COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Last data extracted on 28.10.2020

Substance name: clothianidin (ISO); (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS number: 210880-92-5
EC number: 433-460-1
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	France		MemberState	1
Comment received				
- Substance has a CIPAC number 738 please add it in the identity part. - Minimum purity of active substance as manufactured was 95% w/w and not 93% w/w (p4). - Please add purity (97.6%) of test item used to determine the vapour pressure, the surface tension, the pKa, the water solubility and the partition coefficient.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	2
Comment received				
With regard to the developmental toxicity of clothianidin, I agree with the Applicant Sumitomo Chemical that there are no adverse findings in the rat embryo-foetal developmental toxicity study and that, in the rabbit study, although a small number of foetal findings were recorded at high dose levels, the severe maternal toxicity that occurred at these dose levels precluded a meaningful interpretation of these data. Therefore, these findings are not considered appropriate to justify classification for developmental toxicity. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Clothianidin Report - 27 Aug 2020.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
17.09.2020	United Kingdom		Individual	3
Comment received				
I have worked in the areas of chemical risk assessment and developmental and				

reproductive toxicity for 45 years. As a consultant for the manufacturer, Sumitomo Chemical (UK) Plc, I have assessed the original study reports (rat two-generation, rat and rabbit developmental toxicity, and rat developmental neurotoxicity) and related documentation on Clothianidin and give my opinion concerning EU classification for reproductive toxicity below.

Two-generation study

Reductions in body weight in male and female offspring at birth and during the pre-weaning period were a prominent feature at the highest dose of 2500 ppm Clothianidin in the diet. It is relevant to consider whether the effect on offspring body weight was a consequence of the general systemic toxicity of clothianidin in adults and offspring, or was due to a specific effect on postnatal development.

Adult body weight

Throughout the study, there were reductions in adult male and female body weights of 5-14% in P0 animals and 16-21% in F1 animals at 2500 ppm, compared with controls. These reductions occurred despite increases in feed consumption of 5-30%, depending on the time point in the study. This indicates substantial reduced feed efficiency, which paralleled the reductions in adult body weight.

Offspring body weight

At 2500 pm, the reductions in body weight, evident at birth, increased as the pups reached the stage of consuming solid diet during the preweaning period. By postnatal day (PND) 21 the reductions in body weight were substantial, ranging up to 26% in F1 pups and up to 21% in F2 pups, compared with controls. Reduced offspring body weight continued during the postweaning period.

Given the size of the body weight reductions in adults and offspring at 2500 ppm, it is reasonable to conclude that the effects on body weight in both adults and offspring were most likely directly due to the systemic toxicity of clothianidin and not to any specific effect on development.

Adult organ weights and sperm parameters

There were reductions in some male reproductive absolute organ weights, but not relative organ weights, and effects on sperm motility at 2500 ppm (i.e. reduction in percentage of motile sperm in F1 males and reductions in percentage of progressively motile sperm in both P and F1 males). These were not accompanied by any effects on sperm count, sperm morphology, ability to mate, fertility, or reproductive organ histopathology in either P0 or F1 adult males. It is plausible that the sperm effects are attributable to the observed reductions in adult body weight, as shown by Chapin et al. (1993).

Attainment of puberty milestones

In the female offspring, there was a slight delay of 2.3 days in attainment of vaginal opening (VO) at 2500 ppm. An analysis of covariance for the day of attainment of VO versus female pup body weight on PND 21 (BioSTAT, 2018) indicated that the slight delay in VO at 2500 ppm is attributable to the reduction in female offspring body weight in this group.

In the male offspring, there was a mean delay of 6.7 days in the attainment of preputial separation (PPS) at 2500 ppm. Analysis of covariance for the day of attainment of PPS versus male pup body weight on PND 21 (BioSTAT, 2018) showed that this delay is only partly attributable to the reduction in male offspring body weight. Other reasons contributing to the delay in preputial separation are not immediately apparent. However, the lack of any delay in either VO or PPS in the developmental neurotoxicity study (Argus Research Laboratories, 2000), which used a top dose of 1750 ppm, providing maternal exposures during gestation and lactation that were only 10-30% lower than those in the equivalent periods in the two-generation study at 2500 ppm, shows that in utero and preweaning exposure alone do not have an effect on PPS or VO. This provides further evidence that the effect on PPS seen in the two-generation study is unlikely to be a specific developmental effect.

