Committee for Risk Assessment

RAC

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

Adopted

9 March 2018

9 March 2018

CLH-O-0000001412-86-203/F

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

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

Chemical name: **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**

The proposal was submitted by **Germany** and received by RAC on **30 May 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **19 July 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 September 2017**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Ignacio de la Flor Tejero**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 March 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	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 Aquatic Chronic 3	H351 H412	GHS08 Wng	H351 H412			
Dossier submitters proposal	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	**Retain** Carc. 2 **Add** Repr. 2 **Modify** Aquatic Chronic 1	**Retain** H351 **Add** H361fd **Modify** H410	**Retain** GHS08 Wng **Add** GHS09	**Retain** H351 **Add** H361fd **Modify** H410		**Add** Chronic M-factor = 1	
RAC opinion	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	**Retain** Carc. 2 **Add** Repr. 2 **Modify** Aquatic Chronic 1	**Retain** H350 **Add** H361fd **Modify** H410	**Retain** GHS08 Wng **Add** GHS09	**Retain** H350 **Add** H361fd **Modify** H410		**Add** Chronic M-factor = 1	
Resulting Annex VI entry if agreed by COM	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 Repr. 2 Aquatic Chronic 1	H351 H361fd H410	GHS08 GHS09 Wng	H351 H361fd H410		M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

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.**

HUMAN HEALTH HAZARD EVALUATION

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	**<u>5000 ppm</u>** Higher survival than control (45 vs 36, p<0.05) <u>*Body weight (p<0.01)*</u> ↓ 10% bodyweight (week 51) ↓ 11% bodyweight (week 75) <u>*Organ weights (p<0.01)*</u> ↑ 55% (absolute) and 77% (relative) liver weight ↓ 17% (absolute) and 5% (relative) kidney weight ↑ 19% (absolute) and 35% (relative) adrenals weight <u>*Haematology (p<0.01)*</u> Red blood cell count: ↓ 9% (week 53) Haemoglobin: ↓ 5% (week 53) Haematocrit: ↓ 4% (week 53) <u>*Macroscopic lesions*</u> 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) <u>*Microscopic lesions (p<0.0001)*</u> 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	**<u>5000 ppm</u>** <u>*Body weight (p<0.01)*</u> ↓ 18% bodyweight (week 51) ↓ 24% bodyweight (week 75) <u>*Organ weights (p<0.01)*</u> ↑ 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 <u>*Macroscopic lesions*</u> 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) <u>*Microscopic lesions (p<0.0001)*</u> 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

	vs 24/50 (control) Animals with hypercellularity: 42/50 vs 29/50 (control) **<u>2000 ppm</u>** *<u>Organ weights (p<0.01)</u>* ↑ 29% (absolute) and 36% (relative) liver weight ↓ 10% (absolute) and 5% (relative) kidney weight ↑ 21% (absolute) and 28% (relative) adrenals weight *<u>Macroscopic lesions</u>* 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) *<u>Microscopic lesions (p<0.0001)</u>* 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) **<u>100 and 10 ppm</u>** <u>No treatment related observations</u>	30/50 (control) Animals with hypercellularity: 33/50 vs 22/50 (control) **<u>2000 ppm</u>** *<u>Organ weights (p<0.01)</u>* ↑ 10% relative liver weight *<u>Macroscopic lesions</u>* Animals with enlarged liver: 28/60 vs 10/60 (control) *<u>Microscopic lesions (p<0.0001)</u>* Animals with liver hypertrophy: 47/50 vs 12/50 (control) Animals with hemosiderosis: 41/50 vs 30/50 (control) **<u>100 and 10 ppm</u>** <u>No treatment related observations</u>

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

	Mean (%)	Minimum (%)	Maximum (%)
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 Pymetrozine 98% 0, 10, 100, 1000 and 3000 ppm 0, 0. 4, 3.7, 39.3 and 128 mg pymetrozine/kg bw/day in males 0, 0.4, 4.5, 47.1 and 154 mg pymetrozine/kg bw/day in females 50 animals/sex/dose for carcinogenic potential	**3000 ppm** Higher survival than control (35 vs 20, p<0.05) Decrease of food consumption *Body weight (p<0.01)* ↓ 17% bodyweight (week 50) ↓ 15% bodyweight (week 104) Relative organ weights (p<0.01) Liver: ↑ 43% (interim) and ↑ 13% (terminal) Kidney: ↑ 22% (interim) Testes: ↑ 16% (interim) and ↑ 10% (terminal) Spleen: ↑ 77% (interim) and ↑ 7% (terminal) *Haematology (p<0.01)* Glucose: ↓ 18% (week 53) Albumin: ↑ 12% bodyweight (week 105) Bilirubin: ↑ 63% (week 53) and ↑37% (week 105) *Macroscopic lesions* Animals with mottled liver: 4/80 vs 1/80 (control) Animals with liver cyst: 7/80 vs 2/80 (control)	**3000 ppm** Decrease of food consumption *Body weight (p<0.01)* ↓ 22% bodyweight (week 50) ↓ 24% bodyweight (week 104) Relative organ weights (p<0.01) Liver: ↑ 28% (interim) and ↑ 24% (terminal) Kidney: ↑ 20% (interim) and ↑ 9% (terminal) Ovaries: ↑ 25% (terminal) Spleen: ↑ 50% (interim) and ↑ 33% (terinal) *Haematology (p<0.01)* Phosphorus: ↑ 38% (week 53) and ↑13% (week 105) ALAT: ↓ 55% (week 53) *Macroscopic lesions* Animals with liver masses: 4/80 vs/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)* Animals with liver hypertrophy: 37/60 vs 0/60 (control) Animals with foci of cellular change in liver: 30/60 vs 10/60 (control) Animals with follicular thyroid epithelium hyperplasia: 10/60 vs 2/60 (control)	*Microscopic lesions (p<0.0001)* Animals with biliary cyst: 13/60 vs 2/60 (control) Animals with liver hypertrophy: 40/60 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 *Body weight* ↓ 6% bodyweight (week 50) (p<0.01) ↓ 4% bodyweight (week 104) *Microscopic lesions (p<0.0001)* Animals with liver hypertrophy: 22/60 vs 0/60 (control) Animals with follicular thyroid epithelium hyperplasia: 9/60 vs 2/60 (control)	**1000 ppm** Decrease of food consumption *Body weight* ↓ 4% bodyweight (week 50) (p<0.01) ↓ 7% bodyweight (week 104) *Microscopic lesions (p<0.0001)* Animals with foci of cellular change in liver: 19/60 vs 9/60 (control) (within above the historical control incidence)
	100 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	**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

<u>Tumours of the cerebral meninges</u>

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.

<u>Lung tumours</u>

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).**

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).

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

Supplementary study 3: Oral 28-day study in rat

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[1] 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 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;

[1] 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.

- 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".**

ENVIRONMENTAL HAZARD EVALUATION

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) NOErC = 7.5 mg/L (m)	key[1]	none	Grade (1993); 928272
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 $\leqslant$ 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 $\leqslant$ 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.

Additional references

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

ANNEXES:

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

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