Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N'*-cyano-*N*-methylethanimidamide; (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine

EC Number: -
CAS Number: 135410-20-7; 160430-64-8

CLH-O-0000006797-57-01/F

Adopted

4 May 2020

4 May 2020

CLH-O-0000006797-57-01/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: **acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N'*-cyano-*N*-methylethanimidamide; (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine**

EC Number: **-**

CAS Number: **135410-20-7; 160430-64-8**

The proposal was submitted by **The Netherlands** and received by RAC on **23 October 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands 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 **21 January 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 March 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **4 May 2020** by **consensus**.

Existing Annex VI entry (CLP, Table 3)

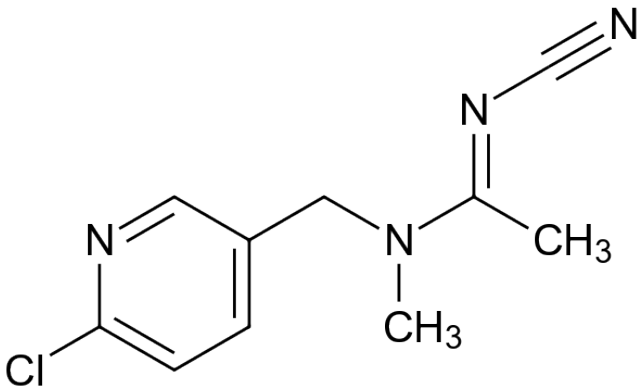
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	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	608-032-00-2	acetamiprid (ISO); (*E*)-N^1-[(6-chloro-3-pyri-dyl)methyl]-N^2-cyano-N^1-methylacetamidine		135410-20-7	Acute Tox. 4* Aquatic Chronic 3	H302 H412	GHS07 Wng	H302 H412			
Dossier submitters proposal	608-032-00-2	acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N*′-cyano-*N*-methylethanimidamide; (*E*)-*N*^{1}-[(6-chloro-3-pyridyl)methyl]-*N*^{2}-cyano-*N*^{1}-methylacetamidine		135410-20-7; 160430-64-8	**Add** Carc. 2 Repr. 2 Aquatic Acute 1 **Modify** Acute Tox. 3 Aquatic Chronic 1	**Add** H351 H361d H400 **Modify** H301 H410	**Remove** GHS07 Wng **Add** GHS06 GHS08 GHS09 Dgr	**Add** H351 H361d **Modify** H301 H410		**Add** M=10 M=100 Oral: ATE = 140 mg/kg bw	
RAC opinion	608-032-00-2	acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N*′-cyano-*N*-methylethanimidamide; (*E*)-*N*^{1}-[(6-chloro-3-pyridyl)methyl]-*N*^{2}-cyano-*N*^{1}-methylacetamidine		135410-20-7; 160430-64-8	**Add** Repr. 2 Aquatic Acute 1 **Modify** Acute Tox. 3 Aquatic Chronic 1	**Add** H361d H400 **Modify** H301 H410	**Remove** GHS07 Wng **Add** GHS06 GHS08 GHS09 Dgr	**Add** H361d **Modify** H301 H410		**Add** M=10 M=10 Oral: ATE = 140 mg/kg bw	
Resulting Annex VI entry if agreed by COM	608-032-00-2	acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N*′-cyano-*N*-methylethanimidamide; (*E*)-*N*^{1}-[(6-chloro-3-pyridyl)methyl]-*N*^{2}-cyano-*N*^{1}-methylacetamidine		135410-20-7; 160430-64-8	Repr. 2 Acute Tox. 3 Aquatic Acute 1 Aquatic Chronic 1	H361d H301 H400 H410	GHS06 GHS08 GHS09 Dgr	H361d H301 H410		M=10 M=10 Oral: ATE = 140 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Acetamiprid is a pyridyl-methylamine, neonicotinid insecticide and is registered for various applications, which has been evaluated in the context of both the Biocidal Products Regulation (BPR) (EU) 528/2012 (ECHA, 2017) and the Plant Protection Product (PPP) Regulation (EC) 1107/2009 (EFSA, 2012). It acts on insects by contact and ingestion, affecting the central nervous system, causing paralysis and death.



Acetamiprid is already included in Annex VI of the CLP Regulation (EC) No 1272/2008 with Index Number 608-032-00-2 (CAS Number 135410-20-7) and classified as Acute Tox. 4*; H302 and Aquatic Chronic 3; H412. The proposed change in the existing entry is due to the presence of a minimum classification and to some new data presented in the Renewal Assessment Report (RAR) on renewal of the approval of the active substance acetamiprid as a plant protection product. In addition, the EFSA Pesticide Peer Review (PPR) no. 146 of 2016 concluded with a recommendation of classification as Carc. Cat. 2 on the basis of the available two-year rat study.

A new developmental neurotoxicity study was submitted for the BPR review and was part of the RAR for the PPP renewal (Anon., originally dated 2003, [2008 (revised report)]). No reproductive or developmental toxicity classifications were proposed during the above reviews. The DS proposed Repr. 2 (H361) for developmental effects based on existing studies. A classification proposal for Carc. 2 was made during the PPP peer review in 2016 but not during the biocidal product regulation review (CAR 2018).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed a change to the Annex VI classification of Acute Tox. 3 on the basis of new study results presented in the RAR (2016). There are now 3 studies available as shown below:

Table 1: *Summary of the Acute oral toxicity studies*

Method, guideline, deviations if any	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
NEW Study In compliance with the EEC B.1 (equivalent to OECD TG 401) 5 Crj: CD (SD) male rats/dose Crj: CD (SD) female rats/dose	Acetamiprid Purity: >99.9% Vehicle: corn oil Homogeneity not mentioned	140-560 mg/kg bw 14 days	Males: 195 mg/kg bw Females: 140-200 mg/kg bw	RAR (2015)
In compliance with the EEC B.1 (equivalent to OECD TG 401) 5 Crj: CD (SD) male rats/dose Crj: CD (SD) female rats/dose	NI-25 (acetamiprid) Purity: 99.9% Vehicle: ion-exchanged water	Males: 100-760 mg/kg bw Females: 70-760 mg/kg bw 14 days	Males: 417 mg/kg bw Females: 314 mg/kg bw	RAR (2015)
In accordance with OECD TG 401 5 Crj: CD (SD) male rats/dose Crj: CD (SD) female rats/dose	NI-25 (acetamiprid) Purity: 99.46% Vehicle: ion-exchange water	Males: 100-510 mg/kg bw Females: 80-510 mg/kg bw 14 days	Males: 217 mg/kg bw Females: 146 mg/kg bw	RAR (2015)

The lowest calculated LD_{50} value in the studies using ion-exchanged water as vehicle is 146 mg/kg bw. The lowest calculated LD_{50} value in the study using corn oil as vehicle is 140 mg/kg bw and the DS proposed classification as Acute Tox. 3.

Comments received during consultation

A number of Member State Competent Authorities (MSCAs) supported the proposed change from Acute Tox. 4 to Acute Tox. 3. There was no disagreement.

Assessment and comparison with the classification criteria

According to the CLP criteria, substances with LD_{50} values falling within the range $50<LD_{50}\leq300$ mg/kg bw/day should be classified as Acute Tox. Category 3 (H301: Toxic if swallowed). There are two studies for acetamiprid in which the lowest LD_{50} value was within this range for female animals; 146 mg/kg bw when ion-exchange water was the vehicle and 140 mg/kg bw when corn oil was the vehicle. Cat. 3 is therefore the appropriate category for acetamiprid.

Overall, in support of the DS, RAC considers a classification as **Acute Tox. 3; H301; with an ATE of 140 mg/kg bw** is warranted for acetamiprid.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Germ cell mutagenicity was not assessed in the CLH dossier and it was also not in the scope of the consultation. However, it was briefly described in the CLH dossier in support of the assessment for the proposed classification of carcinogenicity.

The genotoxic potential of acetamiprid was investigated in a comprehensive range of GLP and OECD guideline compliant *in vitro* and *in vivo* assays. The results of these assays were summarised in Tables 13 and 14 of the CLH report.

Acetamiprid did not induce point mutations in bacteria *in vitro*. The mammalian gene mutation assay was also negative. Acetamiprid was found to be positive in the chromosomal aberration in vitro assay in CHO cells. Although a positive result was obtained in the *in vitro* chromosome aberration test, further *in vitro* (i.e. UDS *ex vivo/in vitro* using primary rat hepatocytes) and *in vivo* studies (i.e. a mouse micronucleus, [mortalities at the highest tested dosage of 80 mg/kg bw], a bone marrow metaphases analysis in rats, [mortalities as well as reduction of mitotic index, compared to controls observed at the highest dose of 250 mg/kg bw], and an *in vivo* UDS) failed to confirm any genotoxic effect *in vivo*. Information available for metabolites of acetamiprid also suggest they are not genotoxic RAR (2016).

Comments received during consultation

Hazard class not within the scope of consultation.

No comments received during consultation

Assessment and comparison with the classification criteria

Not discussed at RAC.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The dossier presented two long-term toxicity/carcinogenicity studies, which have been evaluated under both the Plant Protection Product and Biocidal Product regulations. Classification for carcinogenicity was not proposed initially by the evaluating competent authority but was proposed during the EFSA Peer Review consultation and therefore presented in the Annex by the DS for discussion by the RAC.

Rat study

In a guideline study, Crl:CD (SD) BR rats/sex (4 weeks old at start) received 0, 160, 400 and 1000 ppm of acetamiprid (in the diet). 60 rats/group/sex were used in each group and 10 were euthanized at 12 months of study. The dietary intake was calculated to deliver 7.1, 17.5 and 46.4 mg/kg bw/day in males and 8.8, 22.6, and 60.0 mg/kg bw/day in female rats. Survival was not affected by treatment. Non-specific clinical signs of toxicity were noted in mid and high dose rats. Mean body weights were statistically significantly reduced in rats at 1000 ppm (and at 400 ppm for females only) at both interim and terminal sacrifice. Triglycerides and A/G ratio were significantly reduced in females at 1000 ppm at 12 and 18 months. Mean relative liver weight was increased in the high dose animals (statistically significant in high dose males). Variations in other organ weights were related to the decreased bodyweight in both males and females at the top-dose and were not considered to be associated to the treatment. Combined microscopic non-neoplastic observations for all animals on study (interim and final sacrifice, and all animals dead or sacrificed at unscheduled dates) did not reveal treatment related effects other than observations identified in the liver (hypertrophy and hepatocyte vacuolation from 400 ppm in males only at terminal sacrifice).

A significant trend increase of mammary gland adenocarcinoma ($p<0.05$) was observed with the Cochran Armitage Trend Test and the Peto test when all animals in the study were combined. Nevertheless, such incidence was not statistically significant with the Fisher Exact test and was within the values for historical control data (28.3% high dose in this study, compared to 14.0% to 28.6% for historical control data (n=6, same laboratory and same period). The incidences are presented in Table 2 (as originally presented by the DS with the 50 animals from the main study added to the 10 animals from the interim sacrifice groups):

***Table 2**: Rat mammary gland observations at terminal sacrifice, n=60.*

Microscopic observations	**dose levels ppm**			
	0	**160**	**400**	**1000**
	Neoplastic lesions			
Fibroadenoma	17/59	15/60	10/60	15/60
Adenoma	1/59	0/60	4/60	3/60
Benign tumour (adenoma or fibroadenoma)	18/59	15/60	14#/60	18/60
Adenocarcinoma	10/59	11/60	16/60	17/60
Any mammary tumour	24/59	21/60	24/60	29/60

This incidence is reported as 12/60 in the table B.6.5.1-7 in the RAR.