It is also relevant to consider whether the delay in PPS could be an endocrine-mediated

effect. Substances with anti-androgenic (or oestrogenic) activity would be expected to show other changes indicative of anti-androgenic (or oestrogenic) activity, either within the same multigeneration study or in other toxicity studies. A review of androgen-related endpoints from all the repeat-dose studies on Clothianidin shows no pattern of endocrine effects, as summarised below.

1. No effect on anogenital distance in F2 pups in the two-generation study.
2. There were reductions in absolute weight of some male sex organs in F1 males in the two-generation study, but relative organ weights were increased, indicating that these changes were secondary to the substantial reductions in body weight. These observations are in accordance with the findings in the 28- and 90-day repeat-dose toxicity studies in the rat (see RAR, 2018, Vol 3, B.6), in which dietary concentrations up to 2500 or 3000 ppm, respectively, were given.
3. There were no effects on male sex organ histopathology in the two-generation study, or in the 28-day, 90-day or 104-week studies (see RAR, 2018, Vol 3, B.6).
4. Timing of PPS and VO:

(i) In the two-generation study, comparison of the slopes of the linear regression lines for day of attainment of PPS and PND 21 pup body weight for the high-dose clothianidin versus control animals shows clear separation of the point clouds for control and treated group data and differing slopes. This pattern is typical of non-anti-androgenic substances (Melching-Kollmuss et al., 2014).

(ii) If there had been anti-androgenic (or oestrogenic) activity by clothianidin in the two-generation study, it would be expected that VO would be accelerated and PPS delayed, but VO and PPS were both delayed.

(iii) There were no effects on the timing of PPS or VO in F1 offspring in the developmental neurotoxicity study.

These results show that clothianidin is unlikely to be anti-androgenic (or oestrogenic). Overall, the delay in PPS is most likely to be attributable to the direct systemic toxicity of clothianidin and is not a specific developmental or endocrine-mediated effect.

In conclusion, the most plausible explanation for the effects observed in the two-generation study is that they are directly caused by, or are secondary to, the systemic toxicity of clothianidin.

Should clothianidin be classified for reproductive toxicity?

None of the effects of Clothianidin appear to be attributable to a direct effect on reproduction or to a specific effect on embryo-foetal or postnatal development.

The effects on offspring body weight seen at the high dose of 2500 ppm Clothianidin in the diet in the two-generation study are most likely attributable to the observed adult/maternal systemic toxicity of Clothianidin, seen throughout the entire study and expressed mainly as reductions in adult body weight and body weight gain, and to direct systemic toxicity to the offspring once they began consuming treated diet during the third week of life.

The reduction in female offspring body weight was shown to account for the slight delay in vaginal opening. In the case of the longer delay in preputial separation at 2500 ppm, the reductions in body weight of the male offspring did not account for all of the effect. Additional reasons for the delay in preputial separation are not readily apparent but a weight-of-evidence interpretation of all the toxicity data on clothianidin shows that it is highly unlikely that the effect is endocrine-mediated; there is no pattern of anti-androgenic or oestrogenic activity in the repeat-dose toxicity studies and analysis of the preputial separation data also supports a non-anti-androgenic mechanism. The most plausible explanation of the delay in preputial separation observed in the two-generation study is that it was also caused by the systemic toxicity of clothianidin.

There was also an isolated effect on sperm motility at 2500 ppm. The most plausible explanation for the reduction in sperm motility is that it is secondary to the reduced adult body weight and epididymis weight. A study by Chapin et al. (1993) supports this interpretation.

Classification in Category 2 for Reproduction requires that the reproductive effects: '…shall

have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects' (ECHA, 2017). In the two-generation rat study, the effects on sperm motility and on attainment of pubertal milestones at 2500 ppm in the diet occurred only in the presence of adult male and female systemic toxicity, including maternal toxicity, and direct systemic toxicity to the offspring, as revealed in the substantial reductions in their body weight. There was no evidence of any endocrine-mediated effects. In the opinion of this reviewer, classification for reproductive toxicity Category 2 is not warranted.

