Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine

EC Number: 433-460-1
CAS Number: 210880-92-5

CLH-O-0000007020-91-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007020-91-01/F

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

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

Chemical name: **clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine**

EC Number: **433-460-1**

CAS Number: **210880-92-5**

The proposal was submitted by **Germany** and received by RAC on **22 July 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **24 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 October 2020**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

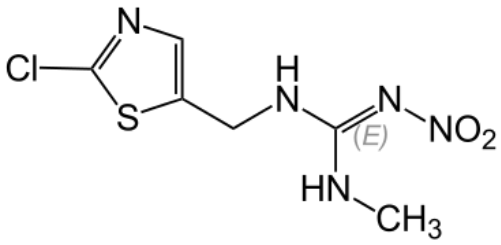
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-307-00-5	clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	433-460-1	210880-92-5	Acute Tox. 4* Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410		M=10	
Dossier submitters proposal	613-307-00-5	clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	433-460-1	210880-92-5	**Modify** Acute Tox. 4 **Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 STOT SE 1	**Retain** H302 H400 H410 **Add** H361fd H370 (nervous system)	**Retain** GHS07 GHS09 **Add** GHS08 **Modify** Dgr	**Retain** H302 H410 **Add** H361fd H370 (nervous system)		**Retain** M=10 **Add** Oral: ATE=389 mg/kg bw M=100	
RAC opinion	613-307-00-5	clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	433-460-1	210880-92-5	**Modify** Acute Tox. 4 **Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 STOT SE 1	**Retain** H302 H400 H410 **Add** H361f H370 (nervous system)	**Retain** GHS07 GHS09 **Add** GHS08 **Modify** Dgr	**Retain** H302 H410 **Add** H361f H370 (nervous system)		**Retain** M=10 **Add** Oral: ATE=390 mg/kg bw M=100	
Resulting Annex VI entry if agreed by COM	613-307-00-5	clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	433-460-1	210880-92-5	Repr. 2 Acute Tox. 4 STOT SE 1 Aquatic Acute 1 Aquatic Chronic 1	H361f H302 H370 (nervous system) H400 H410	GHS07 GHS08 GHS09Dgr	H361f H302 H370 (nervous system) H410		Oral: ATE=390 mg/kg bw M=10 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Clothianidin ((E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine) is an active substance in biocides and plant protection products used as agricultural insecticide. Its chemical structure is shown below:



HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed the classification of clothianidin as Acute Tox. 4, H302 with an ATE = 389 mg/kg bw based on an acute oral toxicity study in mouse performed according to OECD TG 401.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the DS's proposal for classification of clothianidin as Acute Tox. 4, H302.

Assessment and comparison with the classification criteria

The table below summarises all the available studies for assessment of acute toxicity of clothianidin.

Table: Summary of animal studies on acute oral toxicity with clothianidin.

Study	Dose level	Results	Reference
Crl:CD.BR rats 5 rats/sex OECD TG 401 Gavage	1758-2283-2965-3850-5000 mg/kg bw Purity: 96% w/w Vehicle: 5% w/v aqueous gum Arabic	No mortalities at 1758 and 2283 mg/kg bw 2965 and 3850 mg/kg bw: 1 female mortality, no male mortalities 5000 mg/kg bw: 1 male and 1 female mortalities Clinical signs: palpebral closure, ↓ activity, lethargy, ataxia, hunched posture, vocalisation, tremor, piloerection, hair loss, waisted appearance **LD_{50} > 5000 mg/kg bw**	Anonymous 3, 1997a

