Committee for Risk Assessment

RAC

Annex 1

Background document

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

pymetrozine (ISO); (E)-4,5-dihydro-6-methyl-4-(3-pyridylmethylene amino)-1,2,4-triazin-3(2H)-one

EC Number: -
CAS Number: 123312-89-0

CLH-O-0000001412-86-203/F

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

Adopted

9 March 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Pymetrozine (ISO);

(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one

EC Number: **-**

CAS Number: **123312-89-0**

Index Number: **613-202-00-4**

Submitted by: **BAuA**
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Version number: **2.5**

Date: **May 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYMETROZINE (ISO); (E)-4,5-DIHYDRO-6-METHYL-4-(3-PYRIDYLMETHYLENE AMINO)-1,2,4-TRIAZIN-3(2H)-ONE

CONTENTS

1. IDENTITY OF THE SUBSTANCE 4

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 4
1.2 COMPOSITION OF THE SUBSTANCE 5

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING 6

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 6

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 7

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 8

5. IDENTIFIED USES 8

6. DATA SOURCES 8

7. PHYSICOCHEMICAL PROPERTIES 9

8. EVALUATION OF PHYSICAL HAZARDS 9

8.1 EXPLOSIVES 9
8.1.1 Short summary and overall relevance of the provided information on explosive properties 10
8.1.2 Comparison with the CLP criteria 10
8.1.3 Conclusion on classification and labelling for explosive properties 10
8.2 FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES) 10
8.3 OXIDISING GASES 10
8.4 GASES UNDER PRESSURE 10
8.5 FLAMMABLE LIQUIDS 10
8.6 FLAMMABLE SOLIDS 10
8.6.1 Short summary and overall relevance of the provided information on flammable solids 10
8.6.2 Comparison with the CLP criteria 10
8.6.3 Conclusion classification and labelling for flammable solids 10
8.7 SELF-REACTIVE SUBSTANCES 11
8.8 PYROPHORIC LIQUIDS 11
8.9 PYROPHORIC SOLIDS 11
8.10 SELF-HEATING SUBSTANCES 11
8.11 SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES 11
8.12 OXIDISING LIQUIDS 11
8.13 OXIDISING SOLIDS 11
8.13.1 Short summary and overall relevance of the provided information on oxidising solids 11
8.13.2 Comparison with the CLP criteria 11
8.13.3 Conclusion on classification and labelling for oxidising solids 11
8.14 ORGANIC PEROXIDES 11
8.15 CORROSIVE TO METALS 11

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 12

10. EVALUATION OF HEALTH HAZARDS 13

10.1 ACUTE TOXICITY - ORAL ROUTE 13
10.2 ACUTE TOXICITY - DERMAL ROUTE 14
10.3 ACUTE TOXICITY - INHALATION ROUTE 14
10.4 SKIN CORROSION/IRRITATION 14
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 14
10.6 RESPIRATORY SENSITISATION 14
10.7 SKIN SENSITISATION 14
10.8 GERM CELL MUTAGENICITY 14
10.9 CARCINOGENICITY 14
10.9.1 Short summary and overall relevance of the provided information on carcinogenicity 14
10.9.2 Comparison with the CLP criteria 18
10.9.3 Conclusion on classification and labelling for carcinogenicity 23
10.10 REPRODUCTIVE TOXICITY 38
10.10.1 Adverse effects on sexual function and fertility 38

10.10.2 Adverse effects on development ... *41*
10.10.3 Adverse effects on or via lactation ... *45*
10.10.4 Conclusion on classification and labelling for reproductive toxicity ... *45*
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE ... 65
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE ... 65
10.13 ASPIRATION HAZARD ... 65

11. EVALUATION OF ENVIRONMENTAL HAZARDS ... 66

11.1 ACUTE AQUATIC HAZARD ... 66
11.1.1 Acute (short-term) toxicity to fish ... *66*
11.1.2 Acute (short-term) toxicity to aquatic invertebrates ... *67*
11.1.3 Acute (short-term) toxicity to algae or other aquatic plants ... *67*
11.1.4 Acute (short-term) toxicity to other aquatic organisms ... *68*
11.2 LONG-TERM AQUATIC HAZARD ... 69
11.2.1 Chronic toxicity to fish ... *69*
11.2.2 Chronic toxicity to aquatic invertebrates ... *69*
11.2.3 Chronic toxicity to algae or other aquatic plants ... *70*
11.2.4 Chronic toxicity to other aquatic organisms ... *70*
11.3 BIOACCUMULATION ... 70
11.3.1 Estimated bioaccumulation ... *70*
11.3.2 Measured partition coefficient and bioaccumulation test data ... *71*
11.4 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES ... 71
11.4.1 Ready biodegradability ... *71*
11.4.2 BOD_5/COD ... *71*
11.4.3 Other convincing scientific evidence ... *71*
11.4.4 Field investigations and monitoring data (if relevant for C&L) ... *72*
11.4.5 Inherent and Enhanced Ready Biodegradability tests ... *72*
11.4.6 Soil and sediment degradation data ... *72*
11.4.7 Hydrolysis ... *72*
11.4.8 Photochemical degradation ... *72*
11.5 ENVIRONMENTAL TRANSFORMATION OF METALS OR INORGANIC METAL COMPOUNDS ... 73
11.5.1 Summary of data/information on environmental transformation ... *73*
11.6 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION ... 73
11.7 COMPARISON WITH THE CLP CRITERIA ... 73
11.7.1 Acute aquatic hazard ... *73*
11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation) ... *73*
11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS ... 74

12. EVALUATION OF ADDITIONAL HAZARDS ... 81

12.1 HAZARDOUS TO THE OZONE LAYER ... 81

13. ADDITIONAL LABELLING ... 82

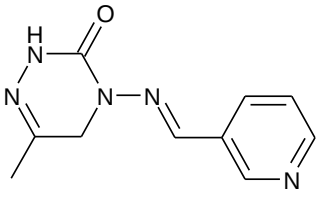
14. REFERENCES ... 83

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYMETROZINE (ISO); (E)-4,5-DIHYDRO-6-METHYL-4-(3-PYRIDYLMETHYLENE AMINO)-1,2,4-TRIAZIN-3(2H)-ONE

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)	(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	pymetrozine
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	123312-89-0
Other identity code (if available)	-
Molecular formula	$C_{10}H_{11}N_5O$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 95.0 %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one	***≥ 95.0 %***	Carc. 2 – H351 Aquatic Chronic 3 – H412	Carc. 2, H351 Aquatic Chronic 3, H412 Acute Tox. 4, H332 Carc. 2, H351 Aquatic Chronic 3, H412 Carc. 2, H351 Aquatic Chronic 3, H412

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**
confidential				

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**
confidential					

Table 5: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**
confidential			

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

	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 state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	***613-202-00-4***	***pymetrozine (ISO) (E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one***	-	***123312-89-0***	Carc. 2 Aq. Chronic 3	H351 H412	GHS08 Wng	H351 H412			
Dossier submitters proposal					**Add** Repr. 2 **Modify** Aq. Chronic 1	**Add** H361fd **Modify** H410	GHS08 Wng **Add** GHS09	**Add** H361fd **Modify** H410		M=1	
Resulting Annex VI entry if agreed by RAC and COM					Carc. 2 Repr. 2 Aq. Chronic 1	H351 H361fd H410	GHS08 GHS09 Wng	H351 H361fd H410		M=1	

Table 7: 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)	*data lacking*	No
Oxidising gases	*data lacking*	No
Gases under pressure	*data lacking*	No
Flammable liquids	*data lacking*	No
Flammable solids	*data conclusive but not sufficient for classification*	Yes
Self-reactive substances	*data lacking*	No
Pyrophoric liquids	*data lacking*	No
Pyrophoric solids	*data lacking*	No
Self-heating substances	*data lacking*	No
Substances which in contact with water emit flammable gases	*data lacking*	No
Oxidising liquids	*data lacking*	No
Oxidising solids	*data conclusive but not sufficient for classification*	Yes
Organic peroxides	*data lacking*	No
Corrosive to metals	*data lacking*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/ irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*harmonised classification (proposed and) available*	Yes
Reproductive toxicity	*harmonised classification proposed*	Yes
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*hazard class not assessed in this dossier*	No
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	*harmonised classification proposed*	Yes
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No data on the discussions during the C&L procedure of the ECB.

During the PPP procedure it was discussed, whether there is a need for classification as a reproductive toxicant (Pesticides peer review meeting 114). The report states:

> *"From the available data, adverse findings in testes were observed mainly in short term studies (effects on hormonal levels and testes at 255 mg/kg bw per day in 28-d rat study; effects in testes at 61 mg/kg bw per day in 28-day dog, at 14 mg/kg bw per day in 90-day dog and at 5 mg/kg bw per day in 1-year dog). In the multigeneration study, there was no effect on reproduction/fertility up to the high dose (110 mg/bw per day), but the testes were not examined histopathologically.*
>
> *The applicant's hypothesis that the testes findings are secondary to systemic toxicity (liver) is not supported by the experts.*
>
> *One expert highlighted that substances have already been classified for effects on reproductive organs without effect on fertility. It was noted that changes were also observed in uterus (dogs and mice).*

> *The majority of experts agreed to propose classification* ***Repr. Cat. 2*** *(H361f Suspected of damaging fertility), on the basis of the overall findings in reproductive organs in rats, dogs and mice."*

During the pesticides peer-review procedure it was discussed, whether there is a need for classification as a developmental toxicant (Pesticides peer review meeting 114). The report states:

> *"Since similar malformations are observed in 2 species, it was mentioned that this could trigger a classification in category 1. Taking into account the high maternal toxicity in one species and the absolute number of findings (limited), the experts agreed to propose* ***Repr. Cat. 2*** *(H361d Suspected of damaging the unborn child)."*

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pymetrozine is an active substance in the meaning of Directive 91/414/EEC (repealed by the Regulation EC 1107/2009).

5. IDENTIFIED USES

Pymetrozine is an insecticide used in agriculture, for ornamental plant and market gardening.

6. DATA SOURCES

Main data source for the evaluation of the toxicological properties of pymetrozine were Volumes 1 and 3 of the revised Renewal Assessment Report (RAR) dated 27 February 2014, which was prepared for the pesticides procedure. It is attached to the CLH dossier in its final version „Final Addendum to the Renewal Assessment Report" (available at EFSAs website). In April 2015, the applicant submitted additional toxicological studies and statements intended to support the preparation of this CLH dossier. These were taken into account and integrated in the dossier. All toxicological studies included in this dossier were evaluated and assessed by the dossier submitter.

7. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or stimated)
Physical state at 20°C and 101,3 kPa	Solid		Visual assessment
Melting/freezing point	217°C	Rodler, 1993	Measured
Boiling point	190°C (decomposition)	Rodler, 1993	Measured
Relative density	1.37	Fueldner, 1995	Measured
Vapour pressure	< 4.2 x 10–6 Pa (25 °C)	Geoffroy, 1993	Measured
Surface tension	69.4 -72.3 mN/m (10 g/l at 20 °C)	Ryser, 1994	Measured
Water solubility	320 mg/L (25 °C; pH 5) 270 mg/L (25 °C; pH 7) 270 mg/L (25 °C; pH 9)	Stulz, 1995	Measured
Partition coefficient n-octanol/water	log Po/w = -0.24 (25 °C; pH 5) log Po/w = -0.19 (25 °C; pH 7) log Po/w = -0.20 (25 °C; pH 9)	Stulz, 1995	Measured
Flash point			Not applicable (melting point > 40 °C)
Flammability	Not highly flammable	Schürch, 1993	estimated
Explosive properties	Not explosive	Schürch, 1993	Measured
Self-ignition temperature	No self-ignition up to 400 °C.		
Oxidising properties	Non-oxidising	Schürch, 1993	Measured
Granulometry	Data lacking		
Stability in organic solvents and identity of relevant degradation products	Data lacking		
Dissociation constant	pKa = 4.06	Jäckel, 1993	Measured
Viscosity	Data lacking		

8. EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A 14	Not explosive		Schürch, 1993

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

There are no effects after burning, shock or friction.

8.1.2 Comparison with the CLP criteria

Data conclusive but not sufficient for classification.

8.1.3 Conclusion on classification and labelling for explosive properties

Pymetrozine has no explosive properties.

8.2 Flammable gases (including chemically unstable gases)

8.3 Oxidising gases

8.4 Gases under pressure

8.5 Flammable liquids

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A 10	Not highly flammable		Schürch, 1993

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

Ignition with a hot platinum wire results in melting of the substance. The molten substance does not sustain a flame.

8.6.2 Comparison with the CLP criteria

Data conclusive but not sufficient for classification.

8.6.3 Conclusion classification and labelling for flammable solids

Pymetrozine is not highly flammable.

8.7 Self-reactive substances

8.8 Pyrophoric liquids

8.9 Pyrophoric solids

8.10 Self-heating substances

8.11 Substances which in contact with water emit flammable gases

8.12 Oxidising liquids

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A 17	Non-oxidising		Schürch, 1993

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

The burning rate of the reference mixture and the test mixture result in non-oxidising properties for Pymetrozine.

8.13.2 Comparison with the CLP criteria

Data conclusive but not sufficient for classification.

8.13.3 Conclusion on classification and labelling for oxidising solids

Pymetrozine has no oxidising properties.

8.14 Organic peroxides

8.15 Corrosive to metals

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification of pymetrozine for physical hazards based on the negative results obtained in three CEC tests (A14, A10 and A17).

Comments received during public consultation

No comments addressing these endpoints were submitted during public consultation.

Assessment and comparison with the classification criteria

RAC notes that pymetrozine:

- Was not explosive in an A14 test (produced no effects after burning, shock or friction);
- Was not highly flammable in an A10 test (test ignition with a hot platinum wire results in melting of the substance and the molten substance does not sustain a flame);
- Was not oxidising in an A17 test.

Therefore, RAC supports the proposal of the DS **not to classify pymetrozine for physical hazards.**

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

This section contains a short summary taken from Volume 1 (chapter 2.6) of the Renewal Assessment Report (RAR), which was written for the pesticides procedure. In case more detailed information on the reported findings is needed, it is referred to the confidential annex to this document or to Volume 3 / chapter B.6 of the RAR.

Following oral administration to rats, pymetrozine was rapidly and almost completely absorbed from the gastrointestinal tract into the general circulation. Independent of the label, maximum concentrations in the blood were reached between 15 minutes and 1 hour after administration at the low dose level (0.5 mg/kg bw) and between 4 and 8 hours after administration at the high dose level (100 mg/kg bw).

The extent of absorption from the intestinal tract (based on renal and biliary excretion and on the amount remaining in the carcass) was about 90 % at the low dose and about 82 % at the high dose, independent of the label.

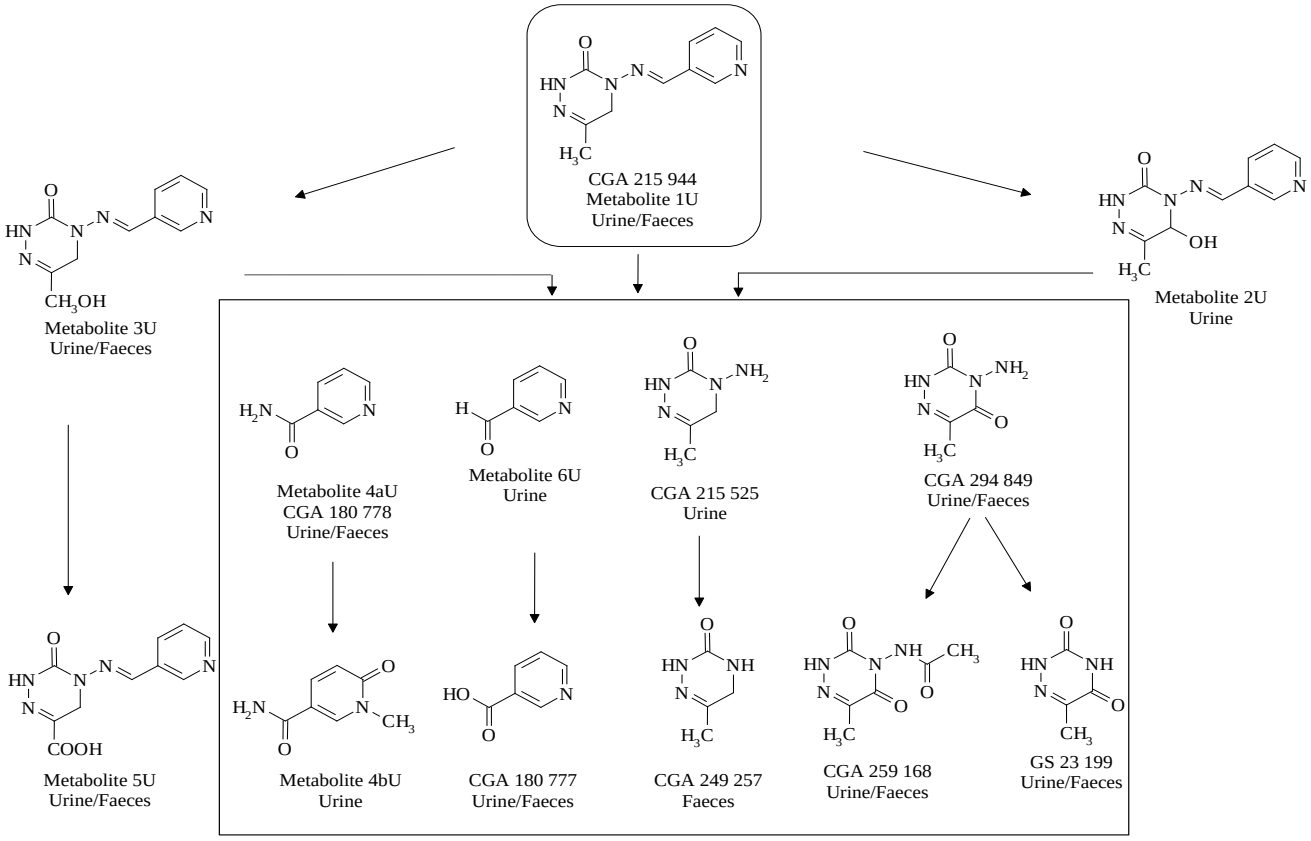
At the high dose, only the fat residues were proportionally higher. The data indicate a saturation of distribution and/or binding processes except on fat where the distribution of radioactivity was unhindered.

Pymetrozine was extensively metabolised and the metabolic pathways were independent of sex, pre-treatment and dose level. The derived metabolic pathways were oxidation reactions (about 19 % of the dose) at the methyl substitute leading to the corresponding carboxylic acid, oxidation reactions (about 7 % of the dose) at the triazine-methylene group leading to the corresponding alcohol, and cleavage reactions between the triazine and the pyridine ring systems (about 20 % of the dose).

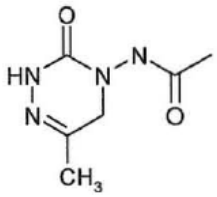
It is concluded that regardless of the dose level and the label position pymetrozine was well absorbed into the systemic circulation, from where it was eliminated in both urine and bile. Elimination from blood and tissues was biphasic. The proposed metabolic pathways in rats are depicted in Figure 1.

The major metabolic pathways proposed for rats are also valid for mice. The metabolic pathways are not influenced by pre-treatment with pymetrozine up to concentrations of 5000 ppm (mice) and 3000 ppm (rat).

Figure 1: Proposed metabolic pathways of pymetrozine in rats



According to a statement by industry (Hadfield, 2011 ASB2012-4624), the chemical structure of metabolite CGA259168 was incorrectly reproduced in the study reports and it would be a metabolite of CGA215525 (instead of metabolite CGA294849). According to the statement, the structure should have been given as:



10. EVALUATION OF HEALTH HAZARDS

This section contains short summaries taken from Vol. 1 (chapter 2.6) of the RAR, which was written for the pesticides procedure. In case more detailed information on the reported effects is needed, it is referred to the confidential annex to this document or Volume 3 / chapter B.6 of the RAR. The additional data/information submitted by industry during the preparation phase of this CLH dossier is also included and taken into account. All studies included in this dossier were evaluated and assessed by the dossier submitter.

10.1 Acute toxicity - oral route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.2 Acute toxicity - dermal route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.3 Acute toxicity - inhalation route

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.4 Skin corrosion/irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.5 Serious eye damage/eye irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.6 Respiratory sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.7 Skin sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.8 Germ cell mutagenicity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.9 Carcinogenicity

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

Two long term toxicity and/or carcinogenicity studies were performed in mice and rats. The results are summarised in Table 12. Further details including method, guideline (and deviations if any), doses, substance purity (if known), species, strain, sex, no of animals/group, study duration, exposure route and a description of the results (including information on incidences and severities of findings and extent of changes relative to controls, etc.) are given in the text below or in the RAR.

Table 12: Summary of chronic toxicity/oncogenicity studies

Study	Dose levels	NO(A)EL	Target organs/main effects	Reference
18-months, mouse OECD TG 451 (1982)	0, 10, 100, 2000, 5000 ppm	100 ppm 11.4 mg/kg bw	Liver, spleen and lung toxicity, body weight reduced liver tumours in males at 2000 ppm and in both sexes at 5000 ppm lung tumours in females at 2000 ppm and in both sexes at 5000 ppm	(Author 3, 1995 TOX9652154)
24-months, rat OECD TG 453 (1982)	0, 10, 100, 1000, 3000 ppm	100 ppm 3.7 mg/kg bw	Liver toxicity, body weight reduced Benign hepatoma (f), malignant adrenal medullary tumour (m) at 3000 ppm	(Author 3, 1995 TOX9652155)

Under the conditions of the study, rats treated with 10 or 100 ppm (equal to 0.357/0.430 mg/kg bw/d or 3.73/4.45 mg/kg bw/d in males/females, respectively) showed no toxic findings throughout the study period. Animals treated with 1000 ppm (equal to 39.3/47.1 mg/kg bw/d) had lower body weights and feed intake. Relative liver, kidney and spleen weights of males were increased at interim sacrifice (but not at terminal sacrifice). Microscopic analysis revealed hepatocellular hypertrophy and thyroid follicular epithelium hyperplasia. At the next higher dose level of 3000 ppm (equal to 128/154 mg/kg bw/d) the following additional findings were observed: lower red blood cell count in week 13, changes in several clinical chemistry parameters (plasma glucose, chloride, albumin, bilirubin, cholesterol, inorganic phosphorous), increased relative liver, kidney and spleen weights in both sexes at interim and terminal sacrifice. Several macroscopic and histopathological findings were observed in liver (cysts, masses, mottled appearance, hypertrophy, foci of change, benign hepatoma), thyroid (follicular epithelium hyperplasia), uterus (dilatation) and adrenals (medullary tumours).

Table 13: Selected incidences of neoplastic microscopic lesions in rats (including data from the animals of the interim sacrifice)

Sex	**Males**					**Females**				
Feeding level, ppm	0	10	100	1000	3000	0	10	100	1000	3000
Liver / Total examined	60	60	60	60	60	60	60	60	59	60
Benign hepatoma +)	2 (3 %)	0	2 (3 %)	0	2 (3 %)	0	0	0	2(3 %)	7 (12 %)***
Adrenal medulla / Total examined	60	60	60	60	60	60	60	60	60	60
Benign medullary tumor	2	0	3	2	1	0	1	0	0	1
Malignant medullary tumor ++)	0	0	1	0	3 (5 %)	0	0	0	0	0
Cereb. meninges / Total examined	60	60	60	60	60	60	60	60	60	60
Benign gran. cell tumor +++)	0	0	0	1	2* (3 %)	1	0	1	0	1

Peto-Test: * = p<0.05; *** = p<0.0001

+) Historical control incidence in females: 0 - 3 % in the conducting laboratory between 1989 & 1993, up to 8 % according to Registry of Industrial Toxicology Animal-data, Hannover, Germany

++) Historical control incidence in males: 0 - 3 % for malignant medullary tumor and 4 - 12 % for benign & malignant medullary tumor in the conducting laboratory between 1989 & 1993.

