Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ACETAMIPRID (ISO); (1E)-N-[(6-CHLOROPYRIDIN-3-YL)METHYL]-N'-CYANO-N-METHYLETHANIMIDAMIDE; (E)-N1-[(6-CHLORO-3-PYRIDYL)METHYL]-N2-CYANO-N1-METHYLACETAMIDINE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: acetamiprid (ISO); (1E)-N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide; (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine
EC number: -
CAS number: 135410-20-7;160430-64-8
Dossier submitter: Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	France	<confidential>	Company-Downstream user	1
Comment received				
The public consultation on CLP classification for acetamiprid let the possibility to comment the proposed CLP classification. Please, find the comments below.				
Dossier Submitter's Response				
Thank you for your comments. We have a general comment, in the CLP table "Retain aquatic chronic 1'' should be read as "Modify aquatic chronic 1".				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
13.03.2019	Germany		MemberState	2
Comment received				
Agreement with the proposal that Acetamiprid should be classified as Carc. 2, H351 based on an increased incidence of mammary gland adenocarcinoma in connection with increased incidence of mammary gland hyperplasia. The proposed classification agrees also with the Conclusion on Pesticide Peer Review EFSA Journal 2016; 14(11):4610.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
The RAC considers the proposed classification as Carc Cat 2 for acetamiprid is a borderline case.				

Date	Country	Organisation	Type of Organisation	Comment number
20.03.2019	United Kingdom	Nisso Chemical Europe GmbH	Company-Manufacturer	3

Comment received

Please see uploaded document reference 1007618.UK0-9906, which includes formatted figures and tables.

We disagree the CLH proposal as Carc Cat 2. No classification should apply.
The proposal is based on an increased incidence of mammary tumours in female rats.
The increased incidence of mammary tumours was 29 tumour-bearing animals in a group size of 60 rats (29/60), vs 24/59 in controls. In pairwise comparison, the groups were not statistically different.

The data owner notes that similar (or greater) incidences of mammary tumours have been assessed at RAC on occasions within the recent past, and anticipates that these data for acetamiprid shall receive equal treatment.

Mammary Gland Histopathology
Dose (ppm) HCD
0 160 400 1000
Fibroadenoma 17/59 15/60 10/60 15/60
Adenoma 1/59 0/60 4/60 3/60
Benign (adenoma or fibroademoma) 18/59 15/60 14/60 18/60
Adenocarcinoma 10/59 11/60 16/60 17/60
(28.3%) 13.3 – 28.6%
Total mammary tumour-bearing animals 24/59 21/60 24/60 29/60

Hyperplasia (1-year interim sacrifice) 3/10 1/10 2/10 2/10
Hyperplasia (terminal sacrifice) 5/23 10/26 10/29 18/29**
Hyperplasia (total) (a) 17/59 13/60 16/60 26/60 0 – 58%
** p <0.01 in comparison with control by Fisher's exact test
(a) Sourced from XXXXX(1999). A report by XXXXX et al (2001) mis-states the statistical significance and did not re-analyse the results reported in XXXXX(1999).

The CLH report notes a continuum between increased mammary gland hyperplasia (statistically significant) at terminal sacrifice, and increased mammary gland adenocarcinoma (not statistically significant). However, the continuum is not continuous as there was no increase in the incidence of (intermediate) adenoma. Further, it must be suspected that the proposed classification is based not on tumour incidence, but on the statistically-significant increased incidence of hyperplasia predominantly in the sub-population surviving to terminal sacrifice. Hyperplasia is not a neoplastic change, is not evidence of carcinogenicity, and is not appropriate for carcinogenicity classification. The incidence of hyperplasia was highly variable between studies at the test facility and there was no treatment related change in the incidence of hyperplasia at the 1-year interim kill. Further, mammary gland hyperplasia was not observed in in any other studies in rats, mice or dogs.

Mammary tumours are a common finding in Sprague-Dawley female rats, and the incidence of mammary tumours in this study remained within the historical control range of this specific test facility, and further within historical control ranges of comparable facilities with

this strain of rat. The high incidence typical of female Sprague-Dawley rats is specifically noted in the "Guidance on the Application of the CLP Criteria", where "even a statistically significant increase within the historical control range may not be providing reliable evidence of treatment-related carcinogenicity". The incidences in this study do not achieve (pairwise) statistical significance. There was no change in tumour latency (the time of first appearance of these tumours in rats is readily detected by palpation).

No mode of action is evident for mammary hyperplasia/ carcinogenicity. Acetamiprid is not genotoxic. An increase in mammary gland hyperplasia in female rats might be taken to imply some form of estrogenic influence. However, acetamiprid shows no estrogenic activity in the US EPA ToxCast ER bioactivity model. This model meets EFSA/ECHA requirements for "sufficiency" of testing for estrogenicity under the 2018 Joint EFSA/ECHA "Guidance for the identification of endocrine disruptors".

(US EPA EDSP21 Dashboard. Available at: https://actor.epa.gov/edsp21/

In summary, there is inadequate evidence to support a treatment-related increase in the incidence of mammary tumours in female rats with acetamiprid. Acetamiprid should not be classified for carcinogenicity.