F-344/BR rats 5 rats/sex Partly TG OECD 401	290-523-1216-2000 mg/kg bw Purity: 96% w/w	♂: **LD_{50} > 1216 mg/kg bw** ♀: **LD_{50} > 523 to < 1216 mg/kg bw**	Anonymous 9, 2002 (acute neurotoxicity range-finding)
(Crl:CD-1(ICR)BR mice 5 mice/sex OECD 401 Gavage	304-380-475-594-742 mg/kg bw Purity: 96% w/w Vehicle: 5% w/v aqueous gum arabic	Male mortalities: 0, 2, 3, 3 and 5 for 304, 380, 475, 594 and 742 mg/kg bw; respectively Female mortalities: 0, 2, 5, 5 and 5 for 304, 380, 475, 594 and 742 mg/kg bw; respectively Clinical signs: ↓ activity, ataxia, tremor, palpebral closure ♂: **LD_{50} = 389 mg/kg bw** ♀: **LD_{50} = 465 mg/kg bw** **Combined ♂/♀ LD_{50} = 425 mg/kg bw**	Anonymous 4, 1997b

Comparison with the criteria

The lowest LD_{50} (389 mg/kg bw) recorded among three available acute oral toxicity studies was found in the mouse study with males. This LD_{50} falls within the LD_{50} range warranting classification within Cat. 4. The LD_{50} value in female mice is slightly higher but also supports the classification within Cat. 4. Rats seem to be more resistant to clothianidin. One rat species yielded a LD_{50} value higher than 5000 mg/kg bw; while a second rat species showed a LD_{50} for females within the range for classification within Cat. 4. Overall, RAC supports the DS's proposal for **classification of clothianidin as Acute Tox. 4, H302** with an **ATE of 390 mg/kg bw.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

DS proposed the classification of clothianidin as STOT SE 1, H370 (nervous system) based on the impairment of motor activity reported in mice starting at 50 mg/kg bw and in rat starting at 100 mg/kg bw. These effects were noted in absence of mortality.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the DS's proposal for classification of clothianidin as STOT SE 1, H370 (nervous system).

One manufacturer company considered the DS's proposal for classification based on neurotoxic clinical signs not to be appropriate because effects representing "significant toxicity" are not seen at dose levels relevant to STOT SE Category 1. Furthermore, the commenter argued that "significant toxicity" is seen at higher dose levels than those relevant for STOT SE classification in Category 2, and that these dose levels are the same as (or are relatively close to) these causing lethality. Furthermore, according to the manufacturer company, the adoption of this classification for clothianidin would be inconsistent with the other neonicotinoid insecticides previously considered by RAC, with special reference to thiamethoxam. The DS replied that clothianidin is a known neurotoxic compound and the observed neurotoxicological findings are

attributable to the treatment. They also replied that the proposal for classification is supported because dose levels causing impairment of motor activity (starting at 50 mg/kg bw in mice and at 100 mg/kg bw in rats) did not induce mortalities.

Assessment and comparison with the classification criteria

In addition to the acute oral toxicity studies summarised in the table above, the DS used two different acute oral neurotoxicity studies in rats and one pharmacological study in rats and mice for assessing the STOT SE hazard.

Acute oral neurotoxicity study in rats (Anonymous 11, 2000)

The study was performed observing U.S. EPA FIFRA, Guideline 81-8(SS) and U.S. EPA. Health Effects Test Guidelines OPPTS 870.6200. Fischer 344 rats (12 animals/sex/experimental group) were treated by gavage with 0, 100, 200 and 400 mg/kg bw of clothianidin (purity 95%) in aqueous 0.5% methylcellulose/0.4% Tween 80.

There were no deaths and no effects on body weight or body weight gain. Results of clinical observations and functional observation battery are shown in the table below. At the top-dose and in both males and females, tremors, decreased activity and ataxia were observed. Decreased activity, pin-point pupils, an uncoordinated righting response and decreased body temperature were observed in a number of animals at 400 mg/kg bw and in a few animals at 200 mg/kg bw. Tremors and uncoordinated gait were also observed in high dose animals. A few males showed decreased activity at 100 mg/kg bw but no other signs were noted at this dose level. There were no treatment related effects on remaining endpoints, including forelimb and hindlimb grip strength and landing foot splay. In general, all findings were restricted to the day of treatment. Most endpoints revealed substantial effects at the top dose in both males and females (tremors, hypoactivity, decreased arousal, miosis following light stimulus, decreased aerial righting response, hypothermia), and in addition, gait incoordination and reduced approach response in the males. Effects on arousal were detected at 100 mg/kg bw and above in the males, and at 200 mg/kg bw and above in the females. Biologically significant dose related reductions in motor activity were observed after dosing on day 0 in all male dose groups and in females at 200 and 400 mg/kg bw. There were no treatment related macroscopic or microscopic findings or effects on body weight and brain weight at termination in clothianidin exposed groups.

