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**

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United Kingdom

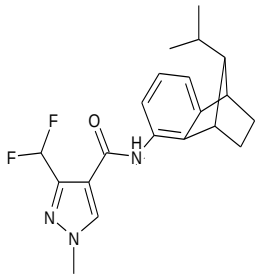
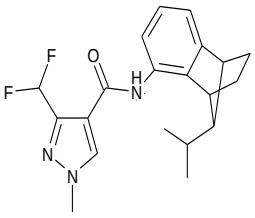
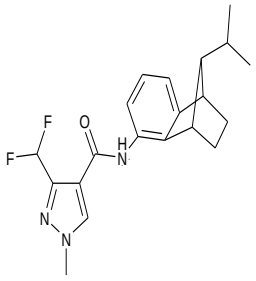
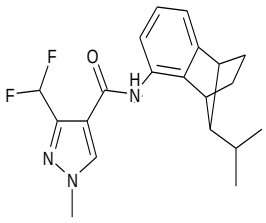
Version number: 2 **Date: August 2019**

CONTENTS

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*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 [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	$C_{20}H_{23}F_2N_3O$
Structural formula	 syn*(1*R,*4*S,*9*R*)-enantiomer* *78-100%*  syn*(1*S,*4*R,*9*S*)-enantiomer*  anti*(1*R,*4*S,*9*S*)-enantiomer* *0-15%*  anti*(1*S,*4*R,*9*R*)-enantiomer*
SMILES notation (if available)	-

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 CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
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 *(Syn-isomer)*	**>78 - <100%**	**Not listed**	**-**[#]
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 *(Anti-isomer)*	**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 CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**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 diasteroisomers (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.

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-*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	-	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-*N*-	-	88168-58-1*	Repr. 1B	H360D	GHS07	H360D	-		-

entry if agreed by RAC and COM		[(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			Skin Sens 1B Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS08 GHS09 Dgr	H317 H410		M =10 M =10	

*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.

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 ºC **Anti:** 144.5 ºC	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 > 130ºC	-	-
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%$

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%[$]
Self-ignition temperature	No self-ignition up to a temperature of 400 °C. The substance melted during the study.	Jackson, 2008a & b	EEC method A16 Purity 95.5%[$]
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%[$]
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data. Solubility in other solvents is acetone 314g/l at 25°C dichloromethane 303g/l at 25°C ethylacetate 179g/l at 25°C hexane 1.17g/l at 25°C methanol 119g/l at 25°C octanol 44.1g/l at 25°C toluene 77.1g/l at 25°C	Weissenfeld 2008f	CIPCA MR 157.6 Purity 95.5%[$]
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.

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 ºC. In the OECD 103 studies conducted with the syn and anti isomers, exothermic decomposition was noted from 261 ºC (with 192 J/g) and 274 ºC (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.

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.

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.

Comparison of the toxicokinetics of the 93:7and 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	Limit test 1/1 at 2500 mg/kg bw killed in extremis Main test 1/1 at 175, 1/1 at 275 & 3/3 at 2000 mg/kg bw survived **LD_{50} >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 animals 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 *in extremis* 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 LD_{50} 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	Limit test 1 female at 2000 mg/kg bw was killed *in extremis* Main test 5/7 killed *in extremis* at 2000 mg/kg bw, 4/4 at 550 & 1/1 at 175 mg/kg bw survived **LD_{50} ≤ 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)	**SYN534969 (pure syn)** 5/5 survived at 2000 mg/kg bw **LD_{50} >2000 mg/kg bw** **SYN534968 (pure anti)** 1/1 at 2000 mg/kg bw killed *in extremis* 2 hours post-dose 3/3 at 550 mg/kg bw killed *in extremis* 1-2 hours post-dose 3/3 survived at 175 mg/kg bw **LD_{50} = 310.2 mg/kg bw** (95% confidence interval 175-550 mg/kg bw) **Isopyrazam (50:50 syn:anti)** 1/1 at 2000 mg/kg bw killed *in extremis* 2 hours post-dose 3/3 at 550 mg/kg bw killed *in extremis* 1-2 hours post-dose 3/3 at 175 mg/kg bw survived **LD_{50} = 310.2 mg/kg bw** (95% confidence interval 175-550 mg/kg)

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 *in extremis*, 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 LD_{50} 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 LD_{50} is likely to be lower and therefore proposes an LD_{50} of $\leq$ 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 *in extremis* 1-2 hours after dosing	Hunched posture, sedation, ventral recumbency
1 female	2000	All killed *in extremis* 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 *in extremis* 1-2 hours after dosing	Ventral recumbency, sedation
1 female	2000	All killed *in extremis* 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 LD_{50} of SYN534969 (pure syn) is > 2000 mg/kg bw, whilst the acute oral LD_{50} 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 LD_{50} 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 LD_{50} 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 (LD_{50}) of a substance is ≤ 2000 mg/kg bw. The acute oral toxicity study on isopyrazam (with a 93:7 syn:anti ratio) gave an LD_{50} 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)

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 LD_{50} 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 LD_{50} 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 LD_{50} 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 LD_{50} value is ≤ 2000 mg/kg bw. In the available study, isopyrazam (syn:anti ratio 93:7) was found to have an LD_{50} 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 LD_{50} 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, HsdBrlHan 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 **LC_{50} = >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 LC_{50} 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 LC_{50}. 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 LC_{50} value of ≤ 5 mg/l air. Since in the available study the LC_{50} 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)

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)

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

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

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.

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)

10.7 Respiratory sensitisation

No studies available

10.7.1 Conclusion on classification and labelling for respiratory sensitisation

Not classified (data lacking)

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

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

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 ≤ 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

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 *S.typhimurium* and *E. coli* Plate incorporation & pre-incubation OECD 471 GLP Callander (2006)	*S.typhimurium* strains TA1535, TA1537, TA98 & TA100 *E.coli* strains WP2 (pKM101) & WP2 *uvrA* (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 *S.typhimurium* and *E. coli* Plate incorporation & pre-incubation OECD 471 GLP Sokolowski (2008a)	*S.typhimurium* strains TA1535, TA1537, TA98 & TA100 *E.coli* strains WP2 (pKM101) & WP2 *uvrA* (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
In vitro 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)

Method, guideline, deviations if any	Test system (organism, strain)	Test substance, syn:anti ratio	Conc. range	Results		Remarks
				+S9	-S9	
In vitro 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
In vitro 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	Assay 1 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
			Assay 2 10 – 20 µg/ml (-S9) & 20 – 50 µg/ml (+S9) 3h exposure (+S9) & 20 h exposure (–S9)	neg	neg	
In vitro 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	Assay 1 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
			Assay 2 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