Additional historical control data (HCD) were presented in the RAR and was considered supportive of the RMS opinion that the finding at the top dose was within the background and not treatment related. This additional information shows that the historical control ranges for mammary adenocarcinomas in female Sprague Dawley (CD) rats are:

- MPI laboratory: 13.3 – 28.6%
- Charles River Laboratories: 0 – 58.3%
- WIL Laboratories: 0 – 37.2%
- Covance Laboratories: 2.2 – 40%

The information (*see also revised data by RAC under Additional Key Elements*) shows that this is a common tumour in female rats, with a large range of normal variability. In the 2-year study in rat with acetamiprid, mammary gland adenocarcinoma was found at 16.9% (18.4%)[1] for the control group, 18.3% (20.4%)[1] for the 160 ppm group, 26.7% (31.9%)[1] for the 400 ppm group and 28.3% (34.7%)[1] for the 1000 ppm group. All these values are within or close to the historical control ranges reported for this tumour type in this strain of rat in the MPI Laboratory (13.3 - 28.6%) where this 2-year study with acetamiprid was performed. The study with acetamiprid was run between 1 Oct. 1991 and 1 Oct. 1993. RAC notes that the HCD for the MPI Lab only included 6 studies which started between 16 Oct. 1991 and 28 July 1994 and ended between 22 Oct. 1993 and 31 July 1996. RAC notes the CRL labs HCD[2] is a more robust database with 24 studies and a range from 0 – 58.3%, with a mean of 24.2% incidence, the initiation dates varied from 1991 to 1996.

The DS concluded that since the HCD came from the same laboratory and were from the correct time period, the reported HCD were appropriate and acceptable. Therefore, the slight increase observed at the top dose level was not considered to be treatment related.

Overall, the increase in adenocarcinoma of the mammary gland of female rats was questioned as it was significant in a trend test but not in the pair-wise comparison with the controls, and no

[1] Revised data by RAC, see "Additional Key Elements"

[2] Giknis & Clifford, March 2001. Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) BR Rats from Control Groups.

decrease in latency period was observed. In addition, the incidence at the highest dose remained within the higher end of the historical control range.

Hyperplasia in the mammary gland was increased significantly in the top dose level females. HCD were provided for this finding, indicating an incidence range of 0.00 – 58.57%. The finding at the top dose level is just outside this range (i.e. 62%) but was interpreted as supportive for the discussion on classification.

Table 3: *Incidence of mammary gland hyperplasia in rats at different interim intervals.*

Dose (ppm)	**0**	**160**	**400**	**1000**
1-year interim	3/10	1/10	2/10	2/10
2-year final	5/23	10/26	10/29	18/29 (62%)*
Unscheduled (determined by calculation)	9/26	2/24	4/21	6/21
Total	17/59	13/60	16/60	26/60 (43%)*

* $p < 0.01$

The mammary tumours were seen in one species and one sex and in the absence of substance related mortality. The high dose was in the range of the MTD. It was suggested that the reduced weight and body weight gain may have been responsible for the absence of an increase in adenoma as feed restriction has been associated with a reduction in the occurrence of fibroadenoma in female SD rats (Keenan *et al.*, 1995).

The DS proposed classification in category 2 based on a treatment-related increase in mammary adenocarcinoma and the possibility that the statistically significant increase in incidence of hyperplasia may represent a continuum indicating, a transition into neoplasia.

Mouse study

An 18-month, guideline compliant study was evaluated in which 60 mice/group/sex were dosed with 0, 130, 400 or 1200 ppm acetamiprid in the diet from which 10/sex/group were sacrificed after 1 year. Surviving animals were sacrificed after 18-months of treatment.

Mortality was not adversely affected and clinical signs of toxicity were non-specific. Mean body weight and mean body weight gains were statistically significantly reduced in animals at the high dose of 1200 ppm. There were some overall reductions in food consumption in high dose males. Haematology parameters were unaffected. Variations in organ weights, regarding decreases in absolute and relative heart and kidney weight, increase in relative pituitary weight and decrease in prostate absolute weight, were considered related to the decreased body weight in both males and females at the top-dose and not associated with treatment.

A statistically significant increase in hepatocellular hypertrophy was seen in mice of the high dose at interim sacrifice. In addition, a statistically significant increase in myeloid hyperplasia of the femoral bone marrow was seen in males at this dose. This was not considered to be treatment-related because of the following:

- No dose response relationship was observed between acetamiprid administration and bone marrow hyperplasia.
- Haematology values were not affected in a way related to bone marrow hyperplasia.
- Chronic administration of the test substance did not increase the severity or the incidence or the effect on bone marrow and there was no progression of bone marrow hyperplasia, as observed at post-mortem examination of the animals at the end of the study.

Table 4a: *Microscopic observation in mice after acetamiprid administration*

Interim (0-12 month) sacrifice								
Dose	**0 ppm**		**130 ppm**		**400 ppm**		**1200 ppm**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Bone marrow Femur: Myeloid hyperplasia	0/10	0/10	1/10	1/10	2/10	0/10	4/10 [1]	0/10
Liver: Centrilobular hypertrophy	0/10	0/10	0/10	0/10	1/10	0/10	8/10 [2]	8/10 [2]
Terminal sacrifice (18 month-scheduled)								
Bone marrow Femur: Myeloid hyperplasia	0/38	0/38	5/42 [1]	5/42 [1]	7/38 [2]	4/38	6/39 [1]	6/43 [1]
Bone marrow Sternum: Myeloid hyperplasia	0/38	0/38	6/42 [1]	6/42 [1]	7/38 [2]	4/38	6/39 [1]	6/43 [1]
Liver: Centrilobular hypertrophy	0/38	0/38	0/42	0/42	0/38	3/38	23/39 [2]	16/43 [2]
Kidney: Chronic progressive nephropathy	32/38	21/38	35/42	24/42	33/38	21/38	24/39	35/43 [1]
Lung: Epithelial hyperplasia	3/38	0/38	4/42	4/42	2/38	1/38	3/39	5/43 [1]

*p < 0.05, ** p < 0.01, compared to control by Fisher's exact test

Table 4b*: Statistically significant incidences of non-neoplastic lesions for all combined animals on study (interim and final sacrifices with all decedent or unscheduled sacrificed animals).*

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**130**	**400**	**1200**	**0**	**130**	**400**	**1200**
Liver: hypertrophy centrilobular	0/60	0/60	2/60	37/60**	0/60	0/60	4/60	26/60**
Kidney: Chronic progressive nephropathy	47/60	45/60	45/60	31/60	33/60	33/60	33/60	48/60**
Bone marrow, Sternum: hyperplasia, myeloid	11/60 S:1/48 D:10/12	10/60 S:7/52 D:3/8	14/60 S:9/48 D:5/12	14/60 S:11/49 D:3/11	4/60 S:0/48 D:4/12	11/60* S:7/52 D:4/8	7/60 S:4/48 D:3/12	8/60 S:6/53 D:2/7
Bone marrow, Femur: hyperplasia, myeloid	10/60 S:0/48 D:10/12	9/60 S:6/52 D:3/8	13/60 S;9/48 D:4/12	13/60 S:10/49 D:3/11	4/60 S:0/48 D:4/12	10/60 S:6/52 D:4/8	7/60 S:4/48 D:3/12	8/60 S:6/53 D:2/7

D=premature death, S=sacrificed
*p < 0.05, ** p < 0.01, compared to control by Fisher's exact test.

Note: the CLH report refers to HCD for this finding (p. 18). This data was considered in the re-review (RAR) when the applicant was required to submit additional HCD on hyperplasia. These were from 3 studies performed from April 1989 – October 1990, January 1991 – July 1992 and June 1991 – December 1992. The 18-month study in mice performed with acetamiprid was performed from October 1991 to April 1993. Therefore, the HCD are from the appropriate time period and from the same laboratory.

The HCD data show that the incidence of hyperplasia in bone marrow of the femur was 0 - 1 in 50 animals (males) and 0/50 (females). For hyperplasia in bone marrow of the sternum the incidence was 0 – 2 in 50 animals (males) and 0/50 in females. The overall combined findings in Table 30 of the CLH report show that in the control groups, myeloid hyperplasia in the sternum was 11/60 (males) and 4/60 (females), which is indeed above the HCD.

The incidence of hyperplasia of the bone marrow in the sternum was **not** statistically significantly increased (CLH Table 29) when all combined animals were considered together at the final sacrifice.

In addition, in males, the incidence of amyloidosis in the terminal sacrifice was increased in several organs at the top-dose and in the adrenal cortex and kidney from 400 ppm. Nevertheless, those incidences did not exceed HCD (except for the nonglandular stomach) and when all mice were considered together, these incidences were not statistically significant.

In conclusion, there were no neoplasms at a statistically significantly increased incidence noted at any dose and it was concluded that acetamiprid is not carcinogenic to the mouse when administrated in the diet for 18 months.

Comments received during consultation

Three MSCAs commented in support of the proposal to classify as Carc. 2 on the basis of a possible increase in mammary tumours in association with a significant increase in hyperplasia.

One manufacturer and two downstream user companies also commented and did not support the proposed classification. These parties disagreed with the DS argument that a continuum between the observed statistically significant mammary hyperplasia and the increase in mammary adenocarcinoma (statistically significant trend) can be the basis for classification for carcinogenicity. Their rational being;

- A continuum has not been demonstrated as adenoma are not increased.
- Hyperplasia was not increased in the interim kill.
- Hyperplasia is a non-neoplastic change, is not evidence of carcinogenicity, and is not appropriate for carcinogenicity classification.
- The HCD incidence of hyperplasia is highly variable at the test facility.
- Mammary gland hyperplasia or any other mammary pathology was not seen in other species tested.

In addition, mammary adenocarcinoma has a high background in SD female rats (see Guidance on the Application of the CLP Criteria) and the incidence of mammary tumours in this study remained within the historical control range for this specific test facility.

Acetamiprid is not genotoxic, there is no apparent mode of action and it is negative in the USEPA ToxCAst ER bioactivity model (no evidence of ED disruption).

Assessment and comparison with the classification criteria

Classification in category 1A is not appropriate in this case as there is no human data. Classification in category 1B is also not considered appropriate as there is only one study in which a possible increase in carcinoma was observed.

Classification in category 2 is therefore considered as evidence was presented which was obtained from a single animal study.

Two studies investigated the long-term oral toxicity/carcinogenicity of acetamiprid in rats and mice.

The argument for classification is based on a statistically significantly increased incidence in mammary adenocarcinoma in the rat 2-year study.

There was an increase in adenocarcinoma of the mammary gland in female rats. This increase in incidence was significant in a trend test, but not in a pair-wise comparison with the controls. The increase in the highest dose (34.7%) was outside the historical control range of the performing laboratory (MPI) of 13.3% - 28.6% and in addition, the incidence on the concurrent control, low and mid groups were 18.4%, 20.4% and 31.9%, respectively. Moreover, an increase in mammary gland hyperplasia (49%) was observed at the highest dose which was statistically significantly increased compared to the concurrent control. HCD were also provided for this finding, indicating an incidence of 0.00 – 58.57%. The finding at the top dose level however was within this range.