Table: Functional observation battery and clinical observations in acute neurotoxicity study of clothianidin in rats. The results are shown as incidence/12 animals. * = statistically significant modifications with ANOVA + Dunnetts's t-test for $p < 0.05$. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw)	**0**		**100**		**200**		**400**	
Sex	**m**	**f**	**M**	**f**	**m**	**f**	**m**	**f**
FUNCTIONAL OBSERVATION BATTERY (incidences per 12 animals)								
Home cage observations:								
Tremors score 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7*	9*
score 2							1	2
Decreased activity score 1	n.d.	n.d.	n.d.	n.d.	1	0	8*	11*
Open field observations:								
Posture:								
standing normally	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7	7
sitting or lying normally							5	5
Tremors score 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10*	6*
score 2							1	5
Gait incoordination	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Arousal:								
sluggish exploratory movements	1	0	3	0	4	2	9*	9*
sluggish minimal movement	0	0	1	0	1	1	0	2
Reflex/Physiologic/Manipulative observations:								
Approach response:								
no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Touch response:								
no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	2
Pupil response on light:								
pin-point constriction	n.d.	n.d.	n.d.	n.d.	1	0	8*	9*
Aerial righting response:								
slightly uncoordinated								
landing on side	0	1	0	1	2	0	2	3
							0	1
Body temperature (°C)	36.5	36.4	36.1	36.3	34.8*	35.1*	32.8*	32.7*
FIGURE-EIGHT MAZE (percentual differences with concurrent controls)								
Motor activity	n.d.	n.d.	↓23%*	-	↓59%*	↓43%*	↓81%*	↓72%*
Locomotor activity	n.d.	n.d.	↓37%*	-	↓62%*	↓45%*	↓88%*	↓83%*

Overall, neurotoxic effects (reduced locomotor activity) were observed in male rats at lowest dose of 100 mg/kg bw and in females at 200 mg/kg bw (reduced locomotor activity, hypothermia).

Acute oral neurotoxicity study in rats (Anonymous 9, 2002)

This study was performed as supplementary to the above-described study (Anonymous 11, 2000). No OECD or EU guidelines were indicated. Animals were sacrificed on day 2 rather than on day 15 of the study; no body weight measurement, and no gross pathology, neither histopathological examination was performed. Fischer 344 rats (12 males/experimental group) were treated by gavage with 0, 20, 40 and 60 mg/kg bw of clothianidin (purity 95.4%) in aqueous 0.5% methylcellulose/0.4% Tween 80.

There were no deaths during the study. No treatment related clinical signs were observed after dosing in any dose group. No treatment related findings were observed in the Functional Observation Battery assessment approximately 4 hours after dosing. No biologically significant dose related reductions in motor activity were observed after dosing on day 0. Overall, there was no evidence of treatment related neurobehavioral changes or signs of toxicity up to the highest dose level of 60 mg/kg bw.

Pharmacological study in rats and mice (Anonymous 14, 2000)

Male CD-1 (ICR) SPF mice were treated with clothianidin (95.5% purity) dispersed in 0.5% arabic gum solution by single gavage at 0, 6.25, 12.5, 25, 50, 75, 100, 200, 225 and 400 mg/kg bw (mice). Treated animals were observed at 1, 2, 4, 6, 8, 12 h and further daily during 1 week to monitor appearance of acute toxic signs and mortalities.

Reductions in spontaneous locomotor activity, tremors and deep respiration were noted at 50 mg/kg bw (table below). Other doses caused a wide array of alterations including, as regard as neurotoxicity, decrease in reactivity, staggering gait, decrease in touch response, decrease in grip strength, inhibition of pinna reflex, inhibition of ipsilateral flexor reflex and inhibition of corneal reflex (table below).