+++) Historical control incidence in males: 0 - 5 % in the conducting laboratory between 1989 & 1993.

Under the conditions of the 18-month study in mice, no adverse effects were reported in groups treated with dietary dose levels of 10 or 100 ppm (equal to 1.24/1.17 or 12.0/11.4 mg/kg bw, respectively, males/females). In animals treated with 2000 ppm (equal to 254/243 mg/kg bw/d), body weight gain was reduced and organ weights of liver, kidney and adrenals were changed. Macroscopic and microscopic findings were observed in liver (masses in males, enlarged organ, hypertrophy and tumours), spleen (enlarged, extramedullary haematopoiesis, haemosiderosis), lung (nodules, tumours above historical control range) and bone marrow (hypercellularity). At the next higher dose level of 5000 ppm (equal to 678/673 mg/kg bw/d), survival of males was increased and feed intake was reduced. Haematology parameters related to red blood cells were reduced. Following additional macroscopic and microscopic findings were observed in liver (nodules, mottled appearance, necrosis) and stomach (inflammation, hyperplasia).

Table 14: Selected incidences of neoplastic microscopic lesions in mice

Sex	**Males**					**Females**				
Feeding level, ppm	0	10	100	2000	5000	0	10	100	2000	5000
Liver / Total examined	50	50	50	49	50	49	50	50	50	50
Benign hepatoma	10	3	12	9	11 (22 %)	4	5	4	1	14* (28 %)
Carcinoma	5	5	5	9** (18 %)	23** (46 %)	0	0	0	0	4** (8 %)
Hepatoma + Carcinoma	15	8	17	18	34** (68 %)	4	5	4	1	18** (36 %)
Lung / Total examined	50	49	49	50	50	49	50	50	50	50
Adenoma +	14	8	11	14	13	6	3	3	9 (18 %)	8 (16 %)
Carcinoma ++	1	1	3	1	0	1	1	5 (10 %)	7 (14 %)	2
Adenoma + Carcinoma +++	15	9	14	15	13	7	4	8	16* (32 %)	10* (20 %)

Peto-Test: * = $p<0.05$; ** = $p<0.001$

+) Historical control incidence in females: 3 - 13 % in the conducting laboratory between 1988 & 1991

++) Historical control incidence in females: 2 - 7 % in the conducting laboratory between 1988 & 1991

+++) Historical control incidence in females: 5 - 18 % in the conducting laboratory between 1988 & 1991

No human data on carcinogenicity are available.

Special mechanistic studies elucidating the formation of liver tumours were conducted in mice and rats.

Subchronic studies on selected biochemical and morphological liver parameters in male mice (Author 8 & Author 5, 1995) showed that pymetrozine was a moderate and largely reversible inducer of foreign compound metabolising liver enzymes and that proliferation of smooth endoplasmatic reticulum (SER) in the liver was stimulated. Based on the specific induction of cytochrome P450 isoenzyme of the gene family CYP3A in the mouse, pymetrozine was addressed as a pregnenolone-16α-carbonitrile-type inducer. The feeding level of 500 ppm was a NOEL in this study.

Liver cell proliferation was studied in male mice administered pymetrozine for up to 42 days (Author 5, 1995). The results demonstrated that, at 2000 and 5000 ppm, the test article induced a sustained but reversible stimulation of hepatocyte cell proliferation and that the observed hepatomegaly in the mouse liver at these high dose levels was the result of hypertrophy and hyperplasia. The 500 ppm feeding level represented a NOEL for this effect.

It was concluded that the reversible biochemical and morphological changes in these studies correlated with mice liver tumours observed in the chronic mouse study at the same dose levels.

A subchronic feeding study in female rats (Author 1, 1996) on selected biochemical and morphological liver parameters and on replicative DNA synthesis in hepatocytes showed that pymetrozine is a weak and reversible inducer of xenobiotic metabolising enzymes, most prominent on glucuronosyl transferase at 1000 and 3000 ppm. Proliferation of smooth endoplasmatic reticulum membranes was observed only at the top dose of 3000 ppm.

The analysis of thyroid hormones indicated a slight stimulation of the thyroid gland by pymetrozine.

The feeding level of 100 ppm was a NOEL in this study. Pymetrozine had no measurable effect on hepatocyte proliferation under the conditions of this study, possibly due to the relatively short duration of administration.

It was concluded that the reversible biochemical and morphological changes in this study correlated with the slight increase in benign liver tumours observed in females in the chronic rat study at the same dose level.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYMETROZINE (ISO); (E)-4,5-DIHYDRO-6-METHYL-4-(3-PYRIDYLMETHYLENE AMINO)-1,2,4-TRIAZIN-3(2H)-ONE

Pymetrozine did not exhibit a tumour promoting potential in liver up to the highest dose level tested of 1000 ppm, but possessed weak promoting activity for the thyroid carcinogenesis at 100 and 1000 ppm under the experimental conditions of a liver and thyroid medium-term bioassay system in rats (Author 7, 1996).

10.9.2 Comparison with the CLP criteria

Table 15 presents the CLP criteria for classification as a carcinogen.

Table 15: Criteria for classification

CLP regulation

A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:
— human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
— animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).
In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
[…]
3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:
(a) Carcinogenicity in humans
The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:
— sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
— limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.
(b) Carcinogenicity in experimental animals
Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.
The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:
— sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
— limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.
3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that

CLP regulation
influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

There are no relevant data from epidemiological studies submitted by Syngeta, hence no classification with Cat. 1A according to CLP regulation is proposed.

Long-term dietary toxicity studies were conducted in rats and mice.

In mice treated for 18 month with pymetrozine, males (treated with 2000 or 5000 ppm) and females (treated with 5000 ppm) showed higher incidences in liver carcinoma and at the higher doses level the incidence of combined liver benign hepatoma and carcinoma was increased in males and females. In females (treated with 2000 or 5000 ppm) higher incidences in lung adenoma and the combined incidence of lung „adenoma + carcinoma“ were observed. Lung carcinoma in females were also increased at 100 and 2000 ppm, however the dose-response was less clear. The tumours in lung and liver were corroborated by masses or nodules observed in these organs.

Pymetrozine induced metabolising enzymes (CYP3A) and proliferation of smooth endoplasmatic reticulum in livers of treated mice. Reversible stimulation of hepatocyte cell proliferation and hepatomegaly in the mouse liver were observed.

In livers of animals treated for 90 days with different dietary doses of pymetrozine, several histological liver changes (organ weight increase, hypertrophy, necrosis, aggregates of lymphocytes) were noted in males and females. No treatment-related histological findings were reported in lungs in this subchronic study.

In rats treated for 2 years with pymetrozine, increased incidences of tumours were observed in top dose (3000 ppm). In males, a significant higher incidence of malignant adrenal medullary tumours was observed (outside the historical control range of the laboratory), although the combined incidence of benign and malignant adrenal medullary tumours was not significantly increased. In females, benign liver hepatoma were significantly increased (outside the historical control range of the laboratory). The incidence of benign granular cell tumours in cerebral meninges of males was also increased but within the historical control range of the laboratory. Findings in liver were corroborated by foci of cellular change in both sexes.

Pymetrozine induced metabolising enzymes (most prominent on glucuronosyl transferase) and proliferation of smooth endoplasmatic reticulum in livers of treated rats. No effect on hepatocyte proliferation was observed. Pymetrozine did not exhibit a tumour promoting potential for the liver up to the highest dose tested of 1000 ppm, but possessed weak promoting activity for the thyroid carcinogenesis at 100 and 1000 ppm under the

experimental conditions using a liver and thyroid medium-term bioassay system in rats. (However, thyroid tumours were not observed in the rat carcinogenicity study.)

According to literature[1] on adrenals findings, „most studies have reported a higher incidence [of pheochromocytomas] in males than in females. […] Both spontaneous and xenobiotic induced pheochromocytomas are less common in the mouse". Further on[2], „pheochromocytoma is a commonly observed tumor in aged rats, particularly in males. Tumors rarely occur before one year of age and the incidence increases thereafter." Hence, it is not unusual, that medullary tumours were observed in males only. Neither in subchronic nor in chronic studies in rats, indications were reported for adverse effects on adrenals. In contrast to this, liver was the target organ in subacute, subchronic and chronic studies in rats.

Taken together, oral administration was used in the studies which is a relevant exposure pathway. The compound was not genotoxic/mutagenic *in vitro* and *in vivo* in the available studies. Neither multi-site response nor reduced tumour latency were reported. For certain combinations of tumour type and dose level either one or both sexes were affected. Only up to a certain extent a consistent pattern of carcinogenicity regarding affected sex or species can be concluded from the available results of the submitted studies. Liver tumours - however of different types – were observed in two species. No ADME data in humans are available; therefore, no comparison is possible with the respective animal data. Body weight in top dose groups in mice and rats were quite low when compared to the respective control groups. No reduction of survival rates was reported.

The available mechanistic data do not explain the occurrence of the observed tumours or the mode of action of their induction. Therefore, it is not possible to analyse the relevance of the observed tumours with the "IPCS framework for analysing the relevance of a cancer mode of action for humans"[3] ("postulate mode of action" would be the first step of the framework). No data are available to show that the tumours observed in experimental animals are not relevant to humans.

Pymetrozine is currently listed in Table 3.1 in CLP regulation with H351.

No "sufficient evidence of carcinogenicity" can be demonstrated when considering the strengths and weaknesses of the available studies and their results. Hence no classification with category 1B according to CLP regulation is proposed.

When balancing the factors for increasing or decreasing the level of concern, and deciding whether there is a "limited evidence of carcinogenicity", it is proposed to keep the classification of pymetrozine for carcinogenic properties (category 2 (H351)).

The CoRMS of the pesticides procedure (Belgium) indicated that hepatic tumours observed in rats and mice and a MoA potentially relevant for humans (stimulation of hepatocyte cell division), might be a reason to trigger higher classification for carcinogenicity.

During the pesticides peer-review procedure it was not discussed, whether there is a need to change the existing harmonised classification.

[1] Rosol et al. (2001): Adrenal gland: structure, function, and mechanism of toxicity, Toxicologic Pathology 29(1):41-48

[2] Paterson et al. (1995): Proliferative lesions of the adrenal glands in rats, in: Guides for toxicologic pathology; available under: http://www.toxpath.org/ssdnc/AdrenalProliferativeRat.pdf [link checked on August 7, 2014]

[3] Boobis et al. (2006): IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans, Critical Reviews in Toxicology, 36:781–792

10.9.3 Conclusion on classification and labelling for carcinogenicity

In summary, classification in Category 2 (H351) is considered appropriate.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to retain the existing classification of pymetrozine as Carc. 2 (H351) based on two carcinogenicity studies (one in rats and one in mice) supported by three mechanistic studies.

The study in mice reported higher incidences than concurrent controls in liver carcinomas and combined benign hepatomas and carcinomas in males and benign hepatomas, carcinomas and combined hepatomas and carcinomas in females. Higher incidences of lung adenoma and carcinoma than in concurrent controls were also reported in females. The carcinogenicity study in mice was supported by mechanistic studies demonstrating that pymetrozine induced metabolising enzymes, proliferation of smooth endoplasmic reticulum, reversible cell proliferation and hepatomegaly.

The carcinogenicity study in rats also reported a significantly higher incidence of malignant adrenal medullary tumours in males and benign liver hepatomas in females (both outside the historical control range).

Comments received during public consultation

Three different commenting Member States (MS) supported the proposal for maintaining the classification of pymetrozine as a carcinogen in category 2

One MS argued that the classification as Carc. 1B might be warranted considering the occurrence of two types of tumours (benign liver hepatoma and/or carcinoma) in two different species (the rat and the mouse), in two sexes (in the mouse). This MS also commented that some metabolites of pymetrozine which are structurally related to triazine show genotoxicity structural alerts and suggested that the absence of genotoxic/mutagenic potential of pymetrozine should not be considered as a strong argument for classification in category 2. A second MS concurred noting that there are no reasons to assume that the effects are not relevant for humans and highlighted the need for additional details on the studies.

Assessment and comparison with the classification criteria

18-month carcinogenicity study in mice

The design, experimental conditions and the main non-neoplastic effects of this study are presented in the Table below.

Table: Summary of the non-neoplastic findings reported in the 18-month carcinogenicity study in mice performed by Gerpach (1995b). The data are from the RAR (2013). There were no adverse effects on mortality, appearance or behaviour at any of the dosage levels.

Method	Males	Females
OECD Guideline 451 Tif:MAGf mice GLP Pymetrozine 98% 0, 10, 100, 2000 and 5000 ppm 0, 1.24, 12.0, 254 and 678 mg pymetrozine/kg bw/day in males 0, 1.17, 11.4, 243 and 673 mg pymetrozine/kg bw/day in females 50 animals/sex/dose for carcinogenicity 10 animals/sex/group for haematological parameters	**5000 ppm** Higher survival than control (45 vs 36, p<0.05) *Body weight (p<0.01)* ↓ 10% bodyweight (week 51) ↓ 11% bodyweight (week 75) *Organ weights (p<0.01)* ↑ 55% (absolute) and 77% (relative) liver weight ↓ 17% (absolute) and 5% (relative) kidney weight ↑ 19% (absolute) and 35% (relative) adrenals weight *Haematology (p<0.01)* Red blood cell count: ↓ 9% (week 53) Haemoglobin: ↓ 5% (week 53) Haematocrit: ↓ 4% (week 53) *Macroscopic lesions* Animals with liver masses: 38/60 vs 16/60 (control) Animals with liver nodules: 7/60 vs 3/60 (control) Animals with mottled liver: 24/60 vs 0/60 (control) Animals with enlarged liver: 14/60 vs 0/60 (control) *Microscopic lesions (p<0.0001)* Animals with liver hypertrophy: 50/50 vs 29/50 (control) Animals with extramedullary haematopoiesis: 44/50 vs 30/50 (control) Animals with hemosiderosis: 35/50 vs 24/50 (control) Animals with hypercellularity: 42/50 vs 29/50 (control) **2000 ppm** *Organ weights (p<0.01)* ↑ 29% (absolute) and 36% (relative) liver weight ↓ 10% (absolute) and 5% (relative) kidney weight	**5000 ppm** *Body weight (p<0.01)* ↓ 18% bodyweight (week 51) ↓ 24% bodyweight (week 75) *Organ weights (p<0.01)* ↑ 26% (absolute) and 53% (relative) liver weight ↓ 7% (absolute) and ↑ 19% (relative) kidney weight ↓ 15% absolute adrenals weight ↓ 32% (absolute) and 12% (relative) spleen weight ↓ 45% absolute thymus weight *Macroscopic lesions* Animals with liver masses: 14/60 vs 3/60 (control) Animals with mottled liver: 18/60 vs 2/60 (control) Animals with liver nodules: 9/60 vs 1/60 (control) Animals with enlarged liver: 28/60 vs 10/60 (control) *Microscopic lesions (p<0.0001)* Animals with liver hypertrophy: 46/50 vs 12/50 (control) Animals with extramedullary haematopoiesis: 46/50 vs 37/50 (control) Animals with hemosiderosis: 43/50 vs 30/50 (control) Animals with hypercellularity: 33/50 vs 22/50 (control) **2000 ppm** *Organ weights (p<0.01)* ↑ 10% relative liver weight

	↑ 21% (absolute) and 28% (relative) adrenals weight *Macroscopic lesions* Animals with liver masses: 5/60 vs 16/60 (control) Animals with mottled liver: 10/60 vs 0/60 (control) Animals with enlarged liver: 14/60 vs 0/60 (control) *Microscopic lesions (p<0.0001)* Animals with liver hypertrophy: 49/50 vs 29/50 (control) Animals with extramedullary haematopoiesis: 38/50 vs 30/50 (control) Animals with hypercellularity: 40/50 vs 29/50 (control) **100 and 10 ppm** No treatment related observations	*Macroscopic lesions* Animals with enlarged liver: 28/60 vs 10/60 (control) *Microscopic lesions (p<0.0001)* Animals with liver hypertrophy: 47/50 vs 12/50 (control) Animals with hemosiderosis: 41/50 vs 30/50 (control) **100 and 10 ppm** No treatment related observations

The treatment had no adverse effect on mortality. However, the number of male mice surviving to termination at 5000 ppm was significantly higher compared to the controls. Animals exposed to 100 and 10 ppm did not show any treatment-related effects. The main non-neoplastic findings found in this study consisted of the following:

- Reductions in interim and terminal bodyweights in males and females treated with 5000 ppm;
- Dose dependent increases in liver weight in males and females dosed at 2000 and 5000 ppm;
- Dose dependent increases in adrenal weight in males treated with 2000 and 5000 ppm;
- Higher incidence of liver masses in males dosed at 2000 and 5000 ppm and in females given 5000 ppm;
- Increased number of liver nodules in animals dosed at 5000 ppm;
- An increased incidence of animals with enlarged livers treated with 2000 and 5000 ppm and mottled livers at 5000 ppm;
- Splenic enlargement in males treated with 2000 and 5000 ppm;
- Significantly increased incidence and severity of liver cell hypertrophy in males and females dosed at 2000 and 5000 ppm.

The neoplastic lesions found in mice are summarised in the table below. The incidence of liver carcinomas and combined liver hepatomas plus carcinomas were statistically significantly higher in males and females dosed with 5000 ppm than in controls. At this dose level the incidence of benign hepatomas in females was also statistically significantly greater than the incidence reported in controls. In addition, the incidence of carcinomas in males treated with 2000 ppm was statistically significantly higher than the incidence in the control group.

Table: Incidences of neoplastic lesions in the 18-month carcinogenicity study in mice performed by Gerpach (1995b). The general design of the study was summarised in the Table above.

	0 ppm	**10 ppm**	**100 ppm**	**2000 ppm**	**5000 ppm**	**HCD***
MALES						
Liver/Total Examined	50	50	50	49	50	
Benign hepatoma	10	3	12	9	11	NP
Carcinoma	5	5	5	**9 (18%) (p<0.001)**	**23 (46%) (p<0.0001)**	NP
Hepatoma + Carcinoma	15	8	17	18	**34 (68%) (p<0.0001)**	NP
Lung/Total examined	50	49	49	50	50	
Adenoma	14	8	11	14	13	NP
Carcinoma	1	1	3	1	0	NP
Adenoma + Carcinoma	15	9	14	15	13	NP
FEMALES						
Liver/Total Examined	49	50	50	50	50	NP
Benign hepatoma	4	5	4	1	**14 (28%) (p<0.05)**	NP
Carcinoma	0	0	0	0	**4 (8%) (p<0.0001)**	NP
Hepatoma + Carcinoma	4	5	4	1	**18 (36%) (p<0.0001)**	NP
Lung/Total examined	49	50	50	50	50	
Adenoma	6	3	3	9 (18%)	8 (16%)	3-13%
Carcinoma	1	1	5 (10%)	7 (14%)	2	2-7%
Adenoma + Carcinoma	7	4	8	**16 (32%) (p<0.05)**	**10 (20%) (p<0.05)**	5-18%

*HCD= Historical control data in the facility in the period 1988-1991 provided in the CLH report; NP = Not provided.

Industry provided additional historical control data (HCD) for a period of 10 years from the test facility that carried out the carcinogenicity studies in mice. The Guidance on the Application of the CLP Criteria (2017) states that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the HCD should be contemporary to the study being evaluated (e.g. within a period of around 5 years). RAC analysed the new data and considered those data generated between 1990 and 1994 as contemporary to the study (in which dosing was performed from May, 1992 until December, 1993) without including the concurrent control values. The new data evaluated by RAC are presented in the Table below.

Table: Historical control data for mouse carcinogenicity in the performing facility provided by the industry after public consultation (1990-1994).

	Mean (%)	Minimum (%)	Maximum (%)
MALES	23.4	10	34
Benign hepatoma	12.5	0	32
Liver carcinoma	26.9	10	36
Hepatoma + liver carcinoma	17.4	6	28
Lung adenoma	17.4	6	28
Lung carcinoma	8.7	2	26
Lung adenoma + carcinoma	27.5	25	33
FEMALES			
Benign hepatoma	2.8	0	6
Liver carcinoma	0.36	0	2
Hepatoma + liver carcinoma	3	0	6
Lung adenoma	6.8	-	-
Lung carcinoma	5.3	-	-
Lung adenoma + carcinoma	11.9	-	-

RAC notes that the tumours in liver and lungs of both males and females mice were highly variable from one study to another. However, as highlighted in the Table above, the incidence of lung adenomas in females exposed to 2000 and 5000 ppm was slightly above the HCD, although no dose-response relationship was observed and the difference with the concurrent control was not statistically significant. A similar situation was found for lung carcinomas for females exposed to 100 and 2000 ppm, but not for animals exposed at the highest dose. This may suggest that these carcinomas were incidental. The incidence of lung adenomas plus carcinomas in females exposed to 2000 and 5000 ppm were statistically significantly higher than corresponding incidences in concurrent controls and slightly or clearly above the HCD at 5000 ppm or 2000 ppm, respectively. However, no clear dose-response relationship was observed for the combined incidences of lung adenomas plus carcinomas.

24-month chronic toxicity/carcinogenicity study in rats

The design, experimental conditions and the main non-neoplastic effects of this study are presented in the Table below.

Table: Summary of the non-neoplastic findings reported in the 24-month chronic toxicity/carcinogenicity study in rats performed by Gerpach (1995a). Data are from the RAR (2013).