Reference:
XXXXX et al (2001) Biological and statistical analysis of mammary gland findings in the chronic rat study on acetamiprid. XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX., Unpublished report No.: RD-00994

XXXXX(1999) Two Year Dietary Toxicity and Oncogenicity Study in Rats. XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX., Unpublished report No: RD-99104.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Acetamiprid CLH Consultation - Supporting docs - NON CONFIDENTIAL.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Acetamiprid CLH Consultation - Supporting docs - CONFIDENTIAL.zip

Dossier Submitter's Response

Thank you for your comments.
The increased incidence of total mammary tumor bearing animals was indeed not much higher compared to the control group. However, there is a statistical significant increase in adenocarcinoma's, which is considered more important than the total number as this includes benign tumors as well. We do think the trend test (indicating statistical significance as opposed to the pair-wise comparison) is at least as relevant as the pair-wise comparison because it also considers more categories (dose levels) and is suggestive of a dose-response.
The increased hyperplasia (at terminal sacrifice) is considered to be supportive (not primary) evidence for a carcinogenic effect. The increased incidence at terminal sacrifice should get more weight as compared to interim sacrifice where changes might not be significantly different yet.
We think that generally, statistical significance within the study itself should be considered more important compared to the historical control range. In this case, the data just fell within the historical control range, while the increased incidence of adenocarcinoma's is statistically significant and treatment related (higher incidence already observed in the lower dose groups). Therefore this should get more weight compared to the historical

control range.
The interpretation of the "continuum" might be different. In our interpretation it refers to the transition of total benign adenomas to adenocarcinoma's.
The mode of action is indeed unclear. However, the final effects can be sufficient for the proposed classification without more information on the mode of action.
Overall we want to comment this is a borderline case. Initially, the RMS of the RAR did not express the opinion for classification as carc. 2. Because EFSA was of the opinion acetamiprid should be classified as carc. 2, we have incorporated this opinion in the classification proposal as this should be thoroughly discussed by RAC.

RAC's response

The RAC agrees with the DS that this is a borderline case as it is based on a marginal increased incidence in a tumour of high background incidence. A significant trend was identified ($p \leq 0.05$) in the Cochan Armitage Trend Test and in the Peto test but no significance was identified by Fischers Exact test. It is noted that there is no increase in hyperplasia or adenoma or any altered foci at the interim sacrifice, whereas this might be expected if the increase in hyperplasia is to be considered as part of a continuum. The DS suggests that they consider a continuum to be progression from adenoma to adenocarcinoma. As no increase in adenoma was demonstrated then progression is not supported according to this rationale.
The RAC agrees priority should be given to use of concurrent control over historical unless the incidence in the concurrent controls is significantly different to the norm. It is also noted that consideration of historical control data can be useful and is important in assessment of studies of carcinogenicity in long term studies especially for tumours of a high background incidence. In conclusion, RAC considers the carcinogenic evidence to be insufficient for classification.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	France	<confidential>	Company-Downstream user	4

Comment received

The proposed CLP classification "Carcinogenicity Category 2" for Acetamiprid is based on 2 observations in the long-term oral/carcinogenicity on rats (1999e), knowing that a long-term oral/carcinogenicity on mice (1999a) concludes to "no carcinogenic effects observed". (CLH report pages 12 to 23)

The 2 observations highlighted for classification are: significant hyperplasia at high dose and increase of adenocarcinoma in the mammary gland.

1/ Hyperplasia is mainly observed at the 2 higher doses of 400ppm and 1000ppm that stands for doses upper to 17.5 mg/kg bw/d (see Table 16 – page 12 of CLH report). For both doses, toxicity to rats is expected, considering the NOAEL of 14.8 mg/kg bw/d determined in the supportive sub-chronic 90 days study on rats (1997d - CLH report page 45). Hyperplasia can be so considered as one of the toxicity signs at these doses. Moreover, as hyperplasia is not a neoplastic change, it is not an evidence of carcinogenicity and so is not a justification of carcinogenicity classification.

2/ The increase of adenocarcinoma in the mammary gland does not appear statistically significant in terms of incidence, considering also the fact that no change in tumor latency are observed (possible observation by palpation).

Moreover, the overall weight of evidence from the in vitro and in vivo studies indicates that