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
In vivo 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
In vivo 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)

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

<table>
<tr><th>Method, guideline, deviations if any,</th><th>Species, strain, sex, no/group</th><th>Test substance, batch, purity, syn:anti ratio</th><th>Dose levels, duration of exposure</th><th>Results
(% change from controls)</th></tr>
<tr><td></td><td></td><td></td><td></td><td>
cell count (RBC) (-5.5%**), ↑ platelet count (10.8%*) in F

↑ GGT in M (49.9%**) & F (42.8%**), ↓ ALP in M (-33.7%**) & F (-30.7%*), ↓ AST in F (-21%*)

↑ liver weight in M (17.5%**) & F (26.4%**)

Pale spots on liver & liver masses in F

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**)

Brown pigment in kidney tubules in F, sinus erythrocytosis in lymph nodes in M

<u>500 ppm</u>

↓ mean BW in F -11%**

↑ GGT in M (-24.8%**), ↓ ALP in M (-17.1%**), ↓ AST in F (-22.2%*)

↑ relative liver weight in F (12%**) and M (5%*)

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**)

Brown pigment in kidney tubules in F

<u>100 ppm</u>

↓ mean BW in F (-5.3%*)

<u>Neoplastic findings</u>

<u>Thyroid</u>
<table>
<tr><td rowspan="2">Parameter</td><td colspan="4">Dietary conc. of isopyrazam (ppm)</td></tr>
<tr><td>0</td><td>100</td><td>500</td><td>3000</td></tr>
<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>
<tr><td colspan="5">Laboratory Historical Control Data[$]:
Follicular cell adenoma (males)
1.6 – 9.6%, 3 studies 2007 - 2009</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any,</th><th>Species, strain, sex, no/group</th><th>Test substance, batch, purity, syn:anti ratio</th><th>Dose levels, duration of exposure</th><th>Results
(% change from controls)</th></tr>
<tr><td></td><td></td><td></td><td></td><td>
Liver
<table>
<tr><td rowspan="2">Parameter</td><td colspan="4">Dietary conc. of isopyrazam (ppm)</td></tr>
<tr><td>0</td><td>100</td><td>500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>52</td><td>52</td><td>52</td><td>52</td></tr>
<tr><td colspan="5">Hepatocellular tumours - Males</td></tr>
<tr><td>Adenoma</td><td>1</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Carcinoma</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td colspan="5">Hepatocellular tumours - Females</td></tr>
<tr><td>Adenoma</td><td>0</td><td>1</td><td>1</td><td>11</td></tr>
<tr><td>Carcinoma</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td colspan="5">Laboratory Historical Control Data [$]:
Hepatocellular adenoma (females)
0 – 1.9%, 3 studies, 2007 – 2009
Hepatocellular carcinoma (females)
0%, 3 studies, 2007 - 2009</td></tr>
</table>
Uterus
<table>
<tr><td rowspan="2">Parameter</td><td colspan="4">Dietary conc. of isopyrazam (ppm)</td></tr>
<tr><td>0</td><td>100</td><td>500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>52</td><td>52</td><td>52</td><td>52</td></tr>
<tr><td colspan="5">Uterine tumours - Females</td></tr>
<tr><td>Adenoma</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Carcinoma</td><td>1</td><td>2</td><td>3</td><td>15</td></tr>
<tr><td colspan="5">Laboratory Historical Control Data [$]:
Uterine adenocarcinoma
1.9 – 7.8%, 3 studies, 2007 - 2009</td></tr>
</table>
Mammary
<table>
<tr><td rowspan="2">Parameter</td><td colspan="4">Dietary conc. of isopyrazam (ppm)</td></tr>
<tr><td>0</td><td>100</td><td>500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>52</td><td>52</td><td>52</td><td>52</td></tr>
<tr><td colspan="5">Mammary tumours - Females</td></tr>
<tr><td>Adenoma</td><td>14</td><td>16</td><td>9</td><td>4</td></tr>
<tr><td>Carcinoma</td><td>2</td><td>7</td><td>5</td><td>0</td></tr>
<tr><td colspan="5">Historical control: not available</td></tr>
</table>
Pituitary
<table>
<tr><td rowspan="2">Parameter</td><td colspan="4">Dietary conc. of isopyrazam (ppm)</td></tr>
<tr><td>0</td><td>100</td><td>500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>52</td><td>52</td><td>52</td><td>52</td></tr>
<tr><td colspan="5">Pituitary tumours - Females</td></tr>
<tr><td>Adenoma</td><td>32</td><td>33</td><td>28</td><td>24</td></tr>
<tr><td>Carcinoma</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td colspan="5">Historical control: not available</td></tr>
</table>
</td></tr>
</table>

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)
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 *Non-neoplastic findings* 3500 ppm ↓ 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 500 ppm ↑ 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 70 ppm No adverse effects *Neoplastic findings* 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

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)

	Dietary Concentration of Isopyrazam (ppm)							
	Males				Females			
Finding	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)**

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

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 [a]excludes animals with selected microscopic findings in the liver
[b]excludes 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)

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.

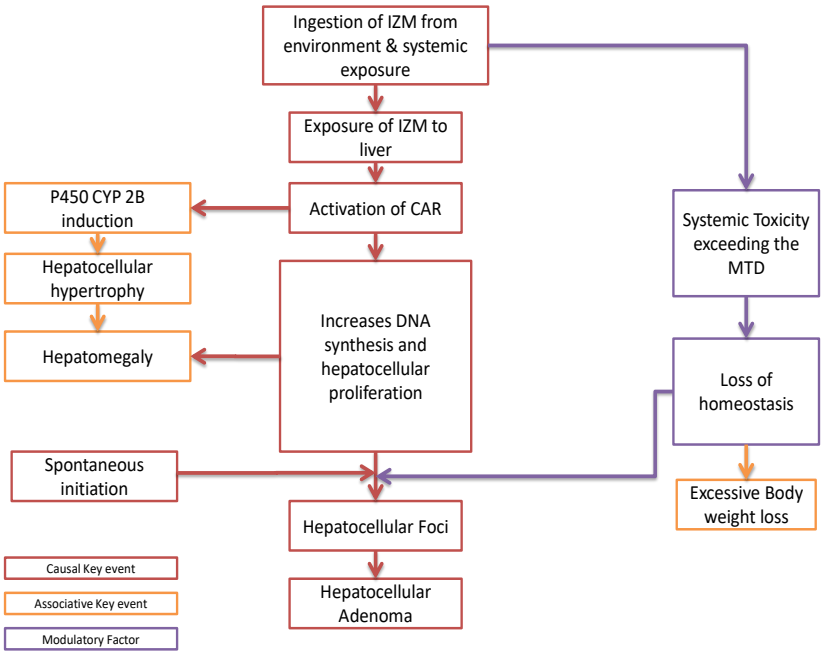
There are various possible mechanistic explanations for this apparent weak carcinogenic response in high-dose female rats, including genotoxicity, PPARα receptor activation, AhR receptor activation, CAR/PXR receptor activation, oestrogenic stimulation, Statins, cytotoxicity, immunosuppression, porphyria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