Method	Males	Females
OECD Guideline 453 Tif:RAIf rats GLP	**<u>3000 ppm</u>** Higher survival than control (35 vs 20, p<0.05) Decrease of food consumption <u>*Body weight (p<0.01)*</u> ↓ 17% bodyweight (week 50)	**<u>3000 ppm</u>** Decrease of food consumption <u>*Body weight (p<0.01)*</u> ↓ 22% bodyweight (week 50)

	↓ 15% bodyweight (week 104)	↓ 24% bodyweight (week 104)
Pymetrozine 98%	Relative organ weights (p<0.01)	Relative organ weights (p<0.01)
	Liver: ↑ 43% (interim) and ↑ 13% (terminal)	Liver: ↑ 28% (interim) and ↑ 24% (terminal)
0, 10, 100, 1000 and 3000 ppm	Kidney: ↑ 22% (interim)	Kidney: ↑ 20% (interim) and ↑ 9% (terminal)
	Testes: ↑ 16% (interim) and ↑ 10% (terminal)	Ovaries: ↑ 25% (terminal)
0, 0. 4, 3.7, 39.3 and 128 mg pymetrozine/kg bw/day in males	Spleen: ↑ 77% (interim) and ↑ 7% (terminal)	Spleen: ↑ 50% (interim) and ↑ 33% (terinal)
	Haematology (p<0.01)	*Haematology (p<0.01)*
0, 0.4, 4.5, 47.1 and 154 mg pymetrozine/kg bw/day in females	Glucose: ↓ 18% (week 53)	Phosphorus: ↑ 38% (week 53) and ↑13% (week 105)
	Albumin: ↑ 12% bodyweight (week 105)	ALAT: ↓ 55% (week 53)
	Bilirubin: ↑ 63% (week 53) and ↑37% (week 105)	
50 animals/sex/dose for carcinogenic potential	*Macroscopic lesions*	*Macroscopic lesions*
	Animals with mottled liver: 4/80 vs 1/80 (control)	Animals with liver masses: 4/80 vs/80 (control)
	Animals with liver cyst: 7/80 vs 2/80 (control)	Animals with mottled liver: 3/80 vs 0/80 (control)
10 animals/sex/group for haematological, biochemical and urine parameters		Animals with liver cyst: 21/80 vs 2/80 (control)
		Animals with nodule in uterus: 3/80 vs 0/80 (control)
		Animals with nodule in ovary: 6/80 vs 1/80 (control)
10 animals/sex/group for interim sacrifice	*Microscopic lesions (p<0.0001)*	*Microscopic lesions (p<0.0001)*
	Animals with liver hypertrophy: 37/60 vs 0/60 (control)	Animals with biliary cyst: 13/60 vs 2/60 (control)
	Animals with foci of cellular change in liver: 30/60 vs 10/60 (control)	Animals with liver hypertrophy: 40/60 vs 2/60 (control)
	Animals with follicular thyroid epithelium hyperplasia: 10/60 vs 2/60 (control)	Animals with foci of cellular change in liver: 35/60 vs 9/60 (control) (slightly above the historical control incidence)
		Animals with follicular thyroid epithelium hyperplasia: 9/60 vs 1/60 (control)
	1000 ppm	**1000 ppm**
		Decrease of food consumption
	Body weight	*Body weight*
	↓ 6% bodyweight (week 50) (p<0.01)	↓ 4% bodyweight (week 50) (p<0.01)
	↓ 4% bodyweight (week 104)	↓ 7% bodyweight (week 104)
	Microscopic lesions (p<0.0001)	*Microscopic lesions (p<0.0001)*
	Animals with liver hypertrophy: 22/60 vs 0/60 (control)	Animals with foci of cellular change in liver: 19/60 vs 9/60 (control) (within above the historical control incidence)
	Animals with follicular thyroid epithelium hyperplasia: 9/60 vs 2/60 (control)	

	100 ppm No treatment related observations **10 ppm** No treatment related observations	**100 ppm** *Microscopic lesions (p<0.0001)* Animals with foci of cellular change in liver: 14/60 vs 9/60 (control) (within above the historical control incidence) **10 ppm** No treatment related observations

Treatment had no adverse effect on mortality, appearance or behaviour. The number of male mice surviving to termination at 5000 ppm was significantly higher compared to the controls. Animals exposed to 100 ppm and 10 ppm showed no treatment-related effects.

The main non-neoplastic findings found in this study were:

- Dose-dependent body weight reductions in males and females dosed at 1000 and 3000 ppm;
- In males and females treated with 3000 ppm, increases in the weight of the following organs: liver, kidney, testes, ovaries and spleen;
- Minor haematological changes in males and females dosed at 3000 ppm;
- Macroscopic alterations (mottled, cyst, masses) in the liver of males and females and in the ovary and uterus of females at the highest exposure level;
- Microscopic alterations (hypertrophy, foci of cellular change) in liver and thyroid (follicular epithelium hyperplasia) of males and females and in the ovary and uterus of females at the highest exposure level and in males dosed at 1000 ppm.

The neoplastic lesions found in rats are summarised in the Table below.

Table: Incidences of neoplastic lesions in the 24-month chronic toxicity/carcinogenicity study in rats performed by Gerpach (1995a). The general design of the study are summarised in the table above.

	0 ppm	**10 ppm**	**100 ppm**	**1000 ppm**	**3000 ppm**	**HCD***
MALES						
Liver / Total examined	60	60	60	60	60	-
Benign hepatoma	2 (3%)	0	2 (3%)	0	2 (3%)	NP
Adrenal medulla / Total examined	60	60	60	60	60	-
Benign medullary tumours	2	0	3	2	1	-
Malignant medullary tumours	0	0	1	0	**3 (5%)**	0-3%
Meninges/Total examined	60	60	60	60	60	-
Benign cell tumour	0	0	0	1	**2 (3%)**	0-5%
FEMALES						
Liver / Total examined	60	60	60	60	59	-
Benign hepatoma	0	0	0	2 (3%)	**7 (12%) (p<0.001)**	0-3% Up to 8% in HCD

						from other facilities
Adrenal medulla / Total examined	60	60	60	60	60	-
Benign medullary tumours	0	1	0	0	1	NP
Malignant medullary tumours	0	0	0	0	0	NP
Meninges/Total examined	60	60	60	60	60	-
Benign cell tumour	1	0	1	0	1	NP
*HCD= Historical control data in the facility in the period 1989-1993; NP = Not provided						

As noted above in the description of the findings from Gerpach (1995b), RAC analysed the HCD data it considered as contemporary without including the concurrent control values. These HCD are presented in the Table below.

Table: Historical control data for rat carcinogenicity in the performing facility provided by the Industry and not described in the CLH report (1990-1994).

	Mean (%)	Minimum (%)	Maximum (%)
MALES			
Benign hepatoma	1.6	0	4
Benign medullary tumours	5.3	2.5	8.2
Malignant medullary tumours	2	0	4.1
Benign meninges tumours	1.2	0	3.3
FEMALES			
Benign hepatoma	0.9	0	4
Benign medullary tumours	1.3	0	4.1
Malignant medullary tumours	0.15	0	1.7
Benign meninges tumours	1.7	0	4

As shown in the Tables above, there was a higher incidence of malignant adrenal medullary tumours (slightly outside the new HCD from the laboratory) in males only. In females, the incidence of benign liver hepatomas was statistically significantly increased and was also outside the HCD from the laboratory. The incidence of benign granular cell tumours in the cerebral meninges of males was also increased compared to concurrent controls, but the incidences were within the historical control range from the laboratory.

Overall, RAC notes that the incidences of tumours in the adrenal medulla and cerebral meninges of both male and female mice were low. However, no clear dose-response relationship was observed for the incidences of these tumours. No human data on carcinogenicity are available. However, the industry conducted special mechanistic studies in mice and rats which aimed to elucidate the mechanism of formation of liver tumours.

Mechanistic study 1: Biochemical and morphological liver parameters in mice

The study is summarised in the CLH report. This study shows that pymetrozine is a moderate and largely reversible inducer of foreign compound metabolising liver enzymes in the male mouse.

Mechanistic study 2: Effects on liver cell proliferation in mice

The study is summarised in the background document. This study shows that at 2000 and 5000 ppm of pymetrozine, the test article induced a sustained but reversible stimulation of hepatocyte cell proliferation and that the observed hepatomegaly in the mouse liver at these high dose levels was the result of hypertrophy and hyperplasia.

Mechanistic study 3: Effects on liver parameters and thyroid hormones in rats

The study is summarised in the CLH report. In this study, the liver enzyme activity profile identified pymetrozine as a weak to moderate inducer mainly of hepatic xenobiotic phase II metabolising enzymes in the female rat. In this study, pymetrozine did not stimulate hepatocyte cell proliferation, but the proliferation of smooth endoplasmic reticulum membranes was observed at the investigated dose of 3000 ppm. The analysis of thyroid hormones indicates a slight stimulation of the thyroid gland by pymetrozine.

Comparison with the criteria

Tumours of the cerebral meninges

Benign cell tumours in the meninges were reported only in rats and not in mice. In male rats, the differences with the concurrent controls were statistically significant, but not in female rats. However, RAC notes that the incidence of benign cell tumours in male rats was low (3%) and below the historical control range from the facility. Thus, RAC considers the benign cell tumours in meninges were incidental and therefore not relevant for classification purposes.

Lung tumours

Lung tumours were reported only in mice and not in rats. There were no statistically significant differences between the incidences of lung adenomas, carcinomas and combined adenomas plus carcinomas of concurrent controls and male mice exposed to pymetrozine. However, in female mice, the incidence of lung adenomas in animals exposed to 5000 ppm was slightly above the HCD of the test facility, although the incidence was not statistically significantly different in comparison with the concurrent control. There were significant differences between the incidences of lung carcinomas in female controls and females exposed to 100 and 2000 ppm, although not with females exposed to the highest dose of 5000 ppm. The incidences of carcinomas at 100 and 2000 ppm were also higher than the HCD of the facility. Therefore, no dose-response relationship was observed regarding the incidence of lung carcinomas. The incidence of combined carcinomas plus adenomas in female mice exposed to 2000 ppm was higher than the incidence in female mice exposed to 5000 ppm of the substance. For both high dose levels, the incidences were higher than the HCD and statistically significantly different from the incidence reported for control animals in concurrent controls.

RAC notes that the incidence of lung adenomas and of combined adenomas, as well as carcinomas in female mice, were very close to the upper limit of the historical control data of the facility, that this tumour type was reported only for a single species and sex and that no dose-response relationship was observed in any case. RAC considers the relevance of these lung tumours equivocal for classification purposes.

Adrenal tumours

Adrenal medullary tumours were reported only in male rats, but not in female rats or in mice. The incidence of malignant tumours (but not of benign tumours) in male rats exposed to the highest dose of pymetrozine was statistically significantly higher than the incidence in concurrent controls and slightly above that of the HCD of the test facility (5% versus 3%).

RAC notes that the incidence of spontaneous malignant medullary tumours is usually higher in male than in female rats and less common in mice (Rosol *et al*. 2001). In addition, pheochromocytoma is a commonly observed tumour in aged rats (Guidance on the Application of the CLP Criteria, 2017). These two elements are consistent with the results described in both carcinogenicity studies.

In conclusion, RAC considers the relevance of malignant medullary tumours equivocal for classification purposes.

Liver tumours

Liver tumours were observed in rats exposed to pymetrozine at a statistically significantly higher incidence than the concurrent controls. In summary, RAC notes the following findings: i) carcinomas in males exposed to 2000 ppm; ii) benign hepatomas in females exposed to 5000 ppm; iii) combined carcinomas and carcinomas plus benign hepatomas in males and females exposed to 5000 ppm. In rats, the differences with the concurrent controls in the incidence of liver tumours were statistically significant only for benign hepatomas in females; however, RAC notes that in the rat study the doses were lower than in the mice study.

RAC considers the liver tumours relevant for classification purposes for the following reasons:

- Data were generated from two independent well conducted (GLP) carcinogenicity studies;
- Tumours were observed in two species (rats and mice) and both sexes (in mice);
- The high incidence of carcinomas and in particular the combined carcinomas plus adenomas in mice, at incidences above the historical control range;
- The incidence of benign hepatomas in female rats was higher than the HCD of the facility where the study was performed;
- The highest dose tested in the rat study (128-154 mg/kg bw/day) was lower than the lowest dose for which the liver tumours appeared in the mouse study (254 mg/kg bw/day), which might justify the lower severity of the tumours in the rat study;
- A dose-response relationship was observed in all the cases;
- The confirmed progression to malignancy;
- The potential relevance for humans of the route of exposure used in the assessed carcinogenicity studies;
- The absence of confounding effects due to excessive toxicity, since at the highest tested dose, only moderate reductions in bodyweight and no clinical signs were reported;
- The correlation between appearance of liver microscopic lesions, (masses, nodules, enlargement), hypertrophy and liver tumours;
- The probable relevance for humans. A potential non-relevant mechanism based on a CAR-mediated activation could not be fully demonstrated because several key events of this MoA were not investigated (Table below). In fact, there were several indications suggesting that potentially relevant mechanisms based on stimulation of thyroid gland (examined in the rat) and hepatocyte proliferation (examined in the mouse) are plausible.

Table: Analysis of key events for the proposed mode of action of pymetrozine in mice and rats.

Key event	Studies in mice	Studies in rats
CAR activation	No	No
Altered gene expression	Yes	Yes
Hypertrophy	Yes	Yes
CYP 2B induction	No	No
Cell proliferation	Yes	No
Altered liver foci	No	Yes
Adenoma/carcinoma	Yes	Yes

According to the Renewal Assessment Report (RAR, 2013), a wide array of reliable *in vitro* tests yielded negative results (*S. Typhimurium* (4 strains) test; *E. Coli* (1 strain) test; gene mutation test in V79 cells; cytogenetic test in Chinese Hamster Cells and auto-radiographic DNA repair test on rat hepatocytes) . The RAR (2013) also reported three reliable, negative *in vivo* tests (two independent micronucleus test in mice and one unscheduled DNA synthesis in mice).

DEREK-QSAR raises an alert for mutagenicity (due to the presence of N-amino heterocycle ring) and an additional alert for carcinogenicity (due to the presence of hydrazine) for the pymetrozine's metabolites CGA 294879 and CGA 215525. The RAR (2013) reported negative results in bacterial mutagenicity test for CGA 215525 metabolite.

In conclusion, due to the negative results in genotoxicity studies for pymetrozine, the negative result for CGA 215525, the structural similarity between CGA 215525 and CGA 294879, RAC considers it unlikely that the carcinogenicity in rat and mice can be attributed to genotoxicity of pymetrozine or its metabolites.

Classification of pymetrozine in Category 1A is not warranted as no human data was reported.

According to the CLP Regulation, the classification for carcinogenicity within Category 1B (presumed to have carcinogenic potential for humans) requires sufficient evidence; this criterion is fulfilled when a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or when an appropriate combination of benign and malignant neoplasms is observed in (a) two or more species of animals; or, (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

As described above, RAC concludes that adrenal, lung and meninges tumours are likely not related to exposure to pymetrozine. Therefore, this combination of benign and malignant tumours should be excluded from the weight of evidence analysis. The liver tumours observed in two species are however considered highly relevant for classification and RAC considers that these tumours clearly fulfil the criteria for carcinogenicity within Category 2 (suspected human carcinogen). RAC concludes that retaining Carc. 2 is appropriate taking into account that pymetrozine is not mutagenic and that there are data, albeit weak or incomplete, suggesting mechanisms potentially non-relevant for humans based on CAR-mediated mode of action, stimulation of thyroid gland (examined in the rat) and hepatocyte proliferation (examined in the mouse) (see background document, in depth analyses by RAC).

Overall, RAC concurs with the DS to retain the classification of pymetrozine as carcinogenic substance within **Category 2 (H351: Suspected of causing cancer).**

Supplemental information - In depth analyses by RAC

Mechanistic study 1: Biochemical and morphological liver parameters in mice

Groups of 5 or 6 Tif: MAGf (SPF) male mice per group were fed dietary with pymetrozine (98 %) at the following dose levels: 0, 10, 100, 500, 2000 and 5000 ppm (0, 1.6, 15.4, 83.5, 325 and 899 mg/kg bw/day) daily for 14 days. Subgroups receiving 0 and 5000 ppm (954 mg/kg bw/d) were kept on a standard control diet for a recovery period of 28 days. Before necropsy the animals were fasted for 16 hours and then killed under carbon dioxide anaesthesia by exsanguination. Afterwards, several biochemical and morphological parameters were investigated.

At the end of the study, body weight was comparable in all experimental groups. Absolute and relative liver weights were reversibly elevated at 5000 ppm up to a maximum of 124 and 136% of control, respectively. In addition, the relative liver weight of animals treated with 2000 ppm was increased to 124% of the respective control in one subgroup.

The major treatment-related biochemical alterations were summarised in the Table below and comprised increased contents of microsomal cytochrome P450 at 2000 (139 % of control) and 5000 ppm (146 % of control); which were paralleled by up to 2-fold increased microsomal CYP3A protein contents at the same dose levels. Cytosolic glutathione S-transferase activity was also induced to 188% of control at the top dose level (Table below). Further alterations regarded as treatment-related consisted in an induced (128% of control at 5000 ppm) microsomal lauric acid 11-hydroxylase activity (Table below), induced microsomal ethoxyresorufin O-de-ethylase activities to 163 and 200% of control at 2000 and 5000 ppm, respectively; an increased content (163% of control at 5000 ppm) of microsomal CYP1A2 protein; as well as induced microsomal 1-naphthol UDP-glucuronosyltransferase activities to about 130% of control at the two highest dose levels (Table below) . After a 28-day recovery period, the observed alterations did not completely return to the control level (Table below).

Ultramorphological analysis revealed that liver of mice treated with 5000 ppm contained a moderate proliferation of smooth endoplasmic reticulum membranes and deposits of particulate glycogen. Peroxisomes in hepatocytes appeared to be smaller but slightly increased in number. The deposits if particulate glycogen were reversible during the 28-days period of recovery (except in 1 animal).

Table: Selected biochemical liver parameters following dietary administration of pymetrozine to male mice. Data taken from RAR (2013). Values are means ± standard deviations of six animals per group. Asterisks indicate results significantly different (two-sided Dunnett's test) from control: *p<0.05, **p<0.01, ***p<0.001

DOSE	Microsomal P450 [nmol/g liver]	CYP3A [nmol/min/g liver]	Cytosolic GST [mmol/min/g liver]	microsomal UDPNAPH [nmol/min/g liver]	LA-11-OH [nmol/min/g liver]
0 ppm	11.9±1.4	1.45±0.37	303±76	664±77	12.4±1.6
10 ppm	12.1±1.4	1.43±0.25	246±47	640±57	11.8±1.9
100 ppm	12.5±1.7	1.15±0.21	285±75	626±97	11.5±2.0
500 ppm	12.8±2.3	1.15±0.33	273±35	622±90	12.9±2.1
2000 ppm	16.5±2.7**	2.36±0.46**	402±0.69	852±114**	15.0±1.2
5000 ppm	17.4±3.5**	2.90±0.74***	570±105***	864±86**	15.9±2.2*

TREATMENT/RECOVERY					
0/0 ppm	10.5±2.0	1.19±0.28	355±25	550±52	9.6±2.3
5000/0 ppm	13.6±2.0*	1.31±0.30	376±91	763±91***	10.3±1.8

In conclusion, **this study shows that pymetrozine is a moderate and largely reversible inducer of foreign compound metabolising liver enzymes in the male mouse.**

Mechanistic study 2: Effects on liver cell proliferation in mice

Subgroups of 5 Tif: MAGf (SPF) male mice per group were kept on daily dietary exposure with pymetrozine (98 %) for 4 days at dosage levels of 0 and 5000 ppm (891.6 mg/kg bw/day), and for 14 and 42 days at dosage levels of 0, 10, 100, 500, 2000 and 5000 ppm (0, 1.6, 15.6, 83.9, 323.4 and 876.7 mg/kg bw/day for the 14 days treatment and 0, 1.6, 13.3, 70.7, 299.9 and 767.1 mg/kg bw/day for the 42 days treatment). Subgroup animals receiving 5000 ppm (1006 mg/kg bw/day) for 14 days were subsequently kept on standard control diet for a recovery period of 28 days. Before necropsy the animals were fasted for 16 hours and then killed under carbon dioxide anaesthesia by exsanguination. The liver of all animals were quickly removed and subjected to morphological and immunochemical determinations (including labelling with appropriate monoclonal antibody of the proliferating cell nuclear antigen (PCNA).

No apparent signs of toxicity were recorded throughout the treatment period and/or recovery period at any test article concentration. Treatment at all dose levels was without an effect on final body weights. Absolute and relative liver weights were slightly increased after treatment with 5000 ppm for 4 days and moderately increased at the same dose level after 14 and 42 days. Slightly increased relative liver weights were observed at 2000 ppm after 14 and 42 days of treatment. Absolute and relative liver weights returned to control levels in the 14-day treatment/28-day recovery animals.

The effects on liver-cell proliferation in these experimental conditions were summarised in the Table below. Upon microscopic investigation, the total number of hepatocyte nuclei per mm^2 was found reduced after treatment with 5000 ppm for 14 and 42 days and after treatment for 42 days with 2000 ppm, indicating hypertrophy. Immunohistochemical staining of liver sections for PCNA revealed a moderate to strong increase in the fraction of DNA synthesising hepatocytes in S-phase upon administration of 2000 and 5000 ppm at all investigated time points, indicating hyperplasia (Table below). These effects were reversible when a 14-day treatment period at 5000 ppm was followed by a 28-day recovery period (Table below).

Table : Effects on liver cell proliferation following dietary administration of pymetrozine to male mice. Data taken from RAR (2013). Displayed are the mean ± standard deviations of total nuclei per mm^2 and mean hepatocellular nuclear labelling indices (LI) expressed as percent labelled nuclei. Values are means of four (group 02) or five animals per group. Asterisks indicate results significantly different (two-sided Mann-Whitney Rank Test) from control: * p< 0.05, ** p< 0.01.

Group	Treatment period (days)	Dietary conc. (ppm)	Total nuclei per mm^2	LI (%)
1	4	0	208.57±21.54	0.08±0.11
2	4	5000	218.59±24.74	0.62±0.12*
3	14	0	209.02±13.64	0.06±0.06
4	14	10	223.25±20.66	0.21±0.25

5	14	100	209.29±15.59	0.15±0.28
6	14	500	221.13±13.64	0.05±0.05
7	14	2000	184.87±22.76	0.37±0.19*
8	14	5000	172.03±10.56*	1.91±0.70**
9	42	0	201.99±15.84	0.08±0.13
10	42	10	211.94±13.34	0.25±0.16
11	42	100	216±28.65	0.04±0.05
12	42	500	197.21±17.02	0.13±0.14
13	42	2000	170.10±20.33*	0.43±0.20*
14	42	5000	167.30±5.72**	0.43±0.35*
15	14/28	5000/0	204.56±22.07	0.08±0.08

In conclusion, the results of this study demonstrate that at 2000 and 5000 ppm, **the test article induced a sustained but reversible stimulation of hepatocyte cell proliferation and that the observed hepatomegaly in the mouse liver at these high dose levels was the result of hypertrophy and hyperplasia.**

Mechanistic study 3: Effects on liver parameters and thyroid hormones in rats

Groups of 5 female Tif:RAIf (SPF) rats were fed in a GLP study with pymetrozine (98%) dietary concentrations of 0, 20, 100, 1000 and 3000 ppm daily for 14 or 42 days (0, 1.5, 8.4, 80.2 and 218.6 mg/kg bw/d for the 14 days treatment and 0, 1.5, 7.6, 72.1 and 207.3 mg/kg bw/d for the 42 days treatment). Subgroups receiving 0 and 3000 ppm (226.3 mg/kg bw/day) for 14 days were kept on standard control diet for a recovery period of 28 days. Additional groups received 0 or 3000 ppm (190.3 mg/kg bw/day) for 4 days. Blood was sampled and plasma was prepared for the determination of the thyroid hormone status from all animals immediately prior to sacrifice. Before necropsy the animals were not fasted. They were killed under ether anaesthesia by exsanguination. The livers of all animals were quickly removed and weighed and processed for morphological (electron microscopy) and biochemical investigations.

The relative liver weights were increased after 14 and 42 days of treatment with 3000 ppm (114 or 123 % of control). This effect was largely reversible (112 % of control). The Table below summarises the biochemical liver parameters investigated in animals treated for 14 days and of the two recovery groups. The major treatment-related biochemical alteration was an increase in UDP-glucuronosyl transferase activity up to 155% and 298% of control at the two highest doses.