acetamiprid is not genotoxic. (CLH report page 12). Consequently, the proposed CLP classification "Carcinogenic Category 2" for Acetamiprid is based on observations that do not fulfil CLP criteria for this classification.
Dossier Submitter's Response
Thank you for your comments, see also our response to comment 3. Absence of carcinogenicity in mice cannot counter observed effects in another species that may be relevant for humans. You mentioned general toxicity is likely at the highest dose levels in the long-term carcinogenicity study based on the information from the sub-chronic toxicity study (anonymous 1997). However, the LOAEL was 800 ppm, just below the highest dose level in the carcinogenicity study. In addition, no significant general toxic effects were observed in the sub-chronic toxicity study. The LOAEL was based on effects on the testis. Also no overt general toxic effects were observed in the chronic carcinogenicity study itself, which should have more weight. A slight decrease in body weight is not sufficient to waive the findings. Hyperplasia could be a response to general cytotoxic or organ damage. However, this could lead to carcinogenic effects as well and should therefore be considered supportive evidence rather than general toxicity. In our opinion, tumor latency is not as important as tumor incidence.
RAC's response
The RAC agrees with the DS that this is a borderline case (see response to 3). Evidence in a 2nd species is not required for Cat 2 and moderate systemic toxicity cannot be considered relevant in analysis of tumour incidence.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Denmark		MemberState	5
Comment received				
Agreement with the RAC opinion, this is in accordance with the EFSA Conclusion.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Germany	<confidential>	Company-Downstream user	6
Comment received				
10.9 CARCINOGENICITY ECHA note – An attachment was submitted with the comment above. Refer to public attachment Evergreen_Expert Statement on C2R2 classification Acetamiprid_final_san.pdf ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Evergreen_Expert Statement on C2R2 classification Acetamiprid_final.pdf				
Dossier Submitter's Response				
Thank you for your comment. Please view our response to comment 3 and 4.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
19.03.2019	France		MemberState	7
Comment received				
FR: Page 12: In one of the two carcinogenicity conducted studies, evidence of carcinogenic effects at high doses, in female rats have been shown. Considering that there was a continuum between hyperplasia (significant at high dose) and increased incidence of adenocarcinoma, FR agrees on the classification proposal: Carc 2, H351 Suspected of causing cancer.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
This is a borderline case for classification as a single tumour type is increased in one of two species tested for carcinogenicity. The evidence is 'limited', ie., the evidence is restricted to a single species, gender and study. In addition, the increase in a tumour which has a high background incidence in the strain of rat used (CD(SD)) is only slightly above the concurrent controls and within the appropriate historical control data. It is debatable if the pattern of hyperplasia and increased adenocarcinoma can be described as a continuum as there is no increase in hyperplasia in the interim sacrifice and the occurrence at termination in the high dose is an increase in occurrence of hyperplasia graded as mild but no increase in severity. There was no increase in adenoma which would be considered a transitional stage to adenocarcinoma. RAC considers the carcinogenic evidence to be insufficient for classification.				

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2019	Spain		MemberState	8
Comment received				
The dossier submitter considers that based on the increase incidence in adenocarcinoma of mammary gland in rats the acetamiprid should be classified as Carc. 2. The increase in adenocarcinoma of the mammary gland in female SD rats was significant in a trend test, but not in a pair-wise comparison with the controls. No decrease in latency period was observed and no excessive toxicity was observed at this highest dose. No carcinogenicity was observed in the mice study. Mammary tumors in female SD rats are known to occur with a high spontaneous incidence (CLP guidance 5.0). In such cases the CLP guidance suggests a comparison with the historical control data. The observed incidence in adenocarcinoma in the highest dose (28.3%) was just within the available historical control range of the performing laboratory of 14.0% - 28.6% (n=6, same laboratory and same period). Overall, the Spanish CA considers that the increase incidence in adenocarcinoma of the mammary gland of female SD rats doesn´t provide reliable evidence of treatment related carcinogenicity and it is rather part of a biological variability in a strain which have a propensity to develop a particular type of tumour spontaneously with potentially high				

incidence. Therefore, in our opinion, acetamiprid does not meet the criteria for classification for carcinogenicity.
Dossier Submitter's Response
Thank you for your comment. Please view our response to comments 3 and 4. The dossier submitter is of the opinion the data suggest the substance may be able to cause carcinogenic effects
RAC's response
See response to comments 3 and 7 above. RAC considers the carcinogenic evidence to be insufficient for classification.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
13.03.2019	Germany		MemberState	9
Comment received				
We agree with the data submitter that "there is some evidence from animal studies on adverse effects on development…". Pub survival was decreased at parental toxic doses. Furthermore, the startle response was decreased in the developmental neurotoxicity study at doses of 45 mg/kg bw and 10 mg/kg bw/day. Therefore, the proposal of the dossier submitter to classify Acetamiprid as Repr. 2, H361d might be agreed. However, it is not that clear if the described effect on startle response is really adverse with regard to further development. It is proposed to report the data on startle response and postnatal mortality in the developmental neurotoxicity study in a table in the CLH report to verify the submitted conclusions and to discuss possible adversity.				
Dossier Submitter's Response				
More elaborate details and evaluation of the neurodevelopmental toxicity study can be found in Annex I, not in the confidential annex. To help verify the submitted conclusions we describe some more details in this response as well. A significant reduction in startle response and pup weights are observed at doses where minimal maternal toxicity is observed (45 mg/kg bw/day). Non statistical significant reductions were observed at 10 mg/kg bw/day. The startle response at PND20 (Vmax) was for males/females (* being stat. sign. different from control): 123*/129*, 157/161, 214/181 (45, 10, 0 mg/kg bw/day, respectively). At PND60 this was 99*/80, 126/80, 210/78 (45, 10, 0 mg/kg bw/day, respectively). The average response (Vave) were for males/females at PND20: 27*/27*, 34/34, 46/40 (45, 10, 0 mg/kg bw/day, respectively). At PND60 this was 22*/16, 29/16, 47/17 (45, 10, 0 mg/kg bw/day, respectively). The historical control range (HCD) 25-75% percentile was at PND20 for males/females 140-188/127-175 and for the minimum-maximum values 103-211/118-200. At PND60 the HCD 25-75% percentile was for males/females 132-185/80-90 and for the HCD minimum-maximum this was 87-247/49-148. The male startle response lie outside the 25-75% percentile HCD ranges, while the female startle responses are borderline within this range. Regarding the postnatal viability: Postnatal survival in the 45 mg/kg/day group was reduced on PND 0 and PND 0-1, attaining statistical significance during PND 0-1 (85.1% survival vs 99% in control). Three females (of which one was the single dead mother) had total litter loss on PND 1 and the only pup born was found dead together with the mother on PND 0. From birth to PND 4, postnatal survival in this dose group was slightly reduced compared to that in the control group.				