Table: General physical condition and behaviour after clothianidin administration to male mice. Value in table includes number of animals with finding out of 3 animals, degree of response (+ = slight, ++ = moderate, +++ = severe) and duration of clinical sign. Observation conducted before, and 0.5, 1, 3, 6 h, 1 d after dosing.

	Dose (mg/kg bw)			
	50	**100**	**200**	**400**
Decrease in spontaneous locomotor activity	$3^{+;0.5\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h,1\ d}$
Tremor	$1^{+;0.5\ h}$	$1^{+;0.5\text{-}1\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h,1\ d}$
Deep respiration	$1^{+;1\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Hypothermia	-	$3^{+;\ 1\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in grooming	-	$3^{+;\ 0.5\ h}$	$3^{++;0.5\text{-}1\ h}$	$3^{++;\ 0.5\text{-}6\ h}$
Mydriasis	-	$2^{+;++\ 0.5\text{-}3\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$2^{+,++,+++;\ 0.5\text{-}6\ h}$
Decrease in reactivity	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{++;\ 0.5\text{-}6\ h}$
Prone position	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Staggering gait	-	$1^{+;\ 0.5\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$3^{+,++,+++;\ 0.5\text{-}6\ h,1\ d}$
Decrease in body tone	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in abdominal muscle tone	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in touch response	-	-	$3^{+;0.5\text{-}1\ h}$	$3^{+;\ 0.5\text{-}6\ h}$
Decrease in grip strength	-	-	$2^{+;\ 0.5\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decreased limb tone	-	-	-	$3^{+,++;\ 1\text{-}6\ h}$
Inhibition of pinna reflex	-	-	-	$3^{+,++;\ 1\text{-}6\ h}$
Inhibition of ipsilateral flexor reflex	-	-	-	$1^{+++;\ 1\ h}$
Inhibition of corneal reflex	-	-	-	$1^{++;\ 1\ h}$
Straub tail	-	-	-	$1^{+;\ 1\ h}$
Skin cyanosis	-	-	-	$1^{++;\ 1\ h}$
Death	-	-	-	$13^{3\ h}$

Clothianidin also caused synergistic effects on tonic flexor and extensor convulsions caused by subthreshold electric stimulations. This synergistic effect was statistically significant from 25 mg/kg bw and from 75 mg/kg bw it affected to 100% of treated animals (table below). Clothianidin also caused significant increases in sleeping time starting at 225 mg/kg bw and in intestinal transfer rate starting at 75 mg/kg bw (table below).

Table: Specific pharmacological effects after clothianidin administration to male mice. * = Statistically different form control for $p < 0.05$. TF = Tonic flexor. TE = Tonic extensor. n.a. = dose level not tested.

		Dose (mg/kg bw)					
	n	**0**	**6.25**	**12.5**	**25**	**75**	**225**
Synergistic effects on convulsions							
Electroshock TF							
TE	10	1	3	2	8*	10*	10*
	10	1	3	2	8*	10*	10*
Sleeping time	8		n.a.	n.a.	-	↑26%	↑63%*
Intestinal transfer rate	8		n.a.	n.a.	↓7.5%	↓60%*	↓78%*

According to the Guidance on the Application of the CLP Criteria substances that warrant classification as STOT SE are those that, based on evidence from studies in experimental animals, can be presumed to have the potential to impair organ function and produce significant toxicity in humans following single exposure. The table below summarises the effects found in the single dose toxicity studies causing nervous system dysfunction and that, therefore, meet these requirements for warranting STOT SE classification.

Table: Summary of neurotoxic effects found in the single dose toxicity studies. All effects were noted in absence of mortality except entries marked with asterisks.