References

Original study reports

Argus Research Laboratories (2000). Developmental Neurotoxicity Study of TI-435 Administered Orally via Diet to CRL:CD® Presumed Pregnant Rats. Performed by Argus Research Laboratories, Inc., Pennsylvania, USA. Study number ARGUS 1120 003, 20 October, 2000.

Bayer Corporation (2000). A Two-Generation Reproductive Toxicity Study With TI-435 in the Sprague-Dawley Rat. Performed by Bayer Corporation Agricultural Division, Kansas, USA, for Takeda Chemical Industries Ltd., Tokyo, Japan. Study Number 98-672-PF, Bayer Report number 109282, March 27, 2000.

Bayer Corporation (2001). A Two Generation Reproductive Toxicity Study With TI-435 in the Sprague-Dawley Rat. Supplemental Submission to Bayer Report No. 109282. Performed by Bayer Corporation Agricultural Division, Kansas, USA, for Takeda Chemical Industries Ltd., Tokyo, Japan. Bayer Report number 109282-1, April 6, 2001.

BioSTAT (2018). Statistical Analysis Report: A two generation reproduction study with TI-435 Technical in the Sprague-Dawley rat. BioSTAT Consultants, Inc., Portage, Michigan, USA.

Published literature

Chapin RE, Gulati DK, Barnes LH, Teague JL (1993). The effects of feed restriction on reproductive function in Sprague-Dawley rats. Fundamental and Applied Toxicology, 20: 23-29.

Melching-Kollmuß S, Fussell KC, Buesen R, Dammann M, Schneider S, Tennekes H, van Ravenzwaay B (2014). Anti-androgenicity can only be evaluated using a weight of evidence approach.. Regulatory Toxicology and Pharmacology, 68: 175-192.

<confidential> BSc PhD DipRCPath, Brighton, UK
17 September 2020

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2020	United States	<confidential>	Company-Manufacturer	4

Comment received

Comment 1

We disagree with the criteria for classification for sexual function and fertility Category 2 as given in Table 25 (page 35).

Specifically, we disagree with the conclusion that in the submitted multi-generation study a delay in sexual maturation in males was observed despite normal (post-weaning) growth following clothianidin administration. As detailed in the expert report and attached to this comment (Section 3; Subsection as indicated by capitalization), the DELAY IN F1 PREPUTIAL SEPARATION at 500 ppm was not statistically significant upon recalculation of the data when body weight of the pups was used as a covariate of analysis as appropriate.

While the effect on preputial separation is still apparent after the appropriate re-analysis of the data using the body weight as a covariate at the high dose, it is likely an artifact of general toxicity and not relevant for reproductive toxicity classification. As further detailed in the attached expert report, pups in the high dose group were likely exposed to concentrations greater than 500 mg/kg/day during their consumption of the 2500 ppm test diet due to their body weight at the time. This dosage of clothianidin is known to cause general toxicity in rodents.

Additionally, we further disagree with the conclusion that the slight effects in SPERM MORPHOLOGY AND MOTILITY observed in the multi-generational study with clothianidin are relevant for human hazard classification. As detailed in the attached expert report (Section 3; Subsection as indicated by capitalization), the leading cause for this slight decrease is likely attributable to general toxicity. Dietary administration of 2500ppm of clothianidin in the multi-generation study resulted in significantly reduced ($p \leq 0.05$) terminal body weights in both the F0 generation males by 9% and in the F1 generation by 19%. Chapin et al. (1997) have previously reported in the literature that reductions in body weight of 10% or more relative to control animals can adversely affect sperm motility in rodents, and this effect in the absence of other findings in sperm is related to general toxicity. Thus, the effect on sperm motility is likely indicative of the general systemic toxicity of clothianidin and should not be relevant for reproductive hazard classification.

Comment 2
We disagree with the criteria for classification for development Category 2 as given in Table 26 (page 36).