Notably, the maternal toxicity at 45 mg/kg bw/day can be considered very minor and it is questionable whether this has influenced the startle response of the pups or the postnatal mortality of the pups. The maternal body weight reduction was statistically significant at GD20, but very minor, <5%). The body weight details were not presented in annex 1 so we have added them here:

Table: Non Gravid maternal body weights neurodevelopmental toxicity study (anonymous 2008)

	Group weights (mg/kg)			
Groups	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	45 mg/kg bw/day
GD0	247	246	247	246
GD9	288	287	286	275*
GD20	385	387	388	370* (-4%)
LD1	284	288	285	265*
LD4	301	307	309	293
LD21	317	317	321	313

Also note that the maternal animals recovered from the lower body weights during lactation.

The single maternal death may be test substance related, but it may also be incidental. Overall, the reduction in auditory startle response seems to occur in the absence of significant maternal toxicity and in a dose-response starting at 10 mg/kg bw/day. Both the RMS and EFSA (opinion 2016) agree that the LOAEL for developmental toxicity should be 10 mg/kg bw/day, which is lower than for maternal toxicity. Whether this effect can be considered sufficiently adverse to warrant classification is not easy to assess and opinions may differ. In the CLP regulation, functional changes to the nervous system are considered sufficiently adverse for STOT RE. The DS therefore believes a reduced startle response is an adverse effect warranting classification for development as well. Overall, we think classification as repro. 2. (H361) is most appropriate.

RAC's response

The attenuation of the auditory startle response is in itself not sufficient to propose the Repr. 2 classification. Indeed, in light of several other parameters showing little to no effect (i.e. a lack of related findings in FOB and neuropathology, and brain morphometry) combined with important neurological indicators not being sufficiently investigated (motor activity, learning and memory evaluation), RAC puts less weight on the relevance of the non-significant effect on the startle response in those animals of the mid dose group of 10 mg/kg bw/day at PND20 but was confined to males only at PND60. However, the apparent dose response indicated by firmer statistical significance on the startle response in top dose animals is considered relevant as part of a weight of evidence for reproductive toxicity classification.
RAC agrees with the DS in that the most adverse developmental effect is the reduced post-natal survival of the pups as observed in the F2 pups of the 2-generation study and in the developmental neurotoxicity study. RAC supports the proposal of the DS for Repr. 2 for development.

Date	Country	Organisation	Type of Organisation	Comment number
20.03.2019	United Kingdom	Nisso Chemical Europe GmbH	Company-Manufacturer	10

Comment received

Please see uploaded document reference 1007618.UK0-9906 and supporting document, Li, A.A. (2015).

CLH Report (p49): There is some evidence from animal studies on adverse effects on development resulting in classification as Repr. Cat. 2 H361d.
General summary of comment: Effects in offspring of both the DNT study and both generations of the 2-generational study occur only in the presence of significant maternal toxicity, characterised by body weight losses and/or markedly decreased body weight gains (up to 60 %) and food intake (up to 16 %). Marked maternal toxicity on both studies occurs at pertinent times for offspring survival and development, particularly post-parturition, and at levels in excess of those reported in many publications documenting the effects of marked maternal toxicity on post-natal development (1, 3, 4, 5, 6, 10, 11, 12, 16), rather than the Carney publication cited in the CLH report, of which methodological differences reduces its relevance to the acetamiprid studies (2, 7, 8, 9). Furthermore, high doses on all generations of both studies were a substantial fraction (up to 70%) of the acute toxicity oral LD50 dose levels; maternal toxicity is therefore consistent with excessive toxicity seen with acetamiprid. The changes seen in survival of the offspring on both studies constitute a secondary, nonspecific, consequence of maternal toxicity and not a direct effect of the substance.