Following the EFSA technical peer review, the increase in mammary gland adenocarcinoma was considered to be substance-related due to what was described as a continuum between hyperplasia and increased tumour incidence. This may be supported by the observation of increased mammary hyperplasia in the highest dose groups. Progression from cell damage to proliferation and then followed by progressive stages from hyperplasia, dysplasia to benign and malignant tumour *in situ* has been demonstrated for mammary epithelia cells e.g., following exposure to 7,12-dimethylbenz[a]anthracene, Al-Dhahrei *et al.*, 2008.

However, while the incidence of mammary tumours is statistically significant in a trend test, the groups are not statistically different by pairwise comparison.

It should be noted that the increased incidence is just outside the range of the HCD for the test house at the appropriate time interval and several other sources of HCD indicate this type of tumour is highly variable in incidence. The in-house (MPI) HCD was limited in that few studies (6) were available. Nevertheless, more comprehensive external collections of HCD were also available where it could be verified that the incident values are all within the historical control ranges for this tumour type in this strain of rat (e.g. CRL laboratory data published in 2001: 0 - 58.3%). The CRL data was more robust, even though originating from different laboratories, and it shows how variable and common this tumour type actually is and prompting the question whether it would be possible to determine if a substance related effect on mammary gland tumours could ever be reliably detected in this strain of rat (figure 1).

This tumour is a common finding in SD rats which is noted in the CLP Guidance where it is stated that "even a statistically significant increase within the historical control range may not be providing reliable evidence of treatment-related carcinogenicity". Additional HCD from other test facilities support this observation.

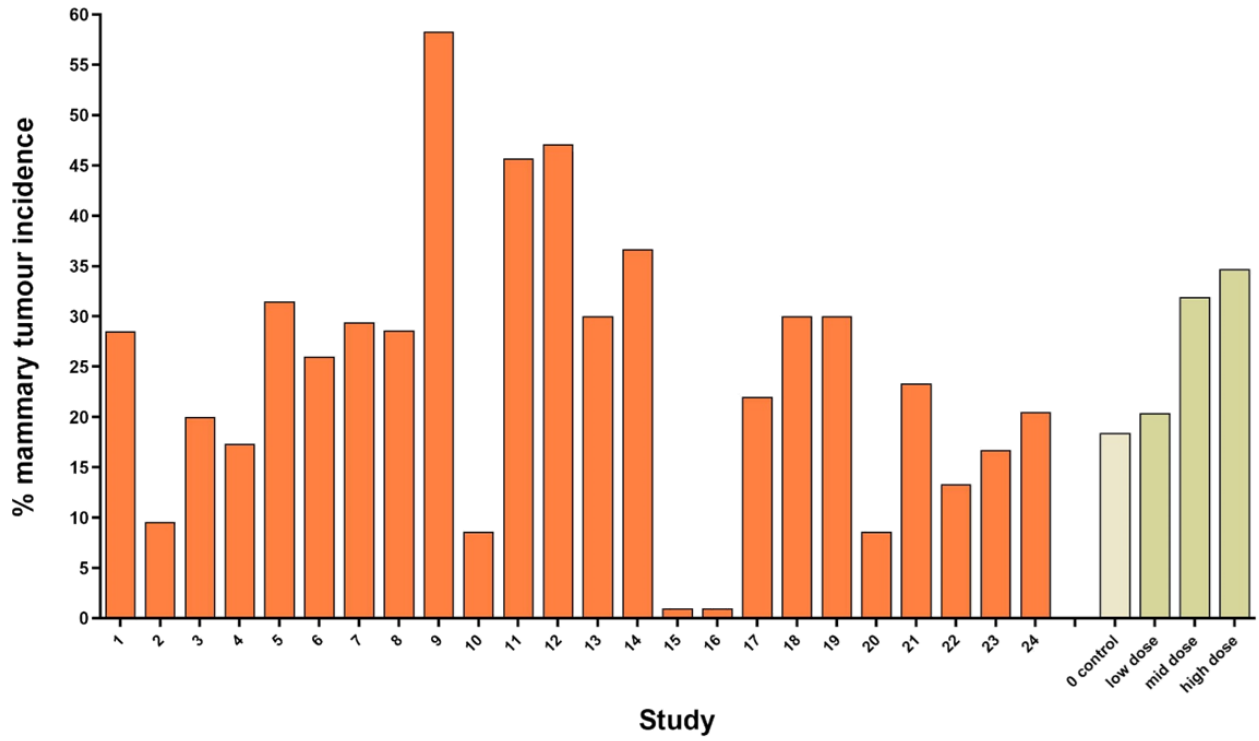


Figure 1: CRL study historical control data taken from Giknis & Clifford, March 2001. Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) BR Rats from Control Groups. This graph illustrates the high background incidence of this tumour type in this strain of rat. 24 studies, initiation from 1992-1996, mean 24.2%: 0 – 58.3%, 1729 animals in total.

No mode of action was apparent from the data presented and the substance was not shown to be genotoxic. In addition, acetamiprid has been assessed in the US EPA ToxCast ER bioactivity model which is considered highly relevant in the assessment of endocrine disruption potential via oestrogenic activity. Acetamiprid shows no oestrogenic activity (US EPA EDSP21).

There was an apparent increased incidence of mammary epithelia hyperplasia but no increase in severity was observed at interim or at terminal sacrifice. The occurrence of mild hyperplasia at terminal sacrifice is not consistent with a continuum from a normal state to malignancy. There was no increase in adenoma. There was no evidence of the expected progression as described by Al-Dhahrei *et al.* (2008), there was no progression from hyperplasia to dysplasia to benign and malignant tumour *in situ* with a clear treatment related effect. The argument that reduced body weight is associated with a reduced incidence of mammary fibroadenoma is not relevant to the observation that increased incidence of adenoma was not observed; these are unrelated pathological entities – fibroadenoma is not part of the continuum referred to above.

The observed tumours were also confined to a single species. No carcinogenicity was observed in the mouse carcinogenicity study.

In conclusion, **RAC considers the carcinogenic evidence to be insufficient for classification.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Acetamiprid was evaluated in a guideline and GLP compliant two-generation study (OPPTS 870.3800, approximating OECD TG 416, 1983; Anon., 1999d) in SD rats in order to assess its

effects on sexual function and fertility. A supplementary study to the main 2-generation study further assessed reproduction in male rats at the top dose to address a lack of male sperm investigations due to technical errors in the primary two-generation study. The effects of acetamiprid on development following exposure during pregnancy were tested in guideline (OECD TG 414; 1981) and GLP compliant pre-natal developmental toxicity studies in rats (Anon., 1997f) and rabbits (Anon., 1997e). Preliminary dose range finding developmental studies in rat and rabbits were also available and briefly described by the DS. A developmental neurotoxicity study in rats (Anon., 2008), with exposure from gestation day 6 until lactation day 21 was also available. The DS also described two peer reviewed journal publications: a repeat dose, non-GLP, non-guideline study by Zhang *et al.* (2011) investigating the effect of acetamiprid on the reproductive function of male mice and also a non-GLP, non-guideline study by Gu *et al.* (2013) on the *in vitro* effects of acetamiprid on spermatozoa, fertilization and preimplantation embryo development.

Effects on sexual function and fertility

Rat 2-gen Study

In a 2-generation reproduction study, acetamiprid (purity 99.9%) was administered to Sprague-Dawley rats (26/sex/group) at concentrations of 100, 280 or 800 ppm (table 7), for at least 10 weeks prior to mating, during mating, throughout gestation and lactation until weaning of the F2 pups. At termination, reproductive capacity evaluations (ovarian follicle count, sperm motility, total sperm count and sperm morphology) were performed on F0 and F1 adults. Necropsy was performed on parents and pups from both generations. Minimal maternal toxicity was evident from a lack of effects on body weight and food consumption parameters.

Table 7: *Mean achieved doses of acetamiprid (mg/kg bw/day) for the F0 and F1 generations*

	Males/pre-mating			**Females/pre-mating**			**Gestation**			**Lactation**		
Dose levels (ppm)	**100**	**280**	**800**	**100**	**280**	**800**	**100**	**280**	**800**	**100**	**280**	**800**
F0	6.5	17.9	51.0	7.6	21.7	60.1	6.8	18.5	50.9	13.2	37.5	108.1
F1	7.5	21.0	63.3	8.4	23.8	72.6	6.6	18.5	55.2	13.7	40.3	105.5

Premating period: week 0 to 10 for F0, and week 0 to 13 for F1 generation. Gestation period: day 0 to 20, lactation period: day 0 to 14. (Rounded values).

General toxicity – Parental toxicity

F0 parents

Mortality and clinical signs: One F0 male of the control group and one F0 female from the low dose group (100 ppm) died during week 2 and on lactation day 18, respectively. No death or clinical signs were attributed to treatment.

Body weight & body weight gain: Lower mean body weight values and food consumption were limited to the top dose groups (800 ppm) throughout the study. Body weight was less than approximately 8% relative to controls at week 10 (premating period) for both sexes. Decreases in mean body weight gain (approximately 8%) and food consumption (approximately 20%, significant) were noted for females in the high dose group over gestation days 0-20.

Organ weights & histopathology: Necropsy examinations did not reveal compound-related changes in F0 adult animals or F1 pups. Organ weight changes comprised increased mean brain-to-body weight percentage (about 10% relative to controls) and decreased kidney-to-brain weight ratio (7.5%) of the 800 ppm F0 females. No changes were observed in the reproductive organs.

No histopathological changes were attributed to treatment and no effect on testes or ovaries were reported. The reproductive assessment of high dose males in the 2-generation study was not concluded because the epididymides removed from these animals autolysed due to an error in thawing prior to analyses. A supplemental study coincident with the 2-generation study was then conducted where 26 males were fed either control chow or chow containing 800 ppm acetamiprid for at least 20 weeks. No compound-related effects were observed on total testicular and epididymal sperm counts or on sperm morphology.

F1 parents

Mortality and clinical signs: Two low dose group (100 ppm) and five top dose group (800 ppm) females experienced total litter deaths. No death or clinical signs were noted in adults of either sex throughout all study phases.

Body weight & body weight gain: Mean body weights were consistently lower in F1 parents and correlated with lower food consumption values in the 800 ppm animals during all study phases. Body weight was less than 12-14% relative to controls at week 10 (premating period, males and females respectively) for both sexes. Decreases in mean body weight gain (approximately 8%) and food consumption (approximately 13%, significant) were noted for females in the high dose group over gestation days 0-20.

Organ weights & histopathology: Necropsy of F1 adult and pup animals did not reveal compound-related changes. No histological changes were attributed to treatment in the F1 animals, and no compound-related effects were observed on reproductive organs or functions, including ovarian follicle-count, evaluation of sperm motility, testicular and epididymal sperm count and sperm morphology of the top dose animals.

Reproductive effects

The DS noted that mating performance, fertility, reproduction parameters and oestrous cycles for both the F0 and F1 adult generations were unaffected by treatment.

