%*), WBC (34%*) & lymphocytes (-36%*) in M, ↓ basophil (57%*) in F ↑ cholesterol (40%***) in F, ↓ ALP (31%***) in M ↑ relative liver weight in M (25%) & F (41%) <u>178.9/181.7</u> ↑ relative liver weight in M (17%) & F (31%) <u>47/46.8 mg/kg bw/d</u> ↑ relative liver weight in F (22%) Pure anti <u>406.5/372.3 mg/kg bw/d</u> Hunched posture and piloerection in F ↓ BW throughout the study in M (-20%) & F (-18%) ↓ FC in F ↑ RBC* in M; ↑ Hb*, ↓ basophil* & APPT** in F ↓ TP** & albumin** in F; ↑ cholesterol**, potassium* & phosphorous* in F, ↓ triglycerides** in M, ↑ GGT* in M & F ↑ relative liver weight in M (23%) & F (38%) <u>181.7/170 mg/kg bw/d</u> Piloerection (F) ↓ BW throughout the study in M (-11%) & F (-17%) ↓ FC in M & F ↑ RBC* & ↓ basophil* & APPT** in F ↓ TP** & albumin** & ↑ cholesterol** in F, ↓ triglycerides* in M, ↑ GGT* in M ↑ relative liver weight in M & F (20%) <u>47/46.8 mg/kg bw/d</u> ↓ BW in F (-4%; day 3)</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
					<p>↓ APPT in F</p> <p>↑ relative liver weight in F (21%)</p> <p>50% syn:50% anti</p> <p>There was a dose-dependent increase in P450 activity <u>456/371.9 mg/kg bw/d</u></p> <p>Hunched posture and piloerection (F)</p> <p>↓ BW throughout the study in M (-14%) & F (-21%)</p> <p>↓ FC in F</p> <p>↑ RBC** & ↓ APPT* in F</p> <p>↓ albumin** & protein* & ↑cholesterol** in F</p> <p>↑ relative liver weight in M (30%) & F (25%) <u>181.1/197.8 mg/kg bw/d</u></p> <p>↓ BW throughout the study in M (-6%) & F (-11%)</p> <p>↑cholesterol* in F</p> <p>↑ relative liver weight in M (18%) & F (34%) <u>44.7/44.6 mg/kg bw/d</u></p> <p>↑ relative liver weight in F (15%)</p> <p>↓ BW in F (-4%; day 2)</p>
<p>90-day oral (dietary)</p> <p>OECD 408</p> <p>GLP</p> <p>Anonymous (2007d)</p>	<p>Rats, HsdRccHan: WIST, males & females, 12/sex/group</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4%</p> <p>Syn:anti ratio: 93:7</p>	<p>0, 300, 1500 & 6000 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 21, 106 & 463 mg/kg bw/d</p> <p>Females: 0, 24, 118 & 484 mg/kg bw/d</p>	<p>Cat 1 = 10</p> <p>Cat 2 = 100</p>	<p>There were no deaths or clinical signs of toxicity</p> <p><u>463/484 mg/kg bw/d</u></p> <p>↓ BW gain (19%**) & final BW in F, ↓ BW in M (-5%)</p> <p>↓ FC in F (-23%**), ↑ FC from wk. 2 in M (21.8%*)</p> <p>↑ cholesterol**, AAT**, GGT** in F; ↑ GGT**, AAT*, CK* in M</p> <p>↑ relative liver weight in M (24%**) & F (19%**)</p> <p>Minimal/slight hepatocellular hypertrophy in all animals</p> <p><u>106/118 mg/kg bw/d</u></p> <p>↓ BW-gain in F (7%*)</p> <p>↓ FC in F (12%*)</p> <p>↑ relative liver weight in M & F (14%**)</p> <p>Minimal/slight hepatocellular hypertrophy in all animals</p> <p><u>21/24 mg/kg bw/d</u></p> <p>↑ relative liver weight in F (14%**)</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
<p>90-day oral (dietary) OECD 408 GLP Anonymous (2008)</p>	<p>Rats, Han Wistar (CrI:WI(Han)), males & females, 10/sex/group</p>	<p>'Isopyrazam' Batch: SMU7DP017 Purity: 96.4% Syn:anti ratio: 93:7 & Batch: SMUDP017 Purity: 90.8% Syn:anti ratio: 70:30</p>	<p>0, 100, 250 & 2000 ppm Equivalent to: <u>93 syn:7 anti</u> Males: 0, 8, 20 & 159 mg/kg bw/d Females: 0, 8, 21 & 163 mg/kg bw/d <u>70 syn:30 anti</u> Males: 0, 10, 24 & 193 mg kg bw/d Females: 0, 9, 24 & 197 mg/kg bw/d</p>	<p>Cat 1 = 10 Cat 2 = 100</p>	<p>There were no deaths or clinical signs of toxicity 93 syn:7 anti <u>159/163 mg/kg bw/d</u> ↓ final BW in F (18%**) ↓ ALP** in M & F ↑ relative liver weight in M (17.9%**) & F (16.8%**), ↑ thyroid weight in M (29%**) Hepatocellular hypertrophy in M & F (8/10), hepatocyte vacuolation in M & F (3/10) <u>20/21 mg/kg bw/d</u> No adverse effects <u>8 mg/kg bw/d</u> No adverse effects 70 syn:30 anti <u>193/197 mg/kg bw/d</u> ↓ BW in F (16%**) ↓ ALP** in M & F ↑ relative liver weight in M (15%**) & F (14.6%**) Hepatocellular hypertrophy in M & F (8/10), hepatocyte vacuolation in M & F (4/10) <u>24 mg/kg bw/d</u> No adverse effects <u>10/9 mg/kg bw/d</u> No adverse effects</p>
<p>90-day neurotoxicity (dietary) OECD 424 GLP Anonymous (2009b)</p>	<p>Rats, HanRcc:WIS T, males & females, 12/sex/dose</p>	<p>Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7</p>	<p>0, 300, 1500 and 6000 ppm Equivalent to: Males: 0, 20, 98 & 382 mg/kg bw/d Females: 0, 25, 114 & 468 mg/kg bw/d</p>	<p>Cat 1 = 10 Cat 2 = 100</p>	<p>There were no deaths or treatment-related clinical signs of toxicity There were no FOB differences or ophthalmoscopy findings There was no effect on brain weight and no treatment related macroscopic or microscopic effects in nervous system tissues <u>382/468 mg/kg bw/d</u> ↓ BW-gain in F** (-28% throughout study) and M* (14% week 1 only) matched by ↓ FC in M & F Locomotor activity: ↓ distance traversed in F & ↓ number of rears in M & F at 13 wks. <u>98/114 mg/kg bw/d</u> No treatment-related effects <u>20/25 mg/kg bw/d</u> No treatment-related effects</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
Mice					
<p>90-day oral (dietary) OECD 408 GLP Anonymous (2008a)</p>	<p>Mice, C57BL/10Jf CD-1, males & females, 10/sex/group</p>	<p>Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7</p>	<p>0, 500, 2500 & 7000 ppm Equivalent to: Males: 0, 76, 391 & 1383 mg/kg bw/d Females: 0, 87, 449 & 1760 mg/kg/bw d</p>	<p>Cat 1 = 10 Cat 2 = 100</p>	<p>There were no treatment-related deaths or clinical signs of toxicity <u>1383/1760 mg/kg bw/d</u> ↓ final BW in M (5%***) & F (8%**) ↑ FC in M & F ↓ A/G ratio* & ↑ AAT** & triglycerides** in F ↑ relative liver weight in M (47%***) & F (59%***) Moderate hepatocellular hypertrophy in M & F <u>391/449 mg/kg bw/d</u> ↓ final BW in M (5%***) & F (6.6%***) ↓ FC in M & F ↑ relative liver weight in M (25%***) & F (32%***) Minimal/slight hepatocellular hypertrophy in M & F <u>76/87 mg/kg bw/d</u> ↑ relative liver weight in M (7.6%*) & F (7.6%*) Minimal/slight hepatocellular hypertrophy in M</p>
Dogs					
<p>90-day oral (capsule) OECD 409 GLP Anonymous (2007)</p>	<p>Dogs, Beagle, males & females, 4/sex/group</p>	<p>Isopyrazam Batch: SMU6AP001 Purity 96.4% Syn:anti ratio: 93:7</p>	<p>0, 30, 100 & 300 mg/kg bw/d</p>	<p>Cat 1 = 10 Cat 2 = 100</p>	<p>There were no deaths <u>300 mg/kg bw/d</u> Transient head wobble, reduced stability & reduced/increased activity in 3 M (days 2/3) ↓ final BW in M (-10%*) & F (-12%**) ↓ FC in M & F** ↑ platelet count** in M (wk. 13), ↑ ALP** in M & F (wks. 4, 8 & 13), ↓ sodium in F** (wk. 13), ↓ cholesterol in F (throughout study) and in M (wk. 13), ↓ urine specific gravity in F ↑ relative liver weight in M (24.5%**) Enlarged liver in M & F (2/sex) <u>100 mg/kg bw/d</u> Transient head wobble, reduced stability & reduced/increased activity in 1 M (days 2/3) ↓ albumin in F (wk. 13) ↑ liver weight in M (12.7%*) <u>30 mg/kg bw/d</u> No adverse effects</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Method, guideline, deviations (if any)	Species, strain, sex, no/group	Test substance, batch, purity, syn:anti ratio	Dose levels, duration of exposure	CLP guideline value for classification (mg/kg bw/d; rat study)	Results (% change from controls)
<p>90-day oral (capsule) OECD 409 GLP Anonymous (2008a)</p>	<p>Dogs, Beagle, males & females, 4/sex/group</p>	<p>'Isopyrazam' Batch: SMU7DP017 Purity: 96.4% Syn:anti ratio: 70:30</p>	<p>0, 10, 30 & 250 mg/kg bw/d</p>	<p>Cat 1 = 10 Cat 2 = 100</p>	<p>There were no deaths <u>250 mg/kg bw/d</u> ↑ salivation in all dogs, abnormal behaviour in 1 M from wk. 4 (sedation, shaking head, buckling of hind limbs, uncoordinated movements & ptosis) Body-weight loss in 1 M (4%) in wk. 1 and 1 F (10%) in wk. 1 ↓ FC in all M in wk1 & 3 F in wk. 1 <u>30 mg/kg bw/d</u> No adverse effects <u>10 mg/kg bw/d</u> No adverse effects</p>
<p>12-month oral (capsule) OECD 452 GLP Anonymous (2008b)</p>	<p>Dogs, Beagle, males & females, 4/sex/group</p>	<p>Isopyrazam Batch: SMU6AP001 Purity: 96.4% Syn:anti ratio: 93:7</p>	<p>0, 25, 100 & 250 mg/kg bw/d</p>	<p>Cat 1 = 2.5 Cat 2 = 25</p>	<p>There were no deaths or treatment-related clinical signs of toxicity <u>250 mg/kg bw/d</u> ↓ BW gain in 3 M (8%, 3% & 7%) and 2 F (6% & 4%) in wk. 1 ↓ BW gain in M (57%*) at wk. 52 ↓ FC wk. 1-6 in M & F ↑ ALP in M* & F** at all-time points, ↑ AAT*, GDH** & ↓ bilirubin** in M at wk. 26 & 52, ↓ albumin** in M & F at all time-points ↑ liver weight in M (52%**) <u>100 mg/kg bw/d</u> ↑ ALP* in M & F at wk. 52, ↓ bilirubin** in M at wk. 26 & 52, ↓ albumin** in M at all time-points ↑ liver weight in M (42%**) <u>25 mg/kg bw/d</u> ↑ ALP* in M at wk. 52</p>

* = statistically significant (p≤0.05), ** = statistically significant (p≤0.01)

FC = food consumption, BW = body weight

Rats

28-days

89:11 syn anti specification

Dietary administration of isopyrazam (89 syn:11 anti) for 28-days resulted in lower final body-weights of males and females at 175/191 mg/kg bw/d. Relative liver weights were increased in both sexes accompanied by histopathological correlates indicative of an adaptive response, comprising centrilobular hepatocellular hypertrophy in all animals at the top-dose. Analysis of cytochrome P450 activity revealed an induction of total cytochrome P450, particularly of 7-pentoxoresorufin O-depentyase (PROD), further suggesting that the

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

observed effects are likely to be an adaptive rather than an adverse response. Slight alterations in certain haematological and biochemical parameters (increased creatinine and creatinine kinase; decreased triglycerides and lymphocyte counts) are not consistent with findings from other repeated-dose toxicity studies and are likely to be a chance finding reflecting normal biological variation.

93:7 syn:anti specification

When isopyrazam (93:7 syn:anti) was administered to rats for 28-days, final body weights were reduced in both sexes at 793/720 mg/kg bw/d and 392/390 mg/kg bw/d. Liver weights were increased at these doses in males and females, accompanied by minimal to slight hepatocellular hypertrophy. Minor alterations in some clinical chemistry parameters (urea, cholesterol and phosphorous in females; triglycerides and creatinine in males) and some haematological parameters (neutrophils and eosinophils in females) were noted; however only the observed changes in cholesterol (increases) and triglycerides (decreases) were consistent with findings from other studies. Overall, in the absence of any adverse histopathology the findings are likely to be an indication of an adaptive response rather than of adversity.

Pure syn & anti and 50:50 specifications

In a further 28-day dietary study, the pure syn and anti isomers of isopyrazam and a 50:50 mixture of both were administered to rats at dietary concentrations of 0, 500 & 2000 ppm. Body weights were reduced in males and females at the mid- and high-doses for both the pure anti and the 50:50 syn:anti groups; however, there was no effect on body weight after administration of the pure syn isomer at any dose. Food consumption was reduced in females of the pure anti isomer group at the mid- and high-doses and in the 50:50 test substance group at the high-dose only. Consistent with other repeated-dose studies, signs of an adaptive response were observed in the liver. With regard to liver weight increases, effects were generally consistent across all test-substance groups (see table below):

Table 61: Liver weight changes (relative to body-weight)

Test substance	Sex	Dietary Concentration of Test Substance (ppm)		
		500	2000	5000
'Isopyrazam' (50% syn:50% anti)	Male	No effect	+18%	+30%
'Isopyrazam' (50% syn:50% anti)	Female	+15%	+34%	+25%
SYN534969 (pure syn epimer)	Male	No effect	+17%	+25%
SYN534969 (pure syn epimer)	Female	+22%	+31%	+41%
SYN534968 (pure anti epimer)	Male	No effect	+20%	+23%
SYN534968 (pure anti epimer)	Female	+21%	+20%	+38%

Minimal to moderate hepatocellular hypertrophy was noted in all test-substance groups (at all doses in males and at the mid- and high-dose in females). Serum cholesterol was increased in females at the top-dose of all three test substance groups, whilst GGT activity was increased in the pure anti group only (high-dose females and mid- and high-dose males). Other small but statistically significant changes in clinical chemistry and haematological parameters were inconsistent and were not related to treatment with isopyrazam. Liver enzyme investigations again revealed dose-related increases in the activity of total cytochrome P450 (PROD and EROD) to a similar extent across all three test-substances, with the most marked increases being in PROD activity. With regard to the liver, from this comparative study it can be seen that the short-term effects of the pure syn and anti isomers and the 50:50 mixture of both are similar with regard to both qualitative and quantitative effects.