Species	Effect	Dose (mg/kg bw)	Incidence	Classification (mg/kg bw)
Rat	Motor and locomotor activity	100	↓23-37% males	STOT SE 1 ≤ 300
Rat	Motor and locomotor activity	200	↓59-62% males ↓43-45% females	STOT SE 1 ≤ 300
Mouse	Decrease in spontaneous locomotor activity	50 100 200	3/3 slight 3/3 severe 3/3 severe	STOT SE 1 ≤ 300
Mouse	Tremor	50 100 200	1/3 slight 1/3 slight 3/3 severe	STOT SE 1 ≤ 300
Mouse	Decrease in reactivity	100 200	1/3 slight 3/6 severe	STOT SE 1 ≤ 300
Mouse	Decrease in grip strength	200	2/3 slight	STOT SE 1 ≤ 300
Rat	Tremor (home cage)	400	7/12 males 9/12 females	STOT SE 2 ≤ 2000
Rat	Decreased activity	400	8/12 males 11/12 females	STOT SE 2 ≤ 2000
Rat	Tremors (open field)	400	10/12 males 6/12 females	STOT SE 2 ≤ 2000
Rat	Sluggish exploratory movements	400	9/12 males 9/12 females	STOT SE 2 ≤ 2000
Rat	Motor and locomotor activity	400	↓81-88% males ↓72-83% females	STOT SE 2 ≤ 2000
Mouse*	Decrease in spontaneous locomotor activity	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Tremor	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Decrease in reactivity	400	3/3 moderate	STOT SE 2 ≤ 2000
Mouse*	Inhibition of pinna reflex	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Decrease in grip strength	400	3/3 severe	STOT SE 2 ≤ 2000

Comparison with the criteria

In addition to the affects reported in the table above, it is also described that mice exposed to clothianidin experienced tonic flexor and tonic extensor convulsions after a subthreshold electrical stimulation. These effects were noted at 25, 75 and 225 mg/kg bw. RAC notes that this effect cannot be considered as a toxic effect but is indicative that the nervous system is altered at concentrations warranting classification as STOT SE 1 as a consequence of clothianidin exposure.

RAC also notes that in the table above, the concentrations supporting STOT SE 1 are always above 30 mg/bw and therefore no SCL is needed.

No narcotic effects or respiratory tract irritation were reported in the single dose toxicity studies and therefore RAC notes that classification as STOT SE 3 is not supported. However, in a weight of evidence assessment, RAC supports the DS's proposal for **classification of clothianidin as STOT SE 1, H370 (nervous system).**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of clothianidin for effects on sexual function/fertility mainly based on the delayed sexual maturation observed in males in the 2-generation reproduction toxicity study in rats. The DS also proposed classification of clothianidin for development based on the high incidences of stillbirth and reduction of perinatal viability observed in both F1 and F2 offspring (in absence of severe maternal toxicity) in this same multi-generation study. Overall, DS proposed classification of clothianidin as Repr. 2, H361fd (Suspected of damaging fertility and unborn child).

Comments received during consultation

During the Stakeholder consultation, reproductive toxicity received a wide range of comments. These comments, together with the answers provided by the DS are summarised in the table below. See the RCOM, Annex 3 and additional information provided by commenters for more detailed information.

In addition to the comments presented in Annex 3, on August 24, 2021 RAC received a new position paper entitled: "*Clothianidin- the delay in preputial separation at 2500 ppm was due to general toxicity and does not represent selective reproductive toxicity*". As indicated in the title, this position paper rejected that the seven-day delay in sexual maturation was a real effect of clothianidin but was in fact the result of general toxicity and not due to any selective effect on reproduction (fertility). According to this paper, delays in preputial separation in male rats can be correlated with decreased body weights at weaning (post-natal day 21) because the highest exposure of the F1 generation occurs just prior to, and for the first week, post-weaning. The applicant estimated that the dose in F1 pups at weaning would have been 525 mg clothianidin/kg bw/day rather than the 161 mg clothianidin/kg bw/day estimated for the Parental animals fed with 2500 ppm. According to the applicant this dose would have caused a notable reduction in food consumption together with the corresponding bodyweight reduction; which would have been responsible for the delay in preputial separation. The Applicant also described that in other cases where delay in preputial separation have been reported it was caused by increased levels of free glucocorticoids (that have been found in foetuses and pups exposed to food restriction) or caused by an effect on the hormone leptin (involved in the control of food intake and in body-weight homeostasis).