We support the view that there is no adverse effect at 10 mg/kg bw/day on the DNT study. The precautionary lowering by EFSA of the NOAEL to 2.5 mg/kg bw/day, was due to variability of the other neurodevelopmental data, since unfounded by HCD, and does not constitute evidence of adversity. The isolated change in startle response amplitude at 10 mg/kg bw/day, was not statistically different from Controls; remained within both laboratory and industry HCD ranges; had no associated change in the reflex time or habituation to the stimuli; nor any concomitant functional or physical indications of neurodevelopmental toxicity. This substantiates the EFSA opinion that (despite disputing the NOAEL), acetamiprid is not sufficiently toxic as for reproduction Category 2. A recent publication examining the potential of different neonicotinoid insecticides as neurodevelopmental toxicants, including acetamiprid, also corroborates this view (14). We consider that no classification for developmental toxicity is warranted.
Specifically:
Question: Are effects on post-natal survival secondary to maternal toxicity or a direct effect of acetamiprid?
CLH Report: Evidence of effects of food restriction and maternal body weight on post-natal mortality are inconsistent due to the publication by Carney et al (2004).
Comment: In contrast to the Carney publication (2), there is a large body of evidence which acknowledges the effect of reduced maternal body weight on post-natal survival and development (1, 3, 4, 5, 6, 16) which is also exacerbated intergenerationally (10). Furthermore, differing from many feed-restriction studies, and those on the DNT and 2-generational studies, animals in the Carney publication did not have ad libitum access to food. Differences in feeding regimen causes different effects on body weight gain, circadian hormonal patterns and maternal behaviour patterns, even when total food intake is similar (7 and 8), with many contradictory findings of calorie restriction studies attributable to methodological differences (9). These differences in study design methodology reduce the

relevance of the findings in the Carney publication to those identified in the DNT and 2-generational studies.

By using a weight of evidence approach, there are far more publications which recognise the impact of maternal toxicity on the maternal behaviours necessary for offspring to thrive, compared with this single publication which raises doubt, particularly when the feeding methodologies are different.

CLH Report: Changes in maternal body weight of females given the highest doses acetamiprid are of only limited effect.
Comment: Comparing maternal toxicity for the total gestation or lactation periods severely underestimates different vulnerabilities within these periods, as reflected by changing nutritional demand with normal feeding behaviour and known adversity to perturbation in maternal behaviour, particularly affecting the neonatal period (5, 11, 16). Changes in excess of 20 % reductions in body weight gain and any body weight losses are recognised industry-wide to constitute excessive maternal toxicity (12). The 2-generational study with acetamiprid achieved up to 44 % less weight gain over Days 0 to 4 of the parental lactation period and actual body weight loss over the same period of the F1 lactational phase. The F1 generation also had 60 % less body weight gain from Lactation Days 14 to 17. Changes in body weight were pursuant of decreases in food intake (16 % and 14 % less over Days 0 to 4 of the parental and F1 generation, respectively). On the DNT study 16 % less body weight was gained during gestation and considering the individual data of the litters with all offspring lost, up to 36 % less weight was gained in comparison to the mean Control. Furthermore, 39 % less food was eaten during gestation Days 6 to 9.

The differences in body weight and food intake seen on both the DNT and 2-generational studies are of a magnitude which constitute significant maternal toxicity which had a secondary consequence on post-natal survival and, as such, do not constitute a direct effect on development. On this basis, classification of acetamiprid for development is not warranted. This interpretation is in concordance with the EFSA Conclusion, as documented in the peer review of the pesticide risk assessment which stated that acetamiprid 'is not as toxic for reproduction category 2' (13).

Question: Are the changes in startle response at 10 mg/kg bw/day related to treatment with acetamiprid?
CLH Report: there was a decrease in startle response in the developmental neurotoxicity study at 10 mg/kg bw/day, enough to constitute an adverse effect justifying a precautious lowering of the NOAEL to 2.5 mg/kg bw/day
Comment: We disagree with the CLH proposal that there was a treatment-related decrease in startle response at 10 mg/kg bw/day. It should be understood that the startle response measured reaction time as a measure of neuronal function, and subsequent habituation to repeated startle, as an indicator of learning and memory. "Amplitude" is the force with which the animal responded. At 10 mg/kg bw/day, the startle response amplitude (i.e. how strong the response was to the stimuli) appeared decreased but the startle time (Tmax) and habituation was not. The biological and toxicological relevance of this putative change in amplitude, in the absence of any effect on Tmax or habituation, is questionable. In addition, the difference in startle amplitude was not statistically different from concurrent Controls and remained within both the laboratory's historical Control data range, and within the variability seen in Control data compiled by the US EPA from numerous subsequent DNT

studies, submitted to the US EPA after the acetamiprid DNT study (14). In addition, there were no concomitant neurodevelopmental delays in any of the other functional assessments at 10 mg/kg bw/day (motor activity, learning and memory assessments), nor any changes in the brain neuropathology or morphometry at any dose level. This change in the amplitude of the startle response is within normal biological variability seen in laboratory animals and should therefore be concluded not to be treatment related.

CLH Report: the DNT study conducted for acetamiprid misses certain study data (motor activity, learning and memory assessments) to enable a change in startle amplitude to be contextualised.
Comment: The DNT study conducted did not miss any guideline compliant data and included temporal assessments of acetamiprid effects on motor activity, learning and memory acquisition. On initial review, US EPA initially deferred decisions on whether acetamiprid affected these parameters due to uncertainty on the extent of variability in these data and whether the data set was sufficiently robust for assessment. This uncertainty was incorporated in the later EFSA opinion, where the NOAEL was on a precautionary basis set to 2.5 mg/kg bw/day until further data was available. US EPA has since compiled a wider database of Control data for these end points, demonstrating that these biological data are inherently variable and that the acetamiprid data are in fact, normal in their variability. US EPA has subsequently concluded that the neurodevelopmental data set is complete for acetamiprid, with robust, guideline compliant studies for developmental neurotoxic assessments (15).