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, NR1I) 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 µM Phenobarbital: 10 – 1000 µM 96-hours' exposure CYP2B activity 3 replicates Proliferation 5 replicates, 1500 hepatocytes/conc ATP depletion 6 replicates	**Cytotoxicity assay**	Cytotoxicity of isopyrazam demonstrated by ATP depletion from 65 µM (effects observed at non-cytotoxic concentrations)	Ellcombe (2011)
		Enzyme Induction CYP2B activity measured by PROD (pentoxyresorufin-O-dependentylase) CYP2B/CYP3A activity measured by BROD (benzyloxyresorufin-O-debenzylase)	**Isopyrazam:** ↑↑ PROD (6.6- & 4.1-fold increase at 10 & 30 µM) ↑ BROD (1.4- to 2.2-fold increase at 3 – 30 µM) **Phenobarbital:** ↑↑ PROD (4.0- to 5.8-fold increase at 100 & 1000 µM) ↑ BROD (2- to 2.6-fold increase at 100-1000 µM)	
		Replicative DNA Synthesis	**Isopyrazam:** ↑ hepatocyte proliferation (at all concentrations, maximum 2.6 times control at 3 µM) **Phenobarbital:** ↑ hepatocyte proliferation (2.4-fold at 1000 µM)	

In cultured rat hepatocytes isolated by *in situ* perfusion, treatment with isopyrazam at concentrations of 10 and 30 µM resulted in a 6.6- and 4.1-fold increase in PROD activity; for comparison purposes, phenobarbital at concentrations of 100 and 1000 µM 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 µM 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 µM (the increase with phenobarbital was 2.4-fold at 1000 µM).

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 µM Phenobarbital: 10 – 1000 µM 96-hours' exposure CYP2B activity 3 replicates Proliferation 5 replicates, 1500 hepatocytes/conc ATP depletion 6 replicates	**Cytotoxicity assay**	Cytotoxicity of isopyrazam demonstrated by ATP depletion from 65 µM (effects observed at non-cytotoxic concentrations)	Ellcombe (2011a)
		Enzyme Induction	**Isopyrazam:** ↑ PROD (max. 1.7-fold at 30 µM) ↑↑ BROD (2.9-, 3.8-, 4.2- & 4.9-fold at 1, 3, 10 & 30 µM) **Phenobarbital:** ↑ PROD (1.8-fold at 1000 µM) ↑↑ BROD (6.3-fold at 1000 µM) 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 µM whilst BROD activity was increased at 1, 3, 10 and 30 µM 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 µM 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 Measurements: **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 6β-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** *3-days* ↓ BW gain (-45%*), ↓ FC, ↑ liver weights (16%**), ↑ mitosis *7-days* ↓ BW gain (-25%**) ↑ liver weights (15%**), centrilobular hypertrophy (5/10 minimal) *14-days* ↑ liver weights (24%**), centrilobular hepatocellular hypertrophy (5/10 minimal, 5/10 mild) **58 mg/kg bw/d** *3-days* ↑ liver weights (20%**) *7-days* ↑ liver weights (6%**) *14-days* ↑ liver weights (10%**) Enzyme induction There were no treatment related effects on peroxisome proliferation, CYP3A or CYP4A **327 mg/kg bw/d** *3-days* Total P450: ↑ 1.6-fold CYP2B (PROD):↑ 346-fold CYP1A (EROD): ↑ 2.1-fold *7-days* Total P450: ↑ 1.5-fold CYP2B (PROD):↑ 162-fold CYP1A (EROD): ↑ 2.5-fold

Type of study/data, aim of study	Test substance, test system	Conc/dose, replicates, duration of exposure	Aim, results/remarks
			14-days Total P450: ↑ 1.6-fold CYP2B (PROD):↑ 340-fold CYP1A (EROD): ↑ 2.7-fold **58 mg/kg bw/d** *3-days* Total P450: ↑ 1.8-fold CYP2B (PROD):↑ 303-fold CYP1A (EROD): ↑ 3.3-fold *7-days* Total P450: ↑ 1.5-fold CYP2B (PROD):↑ 110-fold CYP1A (EROD): ↑ 2.5-fold *14-days* Total P450: ↑ 1.5-fold CYP2B (PROD):↑ 209-fold CYP1A (EROD): ↑ 2.4-fold
			Proliferation
			↑ 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 There was no evidence of cell proliferation at 500 ppm at any time-point

* 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 PPARα 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 6β-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.

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-pentoxyresorufin O-depentylase (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 *in vitro* investigations of genetic toxicity (bacterial mutation - Callander 2006 and Sokolowski 2008; in vitro cytogenetics- Fox 2006a and Bohnenberger 2008; Mammalian cell gene mutation Clay 2006 and Wollny 2008) and two *in vivo* tests to investigate genetic toxicity (rodent micronucleus - Fox 2006b and rodent liver UDS - Fox 2006c).
PPARα 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 *in vitro* (Toole 2011) or uterotrophic *in vivo* 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.