Offspring effects

F1 pups

Viability and weaning indices were unaffected by treatment up to the top dose of 800 ppm, and values were well within HCD for the performing laboratory. However, at the top dose of 800 ppm, mean numbers of live pups/litter with live pups showed a very small but significant reduction on days 14 and 21, this was of questionable biological significance. Pup body weights were affected: in-utero to a small extent (< 7% reduction relative to controls) but post-natal growth of the 800 ppm were significantly decreased throughout lactation, showing reductions of between 11-24% across both sexes relative to the mean control bodyweight.

Landmark and Pubertal attainment

Anogenital distance was not recorded for F1 pups. Landmark data for preputial separation and vaginal opening (mean age in days of pups in a litter reaching the criterion) were described as significantly increased but by how much was not included in the RAR. The DS considered that these effects correlated with the decreased body weights of the pups. The mean age of reaching preputial separation was also significantly increased for the 280 ppm male F1 pups. Necropsy examinations did not reveal compound-related changes in F0 adult animals or F1 pups.

F2 pups

Viability and weaning indices were seriously affected by treatment at the top dose of 800 ppm (see Table 33, CLH report); the values were outside historical controls for the performing laboratory. Pup mortality (not including those culled) from PND 0-21 was greatly increased. Male and female pup body weights were also affected in the second generation in-utero to a small extent (approximately a 7% reduction relative to controls) but post-natal growth of the 800 ppm F2 pups were significantly decreased throughout lactation, showing reductions of between 15-22% across both sexes relative to the mean control bodyweight.

Landmark and Pubertal attainment

Anogenital distance was not affected by treatment. Landmark data for preputial separation and vaginal opening was unavailable because the F2 pups were sacrificed on LD21. The DS noted in the 800 ppm group, in-utero growth, post-natal growth, and body weight throughout lactation, were significantly decreased following treatment. The mean age for reaching eye opening in the 800 ppm F2 pups (in days) was significantly increased and correlated with their decreased body weights. Necropsy of F1 adult and F2 pup animals did not reveal compound-related changes.

The DS concluded that dietary exposure of rats to acetamiprid throughout two generations did not result in effects on reproductive performance or fertility. The DS did not propose classification.

Repeat dose studies and two studies from the peer reviewed public literature.

The DS noted several repeated dose studies which indicated limited effects on sexual organs following exposure to ≥ 1000 ppm in the presence of general toxicity. No specific information was presented on how fertility or mating might be affected, just that there were increases in relative testis weight in rats; decreases in prostate absolute weight in mice; significant increases in relative testis weight and a decrease in absolute and relative ovary weights in an additional mouse study:

- 90-day rat oral toxicity study: ↑ relative testis weight (+13%/+19% at 800/1600 ppm), not significant. ↓ body weight relative to controls was -14%/-13% at 800/1600 ppm), significant (Anon. 1997f).
- 90-day mouse oral toxicity study: ↑ relative testis weight (+37% at 3200 ppm, significant. ↓ in absolute and relative ovary weight was -65%/-52% at 3200 ppm / 466 mg/kg bw/day), significant (Anon., 1992).
- 18-month mouse dietary carcinogenicity study: ↓ prostate absolute weight in the interim and terminal sacrifice groups (-39%/-23% at 1200 ppm / 186 mg/kg bw/day), significant (Anon., 1999a).

Two studies from the peer reviewed public literature

Two studies from the public literature were included in the acetamiprid RAR and described in detail by the DS in the CLH report.

1. Oxidative stress

Role in acetamiprid-induced impairment of the male mouse reproductive system – Zang *et al.*, (2011), a non-guideline and non-GLP study.

The method and the results in the study by Zhang *et al.* (2011) are well described and indicate that effects on male reproductive function may occur following gavage exposure to 30 mg/kg

bw/day (acetamiprid purity >97%) to groups of 10 Kunmin male mice per treatment over 35 days.

Compared to the controls, acetamiprid decreased body weight gain (-38%) and the relative weight of the testis and epididymis (-17%), seminal vesicles and prostate gland (-17%) ($p < 0.05$). Compared to the controls, serum testosterone level decreased in the acetamiprid only group ($p < 0.05$). Vitamin E significantly ameliorated these effects.

Compared to the control group, acetamiprid decreased sperm number (-76%), viability (13%) and motility (52%) ($p < 0.05$), with a significant increased rate of acrosome deformity ($p < 0.05$). Vitamin E reduced these adverse effects.

Histological investigations showed various stages of spermatogenesis in testes of the control group mice; Leydig cells were abundant in the interstitium. In the acetamiprid only group, there was vacuolization of the seminiferous tubules and the number of spermatids and interstitial Leydig cells were clearly decreased. There was degeneration of the mitochondria and endoplasmic reticulum within the Leydig cells.

Acetamiprid increased malondialdehyde (MDA) and nitric oxide (NO) concentrations compared to the controls ($p < 0.05$). Vitamin E also ameliorated these effects.

While these results suggest a possible mode of action, they are not definitive and the DS did not consider their impact with regard to sexual function and fertility classification. The DS concluded the results in this study were different from the 13-week repeated dose diet study in mice, which tested at much higher dose levels up to approximately 450 mg/kg bw/day (Anonymous, 1992). According to the DS, the results obtained by Zhang *et al.* were due to different impurities and were not representative for the form of acetamiprid marketed in Europe.

2. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development *in vitro* – Gu *et al.*, (2013). PLoS one July 2013, Volume 8, Issue 7. Non-guideline and non-GLP study.

The DS described a series of *in vitro* mechanistic studies investigating and comparing the effects of acetamiprid, imidacloprid and nicotine (at concentrations of 500 µM and 5 mM), on fertilization and embryonic development up to the pre-implantation stage. Female B6D2F1 (C57BL/6xDBAx2) strain mice were used as oocyte donors and male B6D2F1 mice were used as semen donors.

> Test 1: Effect of chemical exposure on sperm function. The motility of spermatozoa showed no obvious difference from that of controls. The percentage of DNA fragmented spermatozoa was similar across all treatment groups.
>
> Test 2: *In vitro* fertilization performed with the pre-treated spermatozoa. In the presence of toxicant at levels up to 5 mM, all treated spermatozoa retained their potential to fertilize oocytes. All fertilized oocytes survived without evident changes in cell morphology. However, during the culture process, embryos originating from spermatozoa pre-treated with the highest level of test substance, adversely decreased the rates of pronucleus formation (fertilization), the first cleavage and morula/blastocyst formation, compared to those of non-treated controls. Acetamiprid had the weakest effect compared with the other tested compounds. *In vitro* fertilisation performed with normal untreated spermatozoa where the test substances were included in the incubation medium showed similar effects. Direct exposure of nicotine, imidacloprid or acetamiprid had harmful effects in the order of nicotine > imidacloprid > acetamiprid.

In conclusion, the DS did not consider this study to be of much value. The main criticism centred around the *in vitro* concentrations used in the experiment which were considered to be of no relevance to actual *in vivo* gamete exposures.

DS Conclusion on sexual function and fertility

No effects on fertility were observed in the available 2-generation study. Effects on reproductive organs were sometimes observed in the 2-generation study and in the repeated dose studies. These effects were mostly limited to reductions in absolute organ weights such as testis and epididymis in the presence of reduced body weights. As no such effects were observed on relative organ weights and no histopathological effects were observed, the reduced absolute organ weights were considered secondary to the general toxicity and did not warrant classification. The *in vivo* study by Zang *et al.* (2011) was considered to conflict with the results from the 90-day and 18-month studies in mice. The DS questioned whether the active substance used was representative for the form of acetamiprid marketed in Europe. The *in vitro* studies by Gu *et al.* (2013) were not considered relevant from an *in vivo* exposure point of view. No classification for effects on sexual function and fertility was proposed by the DS.

Developmental toxicity

As regards the two-generation study, the DS concluded (see above) that the developmental effects noted in the two-generation reproduction toxicity study (reduced body weight during and at the end of lactation period in two generations of both sexes) did not provide sufficient evidence for classification for developmental toxicity. However, the reduced survival index in the offspring at the highest dose level was recognised as a factor in a weight of evidence for developmental classification.

Developmental toxicity was primarily investigated in the rat and the rabbit in GLP and OECD TG 414 (1981) guideline compliant studies.

Rat preliminary study

In a rat preliminary gavage dosing study (Anon., 1997f), mated rats were treated with acetamiprid (purity 99.46%) at dose levels of 0, 18, 35 and 70 mg/kg bw/day. Treatment-related reductions in parental body weight and food consumption were observed at 35 and 70 mg/kg bw/day. No offspring anomaly was noted at any dose level. Based on these results the following dose levels were selected for the main study: 5, 16, 50 mg/kg bw/day.

Rat main developmental study

The test substance was administered orally by gavage at doses of 0, 5, 16 and 50 mg/kg bw/day to groups of 24 mated female rats/group, for a period of 10 days from GD6 to 15. No treatment-related changes were noted in dams from dose groups with less than 50 mg/kg bw/day.

Maternal toxicity

There was no mortality or clinical signs associated with treatment. There were reductions in the body weight gain during days 6 to 15 of gestation at the highest dose level; this was significantly ($p < 0.01$) decreased at 50 mg/kg bw/day (24.8 g) compared to control (42.1 g). There was no effect on body weight gain after GD15.

Statistically significant but small increases of liver weights and their body weight ratios and kidney/body weight ratios were also observed in the high dose group.

The number of total implantations, corpora lutea and live foetuses were not affected by treatment. The number of resorbed foetuses was significantly increased at 16 mg/kg bw/day but this finding did not show a clear dose-related trend.

Table 8: *Summary of findings in the Dams (based on Table 43, CLH report).*

Dose (mg/kg bw/day)	**0**	**5**	**16**	**50**
No. of females/group	24	24	24	24
No. pregnant (%)	23 (95.8)	23 (95.8)	24 (100)	23 (95.8)
Uterine examination:				
No. of dams examined	23	23	24	23
No. of corpora lutea/dam	20.7	19.4	20.8	20.1
No. of live foetuses/dam	15.0	14.8	14.8	14.1
No. of dead/resorbed foetuses and percentage	12 (3.4)	16 (4.5)	**25 (6.6)***	18 (5.3)
Mean pre-implantation loss (%)	23.6	19	21.8	26.2
Mean post-implantation loss (%)	3.4	4.4	6.7*	7.3

*Significantly different from control, $p < 0.05$
**Significantly different from control, $p < 0.01$

Foetal anomalies

Visceral findings: There were no test article related effects.

External findings: There were no test article related malformations. In the high dose group, there were 3 and 5 incidences of the variations placental and subcutaneous haemorrhage.

Skeletal findings: There were no skeletal malformations linked to acetamiprid exposure. Statistical significance was observed at 50 mg/kg bw/day, concerning the shortening of the thirteenth rib (variation). There was no clear dose response for this effect. All other anomalies occurred at incidences similar to controls.

The DS concluded there was no evidence for teratogenicity or foetotoxicity in the rat even at the highest dose of 50 mg/kg bw/day (findings summarised in Table 44 of the BD).

Rat developmental neurotoxicity study

In the developmental neurotoxicity study by Anon. (1999, report final revision date 2008), pregnant female Sprague-Dawley rats (25/group) were exposed by gavage from gestation day 6 through lactation day 21 daily. The dose groups were 0, 2.5, 10 and 45 mg/kg bw/day. Maternal toxicity was observed at the dose level of 45 mg/kg bw/day by a single mortality and reductions in body weight gain and food consumption (around 15% and 12% respectively, when compared to control).