- *Table : Biochemical parameters in female rats dosed with pymetrozine.* Data taken from RAR (2013). Displayed are the mean ± standard deviations for 6 animals per group. Asterisks indicate results significantly different (two-sided Dunnett's test) from control: *p<0.05, **p<0.01, ***p<0.001

DOSE (ppm)	**EROD [nmol/min/g liver]**	**PROD [nmol/min/g liver]**	**UDP-GT [nmol/min/g liver]**	**GST [mmol/min/g liver]**
• 14-day TREATMENT				
0	1.97±0.49	0.087±0.014	1320±193	115± 7
20	1.57±0.28	0.068±0.021	1177±217	109±12
100	2.18±0.67	0.070±0.019	1338±186	118±14
1000	2.20±0.58	0.108±0.022	2044±236***	133±11
3000	6.93±1.77***	0.230±0.067***	3936±542***	195±14***
14-day TREATMENT/28-day RECOVERY				
0/0	2.33±0.22	0.090±0.013	1257±69	104± 22
3000/0	2.44±0.54	0.056±0.012**	1252±198	100± 15

Further biochemical alterations comprised induction of ethoxyresorufin O-deethylase, pentoxyresorufin O-dependentylase and glutathione S-transferase activities to 352%, 264% and 170% of control at 3000 ppm, respectively. Ethoxyresorufin O-de-ethylase activity was paralleled by a dose-dependent increase in CYP1A2 protein up to 517% of control and also the occurrence of the CYP1A1 band at the highest dose as detected with the monoclonal antibody specific for rat CYP1A isoenzymes.

All treatment related effects on biochemical liver parameters were reversible after the 28-day recovery period.

Ultramorphological investigation of the liver, performed in three animals each of the highest dose and control group from the 14 days treatment and 14 days treatment/28 days recovery groups, revealed a moderate reversible proliferation of smooth endoplasmic reticulum.

Assessment of replicative DNA synthesis in hepatocytes gave no indication for a stimulation of liver cell proliferation.

TSH (thyroid stimulating hormone) plasma concentration was increased after 14 days treatment to 205% and 171% of control and T3 was slightly increased after 42 days treatment to 139% and 133% of control, each at 1000 and 3000 ppm, respectively. This indicates a slight stimulation of the thyroid gland by pymetrozine. The effects of pymetrozine on plasma concentrations of thyroid hormones were summarised in the table below.

*Table: Plasma thyroid hormones concentrations in female rats treated with pymetrozine. D*ata taken from RAR (2013). Displayed are the mean plasma concentration ± standard deviations of thyroid stimulating hormone (TSH) as well as T4, T3 and rT3. Values are means of five animals per group. Asterisks indicate results significantly different (two-sided Dunnett's test) from control: *p<0.05, **p<0.01, ***p<0.001

DOSE [ppm]	TSH [ng/ml]	T4 [ng/ml]	T3 [ng/ml]	rT3 [ng/ml]
4-DAY TREATMENT				
0	7.98±0.49	29.4±10.2	0.933±0.093	0.123±0.025
3000	11.81±6.05	30.1±6.0	0.833±0.103	0.119±0.036
14-DAY TREATMENT				
0	7.39±1.29	31.7±4.9	0.844±0.076	0.107±0.020
20	9.71±2.12	28.3±2.2	0.803±0.060	0.125±0.018
100	9.61±1.79	28.4±11.5	0.784±0.155	106±0.036
1000	15.15±4.41***	33.9±13.8	0.860±0.061	0.145±0.030
3000	12.62±3.54**	31.0±8.4	0.885±0.130	0.114±0.029
42-DAY TREATMENT				
0	8.32±1.63	34.9±10.1	0.784±0.209	0.140±0.034
20	10.83±2.66	31.9±7.8	0.848±0.034	0.118±0.022
100	10.03±2.69	30.5±4.3	0.933±0.084	0.146±0.025
1000	7.56±2.07	41.7±2.8	1.089±0.163*	0.158±0.031
3000	8.96±1.79	30.2±4.1	1.044±0.140*	0.130±0.017
14-DAY TREATMENT/28-DAY RECOVERY				
0/0	8.32±1.63	34.9±10.1	0.784±0.209	0.140±0.034
3000/0	9.62±1.48	25.6±3.3	0.913±0.115	0.115±0.040

In conclusion, the liver enzyme activity profile identify **pymetrozine as a weak to moderate inducer mainly of hepatic xenobiotic phase II metabolising enzymes in the female rat.** In this study pymetrozine did not stimulate hepatocyte cell proliferation but the proliferation of smooth endoplasmic reticulum membranes was observed at the investigated dose of 3000 ppm. The analysis of thyroid hormones indicates a slight stimulation of the thyroid gland by pymetrozine.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

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

The reproductive toxicity of pymetrozine was assessed in a multi-generation study in rats. The results of this study are summarised in Table 16. Further details including method, guideline (and deviations if any), doses, substance purity (if known), species, strain, sex, no of animals/group, study duration, exposure route and a description of the results (including information on incidences and severities of findings and extent of changes relative to controls, etc.) are given in the text below or in the RAR.

In the 2-generation study the highest dose level was toxic for both parents and offspring. The parental and offspring body weights were reduced, eye opening was slightly delayed in pups, and histopathology of adults revealed changes in liver, spleen, and the pituitary. No effects on reproduction or fertility were reported up to the highest dose tested.

Histological findings in gonads and (slight) changes in hormone levels were reported in repeated-dose toxicity studies in rats and dogs (Table 17).

No human data on adverse effects on sexual function and fertility are available.

Table 16: Summary of reproductive toxicity studies

Study	Dose levels	NO(A)EL	Target organs/main effects	Reference
2-generation study in rats OECD TG 416 (1983)	0, 20, 200, 2000 ppm	<u>parents & offspring:</u> 200 ppm (13.92/15.98 mg/kg bw/d) <u>reproduction:</u> 2000 ppm (126.9/151.6 mg/kg bw/d)	reduced bw at 2000 ppm in parents and offspring; target organs: liver, spleen, pituitary; delayed development in pups no effects on reproduction or fertility	(Author 2, 1993 TOX9652156)

Comparison with the CLP criteria

Table 17: Toxicological results concerning adverse effects on sexual function and fertility

Toxicological result	CLP criteria
2-generation reproduction study in rats, pymetrozine administered via diet (Author 2, 1993 TOX9652156): No effects on fertility or reproduction observed up to highest dose tested (2000 ppm, 126.9/151.6 mg/kg bw/d)	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
28-d studies in rats: 600 mg/kg bw/d (1992 ASB2012-4619): reduced spermatogenesis in testes, reduced spermatozoa in epididymis 162.8 mg/kg bw/d (3000 ppm) (Author 4, 1998 TOX9851465; Author 4, 1998 TOX9851464): rounded spermatids 691 mg/kg bw/d (10000 ppm) (Author 3, 1991 TOX9652142): reduced spermatozoa in epididymis 254.7 mg/kg bw/d (5000 ppm) (Author 4, 1998 TOX9851465; Author 4, 1998 TOX9851464): hormone changes (testosterone, dihydrotestosterone, luteinising hormone, T4), testes (weight increase; in one animal: unilateral atrophy of testis & seminiferous tubular atrophy) and affected spermatogenesis (preleptotene & pachytene spermatocytes decreased) **28-d studies in dogs:** ~ 50 mg/kg bw/d (2500 ppm) (1991 TOX9652143): no relevant findings up to highest dose tested 61 mg/kg bw/d (2000 ppm) (Author 4, 1998 TOX9851466): low dihydrotestosterone levels, testes (histological findings in one animal: giant cell formation, decrease of sperm, degenerated spematogenic cells) **90-d (tox) studies in rats & mice:** No relevant findings up to highest dose tested (~360 or ~1000 mg/kg bw/d, respectively) **90-d study in dogs:** 14 mg/kg bw/d (500 ppm) (1992	

Toxicological result	CLP criteria
TOX9652145; 1992 ASB2012-4620; 1995 TOX9650867): tubular atrophy of testis, reduced spermatogenesis 53/60 mg/kg bw/d (2500 ppm): additionally, uterine atrophy **1-yr study in dogs:** 5 mg/kg bw/d (200 ppm) (1994 TOX9652153): testis (wt ↓) ~27 mg/kg bw/d (1000 ppm): unilateral tubular atrophy in one male, bilaterally spermatic giant cells in testicular spermatogenic epithelium **2-yr study in rats and 18-mo study in mice:** No relevant findings up to highest dose tested (~130 or ~670 mg/kg bw/d, respectively)	

In the submitted multigeneration study no findings with relevance for a classification for adverse effects on sexual function and fertility were reported.

Histological findings in testes accompanied by other toxic effects were observed in dogs and rats. The testes findings were most pronounced at the highest dose groups. However, some of the available studies have limitations in the group size or the set of parameters evaluated. Nevertheless, the evaluated parameters seemed to be assessed appropriately.

There are no epidemiological data to evaluate effects on fertility, hence pymetrozine cannot be placed in category 1A.

Overall, in several studies histological indications for adverse effects on fertility (spermatogenesis) were reported in low incidences, however, they were observed at dose levels inducing systemic toxicity[4]. Additionally, the findings observed in repeat-dose studies could not be corroborated in the 2-year study in rats or the multigeneration study in rats, however (slightly) lower dose levels were administered in the latter studies.

It seems that systemic effects of toxicity which were described in the study reports were not severe enough to induce non-specific findings in testes and to render the observed histological findings as non-specific and non-relevant findings for classification. No data are available to assess whether the testes effects in dogs might have an adverse impact on mating success.

It should be noted, that the rat (multigeneration study) is a limited / poor model to assess certain adverse effects on fertility, because an impact on fertility rate in rats is observed only after severe reductions of sperm numbers

[4] ECHA: Guidance on the application of the CLP criteria, Version 3.0 November 2012 (Section 3.7.2.2.1.1, p. 324): „Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes.

There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity."

(e.g., Working, 1988[5]), whereas in humans fertility rate is reduced already by less extensive reductions in sperm numbers. The value and relevance of histological evaluation of testes is also emphasised in the OECD test guideline 443 and some RAC opinions on harmonised classification of chemicals.

Taking into account the relatively low incidences which were observed in several studies, DS sees "some evidence" but not a "clear evidence" for adverse effects on reproduction and therefore, proposes a classification with category 2 (H361f).

10.10.2 Adverse effects on development

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

The reproductive toxicity of pymetrozine was assessed in two teratology studies in rats and rabbits. After the PPP procedure, DS was informed that EPA had evaluated a developmental neurotoxicity (DNT) study with pymetrozine in rats. During preparation phase of the current CLH dossier this study was provided by Syngenta and evaluated by the DS.

The results of these studies are summarised in Table 18. Further details including method, guideline (and deviations if any), doses, substance purity (if known), species, strain, sex, no of animals/group, study duration, exposure route and a description of the results (including information on incidences and severities of findings and extent of changes relative to controls, etc.) are given in the text below or in the RAR.

The two teratology studies in rats and rabbits revealed developmental changes at dose levels at which maternal toxicity was also apparent.

Under the conditions of the rat developmental toxicity study, slight maternal toxicity was reported at dose levels of 300 and 100 mg/kg bw/d (reduced feed intake and body weight (gain)). At animals dosed with 300 mg/kg bw/d, four foetuses in three litters with displaced pubic bones (classified by the study director as malformation) and several foetuses with anomalies or variations were observed. At a dose level of 100 mg/kg bw/d, an increased incidence of variations (dumbbell-shaped cervical vertebral centres) was observed. No effects were observed in animals treated with 30 mg/kg bw/d. The NOAEL for maternal and developmental effects was at 30 mg/kg bw/d.

In the study report the pelvic findings reported at 300 mg/kg bw/d were described as follows:

- Female 75 / Foetus 15: Displaced pubis; present; left. Left pubic bone displaced parasagittally and rotated ca. 60 deg.
- Female 79 / Foetus 3: Displaced pubis; present; left. Left pubic bone displaced parasagittally and rotated ca. 30 deg.
- Female 80 / Foetus 9: Displaced pubis; present; bilateral. Both pubic bones displaced parasagittally and rotated ca. 30 deg.
- Female 80 / Foetus 13: Displaced pubis; present; bilateral. Both pubic bones displaced parasagittally and rotated ca. 30 deg.

Under the conditions of the rabbit developmental toxicity study, administration of 125 and 75 mg/kg bw/d of the test compound induced toxicity in does (mortality, reduced feed intake and body weight gain). In foetuses of these dose levels, external and skeletal examination revealed several findings (altered position of forelimb,

[5] Working (1988): Male reproductive toxicology: comparison of the human to animal models, Environmental health perspectives 77, 37-44

fused sternebrae, reduced pubis, poor ossification of several bones and occurrence of 13th rib). The NOAEL for developmental and maternal effects was 10 mg/kg bw/d.

In the study report the reduced pubis reported at 75 mg/kg bw/d (females 44 and 53) and 125 mg/kg bw/d (females 66 and 73) were described as follows:

- Female 44 / Foetus 4: Reduced pubis; present; bilateral.
- Female 53 / Foetus 6: Reduced pubis; present; bilateral.
- Female 66 / Foetus 3: Reduced pubis; present; bilateral.
- Female 73 / Foetus 5: Reduced pubis; present; right.
- Female 73 / Foetus 6: Reduced pubis; present; right.

It is important to note that changes in the pelvis occurred in both species. Interestingly, defects of this kind have not been recorded in historic controls in rabbits and only in low incidences in rats.

In the DNT study only two dose groups were evaluated: Due to excessive maternal toxicity (body weight loss, lower feed consumption, clinical signs) and high pup mortality the highest dose group of 2500 ppm was terminated before schedule. At the mid dose level of 500 ppm decreased body weight gain of ca 10 % was reported in dams but did not gain statistical significance, therefore this finding was considered not adverse. When compared to multi-generation study, the high level of maternal toxicity at the top dose is noted.

Changes in brain morphometry in F1 animals were observed in all evaluable dose groups starting at the lowest dose group of 100 ppm (equal to 8.1 mg/kg bw per day). Neonatal mortality including complete litter losses was reported at 500 ppm (equal to 38.7 mg/kg bw per day). No further effects on development, neurological or behavioural parameters were observed based on the information given in the study report.

No human data on adverse effects on development are available.

Table 18: Summary of developmental toxicity studies

Study	Dose levels	NO(A)EL	Target organs/main effects	Reference
Teratology study, rat OECD TG 414 (1981)	0, 30, 100, 300 mg/kg	maternal: 30 mg/kg foetal: 30 mg/kg	maternal toxicity and developmental changes (pelvis, delayed ossification) at 100 and 300 mg/kg	(Author 2, 1992 ASB2012-4617)
Teratology study, rabbit OECD TG 414 (1981)	0, 10, 75, 125 mg/kg	maternal: 10 mg/kg foetal: 10 m/kg	maternal toxicity, embryotoxicity, foetotoxicity and developmental changes (pelvis, fused sternebrae, delayed ossification) at 75 and 125 mg/kg	(Author 2, 1992 ASB2012-4618)
Developmental neurotoxicity study in rats US EPA OPPTS 870.6300 (1998)	0, 100, 500 ppm, 2500 ppm 0, 8.1, 38.7, 173.1 mg/kg bw/d	maternal: 500 ppm (38.7 mg/kg bw/d) foetal: < 100 ppm (8.1 mg/kg bw/d	At 2500 ppm termination due to severe maternal and pup toxicity. At 500 ppm non-significant decreased maternal body weight gain, reduced feed consumption post partum d 1-5 without influence on body weight post partum and possibly related to cannibalism. At 100 ppm and above brain morphometry changes at PND 12 and 63. At 500 ppm increased pup mortality PND 1 – 5	(Author 6, 2003 ASB2015-3677)

Comparison with the CLP criteria

Table 19: Toxiclogical results concerning adverse effects on development

Toxicological result	CLP criteria
Teratology study in rats (Author 2, 1992 ASB2012-4617): Displaced pubis bones, thickened ischium of pelvis, asymmetrically shaped sternebrae and poor ossification in several digit bones at 300 mg/kg bw/d Lower body weight gain and feed intake at 100 and 300 mg/kg bw/d	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Developmental neurotoxicity study in rats (Author 6, 2003 ASB2015-3677): Brain morphometry changes in all dose groups from 8.1 mg/kg bw per day Pup mortality on PND 1-5 at 38.7 mg/kg bw per day severe maternal and pup toxicity at 173.1 mg/kg bw per day	
Teratogenicity study in rabbits (Author 2, 1992 ASB2012-4618): Post implantation loss, reduced pubis at 75 and 125 mg/kg bw/d Fused sternebrae and several variations at 125 mg/kg bw/d 2 dams died in 125 mg /kg bw/d dose group (GD 16 and 19), one was sacrificed in control group (GD 16) Slight body weight loss in top dose group and lower body weight gain in mid dose group during treatment period	

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according to CLP regulation is not possible.

The prenatal developmental toxicity was investigated in rats and rabbits and a developmental neurotoxicity study in rats complying to international test guidelines and GLP.

In rats, findings in offspring included displaced pubic bones (classified by the study director as malformation) and several foetuses with anomalies or variations in the top dose group. In mid dose group, increased incidence of variations (dumbbell shaped cervical vertebral centres) was observed. At the same dose levels, reduced feed intake and body weight (gain) were observed in dams.

In rabbits, altered position of forelimb, fused sternebrae, reduced pubis, poor ossification of several bones and occurrence of 13th rib were observed in offspring in top and mid dose group. In does, lower body weight gain was observed during the treatment period in mid dose group and body weight loss during the treatment period and mortality were observed in top dose group.

According to the website "www.devtox.org", the findings "small pubis" (incompletely developed structure, or less than normal in size), "malpositioned pubis" (not occurring in the proper position and/or orientation) and "misshapen pubis" (abnormally shaped [Not to be used to describe sites of incomplete ossification]) are considered malformations. Whereas, the finding "misaligned pubis" (abnormal relative position of structures on opposite sides of a dividing line or about the centres or axis) is not considered a malformation.

In addition to the data presented above and in the confidential annex, no further data/information is included in the study reports, which would allow judging the functional impact of the pubis dysplasia. However, changes in pubis alignment or position may affect posture, gait and can cause pain. Hence, in humans, such diagnoses are considered malformations if they are induced during pregnancy, or can lead to severe disability if they are acquired later on.

While the standard reference point for the evaluation of treatment responses shall be concurrent control data, historical control data may be helpful in the interpretation of particular developmental studies. Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions in the same laboratory and shall be from contemporaneous studies (adapted from regulation (EU) No 283/2013).

The Guidance on the Application of the CLP Criteria (Version 4.1, June 2015, section 3.6.2.3.2., p. 376) explains only the use of historical control data regarding the evaluation of tumour findings. But the general idea would by applicable to support the evaluation of malformations, too, *mutatis mutandis*:

> *"Use of historical control data should be on a case by case basis with due consideration of the appropriateness and relevance of the historical control data for the study under evaluation. In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. It is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). Historical data older than this should be used with caution and acknowledgement of its lower relevance and reliability (RIVM, 2005; Fung et al, 1996; Greim et al, 2003)."*

It appears that the historical control data summarised by Author (1996) and Anon. (1997) (Tables B.6.6-13 and B.6.6-14 in the confidential annex or in the RAR) are from a relevant time period for a study conducted in 1991 (in-live phase).

In the available DNT study in rats, changes in brain morphometry were reported in the low dose group and above. Effects on brain morphometry in this study were also considered as adverse by US EPA. Additionally, higher neonatal mortality was observed in the mid dose group. Findings in low and mid dose groups were not accompanied by maternal toxicity.

Increased neonatal mortality was not reported in the multi-generation study in rats, despite the higher dose levels in the latter study.

Manifestations of developmental toxicity seen in developmental toxicity studies in rats and rabbits were accompanied by maternal toxicity. Abortion was observed in one (top dose) rabbits only. Several does had resorptions / post-implantation loss. Additionally, mortality was observed in top dose rabbits. Even though pubis was affected in rats and rabbits, dissimilar malformations were observed in the available studies.

The information from the DNT study in rats, which was received during preparation of this CLH dossier supports a classification as a developmental toxicant. Changes in brain morphometry were seen already in the low dose offspring. Additionally, higher neonatal mortality was observed in the mid dose group.

According to regulation (EC) No 1272/2008 major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency.

ECHA's Guidance on the application of the CLP criteria (Version 3.0 November 2012, Section 3.7.2.2.1.1, p. 325) cites the CLP regulation: "3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. …".

No information is available to confirm that the observed effects on offspring have to be regarded as secondary non-specific consequences of maternal toxicity. Additionally, according to the study report, findings in low and mid dose groups of the DNT study were not accompanied by maternal toxicity.

In summary, classification in Category 2 (H361d) is considered appropriate, also taking into account the limited increase in incidences in developmental toxicity studies. The CoRMS of the pesticides procedure (Belgium) indicated that pelvic effects observed in rats and rabbits, might be a reason to trigger higher classification for developmental toxicity.

10.10.3 Adverse effects on or via lactation

No data are available to judge whether there are specific effects on or via lactation (H362).

10.10.4 Conclusion on classification and labelling for reproductive toxicity

In summary, classification in Category 2 (H361fd) is considered appropriate.

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal The DS summarised in the CLH report the following studies for the assessment of sexual function and fertility impairments: • A 2-generation reproduction toxicity study in rats where no effects on reproduction or fertility were found up to the highest dose tested (which was toxic for both parents and offspring); • A 28-day toxicity study in rats reporting alterations in spermatogenesis in testes and spermatozoa in the epididymis, rounded spermatids and alterations in hormones and testes; • A 28-day toxicity study in dogs reporting low dihydrostestosterone levels and histological changes in the testes (of 1 animal); • A 90-day toxicity study in dogs reporting tubular atrophy of the testis and reduced spermatogenesis; • A 1-year toxicity study in dogs reporting unilateral tubular atrophy in one male and bilaterally spermatic giant cells in testicular spermatogenic epithelium.

The DS noted that, the rat is a poor model for studying certain effects on fertility because an impact on fertility rate in rats is observed only after severe reductions of sperm capacity, whereas in humans the fertility rate is reduced already by a less extensive reduction in sperm numbers. From these considerations, the DS proposed to classify pymetrozine as toxic to reproduction category 2 (H361f: Suspected of damaging fertility).

The DS summarised the following studies in the CLP report for the assessment of development:

- One teratology study in rats reporting maternal toxicity and developmental changes (displaced pubic bones, thickened ischium of the pelvis, asymmetrically shaped sternebrae and poor ossification in several digit bones)
- One teratology study in rabbits reporting post implantation loss, reduced pubis at two different doses, fused sternebrae and several variations at the highest dose, always concurrently with maternal toxicity (including mortality at the highest dose)
- One developmental neurotoxicity study in rats reporting changes in brain morphometry in all groups (including those without maternal toxicity).

The DS proposed classification of pymetrozine as toxic to reproduction category 2 (H361d: Suspected of damaging the unborn child), mainly on the basis of morphometric changes reported in the neurodevelopmental study. This was supported by the other developmental toxicity effects reported (concurrently with maternal toxicity) in the developmental toxicity studies in rats and rabbits.

Comments received during public consultation

Five MS supportedCategory 2 on the same basis as argued by the DS. Four MS supported Category 2 for development, although one also requested discussion of category 1B. Another MS supported 1B for development because no maternal toxicity was observed in the low- and mid-dose groups in the neurodevelopmental toxicity study. This MS further argued that neither the severity of maternal toxicity nor any specific mode of action can support consideration of the observed abnormalities as secondary to the maternal toxic effects.