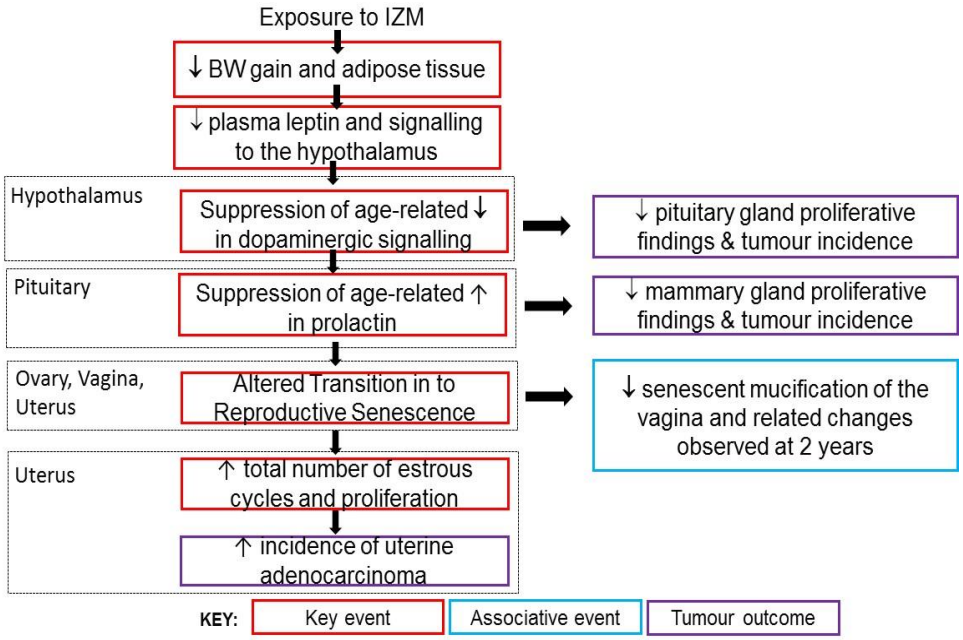
10.10.3.2 Uterine endometrial adenocarcinoma

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

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 *Control* Age related ↑ ↑ in prolactin and leptin *3000ppm (isopyrazam)* ↓ 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 *500ppm (isopyrazam)* ↓ 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

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 *in situ* hybridisation Anonymous (2015b)	Formalin fixed paraffin embedded (FFPE) tissues from the 2-year rat carcinogenicity study	0, 500 & 3000 ppm RNA Scope *in situ* hybridisation (ISH) To quantify mRNA of tyrosine hydroxylase (TH) – enzyme in dopamine synthesis- measured in tuberoinfundibular dopaminergic (TIDA) neurons of the hypothalamus Immunohistochemistry (IHC) To quantify prolactin (PRL) protein expression in the anterior pituitary (PRL IHC) & to quantify dopaminergic neurons in the hypothalamus (TH IHC) Quantitative image analysis 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 *in situ* 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	RNA Scope *in situ* hybridisation (ISH) To quantify tyrosine hydroxylase (TH) mRNA in the arcuate nucleus (ARC) and median eminence (ME) areas of the TIDA Immunohistochemistry (IHC) 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

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

[a]Data 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 (*in vitro*)			
Stably transfected human oestrogen receptor-α transcriptional activation, *in vitro* Non-GLP compliance (abides to GLP principles) Toole (2011)	Isopyrazam Batch: SMU6AP001 Purity: 96.4% (w/w) Syn:anti ratio: 93:7 hERα-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 (17α-estradiol and 17β-estradiol) gave the expected response **Isopyrazam shows no estrogenic potential *in vitro* under the conditions of this study**
Oestrogenicity (*in vivo*)			
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 (17α-ethynylestradiol) gave the expected result **Isopyrazam shows no estrogenic potential *in vivo* under the conditions of this study**
Dopamine agonist/effects on the dopamine transporter (*in vitro*)			
Isopyrazam – effects on dopamine transport *in vitro* 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 µM (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 µM **Isopyrazam is an inhibitor of dopamine transport *in vitro* at a concentration of 100 µM**

Type of study/data	Test substance, test system	Methods	Aim, results/remarks
Isopyrazam – *in vitro* 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 *in vitro* 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 *in vitro* at a concentration of ≥ 30 µM**
Isopyrazam and CSCD459488 – effects on dopamine transport and dopamine D2 receptor ligand binding *in vitro* 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 *in vitro* 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 *in vitro***
Dopamine agonist/effects on the dopamine transporter (*in vivo*)			
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 *in vivo* (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 *in vivo***

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 (ERα and ERβ) 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 [^{3}H]-dopamine into the synaptosomes by 100.33%. As an inhibition of uptake of ≥ 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 supress 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 17β-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)

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
		F_0 generation *3000ppm* ↓ BW in M (-11%**) & F (-8%**), ↓ FC, ↑ liver weights in M (18%**) & F (29%**), ↓ ovary weight (-13%**), ↓ uterus + cervix weight (-27%**), hepatocellular hypertrophy *500ppm* ↓ BW in F (-2%*), ↓ FC, ↑ liver weights in F (8%*), hepatocellular hypertrophy *100ppm* No adverse effects F_1 generation *3000ppm* ↓ BW in M (-5%**) & F (-6%**), ↓ FC, ↑ liver weights in M (27%**) & F (31%**), ↓ ovary weight (-27%**), ↓ uterus + cervix weight (-39%**), hepatocellular hypertrophy *500ppm* ↓ BW in F (-8%**), ↓ FC, ↑ liver weights in M (10%**) & F (15%**), hepatocellular hypertrophy *100ppm* ↑ liver weights in F (10%*)
		Fertility
		F_0 generation No adverse effects F_1 generation No adverse effects

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results
		Offspring toxicity
		F1 generation *3000 ppm* ↓ 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%*) *500 ppm* ↑ liver weights in M (9%**) & F (6%*), ↑ thymus weights in F (5%*) *100 ppm* ↑ thymus weights in F (4%*) F2 generation *3000 ppm* ↓ total litter size (-12%*), ↓ BW-gain during lactation, ↓ BW at weaning in M (-15%**) & F (-16%**), ↑ liver weights in M (23%**) & F (32%**) *500 ppm* ↑ liver weights in M (9%*) & F (14%**) *100 ppm* No adverse effects

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, premating	8	41	250
F_0 parental females, premating	9	47	277
F_0 parental females, gestation	7	37	217
F_0 parental females, lactation	25	119	700
F_1 parental males, premating	9	48	288
F_1 parental females, premating	10	50	301
F_1 parental females, gestation	8	41	239
F_1 parental females, lactation	24	129	774

After 10-weeks' exposure, F_0 animals were mated to produce the F_1 generation. After weaning, the F_1 parental animals were selected and similarly mated following 10-weeks' post weaning exposure to produce

the F_2 generation (with non-selected pups being killed at weaning); the study was concluded when the F_2 generation pups were approximately 4 weeks old.

Parental toxicity

Table 42: Fertility and reproductive performance in isopyrazam treated F_0 and F_1 generations

Observation	Dietary Concentration of Isopyrazam (ppm)			
	0	**100**	**500**	**3000**
F_0 parents, F_1 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
F_1 parents, F_2 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 F_0 and the F_1 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 F_0 generation were 11% and 8% lower than controls, whilst those of the F_1 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 F_0 and F_1 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 F_0 parents. Liver weights were increased in all treated animals of both parental generations. Relative liver weights for the F_0 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 F_1 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 F_1 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 F_0 and F_1 mothers. The relevance of these findings is discussed below.