Specifically, we disagree that clothianidin has evidence of a higher incidence of STILLBIRTH AND DECREASED PERINATAL VIABILITY in the absence of excessive parental toxicity. As summarized in the expert report attached to this comment (Section 3; Subsection as indicated by capitalization), the incidence of stillborn pups in rodents following exposure to clothianidin in the reproductive and developmental studies were all within the normal variation of rodents in the respective testing facilities. As further detailed in the attached expert report, the apparent effect on pup viability at 2500 ppm in the multigenerational study is the result of a calculation error with a dam that was found deceased on PND4. When the pup viability is correctly calculated without the deceased dam, it is clear that there was no effect of clothianidin administration. Thus, neither of these lines of evidence should be used in the developmental hazard classification of clothianidin.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20201006171458958_redacted.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20201006171458958.pdf

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	United Kingdom	Sumitomo Chemical	Company-Manufacturer	5

Comment received

With regard to the developmental toxicity of clothianidin, we agree with the applicant Sumitomo Chemical that there are no adverse findings in the rat embryo-foetal developmental toxicity study and that, in the rabbit study, although a small number of foetal findings were recorded at high dose levels, the severe maternal toxicity that occurred at these dose levels precluded a meaningful interpretation of these data. Therefore, these

findings are not considered appropriate to justify classification for developmental toxicity.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLOTHIANIDIN TCI opinion 12.04.19 FINAL.pdf

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	France		MemberState	6
Comment received				
FR CA agrees with the new classification H361fd Repr. 2				

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	7
Comment received				
The effects observed in the multigenerational study can all be attributed to systemic toxicity and are not a direct effect on reproduction or an effect on embryo-foetal or postnatal development. Classification in Category 2 for Reproduction requires that the reproductive effects: '…shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects' (ECHA, 2017). All of the effects in the multigenerational study occurred at 2500 ppm an exposure that was clearly also producing systemic toxicity. The exposure of rats to 2500 ppm of clothianidin in a multigenerational study resulted in systemic exposures that were clearly toxic and at times during the study probably resulted in the highest exposure achieved in any of the toxicity evaluations of the test material. Based on my review of the data, when considered in its entirety, in the opinion of this reviewer, classification of Clothianidin for reproductive toxicity Category 2 (H361fd) is not warranted. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20201005 Clothianidin DART report.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	8
Comment received				
The effects observed in the multigenerational study can all be attributed to systemic toxicity and are not a direct effect on reproduction or an effect on embryo-foetal or postnatal development. Classification in Category 2 for Reproduction requires that the reproductive effects: '…shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects' (ECHA, 2017). All of the effects in the multigenerational study occurred at 2500 ppm an exposure that was clearly also producing systemic toxicity. The exposure of rats to 2500 ppm of clothianidin in a multigenerational study resulted in systemic exposures that were clearly toxic and at times during the study probably resulted in the highest exposure achieved in any of the toxicity evaluations of the test material. Based on my review of the data, when considered in its entirety, in the opinion of this				

reviewer, classification of Clothianidin for reproductive toxicity Category 2 (H361fd) is not warranted.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20201005 AMH Statement on Clothianidin for ECHA.pdf

Date	Country	Organisation	Type of Organisation	Comment number
04.09.2020	United States		Individual	9

Comment received

Clothianidin is being evaluated for proposed Reprotox hazard. A two-generation rat reproduction study and definitive developmental toxicity studies in both rat and rabbit are available (Anonymous, 1998a,b; Anonymous, 2000). The primary study cited for proposed classification as Repr. 2, H361fd is the rat reproductive toxicity study (Anonymous, 2000). In the Renewal Assessment Report (RAR) for clothianidin (Germany, 2018), the Rapporteur Member State (RMS) noted the following reproductive and offspring effects for the rat reproductive toxicity study of clothianidin:

Reproductive toxicity
- Effects on sperm motility and morphology
- Increased stillborn pup incidence
- Decreased early postnatal viability

Offspring toxicity
- Delayed male sexual maturation
- Delayed vaginal opening at highest dose
- Reduced birth weight and postnatal body weight gain
- Reduced thymus weights

The developmental toxicity studies in the rat and rabbit are not cited as the basis for potential classification. We generally agree with the RMS regarding the studies' NOAELs; but it is our opinion that the rabbit developmental NOAEL should be higher (i.e., 75 mg/kg bw/day), consistent with that called by the study investigators. The RMS considered absence of the intermediate lung lobe and reduced ossification of sternal centers to be of concern for setting the developmental NOAEL; however, these findings are minor variations that occurred in the presence of significant maternal toxicity and the incidence of these two findings are within the expected normal variation for New Zealand rabbits. Thus, they should not drive selection of the developmental toxicity NOAEL for this study.