Industry argued against classification for both fertility and development and presented public comments in response to the CLH proposal, stating that there is no indication of any direct effect of pymetrozine on spermatogenesis, spermatozoa or the testes, in any species tested, with any potential findings being either secondary to general systemic toxicity (in rats) or a commonly occurring background histopathological finding (in dogs). These facts, combined with the lack of any effect on fertility or reproduction in the 2-generation study led the Industry to conclude that there is no evidence that pymetrozine has a direct effect on the reproductive system. They also considered that there is no indication that pymetrozine is associated with a direct effect on foetal development because in the developmental toxicity studies, significant maternal toxicity was observed at the high doses tested in both rats and rabbits. Industry considered that in the developmental neurotoxicity study there was no clear pattern of adverse effects on neurological development and that given the very large number of brain morphometry measures, these few changes have to be considered incidental and non-treatment related. Based on all these considerations, the Industry considered any observed change as secondary to the observed maternal toxicity and hence, that no classification is warranted.

Assessment and comparison with the classification criteria

Fertility

Rat dietary 2-generation reproduction study

This study was compliant with OECD test Guideline (TG) 416 and GLP. Ten weeks after initiation of exposure to the test material at dietary levels of 0, 20, 200 or 2000 ppm pymetrozine (98% purity) the Tif:RAIf (SPF) rats (30 animals per sex and dose level) were paired. Parents were mated 1:1 until positive mating occurred or for 19 days, whichever came first. After weaning and a premating period of 10 weeks, F1 animals were mated to produce the F2 generation. The animals were continuously exposed to the test substance admixed to feed in two successive generations (F0 and F1). Dams were allowed to litter and suckle naturally. Litters were culled to 4 male and 4 female pups, where possible, on day 4 *post-partum*.

F0 generation

In the F0 generation, mean daily test substance intakes were approximately 1 to 4, 10 to 40, and 110 to 440 mg/kg bw/d at 20, 200, and 2000 ppm, respectively.

There were no treatment - related mortalities or clinical signs in parent animals. Body weights were about 10 % lower than controls at 2000 ppm in both sexes from the second week of treatment onwards. Feed consumption was reduced at 2000 ppm in both sexes except in females during the lactation period.

At parental necropsy, females at 2000 ppm had higher absolute and relative liver and spleen weights than controls. No treatment-related macroscopic changes were detected. Microscopic histopathology revealed a minimal hepatocellular hypertrophy in most males and 2 out of 30 females at 2000 ppm and in few males at 200 ppm (Table below). Additionally, minimal to moderate hyperplasia of lymphatic follicles of splenic white pulp was observed in most females at 2000 ppm (Table below).

Table: Incidences of microscopic lesions in F0 generation of the rat dietary 2-generation reproduction study. Data taken from the RAR (2013).

	Males				**Females**			
Feeding level (ppm)	**0**	**20**	**200**	**2000**	**0**	**20**	**200**	**2000**
Liver (Total examined)	30	30	30	30	30	30	30	30
Hypertrophy	0	0	5	27	0	0	0	2
Spleen (Total examined)	30	0	30	30	30	0	30	30
Hyperplasia of lymphatic follicles	0	-	0	0	0	0	0	25

Mating, gestation, fertility, and parturition indices were not affected by treatment (Table below). Likewise, numbers of stillborn pups as well as livebirth, viability, and lactation indices were not affected.

Table: Reproductive performance in F0 generation of the rat dietary 2-generation reproduction study. Data taken from the RAR (2013).

	0 ppm	**20 ppm**	**200 ppm**	**2000 ppm**
Males				
Used for mating [n]	30	30	30	30
Mating index[a] [%]	96.7	86.7	90.0	96.7
Fertility index[b] [%]	82.8	80.8	92.6	96.6
Females				
Used for mating [n]	30	30	30	30
Mated [n]	29	26	27	29
Pregnant [n]	24	21	25	28
With live born pups [n]	23	21	24	27
Mating index[a] [%]	96.7	86.7	90	96.7
Fertility index[b] [%]	82.8	80.8	92.6	96.6
Gestation index[c] [%]	95.8	100	96	96.4
Parturition index[d] [%]	95.8	100	96	96.4
Gestation length [days]	22.2± 0.5	22.3±0.5	22.4±0.5	22.1±0.4
Precoital interval [days]	3.7±3.2	4.2±2.0	4.3±2.7	4.8±3.0
Implantation sites	14.8±3.8	15.4±2.2	14.5±2.9	13.2±2.9

a = Percent animals positively mated (females) or producing positive mating (males)
b = Percent of females pregnant / percent of males producing pregnancy
c = Percentage of females with confirmed pregnancy that resulted in the birth of live pups
d = Percentage of females with confirmed pregnancy that delivered pups

F1-pups

No treatment-related clinical signs in the pups were reported. Litter weights of the F1 generation were reduced at 2000 ppm from the second week of lactation onwards. Eye opening was minimally delayed (by about 0.4 days) at 2000 ppm compared to controls. No treatment-related effects were found for the following parameters: number of litters, total pups born, mean pups per litter, sex ratio, mean number of pups alive and viability and lactation index. The number of stillborn pups was higher in the groups dosed with 200 and 2000 ppm than in controls, although no dose-response was observed (Table below).

Table: Litter data of the F1 generation in the rat dietary 2-generation reproduction study. Data taken from the RAR (2013).

	0 ppm	**20 ppm**	**200 ppm**	**2000 ppm**
Number of litters	23 f	21	24	27
Total pups born	292 f	299	309	316
Mean per litter	12.7 d	14.2	12.9	11.7
Number of stillborn pups	3 f	2	12*	6+
Sex ratio (% females day 0)	46.7	53.5	52.5	51.0
Mean number of pups alive:				
on day 0	12.6 f	14.1	12.4	11.5
on day 4 (post culling)	7.9 f	8.0	7.9	7.5
on day 7	7.8 f	8.0	7.9	7.4
on day 14	7.5 f	7.9	7.7	7.4
on day 21	7.4 f	7.8	7.6	7.4

Viability Index[a]	97.6 f	98.0	97.0	97.1
Lactation Index[b]	93.7 f	97.6	96.1	98.5
Mean pup weight:				
on day 0	6.2 d	6.1	6.6	6.6
on day 4 precull	9.9 d	9.5	10.4	10.0
on day4 postcull	10.0 d	9.6	10.4	10.1
on day 7	16.5 d	15.9	16.9	15.9
on day 14	33.9 d	32.8	33.7	**31.1***
on day 21	57.4 d	56.2	58.9	**52.8***
a: % Pups surviving days 0 to 4; b: % Pups surviving days 4 to 21; d = ANOVA + Dunnett-test; f = chi-square + Fishers exact: * significant at p<0.05; p = 0.508				

F1-parents

In the F1 generation mean daily test substance intakes were similar to the F0 generation. There were no treatment-related mortalities or clinical signs in parent animals. Body weights were about 15 % lower than control at 2000 ppm in both sexes from the start of treatment onwards. Feed consumption was reduced at 2000 ppm in both sexes.

At parental necropsy, females at 2000 ppm had higher relative liver and spleen weights than controls. At 200 ppm, relative liver weights were increased by approximately 10%. No treatment-related macroscopic changes were detected. As shown in the Table below, microscopic histopathology revealed a minimal hepatocellular hypertrophy in males and females at 2000 ppm and minimal to moderate hyperplasia of basophilic cells of the adenohypophysis in males at 2000 ppm. A slight increase of hepatocellular hypertrophy was noted in males treated with 200 ppm.

*Table: Incidences of microscopic lesions in F1 parents in the rat dietary 2-generation reproduction study***.** Data taken from the RAR (2013)

	Males				**Females**			
Feeding level (ppm)	**0**	**20**	**200**	**2000**	**0**	**20**	**200**	**2000**
Liver (Total examined)	30	30	30	30	30	30	30	30
Hypertrophy	0	0	2	26	0	0	0	0
Pituitary (Total examined)	30	30	30	30	30	30	30	30
Hypertrophy of basophilic cells	7	8	7	17	0	0	0	0

Mating, gestation, fertility, and parturition indices were not affected by treatment. Likewise, numbers of stillborn pups as well as livebirth, viability, and lactation indices were not affected (Table below).

*Table: Reproductive performance of the F1 generation in the rat dietary 2-generation reproduction study***.** Data taken from the RAR (2013)

	0 ppm	**20 ppm**	**200 ppm**	**2000 ppm**
Males				
Used for mating [n]	30	29	30	30
Mating index[a] [%]	80	86.7	83.3	96.7
Fertility index[b] [%]	91.7	92.3	96	100
Females				
Used for mating [n]	30	30	30	30
Mated [n]	24	26	25	29

Pregnant [n]	22	24	24	29
With live born pups [n]	21	24	24	29
Mating index[a] [%]	80	86.7	83.3	96.7
Fertility index[b] [%]	91.7	92.3	96	100
Gestation index[c] [%]	95.5	100	100	100
Parturition index[d] [%]	95.5	100	100	100
Gestation length [days]	22.3±0.5	22.2±0.4	22.2±0.4	22.2±0.4
Precoital interval [days]	6.2±3.8	6.0±5.5	7.0±5.3	5.3±4.2
Implantation sites	16.0 ±2.7	14.8 ±4.7	15.5±2.2	14.3±3.8
a = Percent animals positively mated (females) or producing positive mating (males) b = Percent of females pregnant / percent of males producing pregnancy				

F2-pups

There were no treatment-related clinical signs in the F2 pups. Litter weights of the F2 generation were reduced at 2000 ppm from the end of the first week of lactation onwards (Table below). Eye opening was minimally delayed (by about 0.5 days) at 2000 ppm compared to controls. No treatment-related effects were detected for the following parameters (Table below): number of litters, total pups born, mean pups per litter, number of stillborn pups, mean number of pups alive and viability and lactation index.

Table: Litter data of the F2 generation in the rat dietary 2-generation reproduction study. Data taken from the RAR (2013)

	0 ppm	**20 ppm**	**200 ppm**	**2000 ppm**
Number of litters	21	24	24	29
Total pups born	302	310	355	393
Mean per litter	14.4	12.9	14.8	13.6
Number of stillborn pups	3	4	1	2
Sex ratio (% females day 0)	48.2	50.3	50.6	54.5
Mean number of pups alive:				
on day 0	14.2	12.8	14.8	13.5
on day 4 (post culling)	8.0	7.5	8.0	7.7
on day 7	8.0	7.5	8.0	7.6
on day 14	7.9	7.5	7.9	7.4
on day 21	7.9	7.5	7.9	7.4
Viability Index[a]	95.3	97.4	97.2	98.7
Lactation Index[b]	98.8	98.9	98.4	96.9
Mean pup weight	6.1	6.1	6.0	6.3
on day 0	9.6	9.8	9.1	9.1
on day 4 precull	9.7	10	9.3	9.3
on day 4 postcull	16.0	15.5	15.3	**14.7***
on day 7	31.3	30.5	30.5	**28.9****
on day 14	52.2	49.9	49.6	**45.7****
on day 21				
a: % Pups surviving days 0 to 4; b: % Pups surviving days 4 to 21; *: significant at p<0.05; **: significant at p<0.01				

In conclusion, reproductive parameters including gonadal function, mating behaviour, conception, parturition, lactation and weaning, as well as sex organ histopathology were not affected under these experimental conditions following exposure to pymetrozine.

Supplementary study 1: Oral 28-day study in rat

The study was compliant with OECD TG 407 and GLP. Groups of 10 male and 10 female Tif:RAIf (SPF) rats were administered daily dose levels of 0, 10, 100, or 600 mg/kg body weight pymetrozine (98% purity) for 28 days via gavage.

Transient reddening of the ears was noted in 7 males and 5 females at 600 mg/kg bw/d. No compound related mortality was noted. Body weight gains were lower than in the controls at 600 mg/kg bw/day. As a result, terminal mean body weights were 7 % and 10 % below the control values for males and females, respectively. Lower food consumption was noted in males at 600 mg/kg bw/day during the first 2 weeks of the study. Water consumption was increased in females at 600 mg/kg bw/d and a similar trend was also noted in males. Eye examination revealed no changes.

Haematological and blood chemistry changes were also limited to the high dose group of 600 mg/kg bw/d. A mild anaemia was noted in both sexes (7% reduction of red blood cells in males). There were increases of plasma bilirubin (76% in males), albumin (11% in males) and cholesterol (70% in males) and a lower plasma level of chloride in both sexes. Minimally lower plasma glucose levels (13% in males) and increased activities of alkaline phosphatase (46% in males) were noted in males, while minimally lower plasma potassium levels were recorded in females. The urinalysis revealed a slight increase of urine density in males at 600 mg/kg bw/day.

The absolute and/or relative liver weights were increased at 100 mg/kg bw/d (by 10 to 12% above control) and 600 mg/kg bw/d (40 to 72%). Increased kidney to body weight ratios (12 to 23%), increased spleen weights (22 to 42%) and decreased thymus weights (30 to 43%) were recorded for animals of both sexes at 600 mg/kg bw. Mottled livers were seen in males of the 600 mg/kg bw/d group. Microscopic changes consisted in hypertrophy of centrilobular hepatocytes at 100 and 600 mg/kg bw, atrophy of the thymus in males at 100 mg/kg bw/d and in animals of both sexes at 600 mg/kg bw/d, and hyperplasia of the splenic white pulp in both sexes at 100 and 600 mg/kg bw/d. Spermatogenesis in the testes and the number of spermatozoa in the epididymis were found to be reduced in 9 and 7 animals, respectively at 600 mg/kg bw/day. No data about the intensity of such reductions were found either in the CLH report or in the RAR for pymetrozine which was supplied together with the CLH report. The Industry considered in its public comments that the apparent reductions in spermatogenesis and spermatozoa were most likely a secondary consequence of the general systemic toxicity.

Supplementary study 2: Oral 28-day study in rat

A 4-week (28-day) oral toxicity study was conducted in rats to evaluate the effects of pymetrozine on the testis. The test substance (pymetrozine 99.3% purity) was administered to specific pathogen-free (SPF) Sprague-Dawley (Crj:CD) rats (6 animals/group) by incorporating it into the basal diet at levels of 0, 100, 1000, 3000 and 5000 ppm for a period of 4 weeks (28 days).

Neither toxic clinical signs nor deaths were found during the study at the top dose. Body weights were significantly lower than those in the control throughout the treatment period; mean body weights at each week were 83 to 89 % of the control. Food consumption was also lower than the control; overall mean food consumption was 78 % of the control. Organ weight measurements exhibited a significant increase in relative liver (19%), epididymis (22%) and testis (22%) weight. At necropsy, dark colour of the liver was noted in all animals. Histopathologically, centrilobular hepatocellular hypertrophy in the liver was observed in all animals, while treatment-related changes in the testis and epididymis were: testicular atrophy in one exposed animal, tubular atrophy in one exposed and one control animal, mononuclear cell infiltration (bilateral) in the epididymis of 1 exposed animal and decrease of sperm (unilateral) in the epididymis of 1 exposed animal. The morphometric analysis of spermatogonic cells at stage VII of seminiferous epithelial cycle in the testis of male rats revealed some minor effects without a dose-response in preleptotene and pachytene spermatocytes and round spermatid.

The Industry considered in a public comment released in response to CLH-report that these histopathological impairments were indeed due to background observations and normal biological variation.

The blood hormone assay revealed significant decreases in testosterone (1.33±0.59 vs 4.50±4.38 ng/mL in control animals), dihydrotestosterone (0.50±0.11 vs 0.76±0.23 ng/mL in control animals) and luteinising hormone (1.1±0.2 vs 1.6±0.2 ng/mL in control animals), and a significant increase in thyroxine (6.6±1.3 vs 5.1±0.6 µg/mL in control animals). During the EFSA peer-review, the industry argued that the measurements of testosterone and dihydrotestosterone are questionable due to outliers and few measurements. The industry recalculated the means after removing the outlier animals and the new values for testosterone were reported to be 1.67±0.58 vs 2.71±0.38 ng/mL in control animals and, for dihydrotestosterone, to be 0.59±0.08 vs 0.68±0.05 ng/mL in control animals (in all cases for n=5 control animals and n=3 exposed animals).

<u>At 3000, 1000 and 100 ppm group:</u> Effects on testes testis, epididymis or hormone levels were not noted.

<u>Supplementary study 3: Oral 28-day study in rat</u>

The study was compliant with the OECD TG 407. Groups of 5 male and 5 female Tif:RAIf (SPF) rats were given diets containing 0, 100, 500, 2000 or 10000 ppm pymetrozine (100% purity) for 28 consecutive days; corresponding to 10, 55, 200 and 700[6] mg/kg bw/day.

There was no mortality. Clinical signs were only observed at 10000 ppm. They were transient and consisted of red ears in some animals, penis prolapse, and piloerection associated with emaciated condition. At 10000 ppm the animals practically failed to gain any weight. Food and water consumption was markedly reduced at 10000 ppm in both sexes.

Haemoglobin and haematocrit values were minimally decreased in both sexes at 10000 ppm. Regarding organ weights, the increased relative kidney and liver weights at 10000 ppm were

[6] There was a small discrepancy between RAR for pymetrozine and the CLH-report: The former considered an exposure of 1000 ppm to be equivalent to 700 mg/kg bw/day and the latter that this exposure was equivalent to 691 mg/kg bw/day.

considered to be the only treatment-related changes. Histopathological changes were limited to the 10000 ppm group and were:

- Hypertrophy of centrilobular hepatocytes in all animals;
- Minimal multifocal necrosis of the liver in one female;
- Congestion of spleen in all animals;
- Reduced spermatogenesis associated with atrophy of seminiferous tubules in 1 male;
- Reduction of spermatozoa in epididymides in all males (no information was provided to RAC regarding the severity of the reduction);
- Fatty change in the adrenal cortex of most males.

Industry argued in a position paper submitted during the public consultation that rats were not fully mature at the start of the study (approximately 5-6 weeks) and the severe toxicity may have delayed attainment of fully maturity. Industry also considered that apparent reductions in spermatozoa were most likely a secondary consequence of severe general systemic toxicity.

Seven supplementary studies in rats, mice or dogs were presented in the CLH report.

Development

Developmental toxicity study in rats

The study was compliant with OECD TG 414 observing GLP regulations. Nulliparous female Tif:RAIf (SPF) rats (24/group) were mated overnight with proven fertile males of the same stock. Successful mating was determined either by the presence of a vaginal plug or spermatozoa in the vaginal smear. This day was designated day 0 of pregnancy (day 0 p.c.). Mated female were given pymetrozine (98% purity) in an aqueous solution of carboxymethylcellulose (0.5 % w/w) by gavage at daily doses of 0, 30, 100 and 300 mg/kg from day 6 through 15 post-coitum. On day 21 of presumed gestation, females were sacrificed.

There were no treatment-related maternal clinical signs or mortalities. Food consumption was slightly and dose-dependently reduced at 100 and 300 mg/kg during the treatment period (but not at the end of the study).

As shown in the Table below, mean body weights were lower in the 300 mg/kg group than controls during and after treatment, but there were no statistically significant differences compared to controls. Maternal body weight gain was reduced in the 300 mg/kg group from day 6 to 11, achieving only 57 % of the control value. However, there were very large individual differences between the dams in this group (standard deviation: +19 % at 0 mg/kg; +56 % at 300 mg/kg). Furthermore, there was a dose-related, but not statistically significant reduction in mean carcass weight (terminal body weight minus uterine weight). Net body weight changes from day 6 to 21 were dose-dependently reduced at 100 and 300 mg/kg bw/d.

Table: Body weight gain of dams in the developmental toxicity study in rats. Data taken from the RAR (2013)

	Dose (mg/kg bw/day)			
	0	**30**	**100**	**300**
Number of dams	23	22	23	22
Body weight (g); day 0	199.8 d	199.6	198.9	199.1
Body weight (g); day 21	365.6 d	361.5	359.4	350.7
Body weight gain (g), day 6 to 11	24.5 d	23.9	22.2	14.9a**
Body weight gain (g), day 0 to 21	165.8 d	161.9	160.4	151.7
Gravid uterus weight (g)	101.9 d	100.6	103.9	101.9
Carcass weight (g)	263.7 d	260.9	255.4	248.8
Net weight change from day 6 (g)	38.7 d	35.0	29.2 *	22.8 **
d = ANOVA; Dunnet-Test: 5 % (*) or 1 % (**) level; a: (range +29.3 to -3.2).				

One dam receiving 300 mg/kg bw/d had total resorptions. No treatment-related gross necropsy changes were seen. The fertility data are compiled in the Table below.

Table: Fertility data in the developmental toxicity study in rats. Data taken from the RAR (2013)

	Dose (mg/kg bw/d)			
	0	**30**	**100**	**300**
Females assigned Dams pregnant	24 23	24 22	24 23	24 23
Dams with live foetuses on day 21	23	22	23	22
Dams with total resorptions	0	0	0	**1**
Dams delivering early	0	0	0	0
Dams aborting	0	0	0	0

Overall post-implantation losses, number of live foetuses per litter and foetal weights were not affected by the exposure to pymetrozine. The incidence and type of external and visceral findings were also not affected by treatment. The total number of external abnormalities observed in rats in the group treated with 100 mg pymetrozine/kg bw/day shows a significant difference from the control in the Chi^2 statistical test. The corresponding value was, however, not statistically significant in the group treated at the highest dosage (300 mg/kg). One case of omphaloceles (umbilical hernia) occurred only at each of the highest doses levels. This abnormality has also been described in historic controls, although very infrequently (5/7968 foetuses).

The skeletal data are compiled in the Table below. The total number of skeletal malformations is higher in the group which received the highest dosage. The total number of skeletal anomalies was elevated in the group dosed at the highest level (17/157 foetuses compared with 3/158 in the control group). In this case, the higher incidence is not due to a single anomaly. Instead the incidence of many different anomalies is slightly higher (but compared with the control group, each individual finding was not statistically significant).

Table: Developmental toxicity study in rats, skeletal examination data. Foetal data-Skeletal examination (No. of foetuses/No. of affected litters). Data taken from the RAR.

	Dose (mg/kg bw/day)			
	0	**30**	**100**	**300**
No. of foetuses / litter evaluated	158/23	150/22	163/23	157/22
Total skeletal malformations	0/0	0/0	1/0	4**/3
- Displaced pubic bones (1)	0/0	0/0	0/0	4**##/3
Total skeletal anomalies	3/3	4/4	5/5	17**/12
- Thickened ischium of pelvis	0/0	0/0	0/0	2##/2(2)
- Asymmetrically shaped sternebra-5	1/1	0/0	0/0	5#/5
Total Skeletal variations	164/23	151/22	166/23	159/22
- Shortened rib 13 (3)	13/10	11/8	16/11	40#/17
- Absent ossification of metatarsal-1(3)	4/3	6/5	3/3	26**/8
- Absent ossification of prox. phalanges ant. Digit-5 (3)	2/2	2/2	4/3	16**/7
- Absent ossification of post. digit-2 (3)	17/9	9/6	20/10	45**/13
- Absent ossification of post. digit-3 (3)	4/4	3/3	9/7	29**/9
- Absent ossification of post. digit-4 (3)	5/4	5/3	12/9	25**/9
- Absent ossification of post. digit-5 (3)	36/17	29/12	51/18	74**/18
- Dumbbell-shaped thoracic vertebral centres (4)	0/0	1/1	3/3	6*/6
- Dumbbell-shaped cervical vertebral centres (3)	6/4	2/2	17*#/12	6#/5
- Bipartite cervical vertebrae centres (4)	8/7	19*/11	28**/14	23**/12

chi-square + fisher's exat: 5% (*) or 1 % (**) level;
also increased in comparison to historical controls; ## not observed in historical controls
(1 = rare change with low frequency of occurrence and minor severity)
(2 = both of these foetuses also had a displaced pubic bone)
(3 = common variation normally indicating a slight delay in foetal development (ossification))
(4 = all incidences within the range of historical control values)

The incidence of the following skeletal findings was increased at 300 mg/kg bw/day (the effect was statistical significant only with regard to the number of foetuses):

- displaced pubic bone(s) (a defect of this type is not mentioned in the list of defects occurring in historic controls);
- asymmetrically shaped sternebra-5
- thickening of the ischium;
- shortened rib 13, and absent ossification of metatarsal-1 and of proximal phalanges of anterior digit 5 and posterior digits 2 to 5;
- dumbbell-shaped thoracic vertebral centres (this variation lay in the range of historical control values) and was also increased at 100 mg/kg bw/day.
- dumbbell-shaped cervical vertebral centres (also increased at 100 mg/kg bw/day);
- bipartite cervical vertebrae centres this variation lay in the range of historical control values) and was also increased at both 100 and 30 mg/kg bw/day.