90-days

93:7 syn:anti specification

Following administration of isopyrazam (93:7 syn:anti) for 90-days, body weights of female rats were decreased by 19% at 463/484 mg/kg bw/d, along with a decrease in food consumption in both sexes. A similar but less marked effect was noted in females only at 106/118 mg/kg bw/d. Similar to the 28-day rat studies, liver weights were increased in the high-dose group by a magnitude of 24% and 19% in males and females respectively and by 14% in both sexes of the mid-dose group. The liver weight increases were

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

accompanied by minimal to slight hepatocellular hypertrophy in all animals of the mid- and high-dose groups. The only effect noted in the low-dose group (21/24 mg/kg bw/d) was a slight liver weight increase of 14% in females only with no histopathological correlates. Some clinical chemistry parameters (AAT & GGT) were increased in males and females of the high-dose group with cholesterol also being increased in females at this dose. Slight changes in chloride, sodium and phosphorous were inconsistent with the effects of isopyrazam observed in other repeated-dose toxicity studies and so are not likely to be related to treatment.

93:7 and 70:30 syn:anti specifications

In order to compare the repeated-dose toxicity of the 93:7 and the 70:30 syn:anti specifications of isopyrazam, a comparative 90-day dietary study in rats was conducted. Doses of 0, 100, 250 and 2500 ppm equated to 0, 8, 20/21 & 159/163 mg/kg bw/d (93:7 specification) and 0, 10/9, 24 & 193/197 (70:30 specification) in males/females. Body weights were reduced at the top-dose in females only for both the 93:7 (18%) and the 70:30 specifications (16%). Liver weights were increased in males and females to a similar extent (15 – 17 %) across both specifications at the top-dose only, accompanied by hepatocellular hypertrophy in both sexes. Vacuolation of the hepatocytes was noted in males and females at the top-dose of both specifications; when considered in the context of data from other longer-term studies with isopyrazam, this progression of liver effects to signs of adversity would be expected at this dose and duration of exposure. With regard to clinical chemistry, ALP activity was reduced in males (high-dose group) and females (mid- and high-dose groups) of the 93:7 specification, whilst the activity of this enzyme was reduced in males and females in the of the 70:30 specification of isopyrazam (high-dose group only); reductions in ALP activity have not been seen in any other repeated-dose studies and so are likely to be a consequence of natural variation rather than related to treatment. It can be seen that the effects of repeated-dosing of both specifications remains qualitatively and quantitatively similar in rats, when the duration of exposure is increased from 28- to 90-days.

Neurotoxicity study

In a dedicated neurotoxicity study, isopyrazam was added to the diet of 12/sex/dose Wistar rats at dietary concentrations of 0, 300, 1500 and 6000 ppm. This equated to mean intakes of 0, 20, 98 and 382 mg/kg bw/d and 0, 25, 114 and 468 mg/kg bw/d in males and females respectively. All animals survived and there were no treatment-related clinical signs of toxicity. At the top-dose, body-weight gain was reduced in females throughout the study and at the end of the study was 28% lower than controls. In males body-weight gain was reduced in the first week only by a magnitude of 14%. Reductions in food consumption correlated with the body-weight gain reductions at this dose.

There were no changes noted during the FOB assessments; at the 13-week locomotor activity assessment, some scores at 6000 ppm were statistically significantly different than those of controls. In females the distance traversed and the number of rears was reduced (both for an individual 3 minute interval and for the entire 30-minute observation period. In males the number of rears was reduced for an individual 3-minute interval but not for the whole 30-minute observation period. These differences were small, isolated and inconsistent and hence not related to treatment with isopyrazam. Furthermore, there were no macroscopic or microscopic findings in the examined neuronal tissues and brain weights were not affected. There was no evidence of neurotoxicity in this study.

Mice

90-days

Dietary exposure of isopyrazam (93 syn:7 anti specification) to mice for 90-days resulted in reduced body-weight gain throughout the study in both sexes at 1383/1760 mg kg bw/d and at 391/449 mg/kg bw/d; accompanying increases in food consumption resulted in an overall reduction in food utilisation efficiency and slightly lower body weights by the end of the study. Liver weights of the mice were increased in males/females by 47%/59% (high-dose) and by 25%/32% (mid-dose) whilst slight increases (7.6%) were also noted in both sexes of the low-dose group. Hepatocellular hypertrophy was observed in males and females of the mid- and high-dose groups and in males of the low-dose group, ranging from minimal or slight in the lower dose groups to moderate in the high-dose group. Some effects on clinical chemistry parameters were noted in females at the top-dose (albumin/globulin ratio, AAT & triglycerides).

Dogs

90-days

93:7 syn:anti

In dogs, the oral (capsule) administration of isopyrazam (93:7 syn:anti specification; 0, 30, 100, 300 mg/kg bw/d) for 90-days resulted in transient clinical signs of toxicity comprising head wobble and unsteady gait at the mid- and high-dose (males only); there were no treatment related deaths. Body weight was reduced at the high-dose in males (10%) and in females (12%) with an associated reduction in food consumption. Some clinical chemistry, haematology and urinalysis parameters were affected at this dose, with only albumin being affected at the mid-dose. Consistent with the findings in rats and mice, liver weights were increased in dogs, however the effect was less marked and was observed in males only (24.5% at 300 mg/kg bw/d and 12.7% at 100 mg/kg bw/d) and there were no accompanying signs of hepatocellular hypertrophy upon necropsy. Despite liver weight increases only being observed in males the liver appeared enlarged in both sexes of the top-dose group. No adverse findings were noted at the low-dose of 30 mg/kg bw/d.

70:30 syn:anti

When the 70:30 specification was administered to dogs for 90-days (via capsule) at doses of 0, 10, 30 & 250 there were no deaths but clinical signs in one male of the top-dose group included shaking head and uncoordinated movements. Body-weight gain and food consumption were reduced at 250 mg/kg bw/d for the first week only. There were no changes in organ weights at any dose nor were there any unusual findings upon necropsy.

12-months

After 12-months oral (capsule) exposure to isopyrazam (93:7) all dogs survived and there were no treatment-related clinical signs of toxicity. In the high-dose group (250 mg/kg bw/d) body-weight gain was lower in week one in both sexes but was only maintained in males by the end of the study (57%). Food consumption was reduced at this dose in both sexes during the first six weeks of the study. Liver weights were increased in males only at 250 mg/kg bw/d (52%) and at the mid-dose of 100 mg/kg bw/d (42%) which presented without histopathological correlates. Some clinical chemistry parameters were altered at the mid- and high-doses (ALP, AAT, and bilirubin) but only ALP was affected at the low-dose of 25 mg/kg bw/d; there were no other findings in the low-dose group.

10.13.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

A set of standard, well conducted, oral toxicity studies are available in a range of species (rats, mice and dogs) and several isomeric compositions of isopyrazam. All of these studies provided evidence that the oral repeated-dose toxicity of isopyrazam remains consistent regardless of the isomeric composition of the specification, hence the findings can be applied to the specification of isopyrazam for authorisation (which contains a maximum of 15% anti isomer). In all species and specifications reduced body-weight gain and lower food consumption was a feature of isopyrazam exposure. Consistently across all species, the main effect was on the liver, characterised by liver weight increases, being associated (in most cases) by hepatocellular hypertrophy and clinical chemistry changes. The increased liver weights and histopathological correlates were indicative of an adaptive rather than an adverse response. There were no treatment related effects on any other organ.

10.13.2 Additional information on repeated-dose toxicity

The available studies investigating the chronic toxicity and carcinogenicity of isopyrazam are described in detail in section 10.9. Findings from these studies that are relevant to the repeated-dose toxicity of isopyrazam are summarised below:

Table 62: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations
<p>2 Year Dietary Toxicity and Carcinogenicity study in Rats & histological extension</p> <p>OECD 453 GLP</p> <p>Anonymous (2008a) & Anonymous (2009)</p> <p>Rats, HsdRccHn:WIST, M & F</p> <p>12/sex/dose (1-yr)</p> <p>52/sex/dose (2-yr)</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4% (w/w)</p> <p>Syn:anti ratio: 93:7</p>	<p>Doses: 0, 100, 500 & 3000ppm</p> <p>Equivalent to:</p> <p>Males: 0, 5.5, 28 & 173 mg/kg bw/d</p> <p>Females: 0, 7, 35 & 233 mg/kg bw/d</p> <p>52/104 weeks' exposure</p>	<p><u>Chronic phase (12 months)</u></p> <p>There were no treatment-related deaths at any dose</p> <p><u>173/233 mg/kg bw/d</u></p> <p>↑ incidence of scabs (M & F) & subcutaneous masses (M)</p> <p>↓ mean BW: -10.3%** (M) & -18.8%** (F)</p> <p>↓ Hb (-3.4%**), ↑ Platelet count (14%*) in F</p> <p>↑ GGT in M (12.9%**), ↓ ALP in M (-28.9%**)</p> <p>& F (-41.7%**), ↓ AST in F (-34.8%**)</p> <p>↑ liver weight in M (17.2%** & F (14.4%**)</p> <p>Hepatocellular hypertrophy in 12/12** M (5 minimal, 7 slight) & 12/12** F (slight), hepatocyte pigmentation in 6/12* M (5 minimal, 1 slight) & 10/12* F (minimal), minimal hepatocyte vacuolation in 11/12** M & 8/12** F</p> <p><u>28/35 mg/kg bw/d</u></p> <p>↓ mean BW: -3.5%* (F)</p> <p>↑ GGT in M (24.8%**)</p> <p>Hepatocellular hypertrophy in 4/12 M (minimal) & 9/12** F (minimal), hepatocyte vacuolation in 10/12 M** (9 minimal, 1 slight) & 8/12** F (minimal)</p> <p><u>5.5/7 mg/kg bw/d</u></p> <p>No adverse effects</p> <p><u>Carcinogenicity phase (24 months)</u></p> <p><u>173/233 mg/kg bw/d</u></p> <p>↓ mean BW: -13%** (M) & -27%** (F)</p> <p>↓ Hb (-5.4%**), ↓ haematocrit (4.4%**), ↓ RBC (-5.5%**), ↑ platelet count (10.8%*) in F</p> <p>↑ GGT in M (49.9%** & F (42.8%**), ↓ ALP in M (-33.7%** & F (-30.7%*), ↓ AST in F (-21%*)</p> <p>↑ liver weight in M (17.5%** & F (26.4%**)</p> <p>Pale spots on liver & liver masses in F</p> <p>Centrilobular hepatocellular hypertrophy in M (49/52**) & F (50/52**), hepatocyte vacuolation in M (39/52**), centrilobular hepatocellular pigmentation in M (32/52**) & F (46/52**), minimal bile duct hyperplasia in M (12/52**), minimal bile duct fibrosis in M (12/52**), eosinophilic foci in M (32/52**) & F (29/52**)</p>

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations
			<p>Brown pigment in kidney tubules in F, sinus erythrocytosis in lymph nodes in M</p> <p><u>28/35 mg/kg bw/d</u></p> <p>↓ mean BW: -11%** (F)</p> <p>↑ GGT in M (24.8%**), ↓ ALP in M (-17.1%**), ↓ AST in F (-22.2%*)</p> <p>↑ increased liver weight in F (12%**) and in M (5%*)</p> <p>Centrilobular hepatocellular hypertrophy in M (45/52**) & F (49/52**), hepatocyte vacuolation in M (32/52**) & F (18/52**), centrilobular hepatocellular pigmentation in F (49/52**), minimal bile duct hyperplasia in M (19/52**), minimal bile duct fibrosis in M (10/52**), eosinophilic foci in M (23/52**) & F (26/52**)</p> <p>Brown pigment in kidney tubules in F</p> <p><u>5.5/7 mg/kg bw/d</u></p> <p>↓ mean BW: 5.3%* (F)</p>
<p>Chronic toxicity and carcinogenicity in the mouse</p> <p>OECD 451</p> <p>GLP</p> <p>Anonymous (2008b)</p> <p>Mice, C57BL/10J_fCD-1, M & F, 50/sex/group</p>	<p>Isopyrazam</p> <p>Batch: SMU6AP001</p> <p>Purity: 96.4% (w/w)</p> <p>Syn:anti ratio: 93:7</p>	<p>0, 70, 500 & 3500 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 8, 56 & 433 mg/kg bw/d</p> <p>Females: 0, 10, 75 & 554 mg/kg bw/d</p> <p>80-weeks</p>	<p>There were no treatment-related deaths.</p> <p><u>433/554 mg/kg bw/d</u></p> <p>Clinical signs: discharge from the eye of M</p> <p>BW: ↓ BW in M (23%) and F (11%)</p> <p>Organ weights: ↑ liver weights in M (41%) & F (38%)</p> <p>Macroscopic findings: eye discharge in 26/46 M</p> <p>Microscopic findings: hepatocellular hypertrophy in 42/50 M & 47/50 F</p> <p><u>56/75 mg/kg bw/d</u></p> <p>Organ weights: ↑ liver weights in M (14%) & F (7%)</p> <p>Microscopic findings: hepatocellular hypertrophy in 5/50 M & 13/50 F, eosinophilic droplets in gall bladder epithelium in 25/50 F, inflammation in the nasolacrimal ducts in 38/50 M</p> <p><u>8/10 mg/kg bw/d</u></p> <p>No adverse effects</p>

When isopyrazam was administered to rats for 12- and 24-months, overall body-weight gain and final body weights were reduced at the top dose of 173/233 mg/kg bw/d in males/females. Similar to other repeated-dose studies in rats, increases in relative liver weights were observed, to a magnitude of 17.5% in males and 26.4% in females at the top-dose after 24-months' exposure. Histopathological correlates indicative of an adaptive response were again evident at the mid- and high-doses, comprising centrilobular hepatocellular hypertrophy. Additional hepatocellular changes began to appear when the duration of exposure was

increased at doses well above those relevant for classification (hypertrophy, pigmentation and vacuolation along with minimal bile duct hypertrophy and fibrosis). This reflects the findings of the 90-day rat study in which signs of vacuolation began to appear when the dose and duration of exposure were increased. This progression from an adaptive response to actual impairment of liver function resulted in pale spots on the liver, liver masses and an increase in the incidence of hepatocellular adenomas. In mice, progression to liver impairment was not evident in the longer term studies as the only histopathological findings accompanying the reduced final body weights and increased liver weights were hepatocellular hypertrophy and droplets in the gall bladder epithelium.

10.13.3 Comparison with the CLP criteria

Classification for STOT-RE is assigned on the basis of ‘significant’ or ‘severe’ toxicity. Significant is defined as changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant, whilst severe effects are generally more profound and are of a considerably adverse nature, likely to have a significant impact on health. For the purpose of classification, adverse findings should generally be at or below the oral guidance value of 100 mg/kg bw/d (for category 2) or 10 mg/kg bw/d (for category 1) obtained in a 90-day rat study. Equivalent guidance values are available for 28-day and 1-year study and should be extrapolated where appropriate (i.e. increased by a value of 3 for a 28-day study). Adjusted guidance values for categories 1 and 2 are summarised in the table below.