As further weight of evidence, a comprehensive review has subsequently been published since the initial EFSA and EPA opinions were generated on potential of acetamiprid for developmental neurotoxicity (14). This review evaluates whether the neonicotinoid insecticidal class is a neurodevelopmental toxicant comparing data from 6 neonicotinoid insecticides (including acetamiprid). The publication concludes that the neonicotinoids do not selectively affect the developing nervous system, with no common DNT effects or findings associated with the neurodevelopmental effects of nicotine. Instead, findings at higher doses were secondary to systemic toxicity, as demonstrable with acetamiprid.

We conclude that the isolated observation of an apparent decrease in startle amplitude at 10 mg/kg bw/day, which is within background Control data ranges, with no concomitant changes in startle time, changes in startle habituation, motor activity, learning or memory acquisition, or brain neuropathology or morphometric differences, does not demonstrate convincing evidence of neurodevelopmental toxicity and is instead within the normal biological variability for animals at these ages. Accordingly, we concur with the EFSA opinion that acetamiprid is not sufficiently toxic as to justify classification as Repro Cat. 2 and disagree with the CLH Report proposal of Repr. Cat. 2 H361d.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Acetamiprid CLH Consultation - Supporting docs - NON CONFIDENTIAL.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Acetamiprid CLH Consultation - Supporting docs - CONFIDENTIAL.zip

Dossier Submitter's Response

Thank you for your comments.
First we would like to comment that although during some periods significant lower food consumption and body weight gains were observed, these resulted in a very minor reduction in total body weight (<5% difference compared to control) which is considered more important. There are few periods where the body weight gain loss may have been

excessive. We do not agree that a 20% difference in body weight gain is always excessive when the resulting body weight does not change as much. We would regard >50% lower body weight gain for a few days . The periods you refer to are that of the parental generation lactation period and the body weight gain of the F1 during that period. However, the lower pup viability was observed in the F2 pups during the F1 lactation period, mostly during days 0-4. Regardless, the body weight gain in this period of the F1 was -1.55 g in the high-dose group compared to +5.7 g in the control. Because this body weight gain difference is large, the DS considers it possible the lower pup viability of the F2 is related to general toxicity in the F1 during this period. However, as mentioned in the CLH report, Carney et al., did not find any effect of feed restriction on the pup viability during lactation days 1-4 of SD rats (the same strain as used in the 2-generation study). Only the high (50%) feed restricted group had lower pup body weights during lactation days 1-4, but not a lower pup viability. Also note that the overall body weight difference during this period in the 2-generation study was limited to -14%. Overall we consider this supportive evidence while the findings in the neurodevelopmental toxicity study are the main reason for proposing Repr. Cat. 2.
We do not consider the general (maternal) toxicity in the neurodevelopmental toxicity study excessive (smaller reductions in body weight gain and body weight, slightly lower highest dose level). Predominantly based on the neurodevelopmental study, classification as repr. 2 is warranted.
We have screened a few of the articles (other than Carney et al.) stating the influence of feed intake and/or body weight gain on the developing pups. The message of these studies, summaries and book chapters include mostly that there is an influence of food intake and body weight gain on development, but not at what level/when this influence kicks in. Therefore they do not specifically support your claim that certain numbers of food intake or body weight gain might influence the developmental toxicity.
The study by Carney et al., includes numbers on overall food intake and body weight reduction that can influence development, which is the reason why it is particularly usefull for regulatory purposes and therefore referred to in the CLH report. It also seems to be a well performed and valid study.

We do not agree the highest dose is close (70%) to the acute LD50, suggesting all effects are related to maternal toxicity. The acute LD50 is around 140-150 mg/kg bw, while the highest dose tested in the developmental neurotoxicity study was 45 mg/kg bw/day and in the 2-generation study the effective dose was 51 mg/kg bw/day. This is closer to 30% of the LD50 than 70%.
Without more information, we assume the lower startle response is relevant for humans. It is a significant effect, mostly outside HCD (25-75th percentile) range, at least at 45 mg/kg bw/day but in the presence of minor maternal toxicity. There seems to be an effect at 10 mg/kg bw/day as well, suggesting a dose response. Therefore, it seems clear there is an effect and we think it should be considered sufficiently adverse to warrant classification for effects on development. However as mentioned in the CLH report, we do not consider this effect as "very clear evidence" and as a result, we have proposed Repr. 2. (H361d) but not a more stringent classification.

RAC's response

Agrees and supports the comments made by the DS.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	France	<confidential>	Company-Downstream user	11

Comment received

The proposed CLP classification "Reprotoxic Category 2" for Acetamiprid is based on the observations in 2 different studies on rats (CLH report pages 46 and 47):

1/ In the developmental neurotoxicity study (2008), at the higher dose of 45mg/kg bw/d: a decrease in postnatal survival and a startle response in the developmental neurotoxicity are observed but both are linked to a significant toxicity observed for mothers (including body weight changes during the test).