In conclusion, foetal skeletal anomalies and variations consistent with delayed ossification occurred at 100 and 300 mg pymetrozine/kg bw/day.

Developmental toxicity study in rabbits

The study was compliant with the OECD TG 414 and GLP. Nulliparous female rabbits of the Thomae Russian breed, Chbb:HM were injected with a synthetic releasing hormone and one hour later they were artificially inseminated with diluted semen from bucks of the same strain.

The day of insemination was designated day 0 of pregnancy (= day 0 p.c.) The mated females were allocated to groups of 20, randomly, weight stratified. Dosing at 0, 10, 75 and 125 mg pymetrozine (98% purity)/kg bw/day was performed daily on days 7 to 19 p.c. On day 29 of pregnancy, females were sacrificed.

Two pregnant females died at 125 mg/kg on days 16 and 19. The dam that died on day 16 had haemorrhagic perineal discharge on the previous day 15. One dam was sacrificed on day 19 after abortion. In the control group, one dam died on day 16. Food consumption was dose-dependently reduced during the treatment period (but not at the end of it) at 75 and 125 mg/kg bw/day.

Mean body weight gain were dose-related reduced compared with controls in the 75 and 125 mg/kg bw/day groups between days 7 and 19. At the end of the study there was a reduction in body weight of animals dosed with 125 mg/kg bw/day. However, no statistically significant differences were reported at the end of the study in body weight gain, mean gravid uterus weight and mean carcass weight (Table below).

Table: Body weight gain of dams in the developmental toxicity study in rabbits. Data taken from the RAR (2013)

	Dose (mg/kg bw/day)			
	0	**10**	**75**	**125**
Number of dams	16	17	17	13
Body weight (g); day 0	2382 d	2358	2373	2355
Body weight (g); day 29	2633 d	647	2562	2501 *
Body weight gain (g), day 7 to 19	87 d	92	33 *	-29 **
Body weight gain (g), day 0 to 29	251 d	289	189	146
Gravid uterus weight (g)	348	345	334	282
Carcass weight (g)	2284	2302	2228	2219
d = ANOVA; Dunnet-Test: 5 % (*) or 1 % (**) level				

No treatment-related effects on gross necropsy changes were seen. Similarly, there were no treatment-related effects on the following fertility parameters: dams pregnant, dams with live foetuses on day 29 and dams with delivering early. However, one female dosed at 75 mg/kg bw/day and female dosed at 125 mg/kg bw/day presented 1 and 3 total resorption, respectively. One dam dosed at 125 mg/kg bw/day presented abortion.

The Table below summarises the findings from the caesarean sections. The following parameters did not differ between groups: preimplantation losses, pregnant females, corpora lutea/dam, dead foetuses, resorptions/dam, females with litter and implants/dam. Mean litter size was also reduced at 125 mg/kg bw/day. Early resorptions and post-implantation losses were dose-dependently increased in the 75 and 125 mg/kg bw/day.

Table: Developmental toxicity study in rabbits, caesarean section data. Data taken from the RAR (2013)

	Dose (mg/kg bw/day)			
	0	**10**	**75**	**125**
Females pregnant	16	17	18	16
Corpora lutea / dam (mean)	8.3	9.0	8.8	7.8
Implants / dam (mean)	6.8	7.2	6.9	6.3
Pre-implantation loss [%]	20.3	20.5	22.0	20.5
Post-implantation loss [%]	3.7	6.1	13.1	26.3
Dead foetuses	0	0	0	0
Resorptions / dam - Early (mean)	0.3	0.4	0.8	1.8

Resorptions / dam - Late (mean)	0.1	0.1	0.1	0.0
Females with litter	16	17	17	13
Live foetuses / dam (mean)	6.4	6.8	6.5	4.5
Mean pup body weight [g]	38.2	36.8	37.9	37.4
(1) animals that died or had to be sacrificed are excluded: 1 control and 3 high dose dams				

No treatment-related effects on gross necropsy changes were seen. There were also no treatment-related effects on the following fertility parameters: dams pregnant, dams with live foetuses on day 29 and dams delivering early. However, one female dosed at 75 mg/kg bw/day and female dosed at 125 mg/kg bw/day presented 1 and 3 total resorptions, respectively. One dam dosed at 125 mg/kg bw/day presented abortion.

Foetal body weights were not affected by exposure to pymetrozine. The total number of external abnormalities is significantly higher in the group receiving the highest dosage (6/72 foetuses compared with 0/103 foetuses in the control group). At the other dose levels, a significant effect could not be confirmed. Selected foetal external or visceral malformations and anomalies or variations are reported in the Table below. The foetal incidence of forelimb position anomalies was dose-related increased. This anomaly was significantly higher in the 125 mg/kg group and also increased in comparison to historical controls for both the foetal and the litter incidence in the both highest dose groups. All other findings were not regarded as treatment-related.

Table: Developmental toxicity study in rabbits, foetal external and visceral data. Data taken from the RAR (2013)

	Dose (mg/kg bw/day)			
	0	**10**	**75**	**125**
Foetuses evaluated / Litters evaluated	103 / 16	116 / 17	110 / 17	72 / 13
Total external abnormalities	0 / 0	1/ 1	4 / 2	6** / 4
- position anomaly forelimb (A, 1)	0 / 0	1 / 1	4 #/ 2	6 **#/ 4
Total visceral malformations/abnormalities	0 / 0	3 / 2	1 /1	2 / 2
- domed head (A)	0 / 0	1 / 1	0 / 0	0 / 0
- external hydrocephalus (M)	0 / 0	2 / 1	0 / 0	0 / 0
- internal hydrocephalus (M)	0 / 0	1 / 1	0 / 0	0 / 0
- small gall bladder (A)	0 / 0	1 / 1	0 / 0	0 / 0
- small liver (A)	0 / 0	0 / 0	0 / 0	1 / 1
- renal aplasia (M)	0 / 0	0 / 0	1 /1	1 / 1
- ureter aplasia (M)##	0 / 0	0 / 0	1 /1	1 / 1
(M) = malformation, (A) = Anomaly, 1 = flexure of the forepaw at the wrist, chi-square + fisher's exat: ** = p< 0.01; # also increased in comparison to historic controls; ## not observed in historic controls				

Skeletal malformations occurred neither in the groups treated with pymetrozine nor in the control group. Anomalies appeared at the highest dosage with a statistically significantly increased incidence of 28/72. Corresponding changes appeared rarely in the group treated with 75 mg/kg bw/d. Skeletal changes that were considered treatment-related are shaded in the Table below. The increased incidences of total skeletal anomalies and variations at the highest dosage are both clear and statistically significant.

Table: Developmental toxicity study in rabbits, skeletal examination data. Data taken from the RAR (2013)

	Dose (mg/kg bw/day)			
	0	**10**	**75**	**125**
Foetuses evaluated / Litters evaluated	103 / 16	116 / 17	110 / 17	72 / 13
Total Malformations	0 / 0	0 / 0	0 / 0	0 / 0
Total Mean Anomalies	11 / 7	23 / 12	15 / 8	28*# / 11
- fused sternebrae 2-3	0 / 0	0 / 0	0 / 0	6**# / 2
- fused sternebrae 3-4	5 / 4	6 / 3	3 / 3	18**# / 8
- fused sternebrae 4-5	2 / 2	7 / 6	4 / 3	19**# / 8
- reduced pubis	0 / 0	0 / 0	2 ##/ 2	3## / 2
Total Variations	80 / 16	95 / 17	90 / 17	67 / 13
- additional caudal vertebral centres	14 / 10	17 / 10	21 / 13	31** / 10
- additional rib 13	2 / 2	2 / 2	11 / 6	21**# / 7
- poor ossification of metacarpal-1 (1)	1 / 1	2 / 2	2 / 2	10** / 5
- poor ossification of talus (1)	1 / 1	2 / 1	3 / 2	8** / 4
- poor ossification of medial phalanx of anterior digit-5 (1)	9 / 6	19 / 11	14 / 9	22** / 9
(1) = common variation normally indicating a slight delay in foetal development / ossification chi-square + fisher's exat: ** = p< 0.01, * = p< 0.05; # also increased in comparison to historic controls, ## not observed in historic controls				

In conclusion, the incidences of the following skeletal findings were increased at 125 mg/kg bw/day: i) fused sternebrae; ii) additional caudal vertebral centres (variations); and, iii) poor ossification of metacarpal-1, talus, and medial phalanx of anterior digit-5 (variations consistent with a slight delay in foetal development/ossification). However, the incidences of the following skeletal findings were increased at 125 mg/kg and 75 mg/kg: i) reduced pubis (anomaly); and, ii) additional 13th rib(s) (variation).

Developmental neurotoxicity study in rats

In this study, dose levels of 0, 100, 500 and 2500 ppm (approximately 8.1, 38.7 and 173.1 mg/kg bw/day) were administered via diet from day 7 of gestation to day 22 postpartum. Due to excessive toxicity, the high dose group was terminated and no developmental neurotoxicity examination was performed. Maternal toxicity at 2500 ppm included clinical signs (hunched posture, piloerection and subdued behavior) and bodyweight loss. At this dose, there was also high neonatal mortality with 4 out 13 dams with whole litter losses and 31% live born pups either dead or missing by day 5 *post-partum*.

During gestation, maternal body weight gain was around 10% lower at the mid dose level of 500 ppm compared to controls, but this was not statistically significant and consequently was considered to be of no toxicological concern.

At 500 ppm an increase in complete litter losses was noted [2/30 (6.7%), 3/30 (10%), 5/29 (17.2%) for controls, 100 ppm and 500 ppm, respectively]. Moreover, pup mortality (dead or missing, presumed dead) was dose-dependently increased during postnatal days 1-5. Overall, pup mortality seemed comparatively high even at the control level. There were no treatment effects on offspring body weight (gain), food consumption, developmental landmarks, clinical signs, functional observational battery, motor activity, acoustic startle responses, learning and memory or brain weights.

For F1 there were no treatment-related clinical observations and no statistically significant differences from controls in the following parameters: haematology, functional observational battery, developmental landmarks (preputial separation or vaginal opening), locomotor activity and learning and memory.

At the lowest dose level of 100 ppm significant brain morphometry changes were observed (increased thickness of corpus callosum in males on day 63 post-natal and dorsal cortex in females day 12 postnatal). Brain morphometric changes were also observed at 500 ppm as an increased thickness of the *corpus callosum* also on postnatal day 12 in males and of inner granular and molecular layer of the pre-pyramidal fissure in the cerebellum postnatal day 63 in males (Table below):

Table: Morphometric changes observed in the F1 brains of the neurodevelopmental toxicity study in rats. The equipment was calibrated with a graticule or a stage micrometre. However, no units (such as µm) are given in the report, hence the numbers need to be considered as arbitrary units. Data taken from the RAR (2013)

	Males			**Females**		
Dose level (ppm):	**0**	**100**	**500**	**0**	**100**	**500**
D12 dorsal cortex thickness (level 5)	1.02	1.06	1.07	1.00	1.10**	1.09*
D12 corpus callosum thickness (level 4)	0.60	0.64	0.69*	0.62	0.65	0.64
D63 corpus callosum thickness (level 4)	0.32	0.35*	0.36*	0.31	0.33	0.33
D63 Cerebellum pre-pyramidal fissure thickness of inner granular layer	144	162	172*	156	160	162
D63 Cerebellum pre-pyramidal fissure thickness of molecular layer	206.5	209.9	219.8*	198.0	204.4	210.3

Comparison with criteria

There are no appropriate epidemiological studies available on developmental or fertility effects in humans and therefore classification of pymetrozine in Category 1A is not warranted.

<u>Fertility</u>

The rat dietary 2-generation reproduction study displayed no adverse effects on sexual function or fertility. However, a number of repeated dose toxicity studies (but not all) found mild testicular toxicity in rats and dogs. These testicular effects did not always appeare in absence of parental systemic toxicity, ranging from a mild (7-17%) reduction in body weight to mortality and clinical signs.

RAC noted that in some cases these effects were of low incidence, although they were consistently observed in two species (rat and dog) and in studies of different durations (28 and 90 days and 1 year).

RAC also noted that the Guidance on the Application of the CLP Criteria (2017) establishes that *"adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes"*; which might be the case for the effects reported in one of the three 28-day study in rats and in the 90-day study in dogs, but not for

the cases of the two remaining 28-day studies in rats, or the 28-day and 1-year toxicity studies in dogs.

According to the Guidance on the Application of the CLP Criteria (2017) *"it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity"*. Therefore, RAC considers the testicular toxicity reported in the repeated toxicity studies as relevant for classification.

No reduction in fertility was reported in the 2-generation reproduction toxicity study, which reduces the level of concern and therefore *"clear evidence of an adverse effect on sexual function and fertility"* is not met. Therefore classification in Category 1B is not warranted. Alternatively, the relatively low incidence of the testicular effects reported in several repeated toxicity studies is considered by RAC as evidence *"not sufficiently convincing to place the substance in Category 1"* and therefore proposes the classification of pymetrozine within category 2, toxic to reproduction (fertility).

Development

The developmental toxicity study in rats showed statistically significant differences in the number of foetuses (but not number of litters) in several skeletal malformations, abnormalities and especially variations in animals exposed to 300 mg/kg bw/day and in a few skeletal variations in animals exposed to 100 mg/kg bw/day (see Table above on Developmental toxicity study in rats, skeletal examination). The incidences of skeletal malformations and abnormalities were either not observed in the HCD or higher than in the HCD. According to a position paper submitted by the industry during public consultation, a displacement in pubic bones was tentatively categorised as a malformation of minor severity. However, a subsequent study from the same laboratory showed this finding in control groups and the categorisation was downgraded to an anomaly and then to a variant. Moreover, according to this position paper the incidence of this pubic bone displacement (4/150 = 2.7%) was very close to the highest incidence reported in three studies performed in 1992, 1994 and 1997. This position paper also stated that thickening of the ischium was categorised as an anomaly and that, although no clinical signs were observed, certain maternal toxicity was reported, as a reduction of 41% and 25% of corrected body weight change in dams exposed to 300 and 100 mg/kg bw/day, respectively; or reductions of 27% and 10% of body weight change in dams exposed to 300 and 100 mg/kg bw/day, respectively. This toxicity was used by the Industry as argument to suggest that the reported skeletal alterations were indeed secondary to maternal toxicity. The CLP Regulation states that the *"developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity"*. RAC notes that this condition has not meet in this case (unequivocally demonstration that a link between maternal and developmental toxicity) and therefore considers the effects reported in rats as relevant for classification, although RAC also notes that the effects at 300 mg/kg bw/day were reported concurrently with a severe reduction in maternal weight and only in the number of affected foetuses (not in number of litters); which reduces the level of concern.

The developmental toxicity study in rabbits displayed the following skeletal findings at the exposure level of 125 mg/kg bw/day: fused sternebrae and reduced pubis (anomalies), additional caudal vertebral centres, poor ossification and additional 13th rib (variations); while at a dosage level of 75 mg/kg bw/day, reduced pubis and additional 13th rib were also found. On the other hand, there is no robust data for comparing the incidence of the effects reported at 75 mg/mg bw/day with HCD because no data was found for the occurrence of an

additional 13rd rib and for reduced pubis in the RAR, suggests that the effect has not previously appeared in the HCD. During the PC the Industry provided HCD with incidences lower than what was reported in the study, but it was not specifically stated that this data was from the same facility that performed the study. Therefore, RAC does not consider the effects reported in rabbits at 125 mg/kg bw/day relevant for classification and the effects reported in the same species at 75 mg/kg bw/day to be of minor concern.

The neurodevelopmental toxicity study in rats reported morphometric brain changes in the F1 generation. RAC considers these morphometric changes as not relevant for classification since no further effects on motor activity, auditory startle, learning, memory and functional observational battery were observed. However, RAC noted a dose-dependent increase in complete litter losses in this same study (6.7, 10 and 17.2% for control, 100 and 500 ppm, respectively. This effect was considered by RAC as relevant for classification purposes.

In summary, RAC notes an array of developmental effects of minor concern (the skeletal variations and anomalies reported in the developmental toxicity studies in rat and rabbit, the dose-dependent increases in post-implantation loss and early resorptions in developmental toxicity study in rabbit, and the dose-dependent increase in complete litter losses noted in the developmental neurotoxicity study in rats) that considered individually, would probably not trigger classification. However, considering all these effects together, they demonstrate developmental toxicity potential of pymetrozine.

Conclusion

RAC supports the DS's proposal for the classification of pymetrozine as toxic **as Repr. 2; H361fd, "Suspected of damaging fertility and the unborn child".**

Supplemental information - In depth analyses by RAC

Supplementary study 4: Oral 90-day study in rat

Male rats orally exposed during 90 days up to 360 mg pymetrozine/kg bw/day exhibited no detectable testicular toxicity. The study compliant with OECD TG 408 and GLP.

Supplementary study 5: Oral 90-day study in mouse

Male mice orally exposed during 90 days up to 1000 mg pymetrozine/kg bw/day exhibited no detectable testicular toxicity. The study was compliant with OECD TG 408.

Supplementary study 6: Oral 28-day study in dog

Male dogs orally exposed during 28 days up to 50 mg pymetrozine/kg bw/day exhibited no detectable testicular toxicity. The study was compliant with OECD TG 407 and 409.

Supplementary study 7: Oral 28-day study in dog

Groups of 6 male beagle dogs were fed with 0, 100, 500 and 2000 ppm pymetrozine during a period of 4 weeks. Neither toxic clinical signs nor deaths were found during the study. Body weights and food consumption were comparable to the controls. The NOAEL was set at 500 ppm and adverse effects were reported only for the dosage level of 2000 ppm (61 mg/kg bw/day).

At the highest dosage level (2000 ppm = 61mg pymetrozine/kg bw/day), blood biochemical examination revealed a notable increase in alkaline phosphatase (ALP) activity in one animal with relatively severe hepatic lesions. Organ weight measurement exhibited a significant

increase in relative liver weight. Histopathological examination showed inflammatory cell infiltration in the liver in 3 animals. **Another animal showed giant cell formation in the testis, and consequently disclosed decrease of sperm and increase of degenerated spermatogenic cells in the epididymis.**

According to the RAR, there were no statistically significant changes in haematology, blood hormone assay, or necropsy. However, the DS highlighted in the CLH-dossier **that low dihydrotestosterone levels were found in the animals exposed to 2000 ppm**. RAC considers indeed the differences in dihydrotestosterone levels as non-relevant according to the figures found in the RAR of pymetrozine (060±0.15 versus 0.50±0.14 after 2 weeks of exposure and 0.59±0.30 vs 0.55 ±0.19 for 6 animals in all cases).

Industry argued in a position paper submitted during the public consultation that in the assessment of this study it was ignored that in the pre-study measurement these animals generally had lower basal levels of dihydrotestosterone and that testicular observations are common histopathological findings in beagle dogs and one single incidence of these findings cannot be considered to reflect an effect of the treatment.

Supplementary study 8: Oral 90-day study in dog

The study was compliant with OCDE TG 409 and GLP. Groups of 4 male and 4 female Beagle dogs were given diets containing 0, 100, 500, or 2500 ppm pymetrozine for 3 months. These dosage levels corresponded to 3.12/3.24, 13.90/14.55 and 53.43/60.24 mg pymetrozine/kg bw/day for males/females, respectively.

One high dose female dog was sacrificed *in extremis* in week 5. Clinical signs were seen in individual animals of the high dose group. Body weights were affected in the high dose group animals with high inter-individual variation ranging from severe (34% below pre-test) to transient loss to normal development.

Red blood cell parameters were decreased in males and females of the high dose group. Severe anaemia was noted in two high dose females. Liver weights were increased at 500 ppm (absolute) and 2500 ppm (relative) and spleen weights were elevated at 2500 ppm. According to the RAR, tubular atrophy of the testes at 500 ppm together with a **32% reduction in testes weights were noted in animals exposed to 2500 ppm. Also, reduced spermatogenesis (2/4 animals), spermatic granuloma (1/4 animals) and and lymphohistiocytic infiltration (1/4 aniamls) in the epididymis** were reported. According to the Industry these findings are common in beagle dogs and cannot be considered to reflect an effect of treatment, particularly because there was no evidence of a dose-response relationship. During the EFSA assessment the notifier supplied a table with HCD for these testicular impairments; however, RAC noted that according to the RAR, no relevant HCD from the performing laboratory was indeed submitted because the HCD mentioned by the notifier is published literature from other laboratories.

Supplementary study 9: Oral 1-year study in dog

The study was compliant with Guideline 409 and GLP. The test material was administered to Beagle dogs (4 animals per sex and dose level) at dietary levels of 0, 20, 200 or 1000 ppm. The calculated daily average compound intakes were approximately 0.57, 5.33 and 27.9 mg/kg bw/d in males and 0.57, 5.03 and 27.4 mg/kg bw/day in females for the 10, 200 and 1000 ppm feeding levels, respectively.

Treatment had no effect on mortality. A decrease of body weight gain or transient body weight loss was observed in males and females at 1000 ppm. At the end of the study the body weight of treated males was only 94% of the body weight of control animals. After 26 weeks of treatment, one female had a severe anaemia, but all alterations were completely reversible during the following 6 months of treatment. Red blood cell parameters were also slightly decreased in a male of this group.

The liver weights were slightly increased at 200 and 1000 ppm in both sexes. The reduction in the absolute testes weight was statistically significant at 200 and 1000 ppm, although no dose-response relationship was observed because at both dosage levels the reduction was around 17%.

Microscopic findings were limited to animals receiving 1000 ppm. They consisted of myopathy in 2 male dogs, increased inflammatory cell infiltration in the liver in the males associated with a focal fibrosis in one case, and increased splenic or hepatic haemosiderosis in some animals. One of the animals affected by myopathy died of heavy acute bronchopneumonia. Some of the above changes (inflammatory cell infiltration of the liver or increased haemosiderosis and myopathy) were still present at the end of the recovery period. Also, unilateral tubular atrophy was seen in one male; bilateral occurrence of spermatic giant cells in the testicular spermatogenic epithelium as well as atrophy of prostatic glandular tissue of another male were reported.

During the EFSA assessment, the RMS provided a revised assessment of the 1-year dog dietary study, including summary tables with numerical values and the response from the notifier. This new assessment considered that the testicular effects at 200 ppm (unilateral tubular atrophy, bilateral spermatogenic giant cells and atrophy of glandular tissue of the prostate) were within the HCD range. However, RAC noted that according to the RAR, no relevant HCD from the performing laboratory was submitted in the statement. The HCD mentioned by the notifier was published literature from other laboratories.