Table 63: Adjusted guidance values for categories 1 and 2

Duration	Adjusted guidance values (mg/kg bw/d)
28-days	Cat 1 = 30 Cat 2 = 300
90-days	Cat 1 = 10 Cat 2 = 100
12-months	Cat 1 = 2.5 Cat 2 = 25
18-months	Cat 1 = 1.7 Cat 2 = 17
2-years	Cat 1 = 1.25 Cat 2 = 12.5

The decision to classify a substance for STOT-RE is warranted when any of the following effects are observed in humans and/or animals which may indicate significant or severe toxicity:

Effects considered to support classification for specific target organ toxicity following repeated exposure

- (a) *Morbidity or death resulting from repeated or long-term exposure*
- (b) *Significant functional changes in the central or peripheral nervous systems or other organ systems*
- (c) *Any consistent and significant adverse changes in clinical chemistry, haematology or urinalysis parameters*
- (d) *Significant organ damage noted at necropsy*
- (e) *Multifocal or diffuse necrosis, fibrosis or granuloma formation in organs with regenerative capacity*
- (f) *Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction*
- (g) *Evidence of appreciable cell death in vital organs incapable of regeneration*

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

None of the effects observed for isopyrazam at or below the cut of values fulfilled any of these criteria. According to the CLP criteria, the effects that were observed after administration of isopyrazam (at the relevant doses for classification) are not considered to support classification. These findings are summarised below as they relate to the CLP criteria.

Effects considered not to support classification for specific target organ toxicity following repeated exposure

(a) Clinical observations or small changes in body-weight gain

Clinical observations were noted in dogs comprising transient head wobble, reduced stability and increased or decreased activity. Small reductions in body-weight gain were noted in rats (<10%) but not in dogs or mice.

(b) Small changes in clinical biochemistry, haematology or urinalysis parameters

There were no toxicologically relevant changes to clinical chemistry, haematology or urinalysis parameters in any species.

(c) Changes in organ weights with no evidence of organ dysfunction

Liver weight increases were the main feature of isopyrazam exposure; in rats, at the relevant doses for classification into category 2, relative liver weights were increased by up to 30% and 25% in males and females respectively. In mice the magnitude of the liver weight increases was 47% and 59% in males and females respectively after 90-days' exposure, whilst liver weights in male dogs were increased by up to 52% after 12-months' exposure (the liver weights of female dogs were not affected). There were no accompanying signs of organ dysfunction.

(d) Adaptive responses that are not considered toxicologically relevant

The liver weight increases observed in rats and mice after administration of isopyrazam were accompanied by findings indicative of an adaptive response; hepatocellular hypertrophy was noted in both sexes and analysis of enzyme activity showed an increase in total P450 activity (particularly PROD).

(e) Substance induced species specific mechanisms of action

This was not a feature of isopyrazam exposure.

In conclusion, in rats, mice and dogs the only effects observed after the administration of isopyrazam (at the relevant doses for classification) were clinical observations, small changes in body-weight gain, small changes in biochemistry parameters and changes in organ weights (increased liver weights) which were accompanied by histopathological findings indicative of an adaptive response. The only organ affected was the liver, and the isomeric composition of isopyrazam had no influence on the severity or incidence of the findings. There were no observations to support classification; hence it would not be appropriate to classify isopyrazam for STOT-RE.

10.13.4 Conclusion on classification and labelling for STOT RE

Not classified (conclusive but not sufficient for classification)
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RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

DS proposed no classification of isopyrazam. The data package contained several repeated dose toxicity studies (28-day, 90-day, 1-year, 1.5-year and 2-year studies) with preparations of isopyrazam with different *syn:anti* ratios. Three different species (rats, mice and dogs) were used. In his assessment the DS only noted adaptive responses of liver (increases of relative liver weight, hypertrophy and minor alterations in biochemical and haematological parameters).

Comments received during consultation

One MSCA supported the DS's proposal for no classification of isopyrazam for STOT SE.

Assessment and comparison with the classification criteria

Both EFSA and the USA EPA in their opinions, report hepatocellular hypertrophy as a substance related effect in repeated dose toxicity studies of isopyrazam.

According to the US EPA: "*Subchronic and chronic oral toxicity studies in the rat, mouse, rabbit and dog demonstrate that the primary target organ for isopyrazam is the liver (increased organ weight and centrilobular hepatocyte hypertrophy). Liver toxicity is usually accompanied by reductions in bodyweight and food consumption*"

EFSA also identified liver as the target organ of isopyrazam upon short-term and long-term exposure in all species tested: the rat, mouse, dog and rabbit.

Based on data in the CLH report, Tables 5 (See Annex 1), 6 and 7 summarise the main findings in the repeated dose toxicity studies in rats, mice and dogs; respectively.

The database contains three different 28-day oral repeated dose toxicity studies in rats with different isopyrazam preparations (*syn:anti* ratios 93:7, 89:11 and 50:50 and pure *anti* and pure *syn* isomers) (Table 5 – see Annex 1 at the end of the Opinion). The three studies yielded a consistent array of effects consisting in increases of relative liver weight and slight alterations in certain haematological and biochemical parameters. These alterations were in general inconsistent among different studies except for the increases in cholesterol concentrations and decreases in triglyceride concentrations. There were no treatment-related macroscopic necropsy findings. However, treatment-related microscopic changes (hepatocellular hypertrophy) was observed for all three test material ratios. Thus, these changes are likely to be an indication of an adaptive response rather than of adversity.

The two different 90-days oral repeated toxicity studies in rats with different isopyrazam preparations (*syn:anti* ratios 93:7 and 70:30) yielded effects quite similar to those reported for the 28-days studies (Table 5). These are, moderate increases in relative liver weight, moderate changes in blood parameters and hepatocellular hypertrophy. These are considered by RAC as adaptive changes and therefore not warranting classification. The

main difference from the 28-day studies was the moderate increases in thyroid weight (Table 5). However, RAC notes that this effect was not associated to histopathological alterations and was not consistently found in all studies in rats. RAC considers this alteration as incidental and not able to warrant classification.

The neurotoxicity study in rats (Table 5) reported a number of small, isolated and inconsistent effects hence not related to treatment with isopyrazam. Therefore, these effects cannot be considered by RAC for supporting a classification.

The 2-generation toxicity study in rats also reports increases in liver weight (up to 31% at the top dose and up to 10% at the mid and low doses) and increases of incidences in hepatocellular hypertrophy (always minimal to slight) with fine cytoplasmic vacuolation (see Table 21). RAC notes that the effects at the top dose (between 217 and 700 mg/kg bw/day depending whether pre-mating, gestation or lactation periods, see Table 21) occur at doses which are above the guidance value for setting classification based on a 2-generation toxicity study. The increases in liver weight and hepatocellular hypertrophy at mid and low doses were minimal and were considered by RAC adaptive and therefore not sufficient for supporting classification.

Finally, the chronic toxicity study (2-years carcinogenicity study) also showed liver as the target organ of isopyrazam due to increases in liver weight and hypertrophy and vacuolation (Table 5). Again, RAC notes that these changes can be interpreted as adaptive rather than adverse and therefore will not be considered for setting classification as STOT RE. Bile duct also presented microscopic alterations (fibrosis and hyperplasia, Table 5). However, the incidences, with respect to the control, were significantly different only for males and dose-response for these alterations was unclear (especially for hyperplasia). Moreover, some cases were also reported in controls and this effect was not detected in other species or in other studies of different duration in rats. Examination of all these data allows RAC to conclude that these effects on bile ducts are likely incidental and more associated to aging than to isopyrazam exposure. Therefore, bile duct alterations will not be considered for classification.

Overall, RAC notes that no relevant effects for STOT RE classification purposes were detected in the 28-day, 90-day and 2-year repeated dose toxicity studies in rats.

Table 6: Summary for repeated dose toxicity study in mice with isopyrazam.

In all cases the difference with controls were statistically significant for at least $p < 0.05$.

Method	Results	Reference
90-day oral (dietary)	There were no treatment-related deaths or clinical signs of toxicity	Anonymous (2008a)
OECD 408	<u>1383/1760 mg/kg bw/day</u>	
GLP	Moderate hepatocellular hypertrophy in males (10/10) and females (10/10)	
C57BL/10JfCD-1 mice		
		males
		females
	↓ Final body weight	5%
	↓ Albumin/Globulin ratio	-
	↑ Alanine aminotransferase	90%
	↑ Triglycerides	-
	↑ Relative liver weight	47%
10/sex/group		210%
Isopyrazam (batch: SMU6AP001)		59%
	<u>391/449 mg/kg bw/day</u>	

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Purity: 96.4%

93:7 syn:anti

0, 500, 2500
and 7000 ppm

Males: 0, 76,
391 and 1383
mg/kg bw/day

Females: 0, 87,
449 and 1760
mg/kg bw/day

Minimal/slight hepatocellular hypertrophy in males (10/10)
and in females (10/10)

	males	females
↓ Final body weight	5%	6%
↑ Relative liver weight	25%	32%

76/87 mg/kg bw/day

Minimal/slight hepatocellular hypertrophy in males (10/10)

	males	females
↑ Relative liver weight	8%	8%

Chronic toxicity
and
carcinogenicity
in the mouse

OECD 451

GLP

C57BL/10JfCD-1
mice

50/sex/group

80-weeks

Isopyrazam
(batch:
SMU6AP001)

Purity: 96.4%
(w/w)

93:7 syn:anti
0, 70, 500 and
3500 ppm

Males: 0, 8, 56
and 443 mg/kg
bw/day

Females: 0, 10,
75 and 554
mg/kg bw/day

There were no treatment-related deaths.

433/554 mg/kg bw/day

	males	females
↓ Body weight	23%	11%
↑ Liver weights	41%	38%
Eye discharge	24/26	
Hepatocellular hypertrophy	42/50	47/50

56/75 mg/kg bw/day

	males	females
↑ Liver weights	14%	7%
Eosinophilic droplets in gall bladder epithelium	25/50	38/50
Inflammation in the nasolacrimal ducts	38/50	-
Hepatocellular hypertrophy	5/50	13/50

8/10 mg/kg bw/day

No adverse effects

Anonymous
(2008b)

As in the case of rats, the 90-days and 80-week repeated dose toxicity studies in mice reported adaptive effects in liver detected in the form of increases in relative liver weight and, depending of the dose, minimal/slight/moderate hepatocellular hypertrophy (Table 6). RAC does not consider these effects relevant for classification since they can be seen as an adaptive response rather than an adverse effect.

The carcinogenicity study in mice also showed some effects of eosinophilic droplets in gall bladder epithelium of uncertain toxicological significance since they do not observe dose-response and were not noted in rats and dogs. Therefore, this effect was not considered by

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

12-month oral (capsule) There were no deaths or treatment-related clinical signs of toxicity Anonymous (2008b)

OECD 452 250 mg/kg bw/day

GLP		males	females
Beagle dogs	↓ Body weight gain in 3 males	8%, 3%, 7%	-
	↓ Body weight gain in 2 females (week 1)	-	6%, 4%
4/sex/group	↓ Body weight gain (week 52)	57%	
Isopyrazam (batch: SMU6AP001)	↑ Alkaline phosphatase (week 52)	415%	272%
	↑ Alanine aminotransferase (week 52)	294%	-
Purity: 96.4%	↑ Glutamate dehydrogenase (week 52)	830%	-
	↓ Bilirubin (week 52)	48%	-
93:7 syn:anti 0, 25, 100 and 250 mg/kg bw/day	↓ Albumin (week 52)	13%	10%
	↑ Liver weight	52%	-

100 mg/kg bw/day

	males	females
↑ Alkaline phosphatase (week 52)	274%	243%
↓ Bilirubin (week 52)	48%	-
↑ Liver weight	42%	-

25 mg/kg bw/day

	males	females
↑ Alkaline phosphatase (week 52)	223%	-

The 90-day oral repeated dose toxicity study in dogs with the isopyrazam preparation 70:30 *syn:anti* showed only transient clinical effects without apparent liver impairment (Table 7). However, the 90-days and 12-month repeated dose toxicity studies in dogs with the isopyrazam preparation with *syn:anti* ratio 93:7 showed an array of effects compatible with those reported in the repeated dose toxicity studies in rats and mice where liver was detected as the main target organ of isopyrazam. The effects in dogs were increases in relative liver weight together with changes in blood parameters (Table 7). The marked changes in these blood parameters were associated to transaminases and are considered secondary to liver changes. Unlike the studies in rats and mice, liver hypertrophy was not reported in dogs and therefore RAC will not consider hepatic changes for warranting STOT RE classification.

In addition to the effects reported above in rats, mice and dogs, RAC notes that a teratogenicity study in rabbits (Anonymous 2008b) also reports minimal to mild centrilobular hepatocellular hypertrophy/vacuolation in liver at 150 and 500 mg/kg bw/day together with increases in liver weight of 13 and 36%. The top dose of this developmental study (500 mg/kg bw/day) was outside the limit concentration for setting classification based on a developmental toxicity study in rabbits where the exposure is for 22 days. The effects at 150 mg/kg bw/day (minimal hypertrophy and 13% increase in liver weight) are considered by RAC again as indicative of an adaptive liver response and not susceptible to

warrant a classification as STOT RE.

Comparison with the criteria

Classification for STOT-RE is assigned on the basis of 'significant' or 'severe' toxicity. Significant is defined as changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant, whilst severe effects are generally more profound and are of a considerably adverse nature and likely to have a significant impact on health. None of the effects observed for isopyrazam fulfilled any of these criteria. On the contrary, several effects were noted which were specifically considered in the Guidance on the Application of the CLP Criteria as effects not supporting classification as STOT RE. These effects were:

1. *Clinical observations or small changes in body-weight gain.* Clinical observations were noted in dogs comprising transient head wobble, reduced stability and increased or decreased activity. Small reductions in body-weight gain were noted in rats (<10%) but not in dogs or mice.
2. *Changes in organ weights with no evidence of organ dysfunction.* Liver weight increases were the main feature of isopyrazam exposure; in rats, at the relevant doses for classification into category 2, relative liver weights were increased by up to 30% and 25% in males and females respectively. In mice, the magnitude of the liver weight increases was 47% and 59% in males and females respectively after 90-days' exposure, whilst liver weights in male dogs were increased by up to 52% after 12-months' exposure (the liver weights of female dogs were not affected). There were no accompanying signs of organ dysfunction.
3. *Adaptive responses that are not considered toxicologically relevant.* The liver weight increases observed in rats and mice after administration of isopyrazam were accompanied by findings indicative of an adaptive response; hepatocellular hypertrophy was noted in both sexes and analysis of enzyme activity showed an increase in total P450 activity (particularly PROD).

In conclusion, in rats, mice and dogs the only effects observed after the administration of isopyrazam (at the relevant doses for classification) were clinical observations, small changes in body-weight gain, small changes in biochemistry parameters and changes in organ weights (increased liver weights) which were accompanied by histopathological findings (centrilobular hepatocyte hypertrophy) indicative of an adaptive response. Overall, RAC supports the DS's proposal for **no classification of isopyrazam for STO RE.**

10.14 Aspiration hazard

10.14.1 Short summary and overall relevance of the provided information on aspiration hazard

No studies available.

10.14.2 Conclusion on classification and labelling for aspiration hazard

Not classified (data lacking)

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of isopyrazam for aspiration toxicity based on data lacking.