Also, based on reanalysis of the pubertal development data using the PND 21 pup body weights as a covariate in the analysis (i.e., 500 mg/kg bw/day), the NOAEL for offspring toxicity from the rat reproductive toxicity study should be higher than that determined by either the study investigators or the RMS. Further, with regard to classification, we note the following:

- Although both sperm motility and progressive motility were adversely affected in both parental generations at the highest dose tested (2500 ppm), no effect on sperm morphology was observed and no adverse functional effects on fertility were evident. It is likely that the sperm motility findings are indicative of a delay or alteration in sperm development secondary to general systemic toxicity. In the absence of a dose-response or other data showing effects on other sperm parameters and without adverse effects on reproductive function, the sperm motility data should not serve as the basis for classification for reproductive toxicity.
- We find no effect of clothianidin treatment on the incidence of stillborn rat pups. This is consistent with the findings of other studies of clothianidin. Likewise, early postnatal pup viability was not adversely affected by clothianidin. Rather, the slightly lower value reported for the F1 generation at 2500 ppm is due to miscalculation of the value. As such,

increased stillborn pups or reduced early postnatal viability were not evident in the study and should be considered in assessing clothianidin for reproductive toxicity.
• At 2500 ppm, clothianidin had a significant effect on both adult and offspring body weights; significant effects on intrauterine growth were also evident. The effects on pubertal development are likely secondary responses due to general systemic toxicity. The delay in preputial separation at 500 ppm, although statistically significant, is within the expected range of natural variation for the performing laboratory. The delay in vaginal opening at 2500 ppm is not statistically significant when the PND 21 pup body weights are used as a covariate in the statistical analysis.
• The effects on offspring body weights and thymus weights at 2500 ppm occurred in the presence of substantial maternal toxicity and are most likely due to systemic toxicity. As such, they should not be considered when assessing clothianidin for reproductive toxicity. Thus, several findings noted in the RAR list of endpoints for the rat reproductive toxicity study were not affected by clothianidin treatment; the other offspring findings are likely secondary effects due to systemic toxicity and not relevant to an assessment of reproductive toxicity. In conclusion, we do not believe that the classification of clothianidin for reproductive toxicity is warranted based on the available database of DART studies.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment RAC statement - Clothianidin - 3 Sept.docx

Date	Country	Organisation	Type of Organisation	Comment number
28.09.2020	United Kingdom		Individual	10

Comment received

As reproductive toxicologists each with more than forty years of professional experience in the field of developmental and reproductive toxicity, we have reviewed the study reports and related documentation for clothianidin as independent consultants, at the request of Sumitomo Chemicals UK plc. The evaluation below represents our own opinion of the data reviewed.

In the CLH report for clothianidin, a small number of findings in the high dose group (2500 ppm) of the 2-generation reproductive toxicity study in rats were considered to be of concern for classification. It is our opinion that these findings do not constitute a basis for classification of clothianidin as toxic to reproduction (Repro 2) for the following reasons:

1. Reduced sperm motility of between 8 and 13% in both the F0 and F1 males, in the absence of changes in sperm numbers and sperm morphology.