The DS noted the following toxicologically relevant and non-relevant effects:

1. A decrease in pup postnatal survival (45 mg/kg bw/day, PND 0-1); three dams in this high dose group had total litter loss, this significantly decreased the total postnatal survival in this group by 16% compared to the control.
2. During PND 0-1, pups that were found dead, euthanized in extremis or counted as dead due to the death of the dam (dams numbered 7, 16, 4 and 59 in the control, 2.5, 10 and 45 mg/kg bw/day dose groups, respectively) were reported incorrectly in the BD and recalculated here by RAC.
3. A decrease in the pup startle response was observed. The maximum response amplitudes (Vmax) and the average response amplitudes (Vave) in the 45 mg/kg

bw/day group males were significantly reduced compared to the control group values on PND 20 and 60. Maternal toxicity was evident by a significantly lower body weight and body weight gain. A reduction (not statistically significant), was also observed in the mid-dose group of 10 mg/kg bw/day (where no maternal toxicity was observed).

4. When the entire post-weaning period (PND 21-72) was evaluated, mean body weight gain in the 45 mg/kg bw/day group males and females were reduced (less than 10%) and statistically significant compared to the control group values.
5. Time to preputial separation was not affected by the test article at any dose level. The mean days of acquisition were PND 43.6, 43.7, 43.1 and 43.9 for the control, 2.5, 10 and 45 mg/kg bw/day groups, respectively.
6. Time to vaginal opening was not affected by the test article at any dose level. The mean days of acquisition were PND 32.2, 32.3, 32.7 and 32.5 for the control, 2.5, 10 and 45 mg/kg bw/day groups, respectively.

Deficits (attenuation) in the auditory startle response occurred in the top dose F1 males and females without concomitant effects in other functional endpoints, neuropathology or brain morphometry. The only adverse effect observed in the absence of maternal toxicity was the reduced startle response at 10 mg/kg bw/day, although this difference was not statistically significant.

The DS, in annex I to the CLH report, considered the effects on Vmax and Vave in the 10 mg/kg bw/day group as toxicologically significant. The DS agreed with the position stated in the EFSA Peer Review report EFSA (2016) and acknowledged that the EFSA PPR panel took a precautionary and conservative approach when determining the NOAEL (2.5 mg/kg bw/day) for the study. The DS considered the evidence from this study relevant in its opinion on supporting classification for development.

Rabbit preliminary study

In a rabbit preliminary gavage dosing study (Anon., 1997e), pregnant does (4 animals/group) were administered acetamiprid at dose levels of 0, 5, 13, 30 and **75** mg/kg bw/day. Dosing was performed during days 6 to 18 of gestation. All high-dose animals died by day 14. Treatment-related reductions in parental body weight and food consumption were observed at 13 and 30 mg/kg bw/day. No external abnormalities were noted in foetuses at any dose level. Based on these results, the following dose levels were selected for the main study: 7.5, 15 and 30 mg/kg bw/day.

Rabbit main developmental study

The test substance was administered orally by gavage at doses 0, 7.5, 15 and 30 mg/kg bw/day to groups of 17 mated females/dose (Anon., 1997e). Dosing was performed during days 6 to 18 of gestation. Control animals received vehicle only. Based on the results of the preliminary study, the maximal tolerated dose was considered to be 30 mg/kg bw/day.

Maternal toxicity

There was no substance related mortality, no toxic signs or organ weight changes in any dose group (Anon., 1997e). The numbers of total implantations, corpora lutea and live-foetuses did not show any treatment related response. Maternal body weight depression and a statistically significant decrease of food consumption were noted in females from the 30 mg/kg bw/day group. The body weight gain during days 6 to 19 of gestation for the 30 mg/kg bw/day dose group was

negative (-7.3 g). Although not statistically significant, the difference was substantial when compared to the control (+35.2 g).

No treatment-related changes were noted in females from other dose groups.

Table 9: *Summary of findings in the does (based on Table 45, CLH report).*

Dose (mg/kg bw/day)	**0**	**7.5**	**15**	**30**
No. of females/group	17	17	17	17
No. pregnant (%)	12 (70.6)	15 (88.2)	14 (82.4)	14 (82.4)
Uterine examination:				
No. of dams examined	12	12	12	13
No. of corpora lutea/dam	11.0	11.8	11.2	9.3
No. of live foetuses/dam	7.8	8.3	7.8	6.6
No. of dead/resorbed foetuses and percentage	12 (11.4)	10 (8.6)	16 (13.5)	8 (7.3)
Mean pre-implantation loss (%)	20.8	23.2	19.2	26.1
Mean post-implantation loss (%)	11.4	8.6	13.5	7.3

Foetal anomalies

Visceral findings: no test article related effects, though one foetus of the high dose group showed microphthalmia.

External findings: no test article related effects.

Skeletal findings: there were some sporadic, non-statistically significant differences in the type and incidence of skeletal anomalies in the test article treated groups compared to the control group. The DS considered these were spontaneous because there was no statistical significance or dose dependency between the control and dose groups.

The DS concluded there was no evidence for substance related teratogenicity or foetotoxicity.

Other studies

The DS briefly described two rat neurotoxicity studies, an acute oral neurotoxicity study and a sub-chronic (13 weeks) oral neurotoxicity study. There was little relevance to developmental toxicity. There was no evidence of neurotoxicity in the 90 day study which tested up to 1600 ppm (118 mg/kg bw/day and 134 mg/kg bw/day for males and females respectively). The acute study showed some behavioural changes including reduced locomotor activity at 100 mg/kg bw/day and reduced temperature.

DS Conclusion on development

The possible adverse effects of acetamiprid on development were studied in rats as well as in rabbits. In both rats and rabbits, in the presence of limited maternal toxicity, *no teratogenicity or foetotoxicity* was observed at the highest doses tested (50 and 30 mg/kg bw/day, respectively).

In the 2-generation study in rats a reduction in post-natal survival (viability index (PND0-4) and weaning index (PND4-21)) was observed in the F2 high dose animals in the presence of maternal toxicity in the form of reduced body weight. Pup body weights were substantially reduced in both F1 and F2 pups with accompanying delays in preputial separation, vaginal opening, eye opening and pinna unfolding.

In the developmental neurotoxicity study, post-natal survival was also reduced mainly in the early part of the lactation period at the top dose level (45 mg/kg bw/day). In addition, a reduction in post-weaning body weights was also observed. Reduced auditory startle responses in offspring at 10 mg/kg bw/day (observed in the absence of maternal toxicity), were assumed to be related to treatment.

The DS noted the following toxicologically relevant effects in support of its proposal for Repr. 2, H361d:

1. The decrease in postnatal survival observed in the F2 pups of the rat 2-generation study at the top dose of 800 ppm (51 mg/kg bw/day); this effect was not observed in the F1 pups.
2. The decrease in postnatal survival observed in the rat developmental neurotoxicity study at the top dose of 45 mg/kg bw/day at PND 0-1.
3. The decrease in startle response in the developmental neurotoxicity study, significant at the top dose of 45 mg/kg bw/day in males at PND 20 and 60 and observed but not statistically significant at the mid dose of 10 mg/kg bw/day.

Adverse effects on or via lactation

The DS discussed adverse effects on or via lactation in the context of effects seen in four studies:

1. Rat 2-generation study (Anon., 1999d): post-natal growth of the 800 ppm F1 and F2 pups (highest dose tested) were significantly decreased throughout lactation. In addition, a reduction in post-natal survival (viability index and weaning index (PND 4-21)) was observed in the F2 high dose animals in the presence of limited maternal toxicity. Pup body weights were reduced in both F1 and F2 pups with accompanying delays in preputial separation, vaginal opening and eye opening.
2. In the developmental neurotoxicity study (Anon., 2008), exposure from gestation day 6 until lactation day 21 resulted in post-natal effects including reduced survival, reduced body weight gain, reduced body weight ~~at gaining vaginal opening~~ and effects on the startle response at the top dose in the presence of limited maternal toxicity. A possible effect on the startle response was also observed at the mid dose of 10 mg/kg bw/day.
3. Lactating Holstein cows (Anon., 1999b), exposed to acetamiprid for 28 consecutive days showed a dose-dependent and rapid increase of acetamiprid concentration and its IM-2-1 residue in whole milk.
4. Lactating goats (US EPA, 2002) were orally dosed twice daily for 7 days with encapsulated [pyridine-2, 6-^{14}C]-acetamiprid. Less than 1% of the administered radioactivity was found in milk, less than 10% of this being parent compound.

The DS noted that it was unclear whether the observed effects in the 2-generation and developmental neurotoxicity studies were due to exposure via milk or due to *in utero* exposure. There was no cross-fostering study to substantiate effects via lactation. The body weights were already reduced at post-natal day 0, which suggested that the *in utero* exposure at least contributed to the reduced post-natal weight reduction. Some exposure via milk was suggested by the available milk residue studies, but the amount of acetamiprid was very limited and the contribution of the main metabolite IM-2-1 to the toxicity was presumed even lower as the acute oral toxicity for this metabolite was less than that of the parent compound.

The DS concluded no classification for effects on or via lactation because the available data was insufficient to determine if the observed post-natal effects were due to exposure *in utero* or exposure to acetamiprid or its metabolites via milk.

DS Conclusion for Reproductive Toxicity Classification

The DS considered there was sufficient evidence from animal studies on adverse effects on development to propose Repr. 2; H361d.

Comments received during general consultation

Seven comments in total were provided on reproductive toxicity during the general consultation. Four commenting MSCAs supported the proposal to classify acetamiprid as Repr. 2; H361d for effects on development although one of them expressed doubts on where to assign the effects observed.

One MSCA expressed doubts about the described effect on startle response being adverse with regard to further development and asked for additional details on the effect and post-natal mortality from the developmental neurotoxicity study. The DS supplied the details in an annex to the CLH report derived from the latest version of the DAR.

There was one comment from a company-manufacturer, they disagreed with classification for reproductive toxicity. They supplied two detailed reports – one originally targeted at the draft Renewal Assessment Report (RAR) where they requested that their position on the adequacy and interpretation of the developmental neurotoxicity study be represented by the DS (Li, 2015). The second was a report by Exponent (2019) outlining several points on reproductive toxicity and overlapping with points made by Li, (2015). These points were adequately addressed by the DS in the RCOM document and did not represent new data that had to be evaluated further.

There were two comments by company-downstream users. The first commenter stressed that the classification was erroneous because of the major impact by maternal toxicity on the findings. The DS disagreed with this interpretation. The second commenter provided an expert statement on Carcinogenicity and Reproductive Toxicity classification in a pdf document attachment. The DS referred back to its two previous responses on reproductive effects, no new data was submitted.

Assessment and comparison with the classification criteria

Rat 2-generation study

No effects on reproductive performance were observed in the available 2-generation study at dietary concentrations up to 800 ppm. Mating performance, fertility, most reproduction parameters and oestrous cycles for both the F0 and F1 adult generations were unaffected by treatment (tables 10a and 10b). Effects on reproductive organs were not observed in the 2-generation study but were observed at higher concentrations in the repeated dose studies. These effects were mostly limited to reductions in absolute organ weights such as testis and epididymis in the presence of reduced body weights. As no effects were generally observed on relative organ weights and no histopathological changes were observed, the reduced absolute organ weights are considered secondary to the general toxicity and do not warrant classification.