The Industry also argued that testicular observations are common histopathological findings in beagle dogs and one single incidence of these findings cannot be considered to reflect an effect of the treatment and that there was no reduction in relative testis weight; this suggested that the reduction in absolute testis weight simply reflected the lower terminal body weight rather than a direct effect of treatment.

Supplementary study 10: Oral 18-month study in mouse

Male mice orally exposed during 18 months up to 678 mg pymetrozine/kg bw/day exhibited no detectable testicular toxicity (first Table under the carcinogenicity section). The study was compliant with OECD TG 451 and GLP.

Supplementary study 11: Oral 2-year study in rat

Male rats orally exposed during 2 years up to 128 mg pymetrozine/kg bw/day exhibited no detectable testicular toxicity. The study was compliant with OECD TG 453 and GLP.

Summary of supplementary studies

The table below provides a summary of the main testicular findings described in all available repeated dose toxicity studies.

Table: Overall of the main testicular toxicity findings reported in the available repeated dose toxicity studies with pymetrozine. The table is focused on findings reported for males.

Study	Dose	Testicular toxicity	General toxicity (in males)
Oral 28-day study in rat	600	Reduced spermatogenesis in the testes in 9/10 animals Reduced number of spermatozoa in the epididymis in 7/10 animals	↓ 7% bodyweight Mild anaemia ↑40% liver weight
Oral 28-day study in rat	254.7 mg/kg bw/day	↑22% relative testis weight ↑22% relative epididymis weight Testicular atrophy (1/6 animal) Mononuclear cell infiltration in epididymis (1/6 animal) Decrease of sperm in epididymis (1/6 animal). Alterations in hormone levels: • ↓ 71% testosterone ↓ 34% dihydrotestosterone • ↓ 31% luteinising hormone • ↑ 29% thyroxine Alterations in hormone levels (without outsider animals): • ↓ 38% testosterone • ↓ 13% dihydrotestosterone • ↓ 31% luteinising hormone • ↑ 29% thyroxine	↓ 17% bodyweight ↑19% relative liver weight Centrilobular hepatocellular hypertrophy
Oral 28-day study in rat	700 mg/kg bw/day	Reduced spermatogenesis and atrophy of seminiferous tubules (1/5 animals) Reduction of spermatozoa in epididymis in 5/5 males	Transient clinical signs (red ears in some animals, penis prolapse, and piloerection associated with emaciated condition) Practically totally failed to gain any weight
Oral 28-day study in dog	61 mg/kg bw/day	Giant cell formation in the testis and decrease of sperm and increase of degenerated spermatogenic cells in the epididymis (1/6 animals)	Inflammatory cell infiltration in the liver (3/6 animals)
Oral 90-day study in dog	53 mg/kg bw/day	↓32% reduced testes weights Reduced spermatogenesis (2/4 animals), Spermatic granuloma and lymphohistiocytic infiltration in the epididymis (1/4 animals)	Clinical signs (1 female mortality) Severe anaemia

			Body weights with high inter-individual variation
1-year study in dog	28 mg/kg bw/day	↓17% absolute testis weight Unilateral tubular atrophy (1/4 animals) Spermatic giant cells in the testicular spermatogenic epithelium and atrophy of prostatic glandular tissue (1/4 animal)	↓ 6% bodyweight Myopathy in (2/4 males)

10.11 Specific target organ toxicity-single exposure

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.12 Specific target organ toxicity-repeated exposure

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.13 Aspiration hazard

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

11. EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 ACUTE AQUATIC HAZARD

Table 20: Summary of relevant information on acute aquatic toxicity of pymetrozine

Method	Species	Test (endpoint, design, duration)	Results[1]	Key or Supportive study	Remarks	Reference
OECD 203	*Oncorhynchus mykiss*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	key	none	Grade (1993); 928265
OECD 203	*Oncorhynchus mykiss*	Mortality flow-through 96 hours	LC_{50} > 128 mg/L (m)	supportive	none	Boeri (1994); 444-CG
OECD 203	*Cyprinus carpio*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	None	Grade (1993); 928268
OECD 203	*Lepomis macrochirus*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	None	Grade (1993); 928267
OECD 203	*Lepomis macrochirus*	Mortality flow-through 96 hours	LC_{50} > 134 mg/L (m)	supportive	none	Boeri (1994); 443-CG
OECD 203	*Ictalarus punctatus*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	none	Grade (1993); 928266
OECD 203	*Cyprinodon variegatus*	Mortality flow-through 96 hours	LC_{50} > 117 mg/L (m)	supportive	none	Boeri (1994); 446-CG
OECD 202	*Daphnia magna*	Immobility static 48 hours	EC_{50} > 100 mg/L (nom)	supportive	none	Grade (1993); 928270
OECD 202	*Daphnia magna*	Immobility flow-through 48 hours	EC_{50} = 87 mg/L (m)	key	none	Boeri (1994); 442-CG
EPA 72-3	*Mysidopsis bahia*	flow-through 96 hours	EC_{50} = 61.7 mg/L (m)	supportive	none	Boeri (1994); 445-CG
EPA 72-3	*Crassostrea virginica*	Shell deposition flow-through 96 hours	EC_{50} = 3.06 mg/L (m)	key	none	Boeri (1994); 447-CG
OECD 201	*Scenedesmus subspicatus*	Growth inhibition Static 72 hours	EbC_{50} = 47.1 mg/L (m,end) ErC_{50} > 84.6 mg/L (m,end) NOErC = 7.5 mg/L (m)	key	none	Grade (1993); 928272
OECD 201	*Pseudokirchneriella subcapitata*	Growth inhibition Static 120 hours	EbC_{50} = 21.6 mg/L (m,initial) ErC_{50} > 96.7 mg/L (m,initial) NOErC = 6.28 mg/L (m)	key	none	Boeri (1994); 668-CG

[1] Indicate if the results are based on the measured or on the nominal concentration

11.1.1 Acute (short-term) toxicity to fish

The acute toxicity of pymetrozine (CGA 215944 tech.) to rainbow trout (*Oncorhynchus mykiss*), catfish (*Ictalarus punctatus*) , carp (*Cyprinus carpio*) and bluegill sunfish (*Lepomis macrochirus*) has been investigated by exposing fish under static conditions to concentrations between 0 and 100 mg/L of the test

substance followed by a 96 hours observation period. Up to 100 mg/L no mortalities occurred and no other symptoms were observed. Measured initial and final concentrations were between 91 % and 101 % for rainbow trout, 88 % and 104 % for catfish, 83 % and 92 % for carp and 83 and 103 % for bluegill sunfish of the nominal values.

The acute toxicity of pymetrozine (CGA 215944) to rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*) and sheepshead minnow (*Cyprinodon variegatus*) has been investigated by exposing fish under flow-through conditions. For rainbow trout up to concentrations of 128 mg/L no treatment related mortalities or effects were observed. Mean measured test concentrations were between 95 % and 104 % of the nominal values. For bluegill sunfish were no treatment related mortalities or effects reported up to concentrations of 134 mg/L. Mean measured test concentrations were between 91 % and 105 % of the nominal values. For the marine species sheepshead minnow were no treatment related mortalities or effects observed in a concentration range up to 117 mg/L. Mean measured test concentrations were between 88 % and 106 % of the nominal values.

Taking into account all acute studies for fish, where the lowest LC_{50} >100 mg/L was determined, no acute classification is necessary.

11.1.2 Acute (short-term) toxicity to aquatic invertebrates

In an acute static toxicity test with *Daphnia magna*, the 48-hour EC_{50} was determined to be > 100 mg pymetrozine (CGA 215944)/L, the highest concentration tested. The 48-hour NOEC was < 5.8 mg pymetrozine/L, the lowest concentration tested. At the start of the test, the measured concentrations of pymetrozine were in the range 79 to 98 % of the nominal values and at the end of the test were in the range 92 to 103 %. The limit of quantification for this study was not reported. Nominal concentrations were used for the calculation and reporting of results.

In a second study under flow through conditions with *Daphnia magna*, the 48-hour LC_{50} for pymetrozine was 87.0 mg pymetrozine (CGA 215944)/L with 95 % confidence interval of 67.9 – 131.0 mg pymetrozine /L. The 48-hour NOEC was < 19.2 mg pymetrozine /L, the lowest tested concentration. The mean measured concentrations of pymetrozine were in the range 92 to 99 % of the nominal values. The limit of detection in this study was 4.00 mg pymetrozine /L. The results were based on mean measured concentrations.

In another study under flow through conditions with *Mysidopsis bahia*, based on mean measured concentrations, the 96 hour LC_{50} was 61.7 mg pymetrozine /L, with 95 % confidence intervals of 52.4 to 73.1 mg pymetrozine /L. The 96-hour no-observed effect concentration (NOEC) was determined to < 18.7 mg pymetrozine /L, the lowest concentration tested. Mean measured concentrations ranged from 94 to 101 % of nominal values. The limit of detection in this study was 0.5 mg pymetrozine /L. Mean measured concentrations were used for the calculation and reporting of results.

Furthermore, in an acute toxicity test with the oyster *Crassostrea virginica*, the 96 hour EC_{50} was 3.06 mg pymetrozine /L, with 95 % confidence intervals of 2.13 to 4.38 mg pymetrozine /L. The 96-hour NOEC was determined to be 0.768 mg pymetrozine /L. Mean measured concentrations, calculated from the average of all samples, ranged from 94 to 112 % of nominal concentrations. Mean measured concentrations were used for the reporting of the results.

Taking into account all acute studies for invertebrates, where the lowest EC_{50} = 3.06 mg/L was determined, no acute classification is necessary.

11.1.3 Acute (short-term) toxicity to algae or other aquatic plants

The growth inhibition of algae (*Scenedesmus subspicatus*) by pymetrozine was studied in a static system at concentrations between 1.23 and 100 mg/L. The EbC_{50} and NOEbC after 3 days exposure were calculated to be 47.1 mg/L and 7.5 mg/L, respectively. These values are based on measured end concentrations, which were

after 3 days between 57 % and 86 % of the initial concentrations at the lowest and the highest dose level, respectively.

In a second study the toxicity of pymetrozine to the freshwater algae *Selenastrum capricornutum* was evaluated in a static system at concentrations between 6.3 and 100 mg/L. The ErC_{50} and NOErC after 5 days exposure were calculated to be 21.7 mg/L and 6.3 mg/L, respectively. These values are based on measured concentrations; analysis of the test medium showed recoveries between 93 and 100 % at the beginning but less than 70 % of the nominal values at the end of the test period.

Furthermore, the effect of pymetrozine on the growth of the duckweed *Lemna gibba* was studied for 14 days under static conditions at pH 5 and continuous illumination. Nominal test concentrations were 8.5 to 130 mg/L; the initial measured concentrations amounted to 71-84 % of the nominal concentrations (between 6.1 and 109 mg/L) but showed a strong decrease to below the analytical detection limit (5.1 mg/L) after 14 days, indicating some type of absorption or degradation. The EC_{25}, EC_{50} and NOEC values were 68, > 109 and 49 mg/L, respectively. They are determined on basis of effects on the frond production after 14 days exposure and refer to initial measured test concentrations. The NOEC is based on the number of non-chlorotic fronds.

Taking into account all acute studies for algae and aquatic plants, where the lowest ErC_{50} > 84.6 mg/L was determined, no acute classification is necessary.

11.1.4 Acute (short-term) toxicity to other aquatic organisms

No studies with other aquatic organisms in addition to the above mentioned are available.

11.2 LONG-TERM AQUATIC HAZARD

Table 21: Summary of relevant information on chronic aquatic toxicity of pymetrozine

Method	Species	Test (endpoint, design, duration)	Results	Key or Supportive study	Remarks	Reference
OECD 204	*Oncorhynchus mykiss*	growth Flow through 21 days	NOEC = 35.2 mg/L (m)	supportive	none	Grade (1993); 928269
EPA 72-4, OECD 210	*Oncorhynchus mykiss*	ELS Flow through 90 days (60 days post hatch)	NOEC = 11.7 mg/L (m)	key	none	Boeri et al (1995); 504-CG
OECD 202	*Daphnia magna*	Reproduction Semi static 21 days	NOEC = 0.1 mg/L (nom)	supportive	none	Grade (1993); 928271
EPA 72-4, OECD 202	*Daphnia magna*	Reproduction Flow through 21 days	NOEC = 0.025 mg/L (m)	key	none	Boeri et al (1995); 449-CG
OECD 201	*Pseudokirchneriella subcapitata*	Growth inhibition Static 120 hours	EbC_{50} = 21.6 mg/L (m,initial) ErC_{50} > 96.7 mg/L (m,initial) NOErC = 6.28 mg/L (m)	key	none	Boeri (1994); 668-CG
OECD 201	*Scenedesmus subspicatus*	Growth inhibition Static 72 hours	EbC_{50} = 47.1 mg/L (m,end) ErC_{50} > 84.6 mg/L (m,end) NOErC = 7.5 mg/L (m)	key	none	Grade (1993); 928272
EPA 122-2	*Lemna gibba*	Frond number Static 14 days	EC_{50} > 109 mg/L (m,initial) NOEC = 49.2 mg/L (m,initial)	supportive	none	Boeri et al (1995); 669-CG

[1] Indicate if the results are based on the measured or on the nominal concentration

11.2.1 Chronic toxicity to fish

In a first study the chronic toxicity of pymetrozine to rainbow trout has been investigated by exposing fish under flow through conditions for 21 days to pymetrozine concentrations between 0 and 100 mg/L. No mortalities occurred; at the highest applied concentration (120 mg/L, mean measured), however, the rates of weight gain and of length increase were decreased. No other treatment related symptoms were observed. Thus, the LC_{50} was > 120 mg/L, the LOEC and NOEC values are characterized by values of 120 mg/L and 35.2 mg/L, respectively.

As a second study in a fish early life stage test with pymetrozine, egg hatching success was unaffected at all concentrations of pymetrozine tested. No other sub-lethal effects were noted in any test vessel during the test, and the times to hatch, swim up and feeding (day 41) were identical for the controls and all treatments. The NOEC was 11.7 mg pymetrozine /L, the highest concentration tested.

11.2.2 Chronic toxicity to aquatic invertebrates

In a first semi static reproduction test with *Daphnia magna*, the 21-day EC_{50} immobilisation for pymetrozine was 0.6 mg pymetrozine /L, based on nominal concentrations. The 21-day NOEC for reproduction was 0.1 mg pymetrozine /L. The measured concentrations of pymetrozine in the new test media were in the range 63 to

98 % of the nominal values and the measured concentrations in the old media were in the range 72 to 143 %. Nominal concentrations were used for the calculation and reporting of the results.

In a second reproduction test with *Daphnia magna* under flow through conditions, the 21-day LC_{50} for pymetrozine was 0.0735 mg pymetrozine /L with 95 % confidence interval of 0.064 to 0.0847 mg pymetrozine /L, based on mean measured concentrations. The 21-day NOEC reproduction was 0.0251 mg pymetrozine /L. The measured concentration of the test item in the test media were in the range 78 to 94 % of the nominal values and concentrations were stable during the test. Measured concentrations were used for the calculation and reporting of the results. Survival of the parent animals was 98 % in the control. Survival in the 0.0251, 0.0515, 0.102, 0.234 and 0.462 mg pymetrozine /L treatment groups was 98, 80, 25, 0 and 0 %, respectively, at test termination.
The first brood juveniles were observed on day 9 in the control. The mean time to first brood was significantly different from the control at ≥ 0.102 mg pymetrozine /L. The mean number of juveniles per surviving adult showed a statistically significant inhibitory effect on the reproduction of *D. magna* over 21 days at concentrations of 0.0515 mg pymetrozine /L and above.

Taking into account all long term studies for invertebrates, where the lowest NOEC= 0.0251 mg/L was determined, a long term classification is necessary.

11.2.3 Chronic toxicity to algae or other aquatic plants

The growth inhibition of algae (*Scenedesmus subspicatus*) by pymetrozine was studied in a static system at concentrations between 1.23 and 100 mg/L. The EbC50 and NOEbC after 3 days exposure were calculated to be 47.1 mg/L and 7.5 mg/L, respectively. These values are based on measured end concentrations, which were after 3 days between 57 % and 86 % of the initial concentrations at the lowest and the highest dose level, respectively.

In a second study the toxicity of pymetrozine to the freshwater algae *Selenastrum capricornutum* was evaluated in a static system at concentrations between 6.3 and 100 mg/L. The ErC50 and NOErC after 5 days exposure were calculated to be 21.7 mg/L and 6.3 mg/L, respectively. These values are based on measured concentrations; analysis of the test medium showed recoveries between 93 and 100 % at the beginning but less than 70 % of the nominal values at the end of the test period.

Furthermore, the effect of pymetrozine on the growth of the duckweed *Lemna gibba* was studied for 14 days under static conditions at pH 5 and continuous illumination. Nominal test concentrations were 8.5 to 130 mg/L; the initial measured concentrations amounted to 71-84 % of the nominal concentrations (between 6.1 and 109 mg/L) but showed a strong decrease to below the analytical detection limit (5.1 mg/L) after 14 days, indicating some type of absorption or degradation. The EC_{25}, EC_{50} and NOEC values were 68, > 109 and 49 mg/L, respectively. They are determined on basis of effects on the frond production after 14 days exposure and refer to initial measured test concentrations. The NOEC is based on the number of non-chlorotic fronds.

11.2.4 Chronic toxicity to other aquatic organisms

No studies with other aquatic organisms in addition to the above mentioned are available.

11.3 BIOACCUMULATION

11.3.1 Estimated bioaccumulation

No estimation was performed for pymetrozine, because of log POW -0.19 at 25°C, pH 7.

11.3.2 Measured partition coefficient and bioaccumulation test data

Studies ascertaining the bioconcentration potential of pymetrozine were not conducted since the physico-chemical properties of pymetrozine (log POW -0.19 at 25°C, pH 7) and its more polar metabolites (log POW << 3) indicate that the inherent potential for bioconcentration is low.

11.4 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES

Table 22: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
OECD 301 B	Not readily biodegradable (2% degradation within 29 days)	key	none	Grade, R. (1995)

11.4.1 Ready biodegradability

Pymetrozine has shown a biodegradation of 2 % in 29 days in a test according to OECD 301 B and has therefore to be regarded as not readily biodegradable.

11.4.2 BOD_5/COD

BOD5/COD tests are not available.

11.4.3 Other convincing scientific evidence

The behaviour of pymetrozine in two water/sediment-systems (Pond Fröschweiher, Rhine Möhlin) has been investigated in two studies with different radioactive labelling of the parent compound (Reischmann 1995a, Schulze-Aurich 1996b). The degradation rates have been recalculated in the study of Carnall & Ford (2011) following the latest FOCUS kinetic guidance.

A mineralisation of [triazinyl-6-14C]-labelled pymetrozine was measured with 25 % and 23 % AR after 361 days in the pond Fröschweiher and the Rhine river Möhlin systems, respectively. A comparable mineralisation was measured for the [pyridinyl-5-14C]-labelled pymetrozine with 29 % and 32 % AR after 344 days in the pond Fröschweiher and the Rhine river Möhlin systems, respectively.

43 % non-extractable residues of the [triazinyl-6-14C]-labelled pymetrozine were detected after 361 days in both water/sediment-systems, and 21-23 % non-extractable residues of the [pyridinyl-14C]-labelled pymetrozine were detected after 344 days in the pond Fröschweiher and the Rhine river Möhlin systems, respectively.

The degradation rates of the different labelled active substance pymetrozine in the two water-sediment systems have been recalculated in the study of Carnall & Ford (2011) following the latest FOCUS kinetic guidance. The resulting half-lives are summarised in table below. Pymetrozine dissipated with normalised half-lives (recalculated SFO from FOMC-DT90/3.32) between 7.4 and 13.1 days from the water phase. For the sediment phase SFO half-lives between 265 and 425 days were calculated. Normalised DegT50 values (recalculated SFO from DFOP kslow) between 315 and 495 days were calculated for the total water/sediment systems. Geometric mean DT50 values from the different pymetrozine labelling were calculated by the RMS for the water and sediment compartments and the total systems of each water/sediment system. Finally, overall geometric mean DT50 values of 9.5 days, 312 days and 358 days were derived for the water phase, sediment phase and the total systems, respectively and may be used for further risk assessment.

Table 23: DT50 values at level PI for pymetrozine in two water/sediment systems

Parent	**Distribution** (max in water 98 % after 0 days, max. in sediment 70 % after 28 d)												
Water / sediment system	**pH water phase**	**pH sed.**	**t. [°C]**	**DT_{50} [d]**	**DT_{50} [d] geo-mean**	**χ^2 [%]**	**DT_{50} [d]**	**DT_{50} [d] geo-mean**	**χ^2 [%]**	**DT_{50} [d]**	**DT_{50} [d] geomean**	**χ^2 [%]**	**Method of calcula-tion**
				total system			**water**			**sediment**			
pond Fröschweiher (pyridinyl)	8.2	6.8	20	495	395	5.4	8.5	7.9	1.9	425	346	7.3	Re-calculated SFO for system: DFOP kslow for water: FOMC DT_{90}/3.32 for sediment: SFO
pond Fröschweiher (triazinyl)	8.2	6.8	20	315		5.8	7.4		2.2	282		7.0	
Rhine, Möhlin (pyridinyl	8.4	7.1	20	289	325	3.4	9.8	11.3	2.9	265	282	4.4	
Rhine, Möhlin (triazinyl)	8.4	7.1	20	365		3.7	13.1		7.8	299		6.2	
Geometric mean (n = 2)					**358**			**9.5**			**312**		

11.4.4 Field investigations and monitoring data (if relevant for C&L)

Field investigations and monitoring data are not available.

11.4.5 Inherent and Enhanced Ready Biodegradability tests

Inherent and enhanced biodegradability test data are not available.

11.4.6 Soil and sediment degradation data

Information on ultimate biodegradability in soil and sediment is not available.

11.4.7 Hydrolysis

Experiments to investigate the hydrolysis of pymetrozine under sterile conditions showed that the active substance was stable under neutral (pH 7) and alkaline conditions (Kirkpatrick 1995a/b, McDonald 1996). Under acidic conditions, an equilibrium of pymetrozine and its hydrolysis products CGA215525 and CGA300407 was quickly reached. CGA215525 and CGA300407 were the only major metabolites in those studies, which may account up to 40 % and 60 % at pH 5, respectively. The hydrolysis half-lives for pymetrozine are about three hours at pH 1, 5-10 days at pH 5, approximately two years at pH 7. The major hydrolysis products are considered to be hydrolytically stable over a period of 30 to 35 days.

11.4.8 Photochemical degradation

The photolysis of [pyridinyl-^{14}C]- and [triazinyl-^{14}C]-pymetrozine was investigated in a study of Dixon & Gilbert (2011c) following OECD 306 in sterile, aqueous phosphate buffer at pH 7 under artificial continuous irradiation for up to 2 days using light from a Xenon lamp (> 290 nm) with light intensity of 25 W/m^2, equivalent to 1 day of UK/US summer sunlight for 24 hours continuous irradiation, with SFO DT_{50} values of < 1 days. In two previous studies, which were per-reviewed in the DAR (2004), the estimated environmental relevant half lives were 6.8 and 4.3 days for pyridinyl- and triazinyl-labelled pymetrozine at 40 °N, respectively. In the dark controls no degradation was observed.