Throughout the clothianidin study, body weights of F0 males at 2500 ppm were consistently reduced by up to 10% and those of F1 males by 16 – 20%, despite increased food consumption throughout both generations, indicative of impaired food utilization and possible nutritional deficiency. Chapin et al. (1993) manipulated the body weights of male (and female) Sprague-Dawley rats from 10 weeks of age by food restriction, such that body weights were reduced by 10, 20 or 30% of the unrestricted bodyweight. They found that although the numbers of sperm in the cauda epididymis and the number of homogenisation-resistant spermatids in the testis were unaffected, epididymal sperm motility was reduced by 6 - 9% irrespective of the degree of reduction in body weight. The extent of body weight reduction and the reduction in sperm motility of between 8 and 13% in the clothianidin study was in general agreement with the findings of Chapin et al. (1993). As such, this finding should be considered to be a manifestation of impaired nutrition rather than a specific reproductive toxicity finding.

The slight reduction in sperm motility had no adverse effect upon the fertility of either the F0 or the F1 males

2. An increase in the numbers of stillborn pups and reduced early viability index of pups

In both the F1 and the F2 generations there appeared to be a very slight dose-related increase in the number of stillborn pups, with the numbers of stillborns at 2500 ppm achieving statistical significance compared with the concurrent control values. However, when compared with the historical control data for this strain of rat, the concurrent control values were at the bottom end of the historical control range, whilst the numbers and percentage of stillborn pups in all treated groups remained within the historical control ranges (see Text Table 1).

In terms of litters containing stillborn pups, there was a slight increase at 2500 ppm in the F1 generation compared with the concurrent controls but both the number and percentage of litters remained well within the historical control ranges. For the F2 generation, no dose-relationship was apparent in either the number or percentage of litters affected and again the values were within the historical control ranges (see Text Table 1).

Live birth index was unaffected at all dose levels in both generations and all values were within the historical control ranges.

Text Table 1: Incidence of stillbirths and Live Birth Index, F1 and F2 generations

No. stillborn pups
Dose level (ppm) 0 150 500 2500
F1 generation:
Total no. pups delivered (Litters) 325 402 (29) 398 (28) 386 (29)
No. (%)stillborn pups 0 (0) 3 (0.7) 6 (1.5) 9*(2.3)
No. (%) litters with stillborn pups 0 (0) 3 (10.3) 3 (10.7) 6 (20.7)
Live birth index % 100 99.1 97.4 96.5
F2 generation
Total no. pups delivered (litters) 316 (23) 342 (25) 261 (20) 352 (28)
No. (%) stillborn pups 1 (0.3) 3 (0.9) 8 (3.1) 13*(3.7)
No. (%) litters with stillborn pups 1 (4.3) 3 (12) 5 (25) 4 (14.3)
Live birth index 99.8 99 99 97
Historical control data ranges 1989 – 1997
No. studies: F1: 18, F2: 15. No. pups/litters: F1: 6075/448, F2: 4772/377
No. stillborn pups: F1: 0 -16 F2: 0 – 13
% stillborn pups F1: 0.0 - 3.9 F2: 0.0 - 3.7
No. litters with stillborn pups F1: 0 - 9 F2: 0 - 6
% litters with stillborn pups F1: 0 - 33 F2: 0 – 28
Live birth index % F1: 97 - 100 F2: 96 – 100
Data taken from study report: Table 17 Summary of litter data and from Laboratory Historical Control Data report. *$p<0.05$ Chi squared test,

At 2500 ppm the viability index of the F1 pups, i.e. survival to post-natal day (PND) 4, was reported as being slightly reduced compared with the concurrent control value (92.4% compared with 99.2%). However, there was a discrepancy in the way the value was calculated at 2500 ppm. One dam in this group died post partum, having given birth to 14 live pups. Consequently her litter was unable to survive to PND 4. Nevertheless, the loss of these 14 pups was included in the calculation of viability index. If this litter is excluded from the calculation, the viability index at 2500 ppm increases to 95.7%. The historical control range for viability index of F1 pups ranged from 86 – 100% with a mean value of 96.9%. Viability index of the F2 pups at 2500 ppm was similar to that of the controls (97% vv. 98%).

It is considered, therefore, that these minimal variations in pup survival are of insufficient

magnitude to form a basis for classification.