Offspring toxicity

Table 43: Key litter data

Observation	Dietary Concentration of Isopyrazam (ppm)			
	0	100	500	3000
F_0 parents, F_1 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**
F_1 parents, F_2 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)

[a]Historical 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 F_1 (-13%*) and F_2 (-12%*) generations; the number of implantation sites in the F_0 and F_1 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 F_1 and F_2 generations respectively.

Similarly to the parental generations, liver weights of the F_1 and F_2 offspring killed at weaning were increased by 23-32% (3000ppm) and 6-14% (500ppm). Thymus weights were increased in the F_1 but not in the F_2 females; the lack of consistency across generations suggests that this is a chance finding. In F_1 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	***Maternal toxicity*** 250 mg/kg bw/d 1 female killed *in extremis* 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) 75 mg/kg bw/d No treatment-related effects 20 mg/kg bw/d No treatment-related effects ***Developmental toxicity*** 250 mg/kg bw/d ↑ 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%**) 75 & 20 mg/kg bw/d 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	***Maternal toxicity*** There were no treatment-related deaths 200 mg/kg bw/d 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** 75 mg/kg bw/d ↓ BW-gain (-13%*), ↓ BW (-5%* on day 22), when corrected for gravid uterine weight ↓BW-gain (-41%**), ↓ FC** 20 mg/kg bw/d

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

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	5(3)* foetuses with 'small eye'(malformation), 10(3)** foetuses with 'slightly small eye' (variation), 17(4) foetuses with intraocular abnormalities consistent with microphthalmia 800 mg/kg bw/d 5(2)** foetuses with 'slightly small eye', 9(3) foetuses with intraocular abnormalities consistent with microphthalmia 600 mg/kg bw/d 2 (litter incidence not provided) foetuses with 'small eye', 9(3)** foetuses with 'slightly small eye', 15(4) foetuses with intraocular abnormalities consistent with microphthalmia
Dose-range finding prenatal developmental toxicity study (gavage) OECD 414 GLP Rabbits, New Zealand White, females, 10/dose Anonymous (2008a)	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	***Maternal toxicity*** 1000 mg/kg bw/d One female killed *in extremis* on day 21 ↓ BW-gain (53%*) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation 700 mg/kg bw/d One female aborted on day 25 ↓ BW-gain (63%**) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation 400 mg/kg bw/d One female killed *in extremis* on day 23, one female aborted on day 25 ↓ BW-gain (41%) & FC, ↓ faeces production, ↑ GGT, ↑ relative liver weight, hepatocellular hypertrophy, centrilobular hepatocellular vacuolation ***Developmental toxicity*** There was no effect on litter size or pre-implantation loss, post implantation loss was ↑ in all treated groups but without statistical significance 1000 mg/kg bw/d ↓ mean male foetal weight (14%*) ↑ incidence of foetuses 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) 700 & 400 mg/kg bw/d No treatment-related adverse effects

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

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

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 foetuses with malformations or variations

Observation	Dose (mg/kg/day)			
	0	**100**	**200**	**400**
Number foetuses 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 foetuses 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 foetuses at 400 mg/kg bw/d 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.

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 foetuses (litters) with malformations, variants and eye malformations or variations

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

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. Informaiton 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 redardation 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/variations 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

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 F_1 and F_2 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 F_0 and F_1 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

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

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)

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

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
28-day oral (dietary) OECD 407 Deviations: No FOB GLP Anonymous (2007c)	Rats, HsdRccHan: WIST, males & females, 5/sex/group (main study) & 6/sex/group (satellite phase)	SYN534969 Batch: SMU6BP001 Purity: 99% **Pure Syn** SYN534968 Batch: SMU6BP001 Purity: 99.5% **Pure anti** 'Isopyrazam' Batch: SMU6CP014 Purity: 98.2% **Syn:anti ratio:50:50**	0, 500, 2000 & 5000 ppm Equivalent to: Pure syn Males: 0, 47, 178.9 & 449.4 mg/kg bw/d Females: 0, 46.8, 181.7 & 458.9 mg/kg bw/d Pure anti Males: 0, 43.8, 170 & 406.5 mg/kg bw/d Females: 0, 44.4, 182.6 & 372.3 mg/kg bw/d 50% syn:50% anti Males: 0, 44.7, 181.1 & 456 mg/kg bw/d Females: 0, 44.6, 197.8 & 371.9 mg kg bw/d	Cat 1 = 30 Cat 2 = 300	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** 449.4/458.9 mg/kg bw/d ↓ 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%) 178.9/181.7 ↑ relative liver weight in M (17%) & F (31%) 47/46.8 mg/kg bw/d ↑ relative liver weight in F (22%) **Pure anti** 406.5/372.3 mg/kg bw/d 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%) 181.7/170 mg/kg bw/d 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%) 47/46.8 mg/kg bw/d ↓ BW in F (-4%; day 3)

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

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)**
90-day oral (dietary) OECD 408 GLP Anonymous (2008)	Rats, Han Wistar (Crl:WI(Han)), males & females, 10/sex/group	'Isopyrazam' Batch: SMU7DP017 Purity: 96.4% **Syn:anti ratio: 93:7** & Batch: SMUDP017 Purity: 90.8% **Syn:anti ratio: 70:30**	0, 100, 250 & 2000 ppm Equivalent to: 93 syn:7 anti Males: 0, 8, 20 & 159 mg/kg bw/d Females: 0, 8, 21 & 163 mg/kg bw/d 70 syn:30 anti Males: 0, 10, 24 & 193 mg kg bw/d Females: 0, 9, 24 & 197 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	There were no deaths or clinical signs of toxicity **93 syn:7 anti** 159/163 mg/kg bw/d ↓ 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) 20/21 mg/kg bw/d No adverse effects 8 mg/kg bw/d No adverse effects **70 syn:30 anti** 193/197 mg/kg bw/d ↓ 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) 24 mg/kg bw/d No adverse effects 10/9 mg/kg bw/d No adverse effects
90-day neurotoxicity (dietary) OECD 424 GLP Anonymous (2009b)	Rats, HanRcc:WIST, males & females, 12/sex/dose	Isopyrazam Batch: SMU6AP001 Purity: 96.4% **Syn:anti ratio: 93:7**	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	Cat 1 = 10 Cat 2 = 100	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 382/468 mg/kg bw/d ↓ 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. 98/114 mg/kg bw/d No treatment-related effects 20/25 mg/kg bw/d No treatment-related effects