Comments received during consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes that the hazard aspiration toxicity is not relevant for solids and therefore supports the **no classification of isopyrazam**.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Isopyrazam (referred to in some test reports as SYN520453) is a carboxamide foliar fungicide. Environmental fate and hazard studies have been considered under Directive 91/414/EEC and summarised in the Draft Assessment Report (DAR), 2010 and Additional Report, 2011. The agreed endpoints from the peer review of isopyrazam under Directive 91/414/EEC are also included in the EFSA Conclusion (EFSA Journal 2012;10(3):2600).

Isopyrazam contains two diastereoisomers designated *syn* and *anti*-isomers (referred to in some test reports as SYN534969 and SYN534968 respectively). In turn each diastereoisomer has two enantiomers (refer to section 1).

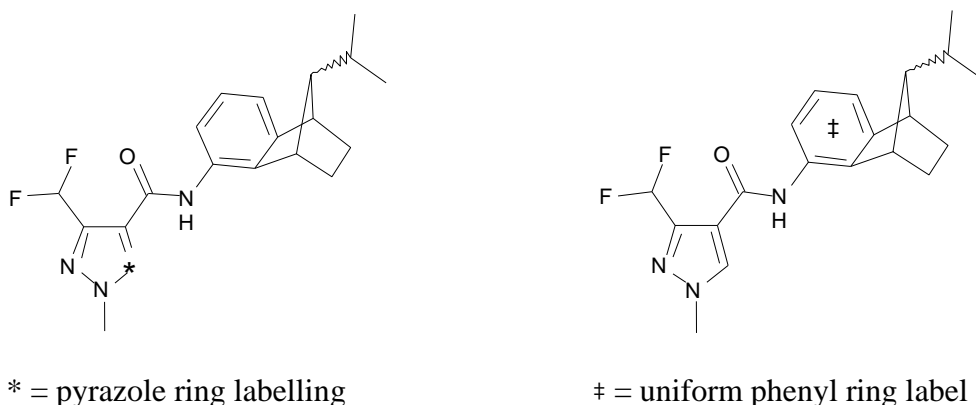
At present there are no data to indicate interconversion of the enantiomers would occur. During review under Directive 91/414/EEC data were presented to support the view that interconversion was unlikely on basis of the structural characteristics of the molecule in relation to the transformations that may occur chemically or enzymatically catalysed under most common environmental conditions.

Both of the isomers are considered to be biologically active. It is noted that aquatic toxicity testing with fish indicates the *anti* isomer may be more ecotoxic (further details below). As the specification for technical isopyrazam covers the range of 78-100% *syn* and 0-15% *anti* available ecotoxicity studies using 70:30 and 90:10 *syn:anti* isomer ratios are considered suitable for hazard classification.

Where available, details of the test item ratio is included.

All radiolabelled studies used ¹⁴C- isopyrazam in a combination of the labels shown in Figure 1.

Figure 1: Structure and radiolabelling positions of isopyrazam



The DAR quotes the measured water solubility of the diastereoisomers as:

- *syn*-isomer (99.5%) 0.00105 g/l at 25 °C and pH 7 (Weissenfeld, 2008b) equating to 1.05 mg/l
- *anti*-isomer (99.6%) 0.00055 g/l at 25 °C and pH 7 (Weissenfeld, 2008c) equating to 0.55 mg/l

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

The Henry's Law Constant for the diastereoisomers have been calculated as follows indicating the substance is unlikely to partition to air for the aquatic environment:

- *syn*-isomer $1.9 \times 10^{-4} \text{ Pa m}^3 \text{ mol}^{-1}$ (Stulz 2008)
- *anti*-isomer $3.7 \times 10^{-5} \text{ Pa m}^3 \text{ mol}^{-1}$ (Stulz 2008)

Isopyrazam is not anticipated to dissociate (Martin, 2007a, 2007b).

Where available information on degradation products is included – details of degradant names and structures are presented in Annex IV. Based on available ecotoxicity data also presented in the Annex, degradation products are not considered more acutely toxic than the parent substance and they are not considered further for classification (see DAR for full study summaries).

11.1 Rapid degradability of organic substances

A summary of available valid information on the fate of isopyrazam is presented in Table 64 below. Endpoints greater than 16 days at study temperature 20 – 50 °C) have not been adjusted to 12 °C as this would not affect the assessment of rapid degradability due results being more conservative.

Table 64: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD Test Guideline 301F (Manometric respirometry test), GLP, isopyrazam: 92.9% purity, 65.6% <i>syn</i> , 27.3% <i>anti</i>	No degradation, day 28	Valid	Seyfried, 2006
Aquatic hydrolysis OECD Test Guideline 111, GLP, isopyrazam 98.8%, approximately <i>syn:anti</i> isomer ratios 90:10	Stable (study temperature 25- 50 °C)	Valid	Berdar and Nicollier, 2007
Aquatic photolysis OECD Test Guideline draft, GLP, isopyrazam >99%	DT ₅₀ : 61 – 64 days summer sunlight at 30 – 50°N (study temperature 25 °C)	Valid	Kuet and Oliver, 2007b
Water/sediment simulation OECD 308, GLP, isopyrazam: 96% purity, 91.3% <i>syn</i> , 8.7% <i>anti</i>	DT _{50 total system} >>1 year at study temperature of 20 °C <1% AR mineralisation after 181 days aerobic systems and after 360 days anaerobic systems	Valid	Stoll and Nicollier, 2008

11.1.1 Ready biodegradability

A ready biodegradation study is available following OECD Test Guideline 301F (Manometric respirometry test) and GLP (Seyfried, 2006). The study was run at ~100 mg/l isopyrazam considered to be above the test item water solubility. No degradation was observed over 28 days

compared to controls and isopyrazam was considered to be 'not readily biodegradable'. Validation criteria were met.

11.1.2 BOD₅/COD

No data

11.1.3 Hydrolysis

An aqueous hydrolysis study (Berdat & Nicollier, 2007) using isopyrazam (approximately *syn:anti* isomer ratios 90:10). The study was conducted to GLP and OECD Test Guideline 111. The study used [¹⁴C-pyrazole]-isopyrazam (radiochemical purity ≥96.1%) at 0.32 mg a.s./l in sterile buffer solutions at pH 4, 5, 7 and 9. Samples were incubated at 50 °C in the dark for 5 days.

Analysis by High Performance Liquid Chromatography (HPLC) UV detector and Thin Layer Chromatography (TLC) indicated that at 50 °C isopyrazam was stable at all tested pHs. A confirmatory test at 25 °C was conducted for 30 days - no significant degradation was seen at any pH or temperature.

Overall, isopyrazam is considered hydrolytically stable.

11.1.4 Other convincing scientific evidence

No data.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Additional data are available and presented in the DAR although these do not impact the hazard classification.

This includes an outdoor microcosm study (Kuet and France, 2008) run to GLP and following elements of OECD and SETAC guidelines (quoted as 13.5 to 53.5 °C water column temperature and natural sunlight). The study calculated a dissipation DT₅₀ of 3 days following Single First Order (SFO) kinetics and a whole system DT₅₀ of 21.2 days (SFO).

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

An aerobic/anaerobic water/sediment study is available following OECD Test Guideline 308 and GLP (Stoll and Nicollier, 2008). The study used isopyrazam (pyrazole-label) and two natural water/sediment systems with water:sediment (dry weight) ratios approximately 4:1 w/w for the river system and 5:1 w/w for the pond system. Physico-chemical properties of the systems are given below in Table 65.

Following equilibration, [¹⁴C-pyrazole] isopyrazam (*syn:anti* isomer ratio 91.3:8.7, i.e. 10.49:1; radiochemical purity ≥96%) was each added in acetonitrile to separate flasks. The ¹⁴C-isopyrazam was applied to the surface water in each vessel to give a nominal initial concentration of 0.04 µg/ml in the water phase.

Flasks were incubated at 20±2 °C in the dark for up to 181 days in the aerobic test systems. Anaerobic systems were set up similarly with a nitrogen flow system and ran for 360 days.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Radioactivity in the water was quantified directly by Liquid Scintillation Counting (LSC) and analysed by HPLC-UV. In all test systems the overall recovery radioactivity ranged from 90.2% to 98.5% of the applied radioactivity (AR).

Table 65: Physico-chemical properties of water/sediment systems in aerobic water/sediment study with isopyrazam

Physical and chemical properties of sediment				
	River		Pond	
Particle size (% w/w):	Aerobic	Anaerobic	Aerobic	Anaerobic
Clay (<2 µm)	11.6	10.5	18.0	19.8
Silt (50-2 µm)	33.4	49.8	75.9	74.6
Sand (2000-50 µm)	55.0	39.7	6.1	5.6
Texture (USDA)	Sandy loam	Loam	Silt loam	Silt loam
pH	7.25	7.34	7.27	7.31
Redox potential (mV)				
Start of acclimation	-358	-414	-449	-446
Start of study	-293	-405	-235	-425
End of study	-213	-474	-220	-496
Organic carbon (%)	1.85	2.01	4.93	5.25
CEC (meq/100 g soil)	13.6	15.8	27.3	25.6
Moisture content	0.843	0.905	2.180	2.126
Carbonate %	24.2	25.0	38.0	36.2
Nitrogen (total, %)	0.14	0.16	0.44	0.44
Phosphorus (total, mg/kg)	422.5	441.6	991.3	975.9
Biomass (mg carbon/100 g soil):				
Initial (start of study)	39.4	not analysed	138.5	not analysed
Final (end of study)	34.4	not analysed	89.0	not analysed
Physical and chemical properties of water				
	River		Pond	
pH	Aerobic	Anaerobic	Aerobic	Anaerobic
At sampling	8.2	9.4	8.1	8.1
Start of acclimation	7.9	8.2	7.4	8.3
Start of study	8.1	8.1	7.7	8.6
End of study	8.6	9.0	8.3	8.8
Redox potential (mV)				
Start of acclimation	20	-273		
Start of study	114	-350		
End of study	20	-453		
Oxygen concentration (%)				
At sampling	8.5	9.4	8.1	8.1
Start of acclimation	5.2	0.2	3.8	0.4
Start of study	5.4	0.04	5.3	0.02
End of study	5.9	<LD	7.0	<LD
Total organic carbon (mg/L)	1.76	2.55	2.13	2.03
Suspended solids (mg/L)	<0.5	<0.5	<0.5	<0.5
Hardness	199	123	188	173

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Isopyrazam dissipated from the water phase in both systems with ~15% remaining in aerobic system and ~25% remaining in the anaerobic system by day 14. During the same period isopyrazam partitioned to sediment with ~69% observed in aerobic sediment and ~62% in anaerobic sediment by day 14.

Minimal mineralisation was observed over the study with <1% AR CO₂ observed in both aerobic and anaerobic test systems.

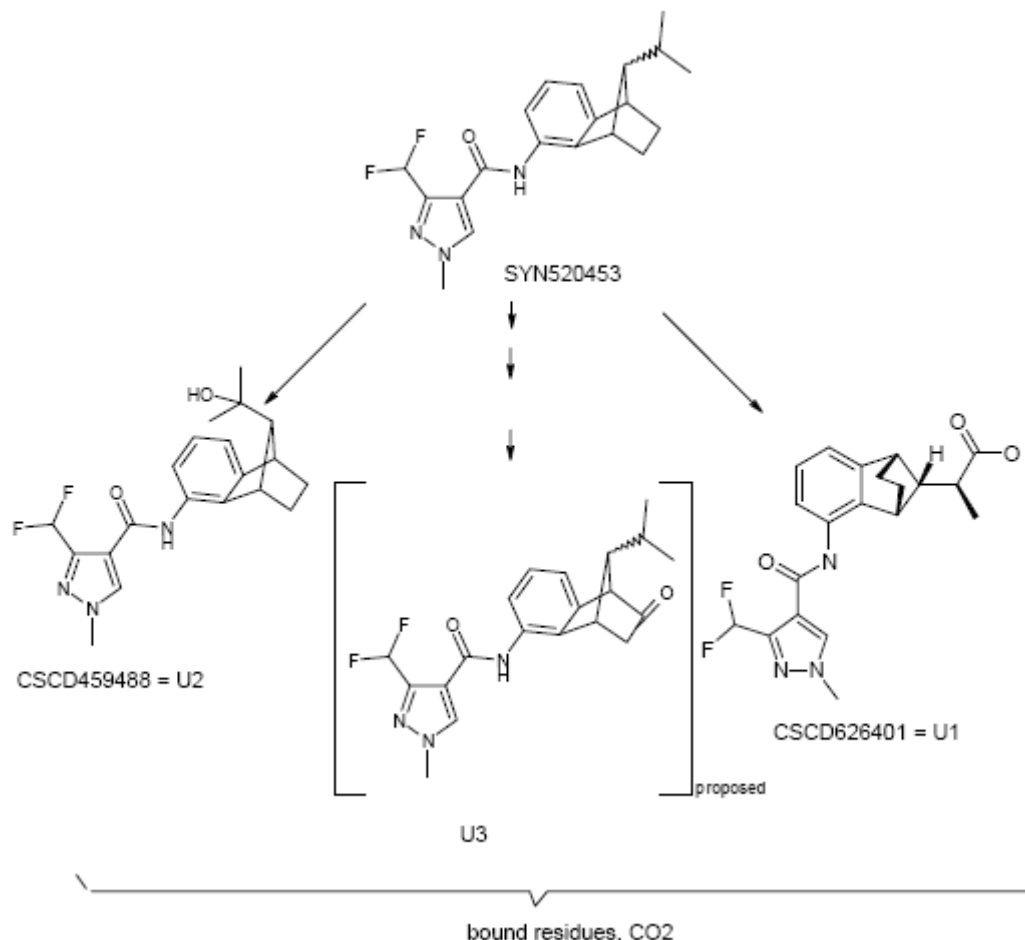
The study report considered total system SFO degradation DT_{50 total system} values were >>1 year for both aerobic and anaerobic test systems.

Analysis under Directive 91/4141/EEC considered that water phase dissipation rates did not appear to conform to SFO kinetics. Consequently, dissipation DT₅₀ values of 1.2 to 1.5 days were calculated for dissipation from the water phase in aerobic systems using First-Order Multi Compartment kinetics.

The *syn:anti* isomer ratio varied during the course of the study but did not appear to consistently increase or decrease. This variation is therefore considered experimental variability.

Low levels of degradants (<5% AR) were observed in both aerobic and anaerobic test systems with two identified (CSCD626401 and CSCD459488).

Figure 2 presents the proposed degradation pathway for isopyrazam in aquatic systems.

Figure 2: Proposed metabolic pathway for isopyrazam in aerobic and anaerobic water/sediment systems

11.1.4.4 Photochemical degradation

An aqueous photolysis study using isopyrazam is available following OECD (draft guidance dated 2000), EPA (Pesticide Assessment Guidelines, Subdivision N, Series 161-2) and Japanese guidelines and GLP (Kuet and Oliver, 2007b).

The study used [^{14}C -pyrazole] and [^{14}C -phenyl] isopyrazam (radiochemical purity $\geq 97\%$) at a concentration of approx. $0.5 \mu\text{g/ml}$. Test solutions were prepared with either sterile buffer (pH 7) or sterile natural water (pH 7) and continuously irradiated at 25°C for up to 29 days with a xenon arc light filtered to restrict the wavelength range to 295 – 800 nm. Mean light energy in the range 300 – 400 nm was 25.17 W.m^2 . The 29 day study duration was considered equivalent to 32 – 34 days summer sunlight at $30 - 50^\circ\text{N}$ assuming 12 hour days.