3. Time of onset of puberty – preputial separation in F1 male pups and vaginal opening in F1 female pups.

At 2500 ppm, for F1 male pups, the mean age at preputial separation (PPS) was statistically significantly delayed from the control mean of 41.2 days to 47.9 days. ($p<0.01$). For control pups mean body weight at PPS was 190.1g but, because of growth retardation, both in utero and post-natally, male pups at 2500 ppm did not achieve a similar mean bodyweight until 47.9 days of age. For F1 female pups, the mean time of vaginal opening (VO) was slightly delayed from the control mean of 32.4 days to 34.7 days ($p<0.01$). In contrast to the male pups, however, the mean weight of treated female pups was lower than that of the control pups at the time of VO, viz. 90.6g compared with 104.6g.

The relationship between male pup body weight and PPS has been investigated by a number of groups, including Ashby and Lefevre (2000) and Melching-Kollmuss et al. (2014). These authors have demonstrated reduced offspring body weight may result in delayed PPS. Engelbregt et al. (2000) investigated the effects on the onset of puberty in male and female rats of intrauterine growth retardation (IUGR) by uterine artery ligation on gestation day17, or post-natal undernutrition (FR) by increasing the size of litters to 20 pups per females on post-natal day 2. Both of these procedures were considered to induce undernutrition, which resulted in growth retardation at birth and in postnatal life, similar to that seen with clothianidin at 2500ppm. They found that for male IUGR pups PPS was delayed from a mean value of 45.8 days in control male pups to 48.1 days whilst that of FR male pups was delayed until 50.4 days. By the time of preputial separation the male IUGR pups had achieved the same mean body weight as control pups but mean body weight of the FR pups was lower. Vaginal opening in IUGR female pups was delayed from a mean value of 36.1 days to 37.4 days but appeared to be less affected for FR pups (mean 36.5 days). At the time of vaginal opening, mean body weight of both IUGR and FR female pups was lower than that of the controls. Their findings, for IUGR in particular, are similar to those recorded for clothianidin.
It is considered, therefore, that the delay in the time of onset of puberty, recorded in the clothianidin study at 2500 ppm, is likely to be a secondary effect related to growth retardation and/or impaired nutrition rather than a direct effect upon sex hormones. If the latter were the case, the delayed PPS would be indicative of an anti-androgenic or oestrogenic effect of the compound, whereas, conversely, the delayed VO would indicate an anti-oestrogenic or androgenic
effect of the compound. There was no evidence, therefore, of a consistent effect on sex hormones. In order to investigate whether there was any endocrine disrupting activity, anogenital distance was measured in the F2 pups and neither male nor female pups showed any differences from the control pups.

With regard to the developmental toxicity of clothianidin, we agree with the CLH Dossier submitter that there are no adverse findings in the rat embryofoetal developmental toxicity study and that, in the rabbit study, although a small number of foetal findings were recorded at high dose levels, the severe maternal toxicity that occurred at these dose levels precluded a meaningful interpretation of these data. Therefore, these findings are not considered appropriate to justify classification for developmental toxicity.

References:

Ashby J., Lefevre P.A. (2000). The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators.

J. Appl. Toxicol. 20 pp. 35-47.

Chapin R.E., Gulati D.K., Barnes L.H., Teague J.L. (1993): The effects of feed restriction on reproductive function in Sprague-Dawley rats.
Fundam. Appl. Toxicol. 20 pp. 23-29.

Engelbregt M.J.T., Houdijk M.E.C.A.M., Popp-Snijders C., Delemarre-van de Waal H.A. (2000): The Effects of Intra-uterine Growth Retardation and Postnatal Undernutrition on Onset of Puberty in Male and Female Rats.
Ped. Res. 48(6) pp. 803-807

Melching-Kollmuss S., Fussell K.C., Buesen R., Dammann M., Schneider S., Tennekes H., van Ravenzwaay B. (2014): Anti-androgenicity can only be evaluated using a weight of evidence approach.
Reg. Toxicol. Pharmacol. 68 pp. 175-192.