2/ In the Two-generation reproduction study (1999d), at the higher dose of 800ppm (i.e. 51mg/kg bw/d): a decrease in postnatal survival is observed for the F2 pups but also linked with a significant decrease of body weight gain and food consumption for mothers. This lets guess a certain toxicity to mothers.

In the supportive sub-chronic 90 days study on rats (1997d - CLH report page 45), the NO Adverse Effect Level (NOAEL) is obtained at 14.8 mg/kg bw/d with no evidence of neurotoxicity and confirms that maternal toxicity is expected for the higher doses of the 2 previous studies, respectively 45mg/kg bw/d and 51mg/kg bw/d.
It is the reason why, due to maternal toxicity, Reprotoxicity is thus highly difficult to be assessed in the same time.

Consequently, the proposed CLP classification "Reprotoxic Category 2" for Acetamiprid is based on observations that do not fulfil CLP criteria for this classification.

Dossier Submitter's Response

Thank you for your comments. We do not believe maternal toxicity has had a major impact on the developing organisms as also explained in our response to comment 10.
The dose in the 90-day study at around 60 mg/kg bw/day is around 20% higher compared to the developmental neurotoxicity study and the 2-generation study. The toxicity at this dose level (lower body weight gain) is therefore also higher (around 20%/33% males/females lower body weight gain). Furthermore, this body weight gain difference is measured over 13 weeks, which is longer than the gestation time and therefore difficult to compare with the developmental neurotoxicity and 2-generation studies. Regarding the lower body weight gain in the 2-generation study on days 1-4 of lactation (F1), please view our response to comment 10.

RAC's response

Supports comments by the DS.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Denmark		MemberState	12

Comment received

DK finds the observed effects (despite maternal toxicity) relevant for classification as Repr 2, and thus agrees with the RAC opinion.

Dossier Submitter's Response

Thank you for your support

RAC's response
Agreed.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Germany	<confidential>	Company-Downstream user	13
Comment received				
10.10 REPRODUCTIVE TOXICITY ECHA note – An attachment was submitted with the comment above. Refer to public attachment Evergreen_Expert Statement on C2R2 classification Acetamiprid_final_san.pdf ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Evergreen_Expert Statement on C2R2 classification Acetamiprid_final.pdf				
Dossier Submitter's Response				
Thank you for your comment. Please see our response to comments 10 and 11.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.03.2019	France		MemberState	14
Comment received				
FR: Page 23: Based on the main adverse effects observed in the 2-generation rat study (i.e. in utero and postnatal growth decrease), FR agrees on the classification proposal: H361d.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Agreed.				

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2019	Spain		MemberState	15
Comment received				
Fertility The Spanish CA agrees with the dossier submitter that the available data do not warrant classification for effects on sexual function and fertility. Development The most adverse developmental effect is the reduced post-natal survival of the pups as observed in the F2 pups of the 2-generation study and in the developmental neurotoxicity study but not in the F1 pups of the 2-generation study. These developmental effects were observed in the presence of maternal toxicity including reduced maternal body weight (gain) and food consumption. We agreed with the dossier submitter that it is considered unclear whether the developmental effects are secondary to the maternal toxicity. Therefore, classification in Repr. Cat. 2 (H361d) is warranted based on the reduced post-natal survival observed.				

Dossier Submitter's Response
Thank you for your support
RAC's response
Agreed.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Denmark		MemberState	16
Comment received				
Agreement with the RAC opinion, this is in accordance with the EFSA Conclusion.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Agreed with MS and DS.				

Date	Country	Organisation	Type of Organisation	Comment number
15.03.2019	Spain		MemberState	17
Comment received				
Acute toxicity - oral route The lowest calculated LD50 value in the studies using ion-exchanged water is 146 mg/kg bw. The lowest calculated LD50 value in the study using corn oil as vehicle is 140 mg/kg bw. As both lowest calculated LD50 values are 50-300 mg/kg bw, acetamiprid should be classified as Acute Tox 3, H301 "Toxic if swallowed".				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Agreed with MS and DS.				

Date	Country	Organisation	Type of Organisation	Comment number
13.03.2019	Germany		MemberState	18
Comment received				
Agreement with the proposal that Acetamiprid should be classified as Acute Tox. 3, H301 based on the lowest calculated oral LD50 values in the range 50-300 mg/kg bw/day. Howev-er, an according ATE should be discussed and harmonised.				
Dossier Submitter's Response				
Agreed, an ATE of 140 mg/kg bw is proposed (lowest LD50 while they were both very similar and there is no specific preference for either study).				
RAC's response				
Agreed with MS and DS.				