Table 10a: *Summary of F0 generation mating and F1 litter body weight and survival data (Mean ± SD).*

Dose (ppm)	Control	100	280	800
No of rats (male/female)	26/26	26/26	25/25	26/26
No. of pregnant females	26	25	25	26
Fertility index (%)	100	96	100	100
Gestation index	100	100	100	100
Gestation length (days)	22.0 ± 0.4	21.8 ± 0.4	22.0 ± 0.0	22.0 ± 0.2
Total no. of pups/litter	13.50 ± 2.10	13.84 ± 1.62	13.44 ± 3.01	13.08 ± 2.06
No. of live pups/litter	13.46	13.68	13.2	12.65
Sex ratio (%) of F1 pups (LD0)	48	52	45	46
Pup viability index during lactation (%)				
Live birth index (%)	100	99	98	97
Viability index (%)	95	99	98	96
Weaning index (%)	96	99	99	94
Pup mortality (not culled) LD0-21	22	5	10	26
No. of total litter losses	1	0	0	0
Male pup bw change				
Mean pup body weights day 0 (%)	100	101.7	102.9	**94.6***
Mean pup body weights day 4 (%)	100	93.5	92.7	**78.5****
Mean pup body weights day 7 (%)	100	95.2	90.7	**76.4****
Mean pup body weights day 14 (%)	100	98.3	94.1	**81.6****
Mean pup body weights day 21 (%)	100	99.6	96.5	**85.0****
Female pup bw change				
Mean pup body weights day 0 (%)	100	100.2	102.3	**93.3***
Mean pup body weights day 4 (%)	100	92.7	90.4	**77.5****
Mean pup body weights day 7 (%)	100	94.2	88.5	**78.4****
Mean pup body weights day 14 (%)	100	99.3	95.5	**84.8****
Mean pup body weights day 21 (%)	100	101.0	98.4	88.8

** $p \leq 0.01$

Table 10b*: Summary of F1 generation mating and F2 litter body weight and survival data (Mean ± SD).*

Dose (ppm)	Control	100	280	800
No of rats (male/female)	26/26	26/26	26/26	26/26
No. of pregnant females	20	24	24	23
Fertility index (%)	77	92	92	88
Gestation index	100	100	100	100
Gestation length (days)	22.1 ± 0.3	22.0 ± 0.4	22.1 ± 0.3	21.8 ± 0.7
Total no. of pups/litter	12.70 ± 4.50	14.50 ± 3.51	14.92 ± 2.24	12.26 ± 4.11
No. of live pups/litter	12.60	13.96	14.50	12.04

Dose (ppm)	Control	100	280	800
Sex ratio (%) of F1 pups (LD0)	53	50	48	51
Pup viability index during lactation (%)				
Live birth index (%)	99	97	97	98
Viability index (%)	94	90	95	**66****
Weaning index (%)	98	**94***	97	**73****
Pup mortality (not culled[1]) LD0-21	20	33	22	**122[2]**
No. of total litter losses	0	2	0	5
Male pup bw change				
Mean pup body weights day 0 (%)	100	98.6	101.1	**92.8****
Mean pup body weights day 4 (%)	100	95.5	99.5	**84.6****
Mean pup body weights day 7 (%)	100	94.0	99.4	**79.9****
Mean pup body weights day 14 (%)	100	94.0	98.7	**78.4****
Mean pup body weights day 21 (%)	100	97.2	98.4	**78.1****
Female pup bw change				
Mean pup body weights day 0 (%)	100	99.2	102.1	**93.8****
Mean pup body weights day 4 (%)	100	95.6	98.2	**84.2****
Mean pup body weights day 7 (%)	100	94.7	99.1	**82.3****
Mean pup body weights day 14 (%)	100	94.2	98.8	**80.8****
Mean pup body weights day 21 (%)	100	97.1	98.1	**81.1****

** $p \leq 0.01$
[1] pup mortality excluding those pups that were intentionally culled according to the study design.
[2] 92 pups from LD0-4 including 1 entire litter loss; 30 pups from LD5-21 including 4 entire litter losses.
HCD range for pup mortality (20 studies, 1994-1998): LD0-4: 1-30; LD5-21: 0-5.

RAC noted statistically significant effects on (i) pup body weight (development, possible lactation effect), (ii) pup survival (development) and (iii) pubertal time of attainment (fertility and sexual function or development).

Maternal Toxicity: There was evidence of limited maternal toxicity. In F0 females there were decreases in mean body weight (8 to 10% relative to controls, significant, ~~P~~p ≤ 0.01) and body weight gain (approximately 8%) and food consumption (approximately 20%, significant p ≤ 0.01) in the high dose group over gestation days 0-20. In F1 females, there were decreases in mean body weight (12 to 14% relative to controls, significant, p ≤ 0.01) and body weight gain (approximately 8%) and food consumption (approximately 13%, significant p ≤ 0.01) in the high dose group over gestation days 0-20.

Effects on pup body weight

The effects on neonatal body weight were significant. F1 pup body weights were affected *in-utero* to a small but significant extent (< 7% reduction relative to controls) and postnatal growth at 800 ppm was significantly decreased throughout lactation, showing reductions of between 11-24% across both sexes relative to the mean control bodyweight. A similar effect on pup body weights was observed for the F2 generation. F2 pup body weights were affected *in-utero* to a small but significant extent (approximately a 7% reduction relative to controls) and postnatal growth at 800 ppm was significantly decreased throughout lactation, showing reductions of between 15-22% across both sexes relative to the mean control bodyweight. It is unclear whether the observed effects were due to exposure via milk or due to *in-utero* exposure. The

fact that the body weights were already reduced at PND 0 suggests that the *in-utero* exposure may have at least contributed to the reduced postnatal weight reduction.

Effects on pup survival

Viability and weaning indices were clearly affected by treatment at the top dose of 800 ppm; these values were outside historical controls for the performing laboratory. Pup mortality (not including those culled) from LD0-21 was greatly increased and is clear evidence of an adverse developmental effect.

Effects on pubertal time of attainment

There was no treatment-related effect on anogenital distance; however, this data was only recorded for F2 pups. Landmark data for preputial separation and vaginal opening (mean age in days of pups in a litter reaching the criterion) were reported for F1 pups only (table 11a), and were significantly increased in the mid dose (males) and high dose groups (both sexes). The HCD from the performing laboratory was as follows:

- Preputial separation: mean = 41.7 days, range 41.1 – 42.4 days, 5 studies (1994-1998)
- Vaginal opening: mean = 32.9 days, range 31.7 – 34.3 days

The mean age for attainment of preputial separation in males was significantly delayed by nearly 5 days in the top dose group compared with the control group. This was outside the performing laboratory historical control values. Note: HCD from several other sources were reported in the original study report. The mean delay in preputial separation in the top dose group remained outside the upper bound limits of the HCD range in all cases.

The mean age of attainment of vaginal opening in females was significantly delayed by about 3 days in the top dose group. This was greater than the mean value for the historical control data but did not lie outside of the HCD range for this endpoint.

RAC notes that there was no effect on time of pubertal attainment in the rat developmental neurotoxicity study (Anon., 2008).

Table 11a*: Summary of F1 litter landmark and pubertal attainment (covariate adjusted mean ± SD).*

Dose (ppm)	Control	100	280	800
Preputial separation	41.56 ± 1.83	42.36 ± 1.05	**43.32* ± 2.54**	**46.48** ± 2.75**
Vaginal opening	31.08 ± 0.91	31.81 ± 1.46	31.80 ± 2.18	**33.98** ± 3.62**
Anogenital distance (mm)[1]	--	--	--	--

* p ≤ 0.05; ** p ≤ 0.01
[1] Not recorded for F1 pups

Note: day of attainment of other landmarks such as eye opening, incisor eruption and pinna unfolding were not affected by treatment with acetamiprid.

Table 11b*: Summary of F2 litter landmark and pubertal attainment (covariate adjusted mean ± SD).*

Dose (ppm)	Control	100	280	800
Preputial separation[1]	--	--	--	--
Vaginal opening[1]	--	--	--	--
Eye opening	14.56 ± 0.69	14.65 ± 0.78	14.35 ± 1.10	**15.44* ± 0.92**
Anogenital distance (mm)				
males:	1.88 ± 0.22	1.85 ± 0.17	1.88 ± 0.19	1.84 ± 0.21
females:	0.70 ± 0.07	0.70 ± 0.09	0.69 ± 0.07	0.69 ± 0.08

Dose (ppm)	Control	100	280	800

* $p \leq 0.05$;
[1] Not recorded for F2 pups, scheduled termination on LD21.

Note: day of attainment of other landmarks such as incisor eruption and pinna unfolding were not affected by treatment with acetamiprid.

Under CLP (Annex I: 3.7.1.3) it is recognised that adverse effects on sexual function and fertility include effects on the onset of puberty. This criterion would appear to be satisfied for acetamiprid, since it significantly delays the time of onset for preputial separation in males and to a lesser extent, vaginal opening in females. The criteria as specified under section 3.7.2 of CLP indicate classification with Repr. 2 may be considered when there is some evidence from animal studies "of an adverse effect on sexual function and fertility".

Reductions in body weight during postnatal development are known to cause delays in the onset of puberty. It is established in the scientific literature that growth rate is of greater importance than arrival at a particular fixed weight in determining the onset of puberty. For the top dose F1 female and male pups there is evidence of a significant delay in growth amounting to about a 15% reduction relative to concurrent controls by PND 21. This indicates a recovery by the pups as the maximum effects were observed during PND 4-14. RAC considers that the delayed pubertal effects seen in males may not only be a direct result of acetamiprid toxicity (and therefore indicative of a direct effect on pubertal attainment and thus fertility), but may also be indicative of a more generalised adverse effect on growth and thus support classification for developmental toxicity.

Rat Oral Developmental Neurotoxicity Study

This 1-generation study (compliant to US-EPA guideline OPPTS 870.6300 (1998)), was designed to determine the potential of the test article, acetamiprid, to induce functional and/or morphological changes to the nervous system, which may arise in the offspring from exposure of the mother during pregnancy and lactation. The test article was administered orally by gavage to three groups of 25 Crl:CD (SD)IGS BR rats once daily from gestation day 6 through lactation day 21, inclusively. Dosage levels were 0, 2.5, 10 and 45 mg/kg bw/day.

There were no significant differences among groups for pregnancy rates, gestation length and parturition, the only exception being the high dose female, which died because of dystocia. At the scheduled necropsy of F0 females on lactation day 21, no internal findings were noted. The mean numbers of implantation sites, numbers of pups born and numbers of unaccounted sites recorded at the scheduled necropsy were unaffected by treatment.

Table 12: *Summary of reproductive performance*

Parameter	Control		2.5 mg/kg bw/day		10 mg/kg bw/day		45 mg/kg bw/day	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Females on study	25		25		25		25	
Females that died	-		-		-		1	
Females allowed to deliver	25		25		25		25	
Non-gravid	2	8	2	8	2	8	-	-
Gravid	23	92	23	92	23	92	25	100
Females with total litter loss	-		-		-		3	12
Females with viable pups	23	100	23	100	23	100	21	84
Gestation length (days – mean value)	21.5		21.5		21.5		21.7	
Implantation sites (mean value)	15.4		15.7		16.2		15.9	
Number born (mean value)	15.1		15.3		15.5		15.5	
Unaccounted sites (mean value)	0.4		0.3		0.7		0.5	

Unlike the results from the rat 2-generation study, the mean numbers of days to acquisition of preputial separation and vaginal opening in the treated group males and females were not affected by F0 maternal test article administration (table 13). There was no evidence of effects on sexual function and fertility.