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	11
Comment received				
We find that a number of the findings noted in the RAR list of endpoints for the rat reproductive toxicity study were not affected by clothianidin treatment. Additionally, the other findings in offspring are likely secondary effects due to systemic toxicity and not relevant to an assessment of reproductive toxicity. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20200828 Clothianidin Repro Evaluation Report.pdf				

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	12
Comment received				
There is no basis for a classification of Category 2 based on the results of these reproductive toxicity studies. The reported criteria for a Category 2 reproduction states "shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects" (ECHA, 2018) is not met based on this expert's interpretation of the multigenerational study. In conclusion, classification for developmental toxicity (Reproductive toxicant 2, H361fd) is not warranted for Clothianidin. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment THT-0383.pdf				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	France		MemberState	13
Comment received				

FR CA agrees with the new classification H302, Acute Tox. 4

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	France		MemberState	14
Comment received				
FR CA agrees with the new classification H370, STOT SE 1 (nervous system)				

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	United Kingdom	SUMITOMO CHEMICAL	Company-Manufacturer	15
Comment received				
The proposal to classify clothianidin for STOT SE in Category 1 (H370) based on clinical signs consistent with neurotoxicity is not considered appropriate. Effects representing 'significant toxicity' are not seen at dose levels relevant to STOT SE Category 1 (≤300 mg/kg bw). Furthermore, while it could be argued that 'significant toxicity' is seen at higher dose levels relevant for STOT SE classification in Category 2 (i.e. in the range >300-≤2000 mg/kg bw), these dose levels are the same as (or are relatively close to) dose levels also causing lethality. Consequently, hazard classification in both the acute oral toxicity and STOT SE classes would represent double classification and should therefore be avoided, in line with the CLP Guidance. Furthermore, adoption of this classification for clothianidin would be inconsistent with the other neonicotinoid insecticides previously considered by RAC, with special reference to thiamethoxam (see Appendix I). ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment STOT SE rebuttal_Final Oct 2020.pdf				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	France		MemberState	16
Comment received				
FR CA agrees with the environmental classification. Please note that on page, 66 first paragraph in section "Short-term (acute) aquatic hazard", it is not 96h-EC50 but 48h-EC50.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2020	United Kingdom	HSE CRD	National Authority	17
Comment received				
Clothianidin (ISO); (EC: 433-460-1: CAS: 210880-92-5 We note that harmonised classifications for other neonicotinoids (e.g. imidacloprid and thiacloprid) are based on data for mayfly, which was a very sensitive test organism to these substances. Has the DS considered if there are available reliable literature data on the aquatic toxicity of clothianidin to mayfly which may be relevant to the classification?				

As a minor comment, we note that the acute key endpoint for Chironomus riparius is based on initial measured concentrations. Test concentrations were not measured at the end of the 48-h exposure period to verify that these remained within 80-120% of the nominal. The acute mysid study (Drottar, MacGregor and Krueger, 2000a) used similar test concentrations and mean measured concentrations are provided in the CLH report. Please can the DS provide information on actual concentrations for this mysid study to support that the test substance is stable from 0 – 48 or 96 hours and support the C. riparius endpoints?

The proposed Aquatic Chronic classification is based on a Chironomus riparius study which included sediment in the test system. While aquatic phase monitoring was conducted indicating loss of the active substance, sediment analysis was not included. Is there further information to help understand test substance concentrations in the sediment phase during the course of the experiment to help consider if the aquatic phase endpoint is reliable for hazard classification?

PUBLIC ATTACHMENTS
1. 20201006171458958_redacted.zip [Please refer to comment No. 4]
2. RAC statement - Clothianidin - 3 Sept.docx [Please refer to comment No. 9]

CONFIDENTIAL ATTACHMENTS
1. 20201006171458958.pdf [Please refer to comment No. 4]
2. 20201005 Clothianidin DART report.pdf [Please refer to comment No. 7]
3. 20201005 AMH Statement on Clothianidin for ECHA.pdf [Please refer to comment No. 8]
4. STOT SE rebuttal_Final Oct 2020.pdf [Please refer to comment No. 15]
5. 20200828 Clothianidin Repro Evaluation Report.pdf [Please refer to comment No. 11]
6. THT-0383.pdf [Please refer to comment No. 12]
7. Clothianidin Report - 27 Aug 2020.pdf [Please refer to comment No. 2]
8. CLOTHIANIDIN TCI opinion 12.04.19 FINAL.pdf [Please refer to comment No. 5]