Radioactivity was quantified directly by LSC and analysed by HPLC, and for selected samples, by TLC.

For the sterile buffer solutions, DT_{50} values were calculated using ModelManager v 1.1 software for the combined dataset of pyrazole and phenyl label tests, as these are effectively replicates. The SFO DT_{50} for the study conditions for both labels was extrapolated to be 54.3 days with an r^2 value of 0.44. This reflects a scattered database of experimental values. The study DT_{50} equates to DT_{50}

values of 61 – 64 days summer sunlight at 30 – 50°N assuming 12 hour days. Two degradants were observed: CSAA798670 (max. 14.8% AR days 15-21) and CSCC210616 (max. 7.4% AR day 15).

Increased photolysis was observed in the sterile natural water samples with an experimental DT₅₀ of 4.2 – 4.9 days, equivalent to 5.2 – 5.9 days summer sunlight at 30 – 50°N assuming 12 hour days. Peak concentration of CSAA789670 was 36.4% at study end (25 days). Peak concentration of CSCC210616 was 20.1 % AR at study end (25 days).

11.1.4.5 Rapid degradation conclusion

Isopyrazam is considered hydrolytically stable at environmentally relevant pH and temperature.

In an OECD Test Guideline 301F study, isopyrazam was considered not readily biodegradable on the basis of 0% mineralisation.

Isopyrazam dissipated rapidly from the water phase to sediment in a water/sediment simulation study (aerobic and anaerobic test systems) using isopyrazam. Minimal mineralisation was observed with <1% mineralisation seen at study termination (day 181 and 360 for aerobic and anaerobic test systems respectively). Total system DT₅₀ values at a study temperature of 20 °C were considered >>1 year. Several aquatic degradants were formed but only at low levels.

Isopyrazam is susceptible to photodegradation. However, it is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore, aquatic photolysis is not considered to meet the criteria for rapid degradation.

A microcosm study is available which supports rapid dissipation with total system DT₅₀ of 21.2 days under natural outdoor conditions (quoted temperature range 13.5 to 53.5 °C under outdoor sunlight).

Overall, isopyrazam is not considered to be rapidly degradable for the purpose of classification according to the CLP criteria.

11.2 Environmental fate and other relevant information

An adsorption coefficient study is available (Elliot and Ricketts, 2006) following GLP and OECD Test Guideline 106. The study used isopyrazam (90:10 *syn:anti*) and 6 soils. Koc values were 2,149 to 4,588 indicating isopyrazam is slightly mobile.

11.3 Bioaccumulation

Table 66: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient <i>n</i> -octanol/water OECD TG107, (shake flask method) isopyrazam <i>syn</i> isomer (99.5%) isopyrazam <i>anti</i> isomer (99.6%)	<i>syn</i> : Log K _{ow} 4.1 at 25 °C, pH 7.3 <i>anti</i> : Log K _{ow} 4.4 at 25 °C, pH 7.8		Weissenfeld, 2008g Weissenfeld, 2008h
Bioaccumulation in fish <i>Lepomis macrochirus</i> OECD 305, GLP isopyrazam: 98.5% purity 69.2% <i>syn</i> , 30.1% <i>anti</i>	BCF _k 406 l/kg BCF _{SS whole fish} 441 l/kg based on ¹⁴ C-residues BCF _{SS lipid normalised whole fish} 374 l/kg based on ¹⁴ C-residues	Flow through, 28 days uptake, 14 days depuration	Anonymous (2007)

11.3.1 Estimated bioaccumulation

No data.

11.3.2 Measured partition coefficient and bioaccumulation test data

The experimental log K_{ow} values for the *syn* and *anti* diastereoisomers of isopyrazam are 4.1 and 4.4 at 25 °C and neutral pH (Weissenfeld, M., 2008g and 2008h).

An experimental aquatic BCF study for radiolabelled isopyrazam is available following OECD Test Guideline 305 and GLP. It was reviewed under EU Directive 91/414/EEC and considered suitable to fulfil the bioaccumulation in fish endpoint. The review noted that the study only employed one test concentration. This was due to test series recommendations at 1% of the acute fish LC₅₀ and a factor of 10 lower which was not analytically feasible. The study BCF results were still considered valid despite this limitation.

The study used a flow-through system with Bluegill Sunfish (*Lepomis macrochirus*) and one exposure concentration; nominally 0.3 µg/l. The exposure period ran for 28 days followed by a 14 day depuration period.

The mean lipid content of fish on days 0, 42 and during steady state was 6.0, 5.0 and 5.9% w/w respectively. The overall mean lipid content was 5.6% w/w and did not differ from that at the start by more ±25%.

The calculated kinetic bioconcentration factor (BCF_k) was 406 l/kg.

The steady state BCF(whole fish) was 441 l/kg based on ¹⁴C-residues.

It is noted that the lipid content at the end of the study was 5.9%. A lipid normalised to 5% whole fish BCF reflecting this would be 374 l/kg based on ¹⁴C-residues.

During the 14 day depuration phase, the levels of [¹⁴C] isopyrazam equivalents in the whole fish decreased rapidly. The calculated DT₉₀ was 1.15 days.

11.3.3 Bioaccumulation conclusion

Isopyrazam has a logKow values of 4.1 and 4.4 for the *syn* and *anti* isomers which are above the CLP threshold of 4. However, an experimental bioaccumulation in fish study is available with the following BCFs:

BCF_k 406 l/kg

BCF_{SS} whole fish 441 l/kg (based on ¹⁴C-residues)

BCF_{SS} lipid normalised to 5% whole fish 374 l/kg (based on ¹⁴C-residues)

As experimental BCF values are below the CLP threshold of 500 l/kg, isopyrazam is not considered to meet the CLP criteria for bioaccumulation.

11.4 Acute aquatic hazard

A summary of available valid information on the aquatic toxicity of isopyrazam is presented in Table 67. A summary of valid information for degradants is also included in Annex IV, Table 1. Based on available data, degradation products are not considered more acutely toxic than the parent substance and are not considered further for classification (see DAR for full study summaries).

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

Further details are presented for studies conducted on the active substance isopyrazam. Both of the isomers are considered to be biologically active. It is noted that aquatic toxicity testing with fish indicates the anti isomer may be more ecotoxic. As the specification for technical isopyrazam covers the range of *syn:anti* isomer ratios 70:30 to 100:0 available ecotoxicity studies using 70:30 and 90:10 *syn:anti* isomer ratios are considered suitable for hazard classification.

Table 67: Summary of relevant information on acute aquatic toxicity

Method	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.066 (mm)	Anonymous 2007a
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.063 (mm)	Anonymous 2005a

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Lepomis macrochirus</i> (Bluegill sunfish)	Mortality	Flow-through	96 hours	LC ₅₀	0.181 (mm)	Anonymous 2007b
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Cyprinus carpio</i> (Common carp)	Mortality	Flow-through	96 hours	LC ₅₀	0.0258 (mm)	Anonymous 2007c
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow-through	96 hours	LC ₅₀	0.0263 (mm)	Anonymous 2007d
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Danio rerio</i> (Zebrafish)	Mortality	Flow-through	96 hours	LC ₅₀	0.3 (mm)	Anonymous 2007e
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow-through	96 hours	LC ₅₀	0.034 (mm)	Anonymous 2006
Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Cyprinodon variegatus</i> (Sheepshead minnow)	Mortality	Flow-through	96 hours	LC ₅₀	0.314 (mm)	Anonymous 2007
Acute toxicity to fish, OECD 203, GLP, purity: SYN534969 <i>syn</i> isomer 98.9%	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.0469 (mm)	Anonymous 2007a
Acute toxicity to fish, OECD 203, GLP, purity: SYN534968 <i>anti</i> isomer 98.6%	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.0092 (mm)	Anonymous 2007b

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Acute toxicity to fish, OECD 203, GLP, purity: SYN534969 <i>syn</i> isomer 99%	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow-through	96 hours	LC ₅₀	0.0817 (mm)	Anonymous, 2007c
Acute toxicity to fish, OECD 203, GLP, purity: SYN534968 <i>anti</i> isomer 98.6%	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow-through	96 hours	LC ₅₀	0.0107 (mm)	Anonymous, 2007d
<i>Daphnia</i> sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Daphnia magna</i>	Acute immobilisation	Static	48 hours	EC ₅₀	0.044 (n) see study details below	Benyon and Richardson, 2007
<i>Daphnia</i> sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 99.7% (86.9% <i>syn</i> , 10.1% <i>anti</i>)	<i>Daphnia magna</i>	Acute immobilisation	Static	48 hours	EC ₅₀	0.13 (mm)	Benyon and Ramsay, 2007
<i>Daphnia</i> sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 99.8% (94.9% <i>syn</i> , 4.93% <i>anti</i>) Guideline adapted for the other species mentioned	<i>Coenagrionidae</i> <i>Crangonyx pseudogracilis</i> <i>Asellus aquaticus</i> <i>Chaoborus</i> sp. <i>Planariidae</i> <i>Cloeon</i> sp. <i>Ostracoda</i> <i>Lymnaea</i> sp. <i>Lumbriculus variegatus</i> <i>Brachionus calyciflorus</i>	Acute immobilisation	Static	48 hours 24 hours	EC ₅₀	>1 (n) >0.74 (mm) >0.775 (mm) >0.730 (mm) >0.750 (mm) >1 (n) >1 (n) >0.9 (mm) >0.955 (mm) >1 (n)	Ashwell and Langridge, 2007
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pseudo-kirchneriella subcapitata</i> *	Cell multiplication inhibition	Static	72 hours 96 hours	ErC ₅₀ NOErC ErC ₅₀ NOErC	>4 (mm) 0.31 (mm) >4 (mm) 0.31 (mm)	Volz, 2005
<i>Lemna</i> sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: isopyrazam (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Lemna gibba</i>	Growth	Static	7 days	ErC ₅₀ NOErC	>0.5 (n) 0.5 (n)	Everett et al, 2007

Notes:

mm refers to mean measured concentrations

n refers to nominal concentrations

*formerly *Selenastrum capricornutum*

Bold value indicates most sensitive acute endpoint relevant to hazard classification proposal

11.4.1 Acute (short-term) toxicity to fish

Twelve valid acute toxicity to fish studies available following OECD Test Guideline 203 and GLP are available and presented below. These studies were reviewed under Directive 91/414/EEC and considered valid. Eight of the studies were conducted with isopyrazam as either 70:30 or 90:10 *syn:anti*. The remaining four studies were conducted with either the *syn* or *anti* isomers.

Study 1 – Anonymous, 2007a

The flow-through study used *Oncorhynchus mykiss* (Rainbow trout) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 5.12, 11.3, 24.8 and 54.5 µg a.s./l. Exposure solutions were prepared with the aid of the solvent tetrahydrofuran (THF) and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 93 to 132% of nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.066 mg a.s./l (95% confidence intervals 0.0271 to 0.120 mg a.s./l) based on mean measured concentrations.

Study 2 – Anonymous 2005a

The flow-through study used *Oncorhynchus mykiss* (Rainbow trout) and isopyrazam (90:10 *syn:anti*) with a nominal exposure range of 6.3, 13, 25, 50, 100 and 200 µg a.s./l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were near nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.063 mg a.s./l (95% confidence intervals 0.033 to 0.151 mg a.s./l) based on mean measured concentrations.

Study 3 – Anonymous, 2007b

The flow-through study used Bluegill sunfish (*Lepomis macrochirus*) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 24.8, 54.5, 120, 264 and 581 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 55 to 95% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.181 mg a.s./l (95% confidence intervals 0.0828 to 0.399 mg a.s./l) based on mean measured concentrations.

Study 4 – Anonymous, 2007c

The flow-through study used *Cyprinus carpio* (Common carp) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 5.12, 11.3, 24.8, 54.5 and 120 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. During review under Directive 91/414/EEC it was noted that the mean length of fish was slightly longer than the test guideline recommendation. However, as there were no mortalities in the controls this was not considered to impact the study. Measured concentrations were 89 to 108% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.0258 mg a.s./l (95% confidence intervals 0.0108 to 0.0528 mg a.s./l) based on mean measured concentrations.

Study 5 – Anonymous , 2007d

The flow-through study used *Pimephales promelas* (Fathead minnow) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 5.12, 11.3, 24.8, 54.5 and 120 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

conditions were considered acceptable. During review under Directive 91/414/EEC it was noted that the fish were fed on the day of study initiation but not during the study period which deviates from the test guideline. However, as the controls were subject to the same conditions with no adverse effects observed and a flow-through system was employed, this was not considered to impact the study. Measured concentrations were 75 to 112% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.0263 mg a.s./l (95% confidence intervals 0.0108 to 0.0563 mg a.s./l) based on mean measured concentrations.

Study 6 – Anonymous, 2007e

The flow-through study used *Danio rerio* (Zebrafish) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 24.8, 54.5, 120, 264 and 581 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 61 to 98% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.3 mg a.s./l (95% confidence intervals 0.201 to 0.458 mg a.s./l) based on mean measured concentrations.

Study 7 – Anonymous 2006

The flow-through study used *Pimephales promelas* (Fathead minnow) and isopyrazam (90:10 *syn:anti*) with a nominal exposure range of 6.3, 13, 25, 50, 100 and 200 µg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 54 to 111% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.034 mg a.s./l (95% confidence intervals 0.017 to 0.039 mg a.s./l) based on mean measured concentrations.

Study 8 – Anonymous, 2007

The flow-through study used the marine species *Cyprinodon variegatus* (Sheepshead minnow) and isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 68, 135, 269, 538 and 1,076 µg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 82.9 to 101% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.314 mg a.s./l (95% confidence intervals were not reported) based on mean measured concentrations.

Study 9 – Anonymous, 2007a

The flow-through study used *Oncorhynchus mykiss* (Rainbow trout) and SYN534969 isopyrazam *syn-isomer* with a nominal exposure range of 13, 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 66 to 153% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.0469 mg a.s./l (95% confidence intervals 0.0186 to 0.0878 mg a.s./l) based on mean measured concentrations.

Study 10 – Anonymous, 2007b

The flow-through study used *Oncorhynchus mykiss* (Rainbow trout) and SYN534968 isopyrazam *anti-isomer* with a nominal exposure range of 0.63, 1.3, 2.5, 5, 10 and 20 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Measured concentrations were 65 to 102% nominal at the start of exposure, 52 to 288% nominal after 48 hours and 62 to 125% nominal at the end of the exposure period. It is noted that at 48 hours the one treatment exhibited significantly higher analytical concentrations compared to nominal. As this treatment was the NOEC, it is not considered to have significantly impacted the LC₅₀ which is based on mean measured concentrations.

The 96-h LC₅₀ was 0.0092 mg a.s./l (95% confidence intervals 0.00759 to 0.0187 mg a.s./l) based on mean measured concentrations.