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					
90-day oral (dietary) OECD 408 GLP Anonymous (2008a)	Mice, C57BL/10Jf CD-1, males & females, 10/sex/group	Isopyrazam Batch: SMU6AP001 Purity: 96.4% **Syn:anti ratio: 93:7**	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	Cat 1 = 10 Cat 2 = 100	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
Dogs					
90-day oral (capsule) OECD 409 GLP Anonymous (2007)	Dogs, Beagle, males & females, 4/sex/group	Isopyrazam Batch: SMU6AP001 Purity 96.4% **Syn:anti ratio: 93:7**	0, 30, 100 & 300 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	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

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)
90-day oral (capsule) OECD 409 GLP Anonymous (2008a)	Dogs, Beagle, males & females, 4/sex/group	'Isopyrazam' Batch: SMU7DP017 Purity: 96.4% **Syn:anti ratio: 70:30**	0, 10, 30 & 250 mg/kg bw/d	Cat 1 = 10 Cat 2 = 100	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
12-month oral (capsule) OECD 452 GLP Anonymous (2008b)	Dogs, Beagle, males & females, 4/sex/group	Isopyrazam Batch: SMU6AP001 Purity: 96.4% **Syn:anti ratio: 93:7**	0, 25, 100 & 250 mg/kg bw/d	Cat 1 = 2.5 Cat 2 = 25	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

* = statistically significant (p=≤0.05), **= statistically significant (p=≤0.01)
FC = food consumption, BW = body weight

<u>Rats</u>

<u>28-days</u>

<u>89:11 syn anti specification</u>

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-pentoxyresorufin O-dependentylase (PROD), further suggesting that the

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

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
2 Year Dietary Toxicity and Carcinogenicity study in Rats & histological extension 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	Doses: 0, 100, 500 & 3000ppm Equivalent to: Males: 0, 5.5, 28 & 173 mg/kg bw/d Females: 0, 7, 35 & 233 mg/kg bw/d 52/104 weeks' exposure	**Chronic phase (12 months)** There were no treatment-related deaths at any dose 173/233 mg/kg bw/d ↑ incidence of scabs (M & F) & subcutaneous masses (M) ↓ mean BW: -10.3%** (M) & -18.8%** (F) ↓ Hb (-3.4%**), ↑ Platelet count (14%*) in F ↑ GGT in M (12.9%**), ↓ ALP in M (-28.9%**) & F (-41.7%**), ↓ AST in F (-34.8%**) ↑ liver weight in M (17.2%**) & 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 28/35 mg/kg bw/d ↓ mean BW: -3.5%* (F) ↑ 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) 5.5/7 mg/kg bw/d No adverse effects **Carcinogenicity phase (24 months)** 173/233 mg/kg bw/d ↓ mean BW: -13%** (M) & -27%** (F) ↓ Hb (-5.4%**), ↓ haematocrit (4.4%**), ↓ RBC (-5.5%**), ↑ platelet count (10.8%*) in F ↑ GGT in M (49.9%**) & F (42.8%**), ↓ ALP in M (-33.7%**) & F (-30.7%*), ↓ AST in F (-21%*) ↑ liver weight in M (17.5%**) & F (26.4%**) Pale spots on liver & liver masses in F 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**)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations
			Brown pigment in kidney tubules in F, sinus erythrocytosis in lymph nodes in M <u>28/35 mg/kg bw/d</u> ↓ mean BW: -11%** (F) ↑ GGT in M (24.8%**), ↓ ALP in M (-17.1%**), ↓ AST in F (-22.2%*) ↑ increased liver weight in F (12%**) and in M (5%*) 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**) Brown pigment in kidney tubules in F <u>5.5/7 mg/kg bw/d</u> ↓ mean BW: 5.3%* (F)
Chronic toxicity and carcinogenicity in the mouse 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>433/554 mg/kg bw/d</u> Clinical signs: discharge from the eye of M BW: ↓ BW in M (23%) and F (11%) Organ weights: ↑ liver weights in M (41%) & F (38%) Macroscopic findings: eye discharge in 26/46 M Microscopic findings: hepatocellular hypertrophy in 42/50 M & 47/50 F <u>56/75 mg/kg bw/d</u> Organ weights: ↑ liver weights in M (14%) & F (7%) 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 <u>8/10 mg/kg bw/d</u> No adverse effects

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

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)

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)

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 diasteroisomers designated *syn* and *anti*-isomers (referred to in some test reports as SYN534969 and SYN534968 respectively). In turn each diasteroisomer has two enantiomers (refer to section 1).

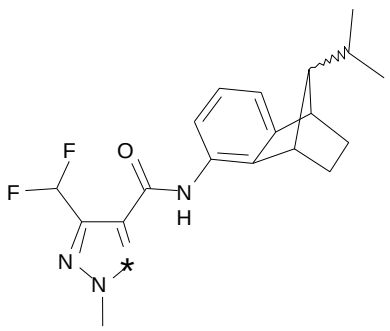
At present there are no data to indicate interconversion of the enantiomers would occur. During review under Directive 91/414/EEC data were presented to support the view that interconversion was unlikely on basis of the structural characteristics of the molecule in relation to the transformations that 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.

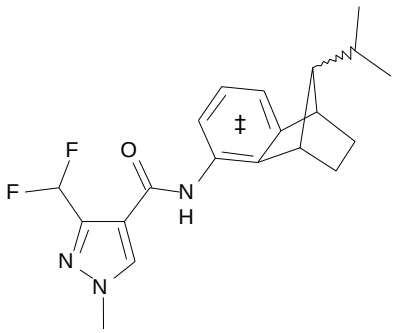
Where available, details of the test item ratio is included.