Table 13*: Summary of F1 litter pubertal attainment.*

Dose (mg/kg bw/day)	Control	2.5	10	45
Preputial separation	43.6 ± 1.01	43.7 ± 1.57	43.1 ± 1.51	43.9 ± 1.77
Vaginal opening	32.2 ± 0.71	32.3 ± 0.95	32.7 ± 1.40	32.5 ± 1.14

* $p \leq 0.05$; ** $p \leq 0.01$

Overall, RAC does not consider the changes in maternal body weight and body weight gain or food consumption as excessive maternal toxicity. The DS did not discuss the pubertal findings in much detail in the CLH report. RAC considers the male pubertal delay in the rat 2-generation study sufficient for discussion on classification for adverse effects on fertility and or development. Classification as reproductive toxicant in Cat. 2 for effects on fertility was proposed for plenary discussion, however it is also recognised that these effects can also reflect the adversity of significantly decreased growth and thus support relevant effects by developmental toxicity. The pubertal findings in the rat developmental neurotoxicity study were also discussed and may simply be a consequence of the different dosing strategy where exposure is after implantation. The effects on pup body weight and survival are best described under classification for development. RAC considered the delays in male puberty as caused by a general developmental delay and thus supporting of the overall evidence for adverse effects on development and supportive of classification in category 2 for development.

Developmental toxicity was primarily investigated in the rat and the rabbit in GLP and OECD TG 414 (1981) guideline compliant studies. In both rats and rabbits, no teratogenicity or foetotoxicity was observed at the top doses tested (50 and 30 mg/kg bw/day, respectively).

In the developmental neurotoxicity study (Anon., 2008), exposure from gestation day 6 until lactation day 21 resulted in postnatal effects including reduced survival, reduced body weight gain, reduced body weight at time of vaginal opening (responses were within the HCD), and effects on the acoustic startle response at the highest dose. It is also noted by RAC that the effects on body weights and survival in these pups were similar to or at least supportive of those in the rat 2-generation study. An important question is whether the effects on auditory startle response (figure 2), represent specific neurotoxicity or developmental toxicity. Also, the sex-specificity of the response (males only), particularly at PND60 remains unexplained.

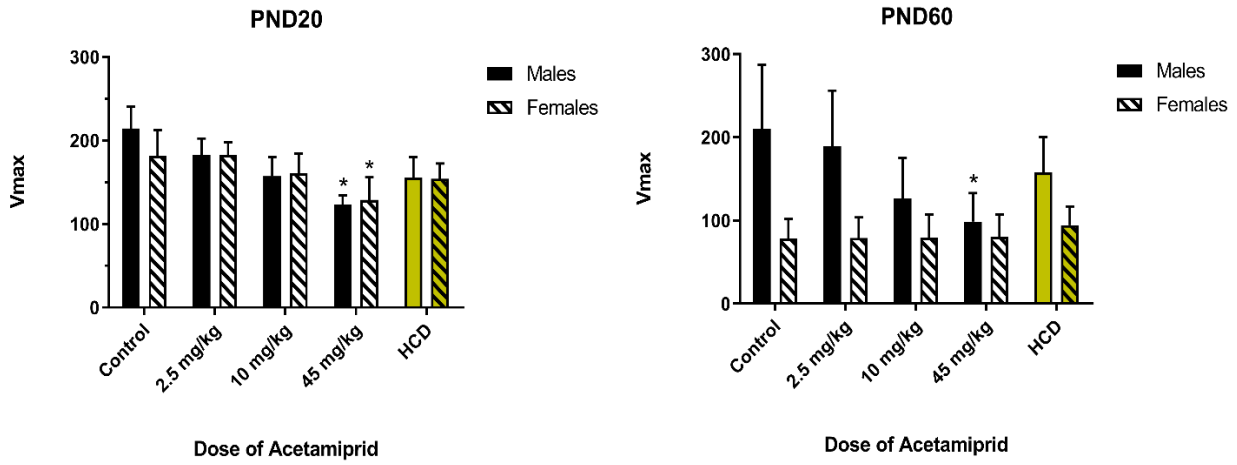


Figure 2: The acoustic startle reflex at PND20 and PND60. Each test session consisted of 5 blocks of 10 trials. There were 10 rats/sex/dose group. Vmax and Vave essentially provide identical information. The PND20 HCD was derived from 19 studies (Oct. 1999 – Jan. 2005). The PND60 HCD was from 22 studies (Oct. 1999 – Jan. 2005). Acetamiprid inhibition of the startle reflex was statistically different from controls in the top dose group only. Error bars are ± 1 s.d.

In summary, the decrease in startle response in the developmental neurotoxicity study, while significant at the top dose of 45 mg/kg bw/day in males at PND20 and 60 and observed but not statistically significant at the mid dose of 10 mg/kg bw/day may not be an indicator of developmental toxicity, it does however suggest an effect such as diminution of motor function. It remains unclear since no recovery group was used in the rat developmental neurotoxicity study to show if the startle response returns to control levels following cessation of exposure. The histopathological investigation failed to show any treatment related changes in the brain.

RAC concurs with the DS in supporting classification in category 2 for developmental effects based on:

- The statistically significant reduction in postnatal pup body weights in the top dose group (800 ppm; 105-108 mg/kg bw/day lactation phase) for both sexes during PND 0-21.
- The decrease in postnatal survival observed in the F2 pups of the rat 2-generation study at the top dose of 800 ppm; this effect was not observed in the F1 pups. This is the most adverse developmental effect noted.
- The decrease in postnatal survival observed in the rat developmental neurotoxicity study at the top dose of 45 mg/kg bw/day on PNDs 0-1.

Overall, RAC considers the reductions in pup body weight, postnatal survival and delayed male rat pubertal attainment sufficient for **classification as Repr. 2; H361d for adverse effects on development.**

Adverse effects on or via lactation

RAC supports the assessment of the DS noting that it was unclear whether the observed effects in the 2-generation and the neurotoxicity developmental toxicity studies were due to exposure via milk or due to *in utero* exposure. There was no cross-fostering study to substantiate effects via lactation. Clear evidence of an adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk could not be demonstrated. **Therefore, no classification for effects on or via lactation is warranted.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Acetamiprid, which belongs to the neonicotinoid insecticides, is currently classified as Aquatic Chronic 3 in Annex VI to the CLP Regulation. The DS proposed to classify the substance as Aquatic Acute Category 1 based on the 48h LC_{50} of 20.7 µg/L for *Chironomus riparius* and in Aquatic Chronic Category 1 based on the 28 day EC_{10} of 0.235 µg/L for *Chironomus riparius* and the substance being not rapidly degradable. The proposed M-factors were 10 (0.0207 mg/L ≤ 1 mg/L) for acute toxicity and 100 (0.0001 mg/L < 0.000235 mg/L ≤ 0.001 mg/L) for chronic toxicity.

Degradation

There was one ready biodegradability test available on acetamiprid (OECD TG 301B, GLP) showing 27% degradation after 28 days, which is below the CLP criteria of 70% after 28 days. Consequently, the DS considered the substance not readily biodegradable.

Hydrolysis of radiolabelled acetamiprid was tested in a GLP study in three buffer solutions of pH 4, 5, 7 and 9 and at temperatures of 22, 35 and 45 °C. The hydrolysis was performed in the dark. At pH 4, 5 and 7 the substance was stable at all test temperatures. At pH 9 the substance became instable at higher temperatures with half-lives of 52.9 days and 13 days for temperatures of 35 and 45 °C. The DS concluded that the substance is stable at environmentally relevant conditions.

In a water/sediment simulation study, radiolabelled acetamiprid was examined in a water-sediment system with sediment from two different locations (Manningtree and Ongar, Essex, UK) for 115 days mainly in the dark at a temperature of 20 °C. The overall recovery was 97.3% of the applied dose. According to the RAR (Volume 3, B8, November 2015), up to 14 days acetamiprid remained the main component of all chromatographic samples from both water and sediment. After this time, both IM-1-4 and IC-0 were the major metabolites. The reassessed (Focus Kinetics) geometric mean half-lives of 23.1 and 31.6 days were calculated for the Manningtree and Ongar whole system, respectively.

The aerobic degradation of radiolabelled acetamiprid in surface water from a natural pond was investigated (OECD TG 309, GLP). The degradation of two concentrations was tested. The total mass balance was between 97.5 and 101.6% of the applied radioactivity. Acetamiprid declined to 6.5% and 11.5% of the applied dose in the low and high dosed systems, respectively. In all

systems, one metabolite (IM-1-4) was detected with a concentration > 10% of the applied dose and the concentration of this metabolite increased to 81.5 and 70.8% in the low and high dose respectively over the 59 days of the incubation. Volatiles did not exceed 1% (≤ 0.57%). Re-evaluation of the half-lives resulted in half-lives of 2.4 and 6.8 days for the low and high dose respectively. The acute toxicity values in RAR (Volume I, November 2015) for the metabolite IM-1-4 were in the range of 10 to 100 mg/L for fish, Daphnia, Chironomus and *Mysidopsis bahia*. No algae data was available. According to the CAR, (Acetamiprid, Product type 18, August 2018) IM-1-4 was hydrolytically stable and there were not enough data available in the water/sediment study and the water degradation study to derive information about the biodegradation of IM-1-4. Based on this information the fulfilment of the criteria for classification as hazardous to the aquatic environment cannot be excluded.

Direct photochemical degradation was investigated with radiolabelled acetamiprid at 25 °C for 30 days with 12-hour light per day. The light intensity was 690 W/m^2 and wavelengths < 290 nm were filtered out. Recovery of the radiolabels was 99.5%. After 30 days, only 0.1-0.2% of the applied dose was retrieved from the volatile traps and 54% of the parent compound was still present in the test solutions. The half-live of the parent was calculated to be 34 days. Degradation products were detected.

Bioaccumulation

There was no fish bioconcentration study available. The measured log K_{ow} was 0.80 (shake flask method). Based on the available Log K_{ow} being below the CLP criterion of ≥ 4, the DS considered the substance to have a low potential for bioaccumulation.