Study 11 – Anonymous, 2007c

The flow-through study used *Pimephales promelas* (Fathead minnow) and SYN534969 isopyrazam *syn*-isomer with a nominal exposure range of 6.3, 13, 25, 50, 100 and 200 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 34 to 93% nominal at the start of exposure, 66 to 231% nominal after 48 hours and 78 to 270 % nominal at the end of the exposure period and results were based on mean measured concentrations.

It is noted that at 48 and 96 hours the one treatment (nominal 6.3µg a.s./l) exhibited significantly higher analytical concentrations compared to nominal. As this treatment was the lowest and below the NOEC, it is not considered to have significantly impacted the LC₅₀ which is based on mean measured concentrations.

The 96-h LC₅₀ was 0.0817 mg a.s./l (95% confidence intervals 0.0743 to 0.158 mg a.s./l) based on mean measured concentrations.

Study 12 – Anonymous, 2007d

The flow-through study used *Pimephales promelas* (Fathead minnow) and SYN534968 isopyrazam *anti*-isomer with a nominal exposure range of 0.65, 1.3, 2.5, 5.0, 10 and 20 µg a.s./l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 55 to 115% nominal at the start of exposure, 35 to 97% nominal after 48 hours and 48 to 99 % nominal at the end of the exposure period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.0107 mg a.s./l (95% confidence intervals 0.00946 to 0.0206 mg a.s./l) based on mean measured concentrations.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Four valid acute toxicity to invertebrate studies available following OECD Test Guideline 203 and GLP are available and presented below. These studies were reviewed under Directive 91/414/EEC and considered valid. The studies were conducted with isopyrazam as either 70:30, 90:10 or 95:5 *syn:anti*. One study included a wide range of non-standard invertebrates although the study appears to be valid.

Study 1 - Benyon and Richardson, 2007

A static acute toxicity to *Daphnia magna* study was conducted with isopyrazam (70:30 *syn:anti*) with a nominal exposure range of 5, 10, 20, 40 80 and 160 µg a.s./l. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 93 to 113% nominal at the start of exposure and 105 to 133% nominal at the end of the exposure period. The study reported a 48-h EC₅₀ was 0.044 mg a.s./l (95% confidence intervals not reported) based on nominal concentrations.

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

It is noted that the analytical measurements were >20% of nominal. During review under Directive 91/414/EEC this was considered conservative. For the purpose of hazard classification, it is noted that an EC₅₀ based on mean measured concentrations would be above the current value and would not impact the classification as this is not the most sensitive endpoint.

Study 2 - Benyon and Ramsay, 2007

A static acute toxicity to *Daphnia magna* study was conducted with isopyrazam (90:10 *syn:anti*) with a nominal exposure range of 12.5, 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 57 to 80% nominal over the study exposure period. The 48-h EC₅₀ was 0.13 mg a.s./l (95% confidence intervals not reported) based on mean measured concentrations.

Study 3 – Ashwell and Langridge, 2007

A static acute toxicity to 9 aquatic invertebrate species was conducted with isopyrazam (95:5 *syn:anti*) following OECD Test Guideline 203 and adapted for each test organism. The following nominal exposure series was employed for all species: 62.5, 125, 250, 500 and 1,000 µg a.s./l. Exposure solutions were prepared with the aid of the solvent acetone and a solvent control was included. Study conditions were considered acceptable although it was noted that oxygen levels declined to 41% saturation in the *Lymnaea* sp test. Given control organisms were not impacted, this is not considered to have impacted the study.

Test item concentrations were verified at 0 and 48 hours with endpoints based on measured concentrations where <80% test item recovery was observed. It is noted that mean measured concentrations were not within >120% of nominal for *Cloeon* sp. and *Ostracoda*. However, 30% and 0% effects were observed for each of these species and they are not the most sensitive endpoints for hazard classification so it is not necessary to update endpoints based on mean measured concentrations.

The *Brachionus calyciflorus* species involved a 24 hour test resulting in a 24-h EC₅₀ of >1 mg/l based on nominal concentrations as analytical verification was not undertaken.

For each species the EC₅₀ is considered above the highest treatment.

Table 68: Summary of endpoints for aquatic invertebrates exposed to isopyrazam (Ashwell and Langridge, 2007)

Organism	48h EC ₅₀ (mg/l)	Based on nominal or mean measured
Coenagrionidae	>1	Nominal - verified
<i>Crangonyx pseudogracilis</i>	>0.74	Mean measured
<i>Asellus aquaticus</i>	>0.775	Mean measured
<i>Chaoborus</i> sp.	>0.730 ^a	Mean measured
Planariidae	>0.750	Mean measured
<i>Cloeon</i> sp.	>1	Nominal
Ostracoda	>1	Nominal
<i>Lymnaea</i> sp.	>0.9	Mean measured
<i>Lumbriculus variegatus</i>	>0.955	Mean measured
<i>Brachionus calyciflorus</i>	Not available	Nominal

^a In 3 tests no effects seen at highest concentration, however effects <50% observed at some lower treatments

Additional information – Kuhl, 2008

A further 48-hour acute toxicity to *Daphnia magna* study is available using isopyrazam (90.8% purity as 63.3% *syn* isomer and 27.5% *anti* isomer) which was run in parallel with a study on a degradant. The isopyrazam 48-hour EC₅₀ was 0.099 mg/l which is based on nominal concentrations as analytical verification was not undertaken. Given this limitation and that a valid acute ecotoxicity study for the species is available, this study is not considered relevant for hazard classification.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

One study each for algae and aquatic plants are available following GLP and appropriate test guidelines. The studies were reviewed under Directive 91/414/EEC and considered valid. The studies were conducted with isopyrazam as 90:10 *syn:anti*.

Study 1 - Volz, 2005

A static algal growth inhibition test using *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available using isopyrazam (90:10 *syn:anti*) following GLP and OECD Test Guideline 201. The nominal exposure range was 0.1, 0.32, 1.0, 3.2, and 10 mg/l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Measured concentrations were 57 to 121 % nominal at the start of the study and 28 to 77% of nominal at the end of exposure (96 hours).

The study reported 72 and 96 hour endpoints based on mean measured concentrations:

- 72-h E_rC₅₀ >4 mg/l
- 96-h E_rC₅₀ >4 mg/l
- 72-h NOE_rC 0.31 mg/l
- 96-h NOE_rC 0.31 mg/l

Study 2 - Everett, 2007

A semi-static 7-day toxicity to *Lemna gibba* study using isopyrazam (90:10 *syn:anti*) is available following GLP and OECD Test Guideline 221. The nominal exposure range was 31.3, 62.5, 125, 250 and 500 µg a.s./l. Validity criteria were met and the test is considered reliable.

The concentrations of the test item in the fresh solutions ranged from 74 to 108 % of the nominal values on day 0, 46 to 87% on day 3 and 96 to 144% on day 5. The concentrations of the test item in the used solutions ranged from 62 to 90 % of the nominal values on day 3, 77 to 112% on day 5 and 75 to 89% on day 7. As the geometric mean measured concentration of the highest test concentration was 83% of nominal, the study used nominal concentrations for reporting the endpoints as follows:

- 7-d E_rC₅₀ (frond number) >0.5 mg/l
- 7-d E_rC₅₀ (dry weight) >0.5 mg/l
- 7-d NOE_rC (frond number) 0.5 mg/l
- 7-day NOE_rC (dry weight) 0.5 mg/l

For the purpose of hazard classification, it would be preferable to present endpoints based on mean measured concentrations. However, no effects were observed during the study and recalculated endpoints would not impact the classification proposal.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

No additional relevant data.

11.4.5 Summary of acute (short-term) toxicity to aquatic organisms

Acute toxicity data are available for fish, invertebrates, algae and aquatic plants using isopyrazam. Acute endpoints for fish and invertebrates are <1 mg/l.

Acute toxicity studies are available using *Oncorhynchus mykiss* and isopyrazam (70:30 and 90:10 *syn:anti*) and *syn* and *anti* isomers individually. The 96-hour LC₅₀s for isopyrazam was 0.063 and 0.066 mg/l. The 96-hour LC₅₀s for the *syn* and *anti* isomers were 0.0469 and 0.0092 mg/l respectively. While these results indicate the *anti* isomer is more ecotoxic to fish, it is noted that isopyrazam under consideration for harmonised classification contains up to 15% of the *anti* isomer. On this basis, it is considered that the LC₅₀ for the *anti* isomer is overly conservative and endpoints based on isopyrazam as a mixture of *syn:anti* isomers are more appropriate for hazard classification.

Therefore, the lowest endpoint is a 96-hour LC₅₀ for *Cyprinus carpio* of 0.0258 mg/l. This study used the *syn:anti* isomer ratio of 70:30 which is considered representative of the substance for harmonised classification.

11.5 Long-term aquatic hazard

Table69: Summary of relevant information on chronic aquatic toxicity

Method	Species	Results	Remarks	Reference
Fish Early-Life Stage toxicity, OECD 210, GLP, purity 98.9%, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	32-day NOEC 0.00287 mg/l (mm) [fry survival]	Valid	Anonymous, 2007f
<i>Daphnia magna</i> Reproduction OECD 202, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Daphnia magna</i>	21-day NOEC [reproduction] 0.013 mg/l (n verified)	Valid	Bätscher, 2007
Freshwater Algal Growth Inhibition, OECD Guideline 201, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pseudo-kirchneriella subcapitata</i> *	72-hour NOErC 0.31 (mm) 96-hour NOErC 0.31 (mm)	Valid	Volz, 2005
<i>Lemna</i> sp. Growth Inhibition Test, OECD Guideline 221, GLP, purity: isopyrazam (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Lemna gibba</i>	7-day NOErC 0.5 (n)	Valid	Everett et al, 2007

Notes:

mm refers to mean measured concentrations

n refers to nominal concentrations

*formerly *Selenastrum capricornutum*

Bold value indicates most sensitive chronic endpoint

11.5.1 Chronic toxicity to fish

Anonymous (2007f)

A flow through chronic toxicity to fish study using isopyrazam (70:30 *syn:anti*) following GLP and OECD Test Guideline 210 is available. The study ran for 32 days reflecting 28 days post hatch. The study used Fathead minnow (*Pimephales promelas*) and the following endpoints were recorded: time to hatch, hatching rate, development rate, survival and growth (length and dry weight). General observations were also recorded. Study conditions were acceptable and study validity criteria were met. The nominal exposure range was 0.7, 1.5, 3.3, 7.3 and 16 µg/l. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Analytical measurement showed that measured concentrations did not remain within 20% of nominal over the study period and ranged from 57 to 113% of nominal and so endpoints were determined based on mean measured test concentrations.

Table 70: presents a summary of study endpoints

Nominal concentration (µg a.s./l)	Mean measured concentration (µg a.s./l)	Hatching success (%) ¹	Fry survival day 5 to test end (%) ²	Overall survival day 0 to test end (%) ³	Mean length (mm) ± SD	Mean dry weight (mg) ± SD
Control	Control	78	81	63	25.2 ± 1.5	53.15 ± 6.4
Solvent control	Solvent control	80	85	68	23.7 ± 0.1	40.66 ± 1.7
0.7	0.52	73	94	69	25.3 ± 0.7	50.35 ± 5.4*
1.5	1.43	86	66*	57	24.6 ± 0.5	47.36 ± 1.5
3.3	2.87	89	83	74	25.4 ± 0.5	50.77 ± 4.0*
7.3	5.49	91	63*	58	23.4 ± 0.7	37.35 ± 3.0
16	13	86	0**	0**	-	-

¹The number of live larvae on the day they are transferred from the egg cups to the test vessels (day 5), expressed as a percentage of the number of eggs added at the start of the test (day 0).

²The number of surviving larvae at the end of the test (day 32), expressed as a percentage of the number of live larvae on day 5.

³The number of surviving larvae at the end of the test (day 32), expressed as a percentage of the number of eggs added on day 0.

*Statistically significantly different from the solvent control (p<0.05)

**Statistically significantly different from the pooled controls

The study reported a 32-day NOEC of 0.00287 mg/l (mean measured) based on fry survival. Review under Directive 91/414/EEC considered that the significant difference between the 0.00143 mg/l treatment and controls may have been influenced by an outlier for one of the 0.00143 mg/l replicates on day 11. On this basis the quoted 32-day NOEC of 0.00287 mg/l was presented which was included in the EFSA Conclusion (EFSA, 2012).

For the purpose of hazard classification the 32-day NOEC is considered to be 0.00287 mg/l.

11.5.2 Chronic toxicity to aquatic invertebrates

Study 1 – Bättscher, 2007

A semi-static chronic toxicity to *Daphnia magna* study using isopyrazam (70:30 *syn:anti*) is available following GLP and OECD Test Guideline 211. The 21 day study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.4, 1.3, 4.0, 13, 40 and 13µg/l. Exposure solutions were prepared with the aid of the solvent DMF and a solvent control was included. Measured concentrations were 84 to 88% of nominal and results were based on nominal concentrations. Validity criteria were met and the test is considered reliable. The study 21-d NOEC_{reproduction} was 0.0013 mg a.s./l based on verified nominal concentrations.

11.5.3 Chronic toxicity to algae or other aquatic plants

See Section 11.4.3 for a previous evaluation of these studies, the chronic endpoints from which are presented below:

Study 1 - Volz, 2005

The chronic endpoints for toxicity to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) using isopyrazam (90:10 *syn:anti*) were as follows based on mean measured concentrations:

- 72-h NOErC 0.31 mg/l
- 96-h NOErC 0.31 mg/l

Study 2 - Everett, 2007

The chronic endpoints for toxicity to *Lemna gibba* study using isopyrazam (90:10 *syn:anti*) were as follows based on nominal concentrations:

- 7-d NOErC (frond number) 0.5 mg/l
- 7-day NOErC (dry weight) 0.5 mg/l

It is noted that some measured concentrations were not within 20% of nominal concentrations. For the purpose of hazard classification, it would be preferable to present endpoints based on mean measured concentrations. However, no effects were observed during the study and recalculated endpoints would not impact the classification proposal.

11.5.4 Chronic toxicity to other aquatic organisms

Two ecotoxicity studies (Mommert (2008), Pfeifle et al (2007)) with *Chironomus riparius* are available and presented in the DAR. These studies employed water-sediment test systems with endpoints based on nominal and initial measured concentrations. Given isopyrazam concentrations declined in the aqueous phases and partitioned to sediment it is unclear if a contribution of the toxicity in this study was due to sediment contact/ingestion. In addition, reliable data are available for standard aquatic test species / test systems which are more sensitive and overall preferable endpoints for hazard classification.

11.5.5 Chronic toxicity to aquatic organisms

Valid long-term ecotoxicity endpoints are available for fish, invertebrates, algae and aquatic plants. NOECs for fish and *Daphnia* are <0.1 mg/l. The lowest endpoint is a 32-day NOEC for *Pimephales promelas* of 0.00287 mg/l.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

Acute toxicity data are available for fish, invertebrates, algae and aquatic plants using isopyrazam. Acute endpoints for fish and invertebrates are <1 mg/l. Therefore, Aquatic Chronic 1 classification is appropriate.

Five acute toxicity to fish 96-hour LC₅₀ endpoints are in the range 0.01 to 0.1 mg/l. This includes:

- studies with *Oncorhynchus mykiss* and isopyrazam (70:30 and 90:10 *syn:anti*) which resulted in 96-hour LC₅₀s for isopyrazam of 0.063 and 0.066 mg/l, and
- studies with *Pimephales promelas* and isopyrazam (70:30 and 90:10 *syn:anti*) exhibiting LC₅₀s for isopyrazam of 0.0263 and 0.034 mg/l.