Assessment and comparison with the classification criteria

The reproductive toxicity of clothianidin was assessed in a 2-generation toxicity study in rats and in two developmental toxicity studies, one in rats and one in rabbits.

2-generation reproduction study in rats

The study was performed following OECD TG 416 and observing GLP. Thirty males and thirty female Sprague-Dawley rats were orally exposed via diet to 0, 150, 500 and 2500 ppm of clothianidin (95.7% purity); which corresponded to 0, 10, 31, 163 mg/kg bw/day and 0, 10, 31, 161 mg/kg bw/day for males and females, respectively.

Adult animals

Significant body weight decreases were observed in both males and females of P and F1 adults at the top dose, while relative feed consumption was mostly increased. In both P and F1 animals,

absolute and relative organ weight changes were in line with the observed body weight loss (see table below).

Table: Effects of clothianidin on body and organ weight in adult animals in the 2-generation reproduction toxicity study in rats. 30 animals per sex per dose; pm: premating; ge: gestation; la: lactation. * = Statistically different form control for p < 0.05. ** = Statistically different form control for p < 0.01. n.d. = dose level tested but not displayed in the CLH report.

	0		150		500		2500	
	P	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
Body weight males	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓8-10%**	↓16-20%**
Body weight females (d7-70) pm (d0,6,13,20) ge (d0,4,7,14,21) la (d14) la	n.d.	n.d.	n.d.	n.d.	↓6.5%*	n.d.	↓9-15%** ↓12-14%** ↓11-18%**	↓16-19%** ↓13-16%** ↓13-15%**
Body weight gain (d0,6,13,20) ge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓17%**	↓8%
Feed consumption males	n.d.	n.d.	n.d.	n.d.	↓4%* wk1 pm	-	↓18%** wk1pm ↑8%* wk3;**wk4,5,8pm	↑16%** wk1-10pm
Feed consumption females	n.d.	n.d.	n.d.	n.d.	↑7.6%* wk10pm	-	↓18% **wk1pm ↑16% **wk8-10pm ↑11%* d6-13ge ↑13%* d14-21la	↑21%* wk1,5-6pm **wk2-4,7-10pm ↑11%* d6-13ge ↑13%* d7-21la
Organ weight								
Thymus males absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓16%* ↓8%	↓29%* ↓13%*
Thymus females absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓35%* ↓32%*	↓41%* ↓32%*
Ovary absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓19%* ↓16%*	↓11% -
Uterus absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓25%* ↓20%*	↓21% ↓10%

Occasional gross pathology findings were reported at the top dose in (i) female P animals: skull malocclusion and ulcer (1/30), kidney adhesion/raised zone (1/30), prolapsed uterus (1/30), vaginal mass (1/30), reduced thymus (2/30); (ii) F1 animals: 1 calculus in kidney (1/30 males), enlarged testicle (1/30 males), testicle with abnormal consistency (1/30 males), thymus oedema (1/30 females), lung discoloration (1/30 females), fluid-filled body (1/30 females) and mammary gland cyst (1/30 females).

The following occasional histopathology findings were noted in P animals at the top-dose: adrenal cortical hyperplasia (1 female), necrosis (1 female), vacuolization (2 females), thymus atrophy (2 females), uterus oedema (2 females), haemorrhage (1 female), cervix congestion (1 female), vagina congestion (1 female), cyst (1 female), abnormal spermatozoa (1 male), epididymis mineralization (1 male) and sperm granuloma (1 male).

In both P and F1 animals, no difference in number and duration of oestrus cycles between treated and control animals were observed on the basis of the smear analyses. Clothianidin did not affect the sperm morphology and total sperm count. However, sperm motility was statistically reduced in F1 animals exposed to 2500 ppm by 11.5%. The progressive sperm motility was reduced by 28.1 and 12.4% in F1 and P animals exposed to 2500 ppm clothianidin (see table below).

There was a delay of 6.7 days in the day of preputial separation among F1 animals exposed to 2500 ppm clothianidin. The delay was of 1.3 days in the F1 animals exposed to 500 ppm (following two tables). Both delays were statistically significant. Vaginal opening in F1 animals exposed to 2500 ppm was also statistically delayed by 2.3 days as regard F1 control animals (see table below).