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

Figure 1: Structure and radiolabelling positions of isopyrazam



* = pyrazole ring labelling



‡ = uniform phenyl ring label

The DAR quotes the measured water solubility of the diasteroisomers as:

- *syn*-isomer (99.5%) 0.00105 g/l at 25 ºC and pH 7 (Weissenfeld, 2008b) equating to 1.05 mg/l
- *anti*-isomer (99.6%) 0.00055 g/l at 25 ºC and pH 7 (Weissenfeld, 2008c) equating to 0.55 mg/l

The Henry's Law Constant for the diasteroisomers have been calculated as follows indicating the substance is unlikely to partition to air for the aquatic environment:

- *syn*-isomer 1.9 x 10^{-4} Pa m_3 mol^{-1} (Stulz 2008)
- *anti*-isomer 3.7 x 10^{-5} Pa m^3 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 ºC) have not been adjusted to 12 ºC 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% *syn*, 27.3% *anti*	No degradation, day 28	Valid	Seyfried, 2006
Aquatic hydrolysis OECD Test Guideline 111, GLP, isopyrazam 98.8%, approximately *syn*:*anti* isomer ratios 90:10	Stable (study temperature 25- 50 ºC)	Valid	Berdat and Nicollier, 2007
Aquatic photolysis OECD Test Guideline draft, GLP, isopyrazam >99%	DT_{50}: 61 – 64 days summer sunlight at 30 – 50ºN (study temperature 25 ºC)	Valid	Kuet and Oliver, 2007b
Water/sediment simulation OECD 308, GLP, isopyrazam: 96% purity, 91.3% syn, 8.7% anti	$DT_{50\ total\ system}$ >>1 year at study temperature of 20 ºC <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 BOD_5/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 ^{o}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 ^{o}C isopyrazam was stable at all tested pHs. A confirmatory test at 25 ^{o}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 ^{o}C water column temperature and natural sunlight). The study calculated a dissipation DT_{50} of 3 days following Single First Order (SFO) kinetics and a whole system DT_{50} 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, [^{14}C-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 ^{14}C-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 rand for 360 days.

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

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 CO_2 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.

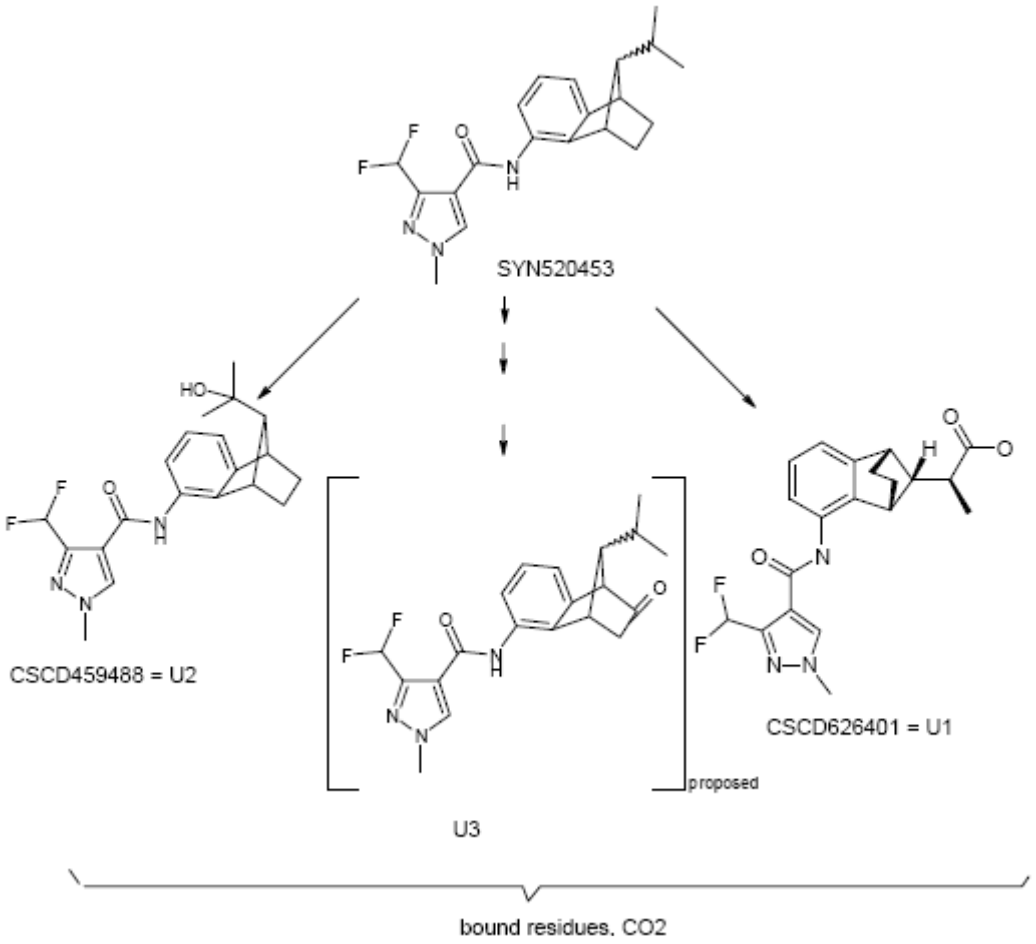
Analysis under Directive 91/4141/EEC considered that water phase dissipation rates did not appear to conform to SFO kinetics. Consequently, dissipation DT_{50} values of 1.2 to 1.5 days were calculated for dissipation from the water phase in aerobic systems using First-Order Multi Compartment kinetics.

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 ≥97%) at a concentration of approx. 0.5 µg/ml. Test solutions were prepared with either sterile buffer (pH 7) or sterile natural water (pH 7) and continuously irradiated at 25 ^{o}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^{o}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 – 50ºN 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 DT_{50} of 4.2 – 4.9 days, equivalent to 5.2 – 5.9 days summer sunlight at 30 – 50ºN 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 DT_{50} values at a study temperature of 20 ºC 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 DT_{50} of 21.2 days under natural outdoor conditions (quoted temperature range 13.5 to 53.5 ºC 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 *n*-octanol/water OECT TG107, (shake flask method) isopyrazam *syn* isomer (99.5%) isopyrazam *anti* isomer (99.6%)	*syn*: Log K_{ow} 4.1 at 25 °C, pH 7.3 *anti*: Log K_{ow} 4.4 at 25 °C, pH 7.8		Weissenfeld, 2008g Weissenfeld, 2008h
Bioaccumulation in fish *Lepomis macrochirus* OECD 305, GLP isopyrazam: 98.5% purity 69.2% *syn*, 30.1% *anti*	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 LC_{50} and a factor or 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 ^{14}C-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 ^{14}C-residues.