In addition, a slightly lower 96-hour LC₅₀ for *Cyprinus carpio* of 0.0258 mg/l is within this classification range.

Further 96 hour LC₅₀ acute toxicity to fish endpoints for the *syn* and *anti* isomers using *Oncorhynchus mykiss* and *Pimephales promelas*:

- *Oncorhynchus mykiss* 96-hour LC₅₀s 0.0469 and 0.0092 (95% C.I. 0.00759 to 0.0187) mg/l
- *Pimephales promelas* 96-hour LC₅₀s 0.0817 and 0.0107 (95% C.I. 0.00946 to 0.0206) mg/l

While these results indicate the *anti* isomer is more ecotoxic to fish, it is noted that isopyrazam under consideration for harmonised classification contains up to 15% of the *anti* isomer. In addition, as acute endpoints using isopyrazam (*syn:anti*) for *Oncorhynchus mykiss*, *Pimephales promelas* and *Cyprinus carpio* are similar and acute endpoints for the *anti* isomer with *Oncorhynchus mykiss* and *Pimephales promelas* are similar, it is unlikely this is due to significant species sensitivity. This is supported by overlapping 95% confidence intervals for 96-hour LC₅₀s.

On this basis, it is considered that the LC₅₀ for the *anti* isomer is overly conservative and endpoints based on isopyrazam as a mixture of *syn:anti* isomers covering multiple fish species are more appropriate for hazard classification.

Therefore, the lowest endpoint is a 96-hour LC₅₀ for *Cyprinus carpio* of 0.0258 mg/l. This study used the *syn:anti* isomer ratio of 70:30 which is considered protective and representative of the substance for harmonised classification. Overall, on the basis of the *Cyprinus carpio* endpoint being in the range 0.01 to 0.1 mg/l an acute M-factor of 10 is appropriate.

Based on available data (see Annex IV), degradation products are not considered more acutely toxic than the parent substance and are not considered further for classification (see DAR for full degradant study summaries).

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Isopyrazam is considered hydrolytically stable at environmentally relevant pH and temperature.

In an OECD Test Guideline 301F study, isopyrazam was considered not readily biodegradable on the basis of 0% mineralisation.

Isopyrazam dissipated rapidly from the water phase to sediment in a water/sediment simulation study (aerobic and anaerobic test systems) using isopyrazam. Minimal mineralisation was observed with <1% mineralisation seen at study termination (day 181 and 360 for aerobic and anaerobic test systems respectively). Total system DT₅₀ values at a study temperature of 20 °C were considered >>1 year. Several aquatic degradants were formed but only at low levels.

Isopyrazam is susceptible to photodegradation. However, it is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore, aquatic photolysis is not considered to meet the criteria for rapid degradation.

A microcosm study is available which supports dissipation in the aquatic environment with a total system DT₅₀ of 21.2 days under natural outdoor conditions (quoted temperature range 13.5 to 53.5 °C under natural sunlight).

Overall, isopyrazam is not considered to be rapidly degradable for the purpose of classification according to the CLP criteria.

Isopyrazam has a logK_{ow} values of 4.1 and 4.4 for the *syn* and *anti* isomers which are above the CLP threshold of 4. However, an experimental bioaccumulation in fish study is available with the following BCFs:

BCF_k 406 l/kg

BCF_{SS whole fish} 441 l/kg (based on ¹⁴C-residues)

BCF_{SS lipid normalised to 5% whole fish} 374 l/kg (based on ¹⁴C-residues)

As experimental BCF values are below the CLP threshold of 500 l/kg, isopyrazam is not considered to meet the CLP criteria for bioaccumulation.

Valid long-term ecotoxicity endpoints are available for fish, invertebrates, algae and aquatic plants. NOECs for fish and *Daphnia* are <0.1 mg/l resulting in a classification of Aquatic Chronic 1. The lowest endpoint is a 32-day NOEC for *Pimephales promelas* of 0.00287 mg/l. This is in the range 0.001 to 0.01 mg/l, so a chronic M-factor of 10 is appropriate for a not rapidly degradable substance.

It is noted that *Pimephales promelas* were not the most acutely sensitive fish species. Considering the most sensitive fish LC₅₀ for *Cyprinus carpio* and the surrogate approach would also result in a chronic M-factor of 10 for a not rapidly degradable substance.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400 – Very toxic to aquatic life

Acute M-Factor: 10

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

Chronic M-Factor: 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Interconversion of the enantiomers is unlikely due to structural characteristics of the molecule in relation to the transformations that may occur chemically or enzymatically catalysed under most common environmental conditions. Both of the isomers are considered to be biologically active.

It is noted that acute aquatic toxicity testing with **fish** based on OECD TG 203 indicates the *anti*-isomer is 5-8 times more ecotoxic than *syn*-isomer (Anonymous, 2007a; Anonymous, 2007b; Anonymous, 2007c, Anonymous, 2007d). As the specification for technical isopyrazam covers the range of 78-100% *syn*- and 0-15% *anti*-isomer, the available eco-toxicity studies that are using data both on the 70:30 and 90:10 *syn:anti* isomer ratios are considered suitable for hazard classification.

The measured **water solubility** of *syn*-isomer (99.5%) is 1.05 mg/L at 25 °C and pH 7 and of *anti*-isomer (99.6%) 0.55 mg/L at 25 °C and pH 7. **Vapour pressure** of *syn*-isomer is 2.4×10^{-7} Pa and 2.2×10^{-8} Pa of *anti*-isomer at 20°C. The **Henry's Law Constant** for the *syn*-isomer 1.9×10^{-4} Pa m³ mol⁻¹ and 3.7×10^{-5} Pa m³ mol⁻¹ for the *anti*-isomer indicate the substance is unlikely to be volatile. Based on the physical-chemical information on the isomers isopyrazam is not expected to dissociate.

Degradation

Abiotic degradation

A valid OECD TG 111 study has been presented. The hydrolytic stability of isopyrazam 90:10 *syn:anti* (0.32 mg/L, radiochemical purity $\geq 96.1\%$) was studied in sterile aqueous buffer solutions at pH 4, 5, 7 and 9 showing **no significant degradation** in 30 and 5 days at respective temperatures of 25 and 50 °C.

A valid aquatic photolysis study with isopyrazam (70:30 *syn:anti*, radiochemical purity $\geq 97\%$) at a concentration of approx. 0.5 µg/mL has been presented. Sterile buffer or sterile natural water at pH 7 continuously irradiated at 25 °C for up to 29 days under a xenon lamp. Sterile buffer solution DT₅₀ values were calculated with ModelManager for the combined dataset of pyrazole and phenyl label tests. The DT₅₀ for the study conditions for both labels was 54.3 days with an r² value of 0.44. The **direct photolysis DT₅₀ equates to 61 - 64 days** summer sunlight at 30 - 50 °N assuming 12 hour days. Two degradants were observed: CSAA798670 (max. 14.8% AR days 15-21) and CSCC210616 (max. 7.4% AR day 15). Faster **indirect photolysis** (due to decontaminants in the natural water test system) was observed in the sterile natural water samples with equated **DT₅₀ of 5.2 - 5.9 days** summer sunlight at 30 - 50 °N assuming 12 hour days. Peak concentration of CSAA789670 and CSCC210616 was 36.4% and 20.1% AR respectively at the end of the study (25 days).

Biodegradation

A valid OECD TG 301F manometric respirometry test (Seyfried, 2006) run above the water solubility at ~100 mg/L of isopyrazam (*syn:anti* isomer ratio 65.6:27.3; purity 92.9%) under aerobic conditions in activated sludge observed no degradation within 28 days compared to

controls and the substance was considered to be '**not readily biodegradable**'.

A valid OECD TG 308 study (Stoll and Nicollier, 2008) measuring the metabolism and rate of degradation of isopyrazam (*syn:anti* isomer ratio 91.3:8.7; radiochemical purity $\geq 96\%$) under aerobic and anaerobic laboratory conditions in aquatic systems is given. Samples of untreated Rhein river water and Rothenfluh natural pond water and their associated sediments (dry weight) ratios approximately 4:1 w/w for the river system and 5:1 w/w for the pond system were equilibrated in flasks for 4 weeks. Flasks with a nominal initial concentration of 0.04 $\mu\text{g/ml}$ of the radiolabelled substance were incubated at 20 ± 2 °C in the dark for up to 181 days in the aerobic and similarly in anaerobic test systems for 360 days. In all test systems the overall recovery radioactivity ranged from 90.2% to 98.5% of the applied radioactivity (AR).

It has been noted that isopyrazam dissipated from the water phase in both systems with $\sim 15\%$ remaining in aerobic and $\sim 25\%$ in anaerobic system by day 14. Partitioning to sediment with $\sim 69\%$ in aerobic and $\sim 62\%$ in anaerobic sediment was also observed. Minimal mineralisation was observed over the study with $< 1\%$ AR CO_2 observed in both aerobic and anaerobic test systems.

The estimations were calculated using ModelManager and FOCUS. The more accurate DT_{50} values of 1.2 to 1.5 days were calculated for dissipation from the water phase in aerobic systems using first order multi compartment kinetics as water to sediment dissipation rates did not appear to conform to single first order kinetics. No sediment dissipation rates were calculated.

Low levels of degradants ($< 5\%$ AR) were observed in both aerobic and anaerobic test systems with two identified (CSCD626401 and CSCD459488).

The river system aerobic and anaerobic DT_{50} were 2 and 8.8 days. The pond system aerobic and anaerobic DT_{50} were 2.6 and 7.1 days respectively. **Total system DT_{50} was $\gg 1$ year** for both aerobic and anaerobic test systems due to slow degradation in the sediment.

Isopyrazam is considered not readily biodegradable and rapidly dissipating from water to sediment with minimal mineralisation in aerobic and anaerobic conditions. Also the DS considered isopyrazam not rapidly degradable for the purpose of classification due to the substance being hydrolytically stable at environmentally relevant pH and temperature; it is susceptible to photodegradation, however it is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify and there is insufficient data to assess it. Based on available information the degradation products of the substance are not considered more acutely toxic than the parent substance.

Bioaccumulation

Experimental $\log K_{ow}$ values of the *syn*- and *anti*-isomers of isopyrazam are 4.1 and 4.4 respectively at 25 °C and neutral pH.

A valid OECD TG 305 aquatic accumulation and elimination study (Anonymous, 2007) for radiolabelled isopyrazam (70:30 *syn:anti*) is available. It is additionally noted the study is considered valid despite only one test concentration (nominally 0.3 $\mu\text{g/l}$ - 1% of the acute fish LC_{50}) instead of two was employed - the factor of 10 lower value was below the limit of detection.

Exposure in a flow-through system to Bluegill sunfish (*Lepomis macrochirus*) ran for 28 days

followed by a 14 day depuration period. Mean measured concentrations (97% of the nominal value) were maintained consistently during the exposure period. The mean lipid content of fish on days 0, 42 and during steady state was 6.0, 5.0 and 5.9% w/w respectively. The overall mean lipid content was 5.6% w/w and did not differ from the start by more than $\pm 25\%$. During the 14 day depuration phase, the levels of [¹⁴C] isopyrazam equivalents in the whole fish decreased rapidly. The calculated DT₉₀ was 1.15 days.

The calculated kinetic bioconcentration factor (BCF_k) was 406 l/kg; the steady state BCF_(whole fish) was 441 L/kg. The lipid content at the end of the study was 5.9%. A lipid normalised to 5% whole fish BCF reflecting this would be 374 l/kg based on ¹⁴C-residues.

The dossier submitter concluded that although the individual logK_{ow} values of the *syn* and *anti*-isomers are above 4, the experimental BCF values of the substance are below the CLP threshold of 500 L/kg, therefore isopyrazam is **not** considered to meet the CLP criteria for **bioaccumulation**.

Aquatic toxicity

A summary of available valid information on the aquatic toxicity of isopyrazam is presented in the following Tables. A summary of valid information for degradants with regards to their ecotoxicity is also given as supplemental information. However, it is noted that based on available data the degradation products are not considered more acutely toxic than the parent substance and are not taken into account for classification purposes.

Acute toxicity

Table 29: Summary of relevant information on acute aquatic toxicity.

Study	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Edpoint	Toxicity (mg/L)	
Fish							
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.066 (mm)	Anonymous 2007a
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow-through	96 hours	LC ₅₀	0.063 (mm)	Anonymous 2005a
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Lepomis macrochirus</i> (Bluegill sunfish)	Mortality	Flow-through	96 hours	LC ₅₀	0.181 (mm)	Anonymous 2007b
Acute toxicity to fish, OECD TG 203, GLP,	<i>Cyprinus carpio</i> (Common)	Mortality	Flow-through	96 hours	LC ₅₀	0.0258 (mm)	Anonymous 2007c

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	carp)						
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow- through	96 hours	LC ₅₀	0.0263 (mm)	Anonymous 2007d
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Danio rerio</i> (Zebrafish)	Mortality	Flow- through	96 hours	LC ₅₀	0.3 (mm)	Anonymous 2007e
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow- through	96 hours	LC ₅₀	0.034 (mm)	Anonymous 2006
Acute toxicity to fish, OECD TG 203, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Cyprinodon variegatus</i> (Sheepshead minnow)	Mortality	Flow- through	96 hours	LC ₅₀	0.314 (mm)	Anonymous 2007
Acute toxicity to fish, OECD TG 203, GLP, purity: SYN534969 <i>syn</i> isomer 98.9%	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow- through	96 hours	LC ₅₀	0.0469 (mm)	Anonymous 2007a
Acute toxicity to fish, OECD TG 203, GLP, purity: SYN534968 <i>anti</i> isomer 98.6%	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Mortality	Flow- through	96 hours	LC ₅₀	0.0092 (mm)*	Anonymous 2007b
Acute toxicity to fish, OECD TG 203, GLP, purity: SYN534969 <i>syn</i> isomer 99%	<i>Pimephales promelas</i> (Fathead minnow)	Mortality	Flow- through	96 hours	LC ₅₀	0.0817 (mm)	Anonymous , 2007c
Acute toxicity	<i>Pimephales</i>	Mortality	Flow-	96 hours	LC ₅₀	0.0107	Anonymous

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

to fish, OECD TG 203, GLP, purity: SYN534968 <i>anti</i> isomer 98.6%	<i>promelas</i> (Fathead minnow)		through			(mm)*	, 2007d
Invertebrates							
<i>Daphnia</i> sp Acute Immobilisation OECD TG 202, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Daphnia magna</i>	Acute immobilisation	Static	48 hours	EC ₅₀	0.044 (n) see study details below	Benyon and Richardson, 2007
<i>Daphnia</i> sp Acute Immobilisation OECD TG 202, GLP, purity: isopyrazam 99.7% (86.9% <i>syn</i> , 10.1% <i>anti</i>)	<i>Daphnia magna</i>	Acute immobilisation	Static	48 hours	EC ₅₀	0.13 (mm)	Benyon and Ramsay, 2007
<i>Daphnia</i> sp Acute Immobilisation OECD TG 202, GLP, purity: isopyrazam 99.8% (94.9% <i>syn</i> , 4.93% <i>anti</i>) Guideline adapted for the other species mentioned	<i>Coenagrionidae</i> <i>Crangonyx pseudogracilis</i> <i>Asellus aquaticus</i> <i>Chaoborus</i> sp. <i>Planariidae</i> <i>Cloeon</i> sp. <i>Ostracoda</i> <i>Lymnaea</i> sp. <i>Lumbriculus variegatus</i> <i>Brachionus calyciflorus</i>	Acute immobilisation	Static	48 hours 24 hours	EC ₅₀	>1 (n) >0.74 (mm) >0.775 (mm) >0.730 (mm) >0.750 (mm) >1 (n) >1 (n) >0.9 (mm) >0.955 (mm) >1 (n)	Ashwell and Langridge, 2007
Algae/other aquatic organisms							
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pseudo-kirchneriella subcapitata</i> *	Cell multiplication inhibition	Static	72 hours 96 hours	E _r C ₅₀ E _r C ₅₀	>4 (mm) >4 (mm)	Volz, 2005
<i>Lemna</i> sp. Growth Inhibition Test OECD TG 221, GLP, purity: isopyrazam (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Lemna gibba</i>	Growth	Static	7 days	E _r C ₅₀	>0.5 (n)	Everett et al., 2007

ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

Notes:

* value not considered for classification, because based solely on anti-isomer at 98.6% purity

mm refers to mean measured concentrations

n refers to nominal concentrations

*formerly *Selenastrum capricornutum*

Bold value indicates most sensitive acute endpoint relevant to hazard classification proposal

Acute toxicity data is available for fish, invertebrates, algae and aquatic plants using isopyrazam as well as the individual *syn*- and *anti*-isomers.