Aquatic toxicity

Table 14: *Reliable aquatic toxicity tests on acetamiprid*

Method	Species	Test material	Results	Remarks	Reference
Fish					
OECD TG 203, GLP Static	*Oncorhynchus mykiss*	Acetamiprid 99.57%	96h LC_{50} > 100 mg/L (nominal)	Measured concentrations 101-104% of nominal (RI = 2)*	Anonymous (1997h)
Flow through toxicity study	*Lepomis macrochirus*	Acetamiprid > 99%	96h LC_{50} > 119.3 mg/L (mean measured)	Measured concentrations 93-99% of nominal (RI = 2)*	Anonymous (1997b)
FIFRA guideline 72-3, GLP Flow-through	*Cyprinodon variegatus*	Acetamiprid Purity: > 99.9%	96h LC_{50} 92 mg/L (mean measured)	Measured concentrations 88-102% of nominal (RI = 2)*	Anonymous (1998c)
OECD TG 210 Static, GLP	*Pimephales promelas*	Acetamiprid Purity: 100%	35d NOEC 19.2 mg/L (mean measured)	Measured concentrations 89-117% of nominal (RI = 2)*	Anonymous (1997g)
Invertebrates					
OECD TG 202 Static	*Daphnia magna*	Acetamiprid (NI-25) Purity: 99.9%	48h LC_{50} 49.8 mg/L (mean measured)	Measured concentrations 94-103% of nominal (RI = 2)*	Saika (1997)
ASTM guideline E-729 and FIFRA 72-2	*Chironomus riparius*	Acetamiprid Purity: 99.3%	**48h LC_{50} 20.7 µg/L** (mean measured)	Measured concentrations 92-110% of	Putt (2003b)

Static, GLP				nominal (RI=2)*	
OPPTS draft guideline 850-1020 Static, GLP	*Gammarus fasciatus*	Acetamiprid Purity: 99.3%	96h LC_{50} 100 µg/L (mean measured)	Measured concentrations 87-100% of nominal (RI = 2)*	Putt (2003a)
FIFRA 72-3 Guideline Flow-through, GLP	*Mysidopsis bahia*	Acetamiprid Purity: 99.9%	96h EC_{50} 66 µg/L (mean measured)	Measured concentrations 98-110% of nominal (RI = 2)*	Anonymous (1998b)
EPA/FIFRA guideline 72-4 Static renewal, GLP	*Daphnia magna*	Acetamiprid Purity: 99.9%	21d NOEC 5 mg/L (mean measured)	Measured concentrations 95-99% of nominal (RI = 2)*	Suteau (1997)
BBA guideline Static, GLP	*Chironomus riparius*	Acetamiprid Purity: 99.9%	**Recalculated: 28d NOEC 0.96 µg/L EC_{10} = 0.235 µg/L** (mean measured)	The study is considered reliable (RI = 2)*	Mc Elligott (1999)
Algae/Aquatic Plants					
OECD TG 201 Static, GLP	*Scenedesmus subspicatus*	Acetamiprid Purity: 100%	72h EC_{50} and NOEC > 98.3 mg/L (mean measured) no effects	Measured concentrations 95-106% of nominal (RI=2)*	Suteau (1996)
FIFRA guideline 122-2 and 123-3 Static, GLP	*Anabaena flos-aquae*	Acetamiprid (N-25) Purity: 99.9%	5d EC_{50} and NOEC > 1.3 mg/L (mean measured) One concentration tested, no effects	Measured concentration 130% of nominal (RI=2)*	Hoberg (1997a)
FIFRA guideline 122-2 and 132-3 Static, GLP	*Lemna gibba*	Acetamiprid Purity: 99.9%	14-d EC_{50} and NOEC > 1.0 mg/L (mean measured) One concentration tested, no effects	(RI = 2)*	Hoberg (1997b)

* Reliability according to Klimisch et al. (1997), since assessment is based on summaries in the DAR, Ri=1 is not given.

Acute toxicity

There were three acute toxicity studies available for fish, four for invertebrates and three for algae. The lowest acute toxicity value was a 48h LC_{50} of 20.7 µg/L 8 (mean measured) for *Chironomus riparius* in a static test. The nominal test concentrations were: 6.3, 13, 25, 50 and 100 µg/L. A total of 140 organisms (5 per replicate, 4 replicates per concentration) were exposed to five concentrations of the test substance, a dilution water control and a solvent control for 48 hours. A 1.0 mg/mL stock solution was prepared by placing 0.25180 g (0.25004 g as active ingredient) of acetamiprid in a 250 mL volumetric flask and bringing to volume with acetone (0.10 mL/L). Mortality was recorded at 0, 24 and 48 hours of exposure. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 hours. At test initiation and test termination, one sample was

removed from each test solution and the control for analysis of test substance concentration. Analytical results showed a mean recovery from 92 to 110% (mean measured concentrations: 6.0, 14, 26, 46 and 110 µg/L). Results were expressed in mean measured concentrations, which is acceptable. The Dossier Submitter notes that the current study setup contains no sediment, thus exposure occurs only via the water.

Chronic toxicity

There was one chronic toxicity study available for fish, two for invertebrates and three for algae.

The lowest chronic toxicity value was from a 28-day static sediment/water system test on *Chironomus riparius.* The study was included in the DAR, but the endpoint has been recalculated because it was expressed as nominal concentration while concentrations drop below the limit of quantification (LOQ) at the end of the study. The test system included 3L glass beakers, 2 cm sediment layer (artificial sediment prepared according to OECD TG 207), and 2.5L reconstituted overlaying water. Samples from the water column were collected for analysis 1 hour, 7, 13 and 28 days after test initiation. No measurements were performed on the sediment. One hour after test initiation analytical verification of the test concentrations in the overlaying dilution water showed that the measured values were close to the nominal concentrations (92-100% recovery). Further on, the test concentrations on day 7 showed that at nominal concentrations 1.3 and 2.5 µg/L the measured concentrations were below the LOQ (1 µg/L). At the higher nominal concentrations of 5 and 10 µg/L, the recoveries were 30 and 45% of the initial measured values, respectively. Following 13 days of exposure, 17% recovery of initial measured value was observed at the highest nominal concentration of 10 µg/L and recoveries at the three lower test concentrations were all below the LOQ. A final analytical verification at test termination on day 28 showed that the recoveries from all of the test concentrations were below the LOQ under the conditions of the test. The results are expressed in nominal concentrations. It was concluded that the 28-day NOEC of acetamiprid for the sediment dwelling life stage of *Chironomus riparius* in a static sediment water system, is estimated to be 5 µg/L, and the 28-day LOEC was observed to be 10 µg/L (nominal concentrations). In the RAR, a recalculation based on the geomean of the measured concentrations was presented. Using the regression equations based on the calculation of the DT_{50} for decline of the concentration of acetamiprid in the water phase for the two highest concentrations, where 2 data points with concentrations below the LOQ are available, estimations were made for the concentrations which were below the LOQ of 1 µg/L. The lowest statistically significant EC_{10} and EC_{20} values were 0.235 and 0.333 µg a.s./L, and the NOEC 0.96 µg/L. The DS notes that the study setup contains sediment. Actual concentrations were determined in the water column, but not in the sediment. However, acetamiprid does not strongly adsorb to soil. Thus, while exposure via the sediment and/or food can occur, it is expected that it mainly occurred via overlaying/pore water. Overall, the results are considered reliable by the DS.

Comments received during consultation

Comments were received from four Member States (MS). Three of them agreed with the proposed classification. One MS had comments relating to the aquatic toxicity tests. They wanted to have more precise information on the mean measured concentrations in the 21 day semi-static *Daphnia magna* NOEC (Suteau, 1997). The issue was clarified by the DS. The MS also had concerns regarding the use of kinetic regressions in the calculation of concentrations in the chronic *Chironomus* test. They also thought it useful to present endpoints using the standard geometric mean measured calculation for analytical periods and ½ the LOQ. This information would be relevant as endpoints using this method would be in the 0.001 to 0.01 mg/L classification range indicating M = 10. They also noted that using the valid acute toxicity to

Chironomus endpoint and the surrogate approach would result in Aquatic Chronic 1, M = 10. The DS answered that geometric mean values calculated from ½ LOQ are 0.6, 0.7, 1.1 and 2.4 µg/L. An EC_{10} based on these values is not available and it should be noted that in the opinion of the DS these values overestimate the actual exposure concentration because at multiple timepoints the measured concentrations are below the LOQ. The DS is of the opinion that the EC_{10} of 0.235 µg/L together with a NOEC of 0.96 µg/L are sufficiently reliable and the most appropriate representation of the toxicity observed in this study. Consequently, they did not consider application of the surrogate approach to be necessary.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the Dossier Submitter to consider acetamiprid as 'not rapidly degradable', based on:

- 27% degradation in 28 days in a ready biodegradability test (OECD TG 301B).
- The substance was not ultimately degraded in the aerobic degradation test (OECD TG 309) in surface water from a natural pond but the half-lives were < 16 days. However, the fulfilment of the criteria for classification as hazardous to the aquatic environment of the degradation product cannot be excluded.
- The substance was hydrolytically stable at environmentally relevant conditions.
- Half-lives of 23.1 and 31.6 days were calculated from the water/sediment simulation test, metabolites were detected but not identified.

Bioaccumulation

There was no fish bioconcentration study available. Based on the measured log K_{ow} of 0.80, RAC agrees with the DS to consider acetamiprid as having a low potential for bioaccumulation.

Acute aquatic toxicity

There were acute toxicity data available for three trophic levels. The lowest acute toxicity value was a 48-h LC_{50} of 0.0207 mg/L (mean measured) for *Chironomus riparius* and RAC agrees with the DS that this value should be used for acute hazard classification.

Chronic aquatic toxicity

There were chronic toxicity data available for all three trophic levels. The lowest chronic toxicity value was from a 28 days static sediment/water system test on *Chironomus riparius.* Originally, it was concluded that the 28 days NOEC of acetamiprid to the sediment dwelling life stage of *Chironomus riparius* in a static sediment water system is estimated to be 0.005 mg/L, based nominal concentrations.

In the RAR, a recalculation based on the geomean of the measured concentrations was presented, as described above. The lowest statistically significant EC_{10} and EC_{20} values are 0.000235 and 0.000333 mg a.s./L, and the NOEC is 0.00096 mg/L. The study setup contains sediment but actual concentrations were only determined in the water column and not in the sediment.

RAC is of the opinion that original NOEC value based on nominal concentrations is not reliable for classification purposes. The same applies to the calculations based on the kinetic regression equations. According to ECHA Guidance on IR & CSR, Chapter R7b, for static tests, where the concentrations do not remain within 80-120% of nominal, the effect concentrations should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test, in static tests.

There are also concerns related to the effect of sediment in the study results. In OECD TG 219 (sediment-Water Chironomid toxicity test) it is indicated as a minimum, samples of the overlying water, the pore water and the sediment must be analysed at the start (preferably one hour after application of test substance) and at the end of the test, at the highest concentration and a lower one. It further indicates that measurements in sediment might not be necessary if the partitioning of the test substance between water and sediment has been clearly determined in a water/sediment study under comparable conditions (e.g. sediment to water ratio, type of application, organic carbon content of sediment). In the case of acetamiprid, there were no information to make such a comparison. The chromatographic samples in the water-sediment test showed that sediment concentrations during the 28-day Chironomus test were likely to be significant. In conclusion, RAC does not consider the test results reliable and they will not be used for chronic hazard classification.

As *Chironomus riparius* is the most sensitive species under acute testing, RAC is of the opinion that in the absence of reliable chronic data for this species, the chronic classification should be based on the surrogate approach.

RAC agrees to use the 48h LC_{50} of 0.0207 mg/L for *Chironomus riparius* for short-term classification. Based on this acetamiprid warrants classification as **Aquatic Acute 1 (H400) with an M-factor of 10**.

The surrogate approach based on the 48h LC_{50} of 0.0207 mg/L for *Chironomus riparius* for a 'not rapidly degradable' substance indicates that acetamiprid warrants classification as **Aquatic Chronic 1 (H410) with an M-factor of 10**.

Additional references

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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).