During the 14 day depuration phase, the levels of [^{14}C] isopyrazam equivalents in the whole fish decreased rapidly. The calculated DT_{90} 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:

BCFk 406 l/kg
BCFSS whole fish 441 l/kg (based on 14C-residues)
BCFSS lipid normalised to 5% whole fish 374 l/kg (based on ^{14}C-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% *syn*, 27.3% *anti*)	*Oncorhynchus mykiss* (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% *syn*, 10.1% *anti*)	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Flow-through	96 hours	LC_{50}	0.063 (mm)	Anonymous 2005a

Acute toxicity to fish, OECD 203, GLP, purity: isopyrazam 92.9% (65.6% *syn*, 27.3% *anti*)	*Lepomis macrochirus* (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% *syn*, 27.3% *anti*)	*Cyprinus carpio* (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% *syn*, 27.3% *anti*)	*Pimephales promelas* (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% *syn*, 27.3% *anti*)	*Danio rerio* (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% *syn*, 10.1% *anti*)	*Pimephales promelas* (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% *syn*, 27.3% *anti*)	*Cyprinodon variegatus* (Sheepshead minnow)	Mortality	Flow-through	96 hours	LC_{50}	0.314 (mm)	Anonymous 2007
Acute toxicity to fish, OECD 203, GLP, purity: SYN534969 *syn* isomer 98.9%	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Flow-through	96 hours	LC_{50}	0.0469 (mm)	Anonymous 2007a
Acute toxicity to fish, OECD 203, GLP, purity: SYN534968 *anti* isomer 98.6%	*Oncorhynchus mykiss* (Rainbow trout)	Mortality	Flow-through	96 hours	LC_{50}	0.0092 (mm)	Anonymous 2007b

Acute toxicity to fish, OECD 203, GLP, purity: SYN534969 *syn* isomer 99%	*Pimephales promelas* (Fathead minnow)	Mortality	Flow-through	96 hours	LC_{50}	0.0817 (mm)	Anonymous , 2007c
Acute toxicity to fish, OECD 203, GLP, purity: SYN534968 *anti* isomer 98.6%	*Pimephales promelas* (Fathead minnow)	Mortality	Flow-through	96 hours	LC_{50}	0.0107 (mm)	Anonymous , 2007d
Daphnia sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 92.9% (65.6% *syn*, 27.3% *anti*)	*Daphnia magna*	Acute immobilis-ation	Static	48 hours	EC_{50}	0.044 (n) see study details below	Benyon and Richardson, 2007
Daphnia sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 99.7% (86.9% *syn*, 10.1% *anti*)	*Daphnia magna*	Acute immobilis-ation	Static	48 hours	EC_{50}	0.13 (mm)	Benyon and Ramsay, 2007
Daphnia sp Acute Immobilisation OECD 202, GLP, purity: isopyrazam 99.8% (94.9% *syn*, 4.93% *anti*) Guideline adapted for the other species mentioned	*Coenagrionidae* *Crangonyx pseudogracilis* *Asellus aquaticus* *Chaoborus* sp. *Planariidae* *Cloeon sp.* *Ostracoda* *Lymnaea* sp. *Lumbriculus variegatus* *Brachionus calyciflorus*	Acute immobilis-ation	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% *syn*, 10.1% *anti*)	*Pseudo-kirchneriella subcapitata**	Cell multipli-cation inhibition	Static	72 hours 96 hours	ErC_{50} NOErC ErC_{50} NOErC	>4 (mm) 0.31 (mm) >4 (mm) 0.31 (mm)	Volz, 2005
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: isopyrazam (89.6% *syn*, 10.1% *anti*)	*Lemna gibba*	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 LC_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} 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

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 LC_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} 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.

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 LC_{50} which is based on mean measured concentrations.

The 96-h LC_{50} 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 LC_{50} which is based on mean measured concentrations.

The 96-h LC_{50} 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 LC_{50} 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 EC_{50} was 0.044 mg a.s./l (95% confidence intervals not reported) based on nominal concentrations.

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 EC_{50} 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 EC_{50} 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 EC_{50} of >1 mg/l based on nominal concentrations as analytical verification was not undertaken.

For each species the EC_{50} 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
Crangonyx pseudogracilis	>0.74	Mean measured
Asellus aquaticus	>0.775	Mean measured
Chaoborus sp.	>0.730[a]	Mean measured
Planariidae	>0.750	Mean measured
Cloeon sp.	>1	Nominal
Ostracoda	>1	Nominal
Lymnaea sp.	>0.9	Mean measured
Lumbriculus variegatus	>0.955	Mean measured
Brachionus calyciflorus	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 EC_{50} 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 and the end of exposure (96 hours).

The study reported 72 and 96 hour endpoints based on mean measured concentrations:

- 72-h E_rC_{50} >4 mg/l
- 96-h E_rC_{50} >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 $E_rC_{50\ (frond\ number)}$ >0.5 mg/l
- 7-d $E_rC_{50\ (dry\ weight)}$ >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 LC_{50}s for isopyrazam was 0.063 and 0.066 mg/l. The 96-hour LC_{50}s 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 LC_{50} 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 LC_{50} 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% *syn*, 27.3% *anti*)	*Pimephales promelas* (Fathead minnow)	32-day NOEC **0.00287 mg/l** (mm) [fry survival]	Valid	Anonymous, 2007f
Daphnia magna Reproduction OECD 202, GLP, purity: isopyrazam 92.9% (65.6% *syn*, 27.3% *anti*)	*Daphnia magna*	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% *syn*, 10.1% *anti*)	*Pseudo-kirchneriella subcapitata**	72-hour NOErC 0.31 (mm) 96-hour NOErC 0.31 (mm)	Valid	Volz, 2005
Lemna sp. Growth Inhibition Test, OECD Guideline 221, GLP, purity: isopyrazam (89.6% *syn*, 10.1% *anti*)	*Lemna gibba*	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**	-	-

[1]The 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).
[2]The 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.
[3]The 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 – Bätscher, 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 (Memmert (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 LC_{50} 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 LC_{50}s 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 LC_{50}s for isopyrazam of 0.0263 and 0.034 mg/l.

In addition, a slightly lower 96-hour LC_{50} for *Cyprinus carpio* of 0.0258 mg/l is within this classification range.

Further 96 hour LC_{50} acute toxicity to fish endpoints for the *syn* and *anti* isomers using *Oncorhynchus mykiss* and *Pimephales promelas:*
- *Oncorhynchus mykiss* 96-hour LC_{50}s 0.0469 and 0.0092 (95% C.I. 0.00759 to 0.0187) mg/l
- *Pimephales promelas* 96-hour LC_{50}s 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 LC_{50}s.

On this basis, it is considered that the LC_{50} 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 LC_{50} 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 DT_{50} values at a study temperature of 20 ^{o}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 DT_{50} of 21.2 days under natural outdoor conditions (quoted temperature range 13.5 to 53.5 ^{o}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 ^{14}C-residues)
$BCF_{SS\ lipid\ normalised\ to\ 5\%\ whole\ fish}$ 374 l/kg (based on ^{14}C-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 LC_{50} 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

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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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