Twelve OECD TG 203 **fish** acute toxicity are presented. Eight of the studies were conducted with isopyrazam as either with 70:30 or 90:10 *syn:anti* isomer ratio; the remaining four with either the *syn*- or *anti*-isomers. Six different fish species of fish (Rainbow trout, Bluegill sunfish, Common carp, Fathead minnow, Zebrafish and Sheepshead minnow) were exposed under flow-through conditions for 96 h. The LC₅₀ values ranged from 0.0092 mg/L to 0.314 mg/L. The results are based on mortality observations and mean measured concentrations.

While results indicate the *anti*-isomer is more ecotoxic to fish, it is noted that isopyrazam contains up to 15% of the *anti*-isomer. In addition as acute endpoints using isopyrazam (*syn:anti*) for *Oncorhynchus mykiss*, *Pimephales promelas* and *Cyprinus carpio* are similar and acute endpoints for the *anti* isomer with *Oncorhynchus mykiss* and *Pimephales promelas* are similar, it is unlikely this is due to significant species sensitivity. This is supported by overlapping 95% confidence intervals for 96-hour LC₅₀ values. On this basis, it is considered that the LC₅₀ for the *anti* isomer is overly conservative and endpoints based on isopyrazam as a mixture of *syn:anti* isomers covering multiple fish species are more appropriate for hazard classification.

The DS therefore concluded that *Cyprinus carpio* flow-through study (Anonymous 2007c) with isopyrazam (70:30 *syn:anti*) should be used for classification. Nominal exposure ranges are 5.12, 11.3, 24.8, 54.5 and 120 µg/L. Exposure solutions were prepared with the aid of the solvent THF and a solvent control was included. Study conditions were considered acceptable. Measured concentrations were 89 to 108% nominal over the study period and results were based on mean measured concentrations. The 96-h LC₅₀ was 0.0258 mg/L (95% confidence intervals 0.0108 to 0.0528 mg/L). There are 4 more studies with isopyrazam at 70:30 or 90:10 *syn:anti* ratio in the same range of toxicity (0.0263-0.066 mg/L) for classification purposes supporting the study selected by DS.

The acute toxicity of isopyrazam to **invertebrates** was studied in valid OECD 202 flow-through tests. Two tests were with water flea and one study included a wide range of non-standard invertebrates despite this the study appears to be valid. The studies were conducted with isopyrazam as either 70:30, 90:10 or 95:5 *syn:anti* ratios. EC₅₀ value of one of the tests (Benyon and Richardson, 2007) was nominal, it was further noted that an EC₅₀ based on mean measured concentrations would be above the chosen sensitive value and would not impact the classification. For the other two the results are given as mean measured concentrations. For the study with the range of non-standard invertebrates (Ashwell and Langridge, 2007) it was noted that mean measured concentrations were not within >120% of nominal for *Cloeon sp.* and *Ostracoda*. However, 30% and 0% effects were observed for each of these species and they are not the most sensitive endpoints for hazard classification so the update of endpoints based on mean measured concentrations was not done. The *Brachionus calyciflorus* species involved a 24 hour test resulting in a 24-h EC₅₀ of >1 mg/L based on nominal concentrations as analytical verification was not undertaken.

One valid study each conducted with isopyrazam as 90:10 *syn:anti* isomer ratio for **algae** and

aquatic plants is available. The study with *Pseudokirchneriella subcapitata* reported 72 and 96 hour endpoints based on mean measured concentrations $E_rC_{50} >4$ mg/L respectively.

As the geometric mean measured concentration of the highest test concentration was 83% of nominal the semi-static 7-day toxicity to *Lemna gibba* study used nominal concentrations for reporting $E_rC_{50} >0.5$ mg/L for frond number and dry weight parameters.

Overall, the DS proposed to use the lowest 96-hour LC_{50} for *Cyprinus carpio* of 0.0258 mg/L based on the study with 70:30 *syn:anti* ratio to classify isopyrazam in the Aquatic Acute 1 category with M-factor 10.

Chronic toxicity

Table 30: Summary of relevant information on chronic aquatic toxicity.

Study	Species	Results	Remarks	Reference
Fish Early-Life Stage toxicity, OECD TG 210, GLP, purity 98.9%, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Pimephales promelas</i> (Fathead minnow)	32-day NOEC 0.00287 mg/L (mm) [fry survival]	Valid	Anonymous, 2007f
<i>Daphnia magna</i> Reproduction OECD TG 211, GLP, purity: isopyrazam 92.9% (65.6% <i>syn</i> , 27.3% <i>anti</i>)	<i>Daphnia magna</i>	21-day NOEC [reproduction] 0.013 mg/L (n verified)	Valid	Bätscher, 2007
Freshwater Algal Growth Inhibition, OECD TG 201, GLP, purity: isopyrazam 99.7% (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Pseudo-kirchneriella subcapitata</i> *	72-hour NOE_rC 0.31 (mm) 96-hour NOE_rC 0.31 (mm)	Valid	Volz, 2005
<i>Lemna</i> sp. Growth Inhibition Test, OECD TG 221, GLP, purity: isopyrazam (89.6% <i>syn</i> , 10.1% <i>anti</i>)	<i>Lemna gibba</i>	7-day NOE_rC 0.5 (n)	Valid	Everett et al., 2007

Notes:

mm refers to mean measured concentrations

n refers to nominal concentrations

*formerly *Selenastrum capricornutum*

Bold value indicates most sensitive chronic endpoint

Chronic toxicity data is available for fish, invertebrates, algae and aquatic plants.

A valid flow-through OECD TG 210 early life stage toxicity *Pimephales promelas* fish study (Anonymous, 2007f) using isopyrazam (70:30 *syn:anti* ratio) running for 32 days reflecting 28 days post hatch is presented. Hatch time and rate, development rate, survival and growth (length and dry weight) were recorded: The nominal exposure range was 0.7, 1.5, 3.3, 7.3 and 16 µg/L. Measured concentrations did not remain within 20% of nominal over the study period and ranged from 57 to 113% of nominal so endpoints were determined based on mean measured concentrations; The resulted 32-day NOEC was 0.00287 mg/L.

A valid OECD TG 211 semi-static chronic toxicity to *Daphnia magna* study (Bätscher, 2007)

using isopyrazam (70:30 *syn:anti* ratio) is available. The 21-day NOEC for reproduction was 0.013 mg/L based on verified nominal concentrations.

A valid study (Volz, 2005) with *Pseudokirchneriella subcapitata* with *syn:anti* ratio 89.6:10.1 reported 72 and 96 hour growth inhibition chronic endpoints based on mean measured concentrations NOEC 0.31 mg/L respectively.

The 7-day nominal chronic endpoints for toxicity to *Lemna gibba* study (Everett et al, 2007) using isopyrazam at 90:10 *syn:anti* ratio were reported as NOEC 0.5 mg/L for frond number and dry weight parameters.

It is noted by the dossier submitter that chronic ecotoxicity studies with *Chironomus riparius* are also available employing water-sediment test systems. Given that isopyrazam concentrations declined in the aqueous phases and partitioned to sediment it is unclear if a contribution of the toxicity in this study was due to sediment contact/ingestion. DS believes reliable data is available from standard aquatic test species/test systems which are more sensitive and considered preferable for hazard classification and thus has not included these results into the CLH report.

The study (Anonymous, 2007f) reporting a 32-day NOEC of 0.00287 mg/L (mean measured) based on fry survival which is also considered the lowest value suitable for hazard classification as Aquatic Chronic 1 with M-factor 10.

Comments received during consultation

Four MSCAs supported the proposed environmental classification Aquatic acute 1, H400 (M=10) and Aquatic Chronic 1, (M=10) based on the available data for the most sensitive species (Fish: *Cyprinus carpio*; 96h-LC50=0.0258 mg/L and *Pimephales promelas* 32-day NOEC=0.00287 mg/L), respectively.

Additional information on the toxicity of a degradation product "metabolite CSCD465008" was given regarding a OECD TG 201 test with *Pseudokirchneriella subcapitata* showing E_yC₅₀ (72 hours) = 22.44 mg/L, E_rC₅₀ (72 hours) = 26.52 mg/L and NOEC (72 hours) = 18 mg/L. This information was noted by the DS.

One MSCA specifically agreed on the approach taken for classification to exclude the studies (Anonymous, 2007b and Anonymous, 2007d) on the *anti*-isomer and only include studies with a representative mixture of the two isomers; it was acknowledged that toxicity testing with fish indicates that the *anti*-isomer may be more ecotoxic than the *syn*-isomer and that isopyrazam contains a mixture of two designated *syn* and *anti*-isomers both considered to be biologically active. This comment was welcomed by the DS.

Another MSCA pointed out the clear difference in toxicity in fish observed between the *syn* and *anti*-isomer where the latter showed higher acute toxicity (1 order of magnitude), also reflected in the outcomes of the 90:10 versus 70:30 *syn:anti* isomer ratios. The same trend is also mentioned to be observed in the *Daphnia magna* studies performed with both ratios. It was noted that studies with algae were only performed with 90:10 *syn:anti* ratio and results might not reflect the true toxicity. The DS agreed with the above and also pointed out the lack of 90:10 *syn:anti* ratio data for the most sensitive species on chronic endpoints. Additionally the DS noted that the application of the order of magnitude observed with fish/invertebrates determined with the 70:30 ratio to the available algal endpoints based on 90:10 ratio, would not impact the classification as they are not lower. Some editorial or/and minor comments were also presented.

Assessment and comparison with the classification criteria

RAC agrees with the DS that isopyrazam can be considered hydrolytically stable in the environment and susceptible to photodegradation.

RAC notes that the degradation data indicates the substance should be considered not readily biodegradable (0% of mineralisation in Seyfried, 2006) and rapidly dissipating to the sediment from water compartment and remaining persistent in the sediment compartment. This is based on the water/sediment simulation study (Stoll and Nicollier, 2008) in aerobic and anaerobic test systems showing total system DT₅₀ at 20 °C >>1 year supported by the results of the microcosm study in the aquatic environment (Kuet and France, 2008) with a total system DT₅₀ of 21.2 days under natural outdoor conditions.

RAC takes into account the note on the available data showing the degradation products are not considered more toxic than the parent substance and agrees with the DS to not take into account the information for classification purposes.

Overall, isopyrazam is not considered to be rapidly degradable for the purpose of classification according to the CLP criteria.

RAC agrees with the DS and concludes that isopyrazam has low bioaccumulation potential based on the available bioconcentration study in Bluegill sunfish showing a BCF_(whole fish) value of 441 L/kg despite the information on the partition coefficients of individual *syn*- and *anti*-isomers (Log Kow values of 4.1 and 4.4 respectively).

RAC agrees with the DS that algae toxicity is not expected to be lower than acute and chronic fish endpoints and would not impact the classification if 70:30 isomer ratio is applied to the available algal endpoints determined with the 90:10 ratio.

The presented acute and chronic algal endpoints were determined with the 90:10 *syn:anti* ratio (72-h ErC₅₀ >4 (mm) mg/L and NOErC 0.31 (mm) mg/L) whereas for fish were 96-h LC₅₀ 0.0258 (mm) mg/L and 32-day NOEC 0.00287 (mm) mg/L and for invertebrate 48-h EC₅₀ 0.044 (n) and 21-d NOEC 0.013 (n-verified) mg/L with 70:30 isomer ratio.

RAC agrees with the DS that based on the available data the *anti*-isomer appears to be more acutely toxic to fish and invertebrates than the *syn*-isomer. As for the chronic toxicity test data is available only for 70:30 *syn:anti* ratio it is not possible to conclude on the chronic toxicity of the individual isomers.

RAC notes the composition of isopyrazam ranging from 78-100% *syn*- and 0-15% *anti*-isomer and agrees with the DS to base the classification of isopyrazam on the available test data using 70:30 and 90:10 *syn:anti* isomer ratios. RAC also notes that the unlikely specie sensitivity based on the test data presented being in the same order of magnitude and the high confidence interval of LC₅₀ values (95%) for the substance itself as for the *syn*- and *anti*-isomers individually.

In conclusion, RAC agrees with the DS that the most sensitive fish LC₅₀ for *Cyprinus carpio* of 0.0258 mg/L results in a classification of **Aquatic Acute 1, H401** with **M = 10** (considering 0.01 mg/L < LC₅₀ < 0.1 mg/L).

RAC agrees with the DS that the lowest chronic endpoint of 32-day NOEC for *Pimephales*

promelas 0.00287 mg/L results in a classification of **Aquatic Chronic 1, H410 with M = 10** as a **not rapidly degradable** substance (considering 0.001 mg/L < NOEC < 0.01 mg/L for non-rapidly degradable substances).

RAC notes if additional data becomes available either on the bioaccumulation potential, the toxicity of the degradation products in the environment and acute or chronic toxicity of isopyrazam or its isomers, the classification could be reconsidered.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

An Ozone Depleting Potential (ODP) is not reported for isopyrazam and it is not listed in Annex I to Regulation (EC) No. 1005/2009. This hazard is therefore not considered further in this Report.

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

Not classified (data lacking)

13 ADDITIONAL LABELLING

None

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ANNEX 2 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPYRAZAM

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15 ANNEXES

ANNEX I (confidential) – Study Summaries (DAR)

ANNEX II – Mode of Action (MoA) and Human Relevance Assessment of the Increased Liver Tumour Incidence

Annex III - Mode of Action Assessment - Uterine Endometrial Adenocarcinoma, Mammary Gland Fibroadenoma and Pituitary Adenoma in the Han Wistar Rat

ANNEX IV - Ecotoxicity degradant information

ANNEX V (confidential) – Full reference list