Date	Country	Organisation	Type of Organisation	Comment number
19.03.2019	France		MemberState	19
Comment received				
FR: Acute Toxicity Page 7: Following the addition of the two acute oral toxicity studies (1998 and 2002), FR agrees on the classification proposal: H301 instead of H302.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agreed with DS and MS				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2019	United Kingdom		MemberState	20
Comment received				
Acetamiprid (EC: N/A; CAS: 135410-20-7/160430-64-8) Chronic toxicity to Daphnia manga: Please can you clarify if the quoted 21-day semi-static Daphnia magna NOEC (Suteau, 1997) is based on mean measured concentrations of fresh solutions or does it reflect measured fresh + expired concentrations? We note the mean measured values are very close to the nominal concentration range which appears unusual if based on analytical measurement of fresh and expired test solutions given observations in other ecotoxicity tests. In addition, the study endpoint appears to be significantly less chronically sensitive than the chronic endpoint for Chironomus despite the two species having acute endpoints in close proximity. Chronic toxicity to Chironomus: The proposed chronic endpoint (28-day EC10 0.000235 mg/l) for Chironomus (McElligott, 1999 and Dossier submitter calculation) is based on concentrations calculated using estimated kinetic regressions. For 3 out of the 4 treatments the kinetic regressions are based on 1 or 2 data points above the LOQ. We are therefore unclear if these regressions and estimated concentrations <LOQ are reliable. We note that significant effects were only observed in the highest treatment. While 3 analytical data points >LOQ are available for this treatment, we are unclear how reliable the overall dose-response curve is and think 95% CI should be presented to aid interpretation. We also think it would be useful to present endpoints (NOEC and EC10 if appropriate) using the standard geometric mean measured calculation for analytical periods and ½ the LOQ where <LOQ is reported. This information is relevant as the RAR text indicates endpoints using this method would be in the 0.001 to 0.01 mg/l classification range indicating M = 10. Finally we note, using the valid acute toxicity to Chironomus endpoint and the surrogate approach would results in Aquatic Chronic 1, M=10.				
Dossier Submitter's Response				
Chronic toxicity to Daphnia manga: In the study report the following is reported: "Analytical verifications of the test				

concentrations prepared on Days 0, 2, 9 and 19 of the test demonstrated all starting concentrations (T0 hour) to be close to the nominal values (percentage recoveries: 95 - 100%). The test substance was also stable in the test solutions as shown by the measured values after 48 or 72 hours of exposure which were close to initial measured values from the same test solutions (percentage recoveries: 95 - 103%). The mean measured concentrations of acetamiprid from all test solutions (T0 and T48 or T72 hours) during the test period were respectively 2, 5, 9, 18, 37 and 74 mg/L. The results of this test are reported in terms of the mean measured concentrations expressed as milligrams of acetamiprid per liter of test solution (mg/L)." We hope that this clarifies your issue.

Chronic toxicity to Chironomus:
The 95% confidence interval for the EC10 of 0.235 µg/L as presetented in the CLH report is 0.183-0.283 µg/L, for clarity it should be noted that the EC10 is based on a reduced dataset of exposure concentrations 0, 0.27, 0.526 and 2.56 µg/L. A statistically significant fit could not be obtained for emergence rate of males and females combined when using the data for all exposure groups. Therefore, the analysis was repeated after omission of the data for the second or third exposure group (0.52 or 0.96 µg a.s./L). A statistically significant fit was obtained for the reduced data set of control and 0.27, 0.52 and 2.56 µg a.s./L, but not for the reduced data set of control and 0.27, 0.96 and 2.56 µg a.s./L. Judging from the normalized width (NW) of the confidence intervals, the estimated EC10 and EC20 values for emergence rate (obtained for the reduced data set containing data for the control and 0.27, 0.52 and 2.56 µg a.s./L) are reliable (i.e. NW <0.5). The EC10 and EC20 values estimated for development rate for all larvae and for males are less reliable (NW ≥0.8).
Geometric mean values calculated from 1/2LOQ are 0.6, 0.7, 1.1 and 2.4 µg/L. An EC10 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 dossier submitter is in the opinion that the EC10 of 0.235 µg/L together with a NOEC of 0.96 µg/L are sufficiently reliable and the most appropriate approach of the toxicity observed in this study. Therefore application of the surrogate approach is not considered necessary.

RAC's response

RAC agrees with the Member States concern on calculation of results in the chronic Chironomus study. In the event of reliable results RAC prefers to use the surrogate approach for chronic classification.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2019	Denmark		MemberState	21
Comment received				
Agreement with the RAC opinion.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.03.2019	Germany		MemberState	22
Comment received				
Proposed harmonised classification and labelling (Table 6): We agree with the proposal of classification for environmental hazards as Aquatic acute 1				

(H400), Aquatic chronic 1 (H410) and acute/chronic M-factor of 10/100.
Dossier Submitter's Response
Thank you for your support
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
19.03.2019	France		MemberState	23
Comment received				
FR: Thank you for this very clear document. We agree with the Aquatic Acute 1 (H400; M-factor=10) and Aquatic Chronic 1 (H410; M-factor=100) classification proposal.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. Evergreen_Expert Statement on C2R2 classification Acetamiprid_final_san.pdf [Please refer to comment No. 6, 13]
2. Acetamiprid CLH Consultation - Supporting docs - NON CONFIDENTIAL.zip [Please refer to comment No. 3, 10]

CONFIDENTIAL ATTACHMENTS
1. Evergreen_Expert Statement on C2R2 classification Acetamiprid_final.pdf [Please refer to comment No. 6, 13]
2. Acetamiprid CLH Consultation - Supporting docs - CONFIDENTIAL.zip [Please refer to comment No. 3, 10]