Two transformation products were identified in the irradiated samples according to the studies of Kirckpatrick (1995a/b) and Dixon & Gilbert (2011c). The metabolite CGA300407 was isolated from the solutions treated with [pyridinyl-^{14}C]-pymetrozine with maximum amounts of 67 to 92 % AR after 2 to 32 days. CGA215525 and CGA249257 were isolated from the solutions treated with [triazinyl-^{14}C]-pymetrozine with maximum amounts of 67 to 71 % AR after 2 to 7 days and a maximum amount of 21 % AR after 38 days, respectively.

In an additional study of Mamouni (2004) the photolysis of [pyridinyl-^{14}C] pymetrozine was investigated in natural pond water at pH 8 following discontinuous irradiation (12 hours light/12 hours dark cycle) up to 29 days using light from a Xenon lamp (> 290 nm) with light intensity of 44 W/m^2. Pymetrozine degraded with a DT_{50} of 15.1 days in the irradiated system, corresponding to 22.6 ± 0.8 days natural summer sunlight at latitudes of 30°N – 50 ˚N. The major transformation product in this additional study was CGA300407 with a maximum amount of 71 % AR after 29 days. No degradation was observed in the dark controls.

11.5 ENVIRONMENTAL TRANSFORMATION OF METALS OR INORGANIC METAL COMPOUNDS

11.5.1 Summary of data/information on environmental transformation

Not relevant

11.6 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION

Other relevant information is not available.

11.7 COMPARISON WITH THE CLP CRITERIA

11.7.1 Acute aquatic hazard

For pymetrozine acute aquatic studies with fish, invertebrates and algae are available. The most sensitive endpoint is an EC_{50} = 3.06 mg/L for *Crassostrea virginica* (cf. chapter 11.1.). A substance has to be classified as **H400** (acute category 1), if the L/EC_{50} is ≤ 1 mg/l. This criterion is not fulfilled for pymetrozine.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

For pymetrozine long-term aquatic studies with fish, invertebrates and algae/aquatic plants are available. The most sensitive endpoint is a NOEC = 0.0251 mg/L for *Daphnia magna* (cf. chapter 11.2.).

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/L is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

The log Pow of pymetrozine is -0.19 at 25°C. So there is no indication for bioaccumulation potential of pymetrozine (cf. chapter 11.3).

Pymetrozine has shown a biodegradation of 2% in 29 days in a test according to OECD guideline 301 B and has therefore to be regarded as not readily biodegradable (cf. chapter 11.4.1). The results of the biodegradation of pymetrozine in water/sediment system and abiotic degradation show that pymetrozine is considered not rapidly degradable (a degradation > 70% within 28 days) for purposes of classification and labelling (cf. chapter 11.4.3).

Therefore pymetrozine has to be classified as **H410** (chronic category 1), as the NOEC is ≤ 0.1 mg/L. The corresponding **M-factor is 1.**

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Pymetrozine should be classified as Aquatic Chronic 1 H410 – "Very toxic to aquatic organisms with long lasting effects" (M = 1) for the environment. This leads to a proposed labelling of H410 (Very toxic to aquatic life with long lasting effects), which triggers the pictogram GHS09 and the signal word "Warning" on the label. The following precautionary statements are indicated: P273, P391 and P501.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pymetrozine is an insecticide used in agriculture, for ornamental plants and market gardening. The proposal of the DS was to change the environmental classification from Aquatic Chronic 3 to Aquatic Chronic 1 based on information produced during the pesticide review programme.

Hydrolysis

Experiments to investigate the hydrolysis of pymetrozine under sterile conditions showed that the active substance was stable under neutral (pH 7) and alkaline conditions (Kirkpatrick 1995a/b, McDonald 1996). Under acidic conditions, an equilibrium of pymetrozine and its hydrolysis products CGA215525 and CGA300407 was quickly reached. CGA215525 and CGA300407 were the only major hydrolysis products in those studies, which may account up to 40 % and 60 % at pH 5, respectively. The hydrolysis half-lives for pymetrozine were about three hours at pH 1, 5-10 days at pH 5, approximately two years at pH 7. The major hydrolysis products were considered to be hydrolytically stable over a period of 30 to 35 days.

Photolysis

The photolysis of the substance was investigated in a study (Dixon & Gilbert (2011c)) compliant with OECD TG 306 resulting in DT_{50} values < 1 day. In two previous studies, the estimated environmental relevant half-lives were 6.8 and 4.3 days for pyridinyl- and triazinyl-labelled pymetrozine. Two transformation products were identified according to the studies of Kirkpatrick (1995a/b) and Dixon & Gilbert (2011c): Transformation product CGA300407 with maximum amounts of 67 to 92 % AR after 2 to 32 days and CGA215525 and CGA249257 with maximum amounts of 67 to 71 % AR after 2 to 7 days and a maximum amount of 21 % AR after 38 days, respectively.

In an additional study by Mamouni (2004), pymetrozine degraded with a DT_{50} of 15.1 days in the irradiated system, corresponding to 22.6 ± 0.8 days natural summer sunlight at latitudes of 30°N – 50°N. The major transformation product in this additional study was CGA300407, with a maximum amount of 71 % AR after 29 days. No degradation was observed in the dark controls.

Ready Biodegradation

Pymetrozine has shown a biodegradation of 2 % in 29 days in a test conducted according to OECD TG 301 B and has therefore to be regarded as not readily biodegradable.

Water-sediment

The behaviour of pymetrozine in two water/sediment-systems (Pond Fröschweiher, Rhine Möhlin) has been investigated in two studies with different radioactive labelling of the parent compound (Reischmann 1995a, Schulze-Aurich 1996b).

Mineralisation of [triazinyl-6-14C]-labelled pymetrozine was measured with 25 % and 23 % AR after 361 days in the pond Fröschweiher and the Rhine river Möhlin systems, respectively. A comparable mineralisation was measured for the [pyridinyl-5-14C]-labelled pymetrozine with 29 % and 32 % AR after 344 days in the pond Fröschweiher and the Rhine river Möhlin systems, respectively.

The degradation rates have been recalculated in the study of Carnall & Ford (2011) following the latest FOCUS kinetic guidance. Geometric mean DT_{50} values of 9.5 days, 312 days and 358 days were derived by the RMS for the water phase, sediment and the total systems, respectively.

Soil and sediment degradation data

Information on ultimate biodegradability in soil and sediment is not available.

Based on all the available information, the DS considered pymetrozine as not rapidly degradable.

Bioaccumulation

No bioconcentration studies with pymetrozine were conducted, as its physico-chemical properties (Log Kow -0.19 at 25°C, pH 7) and its more polar metabolites (Log Kow << 3) indicate that the inherent potential for bioaccumulation is low.

Aquatic toxicity

Acute toxicity

In the following table, the results of the provided ecotoxicological tests from acute studies for three trophic levels are summarised.

Method	Species	Test (endpoint, design, duration)	Results	Key or Supportive study	Remarks	Reference
OECD TG 203	*Oncorhynchus mykiss*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	key	none	Anonymous a (1993);
OECD TG 203	*Oncorhynchus mykiss*	Mortality flow-through 96 hours	LC_{50} > 128 mg/L (m)	supportive	none	Anonymous a (1994);
OECD TG 203	*Cyprinus carpio*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	None	Anonymous b (1993);
OECD TG 203	*Lepomis macrochirus*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	None	Anonymous c (1993);
OECD TG 203	*Lepomis macrochirus*	Mortality flow-through 96 hours	LC_{50} > 134 mg/L (m)	supportive	none	Anonymous b (1994); 443-CG
OECD TG 203	*Ictalarus punctatus*	Mortality static 96 hours	LC_{50} > 100 mg/L (nom)	supportive	none	Anonymous d (1993); 928266
OECD TG 203	*Cyprinodon variegatus*	Mortality flow-through 96 hours	LC_{50} > 117 mg/L (m)	supportive	none	Anonymous c (1994); 446-CG
OECD TG 202	*Daphnia magna*	Immobility static 48 hours	EC_{50} > 100 mg/L (nom)	supportive	none	Grade (1993); 928270
OECD TG 202	*Daphnia magna*	Immobility flow-through 48 hours	EC_{50} = 87 mg/L (m)	key	none	Boeri (1994); 442-CG
EPA 72-3	*Mysidopsis bahia*	flow-through 96 hours	EC_{50} = 61.7 mg/L (m)	supportive	none	Boeri (1994); 445-CG
EPA 72-3	***Crassostrea virginica***	**Shell deposition flow-through 96 hours**	**EC_{50} = 3.06 mg/L (m)**	**key**	**none**	**Boeri (1994); 447-CG**
OECD TG 201	*Scenedesmus subspicatus*	Growth inhibition Static 72 hours	EbC_{50} = 47.1 mg/L (m,end) ErC_{50} > 84.6 mg/L (m,end)	key[1]	none	Grade (1993); 928272

			NOErC = 7.5 mg/L (m)			
OECD TG 201	*Pseudokirchneriella subcapitata*	Growth inhibition Static 120 hours	EbC_{50} = 21.6 mg/L (m,initial) ErC_{50} > 96.7 mg/L (m,initial) NOErC = 6.28 mg/L (m)	key	none	Boeri (1994); 668-CG

[1] Calculated values appear as nominal in the DAR. The value ErC_{50} > 84.6 mg/L was included by the DS in the table, but it does not appear neither in the CLP report nor to the RAR under the pesticide review programme. The DS has not explained the origin of this value.

The most sensitive endpoint is an EC_{50} = 3.06 mg/L for *Crassostrea virginica*. The DS concludes that the substance does not require an Aquatic Acute classification, as $L(E)C_{50}$ is higher than 1 mg/L.

Chronic toxicity

In the following table, the results of the provided ecotoxicological tests from chronic studies for three trophic levels are summarised.

Method	**Species**	**Test (endpoint, design, duration)**	**Results**	**Key or Supportive study**	**Remarks**	**Reference**
OECD TG 204	*Oncorhynchus mykiss*	growth Flow through 21 days	NOEC = 35.2 mg/L (m)	supportive	none	Anonymous e (1993)
EPA 72-4, OECD TG 210	*Oncorhynchus mykiss*	ELS Flow through 90 days (60 days post hatch)	NOEC = 11.7 mg/L (m)	key	none	Anonymous (1995);
OECD TG 202	*Daphnia magna*	Reproduction Semi static 21 days	NOEC = 0.1 mg/L (nom)	supportive	none	Grade (1993)
EPA 72-4, OECD TG 202	***Daphnia magna***	**Reproduction Flow through 21 days**	**NOEC = 0.025 mg/L (m)**	**key**	**none**	**Boeri et al (1995)**
OECD TG 201	*Pseudokirchneriella subcapitata*	Growth inhibition Static 120 hours	EbC_{50} = 21.6 mg/L (m,initial) ErC_{50} > 96.7 mg/L (m,initial) NOErC = 6.28 mg/L (m)	key	none	Boeri (1994)

OECD TG 201	*Scenedesmus subspicatus*	Growth inhibition Static 72 hours	EbC_{50} = 47.1 mg/L (m,end) ErC_{50} > 84.6 mg/L (m,end) NOErC = 7.5 mg/L (m)	key	none	Grade (1993)
EPA 122-2	*Lemna gibba*	Frond number Static 14 days	EC_{50} > 109 mg/L (m,initial) NOEC = 49.2 mg/L (m,initial)	supportive	none	Boeri et al (1995)

m: measured

nom: nominal

The most sensitive endpoint is a NOEC = 0.0251 mg/L for *Daphnia magna* and the proposed classification Aquatic Chronic 1 (M-factor = 1)

Comments received during public consultation

Two MSCAs commented and agreed with the DS's classification proposal.

The first MSCA asked for clarification on the temperature of the quoted DT_{50} values for hydrolysis. They also commented on the *Lemna* study asking whether results for 7 days have been reported and if they were calculated based on growth rate. Furthermore, the use of nominal concentrations in the *Lemna* study was questioned since the test item concentrations in test media declined over the study duration. Finally, the MSCA asked for a 21-day NOEC value based on mean measured concentrations for the *Daphnia* study performed by Grade (1993).

The DS provided the requested information in the response to comments.

In relation to the *Lemna* study (Boeri et al (1995)), the DS indicated that results at 7 days for growth of *Lemna* were not stated in the study report. It clarified that the NOEC was based on growth (frond number increase) at 14 days and that additional information from the study was provided in that the fronds were non-chlorotic. Finally, it explained that the use of mean measured concentrations was preferred, if the concentration declined more than 20% over the study. However in that study, the concentrations of test substance were only measured at the start and at the end (day 14) of the test being the measured concentrations at 14 days for all tested concentrations less than the analytical detection limit (5.06 mg/L). In this case and based on the "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" of EFSA (2015), the study results were only supportive and not relevant for classification.

For *Daphnia* the DS responded that the study triggering the proposed chronic classification (Boeri *et al*., 1995) revealed a NOEC (21 days) value of 0.025 mg/L (mean measured) in a flow-through system. At the supportive study (Grade, 1993) for chronic toxicity to *Daphnia magna*, a NOEC (21 days) of 0.0785 mg/L (mean measured) in a semi-static system was determined.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's assessment that considered pymetrozine as not-readily degradable, the substance biodegraded 2 % in 29 days in a test according to OECD TG 301 B.

The results of the biodegradation of pymetrozine in water/sediment system, soil and abiotic degradation show that pymetrozine is **not rapidly degradable** for purposes of classification and labelling.

In conclusion, RAC agrees with the DS's conclusion that pymetrozine is not rapidly degradable.

Bioaccumulation

RAC agrees with the DS's proposal, with a Log Kow of -0.19 at 25°C, pH 7, that the substance has no potential to bioaccumulate.

Acute Aquatic toxicity

Acute toxicity data are available for all three trophic levels. RAC agrees with the DS that the lowest endpoint corresponds to aquatic invertebrates to a study with *Crassostrea virginica* with EC_{50} = 3.06 mg/L.

Chronic Aquatic toxicity

Chronic toxicity data is available for all three trophic levels. The lowest chronic effect value corresponds to a test with *Daphnia magna* with a determined NOEC = 0.0251 mg/L. No chronic test with the most sensitive species from the acute tests, *Crassostrea virginica,* is available. A lower NOEC could have been obtained if this test was available. Yet, although a chronic NOEC is not available for *Crassostrea virginica*, using the acute EC_{50} of 3.06 mg/L and the surrogate approach would result in Aquatic Chronic 2, which is less a stringent classification than the one based on chronic Daphnia end points.

Conclusion on the aquatic environment classification

Pymetrozine is considered as not rapidly degradable and does not fulfil the criteria that indicate a potential to bioaccumulate.

For pymetrozine acute aquatic studies with fish, invertebrates and algae are available. The most sensitive endpoint is an EC_{50} = 3.06 mg/L for *Crassostrea virginica*. A substance has to be classified as Aquatic Acute 1 - H400, if the $L(E)C_{50}$ is ≤ 1 mg/L. This criterion is not fulfilled, thus, **no acute aquatic classification is proposed**.

Long-term aquatic studies with fish, invertebrates and algae/aquatic plants are also available. The most sensitive endpoint is a NOEC = 0.0251 mg/L for *Daphnia magna* which falls within the 0.01 < NOEC ≤ 0.1 range. As the substance is not rapidly degradable and based on Table 4.1.3 of the CLP Regulation, RAC concludes that pymetrozine fulfils the CLP criteria for classification as **Aquatic Chronic 1 – H410 with an M-factor of 1.**

As this substance is an insecticide, the classification might change in the future if further insect toxicity data becomes available.

Supplemental information - In depth analyses by RAC

Soil degradation data

The Renewal Assessment Report (RAR) of the substance (EFSA 2014) indicates that pymetrozine exhibits low to moderate persistence in soil under laboratory aerobic conditions. It forms various metabolites showing no evidence of ultimate degradation. The same occurs under anaerobic conditions.

Analysis of acute fish tests

An in depth assessment of the acute toxicity tests on fish could not be done due to the short summaries available in the CLP report and the RAR. Therefore, with current data it is difficult to conclude, as the DS has done, which study is the key one for classification. According to the CLH report detail study summary and the RAR all studies presented are valid and relevant for classification.

Analysis of acute invertebrate tests

The DS considers key studies for classification the ones carried out by Boeri (1994) on *Daphnia magna* and *Crassostrea virginica* and gives "supportive status" to the other two studies. RAC does not find a strong reason for considering the study done by Grade (1993) on *Daphnia magna* and Boeri (1994) on *Mysidopsis bahia* not relevant for classification.

The study by Grade (1993) on Daphnia magna is considered valid in both the RAR and the CLH detailed study summary. It fulfils validity criteria of OECD TG 202 and EPA OCSPP 850.1010 Guidelines on Acute testing of Daphnia. No major deviations were found.

The study by Boeri on *Mysidopsis bahia* is also considered valid in both the CLH detail study summary and RAR. The test fulfilled validity criteria according to EPA OCSPP 850.1035: Mysid Acute Toxicity Test. The only deviation found is that the test temperature (22.2-22-6) is lower than the recommended in the Guideline 25±1. This could have affected the organisms, but it does not seem to be the case when looking at the control.

Analysis of the algae studies

According to the RAR, in the Boeri study (1994) the effect values are based on initial measured concentrations and, other than stated in the initial monograph and the CLH, refer to the endpoint biomass. Re-evaluation of the data using the software ToxRat Professional Version 2.10 resulted in a 72-h EyC_{50} of 24.4 mg/L (95 % confidence interval 19.1 – 31.3 mg/L) and a 120-h EyC_{50} of 23.2 mg/L (95 % confidence interval 15.8 – 33.9 mg/L). The effects on growth were shown to remain below 50 % at the highest tested concentration; hence the ErC_{50} is > 96.7 mg/L. The NOEC amounts to 6.28 mg/L for all endpoints and assessment intervals ≥ 72 h. In summary, the new results for yield do not differ significantly from the established 120-h $E(b)C_{50}$ of 21.6 mg/L (95 % confidence interval 11.1 – 42.1 mg/L).

In the other algae study (Grade, 1993) with *Scenedesmus subspicatus* endpoints based on biomass and measured end concentrations. An endpoint base on growth would be more relevant. The Dossier submitter provided and ErC_{50}>84.6 mg/l. Geometric mean concentration should have been used since concentrations disappears. Using final concentrations can be considered a worst case scenario.

Analysis of the Lemna study

RAC agrees in considering the *Lemna* study as only supportive information. The EFSA conclusion "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology"

states that "*When the test concentrations were not maintained and significant residues were not present at the end of the exposure period (or at the end of the renewal period for semi-static design), the validity of the study should be questioned*" and adds "*When only initial and final measurements are available and no concentrations were detected at study end, the use of the LOD or half of LOQ is not supported. This is because it is not known when the concentrations decreased to practically zero (<LOD). The usefulness of such studies in first tier risk assessments should be questioned*."

Analysis of Chronic toxicity on fish

The OECD TG 204 "Prolonged Toxicity Test: 14-Day Study" has been withdrawn due to test design limitations and it is not considered suitable for generating chronic (long-term) toxicity data. The ECHA guidance Chapter R.7b: Endpoint specific guidance Version 4.0 June 2017 on page 30f states "*Only such studies can be regarded as long-term fish test, in which sensitive life-stages (juveniles, eggs, larvae) are exposed. Thus, tests performed according to OECD 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined*." RAC therefore considers the Anonymous e (1993) study with *Oncorhynchus mykiss* non-valid for generating chronic toxicity data. A re-assessment of this study as a prolonged acute study is not possible due to lack of raw data.

Analysis of chronic toxicity to invertebrates

RAC agrees that the Boeri *et al*. (1995) study should be considered the key study for classification. The Grade (1993) study has deviations such as PH variation and time passed until the first brood. Both points are not in fulfilment with the OECD validity criteria 202 Part II. In addition, the number of living offspring produced per parent animal surviving at the end of the test (52) is lower than current requirements for the validity of the test ≥60. Mean measured concentrations are more appropriate since the test substance concentrations vary by more than 20% of the nominal.

Studies on degradation products

Additionally the RAR contains several studies with the degradation products. In most of them, a robust study summary is not provided. In the studies which were carried out with the soil and water/sediment degradation products, all compounds, with the exception of CGA300407, could either be classified as non-toxic for aquatic standard test species or (when effects occurred on algal growth) less toxic than the parent pymetrozine. For CGA300407, stronger effects to fish and daphnids were observed with L(E)C_{50} values of 7.3 and > 20.1 mg/L (30 % immobilisation), respectively (pymetrozine: > 100 and 87 mg/L, respectively), while the toxicity to algae with an EbC_{50} of 15.4 mg/L was in a similar range as for pymetrozine (EC_{50} = 21.6 mg/L). This is additional information, since the substance is not rapidly degradable and the degradation products are not relevant for classification.

12. EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

13. ADDITIONAL LABELLING

None

14. REFERENCES

References cited for the toxicological evaluation:

Germany	2014	Final addendum to the Pymetrozine, Renewal Assessment Report, June 2014
Author 1	1996	CGA 215944 tech. (Pymetrozine): Effects on liver cell proliferation following dietary administration to female rats Report date: 1996-06-28 GLP: Y, published: N TOX9652165
Author 2	1992a	Rat oral teratogenicity - incl Amendment 1 dated 01.03.1996 + 2 dated 07.08.1997 Report date: 1992-09-22 GLP: Y, published: N ASB2012-4617
Author 2	1992b	Rabbit oral teratogenicity - incl amendment 1 dated 01.03.1996 + 2 dated 07.07.1997 Report date: 1992-09-28 GLP: Y, published: N ASB2012-4618
Author 2	1993	CGA 215944 technical: Rat dietary two-generation reproduction study Report date: 1993-11-15 GLP: yes, published: no TOX9652156
Author 3	1995a	CGA 215944 tech.: 24-month carcinogenicity and chronic toxicity study in rats Report date: 1995-10-19 GLP: yes, published: no TOX9652155
Author 3	1995b	CGA 215944 tech.: 18-month carcinogenicity study in mice Report date: 1995-10-30 GLP: yes, published: no TOX9652154
Author 4	1998	Pymetrozine: 4-week oral feeding study in rats (effect on testis) Report date: 1998-04-30 GLP: N, published: N TOX9851464 , ASB2012-4625
Author 4	1998	Pymetrozine: 4-week oral feeding study in rats (effect on seminiferous epithelial cycle in testis) Report date: 1998-05-07 GLP: N, published: N TOX9851465
Author 5	1995	CGA 215944 tech. (Pymetrozine): Effect on replicative DNA synthesis in hepatocytes following dietary administration to male mice Report date: 1995-05-16 GLP: yes, published: no TOX9650888
Author 6	2003	Pymetrozine tech. (CGA 215944): Developmental neurotoxicity study in rats Report date: 21 November 2003
Author 7	1996	Liver and thyroid medium-term bioassay for tumor promotion potential of CGA 215944 Report date: 1996-12-26 GLP: yes, published: no TOX9700527

Author 8 and Author 5	1995	CGA 215`944 tech. (Pymetrozine): Effects on selected biochemical and morphological liver parameters following dietary administration to male mice Report date: 1995-03-29 GLP: Y, published: N TOX9652160

Additional references not cited in the background document
Rosol *et al*. (2001): Adrenal gland: structure, function, and mechanism of toxicity, Toxicologic Pathology 29(1):41-48.

Regarding the references cited for the environmental evaluation, please refer to the RAR (2013).