Table: Gross pathology and histopathology in adult animals in the 2-generation reproduction toxicity study in rats with clothianidin. 30 animals per sex per dose; * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$. n.d. = dose level tested but not displayed in the CLH report. n.a. = dose level not tested.

	0		150		500		2500	
ADULTS	**P**	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
Gross pathology								
Preputial separation (day pn)	n.a.	41.2	n.a.	41.9	n.a.	42.5**	n.a.	47.9**
Vaginal opening (day pn)	n.a.	32.4	n.a.	32.2	n.a.	32.1	n.a.	34.7**
Histo/Cytopathologie								
Sperm morphology			n.a.	n.a.	n.a.	n.a.		
% normal	83.5	69.3					81.9	67.0
% abnormal	15.9	30.0					16.0	29.0
% detached	0.6	0.7					2.1	4.0
Total sperm count			n.a.	n.a.	n.a.	n.a.		
epididymis	146.1	149.5					141.6	133.7
testis	129.9	105.7					129.5	103.8
Sperm motility			n.a.	n.a.				
% motile	82.9	81.7			82.6	79.0	79.2	73.4**
% progressively motile	64.1	59.9			61.5	53.9	56.2**	46.1**
Oestrus cycle			n.d.	n.d.	n.d.	n.d.		
duration (days)	4.2	4.3					4.1	4.4
number	3.3	3.4					3.7	3.4
Oestrus stage (sacrifice)								
dioestrus (n)	18	19	19	18	25	16	24	25
proestrus (n)	2	0	0	3	1	0	1	1
oestrus (n)	9	11	10	9	3	14	2	4
Ovarian follicle count (mean /ovary)								
'non-antral' follicles	12.17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12.22
'antral' follicles	3.94	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.53
corpora lutea	3.71	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.24

Table: F1 male sexual maturation in the 2-generation reproduction toxicity study in rats with clothianidin. ** = Statistically significant different from control for p < 0.01.

Parameter		0 ppm	150 ppm	500 ppm	2500 ppm
F1 males evaluated		32	32	32	32
Day of preputial separation (mean±SD)		41.0±1.27	41.8±1.32	42.5**±1.72	47.8**±2.74
Cumulative achievement	PND 39	3	0	0	0
	PND 40	13	5	4	0
	PND 41	21	14	10	0
	PND 42	28	23	18	0
	PND 43	31	28	21	0
	PND 44	32	31	27	3
	PND 45		32	31	5
	PND 46			32	10
	PND 47				16
	PND 48				23
	PND 49				25
	PND 50				29
	PND 51				30
	PND 52				31
	PND 58				32
F1 males with time of achievement > PND 42		4	9	14	32

Litters

At the top dose, and in both sexes, a time dependent decrease of body weight was observed in both generations (table below). The number of early stillborn were increased at 500 ppm (F2) and at the top dose (F1, F2). All values were within the historical control range, except for the pup incidence at the top dose. Additionally, in the absence of a clear dose-effect relationship for the litter incidence, it was considered that the finding was toxicologically significant only at the top dose.

Decrease of thymus weight was observed at 500 ppm (males) and above (males + females), and a reduction of spleen weight was also noted at the top dose (table: 'Gross pathology and histopathology in adult animals…' above). The decreased thymic and splenic weights were considered compound-related. Occasional gross pathology findings were reported at the top dose in F1 animals. It included one case of hydrocephalus and one case of anophthalmia. All findings in other dose groups were also at low incidence and without dose-response.

Table: Pup data in the 2-generation reproduction toxicity study in rats with clothianidin. * = Statistically significant different from control for p < 0.05. ** = Statistically significant different from control for p < 0.01. n.d. = dose level tested but not displayed in the CLH report.

	0		150		500		2500	
	F1	F2	F1	F2	F1	F2	F1	F2
Litter data								
Observed (day 21)	24	23	29	25	28	20	29	28
Pups missing	2	1	2	4	1	4	4	6
Stillborn pups								
early	0	1	3	3	3	5	6	4
late	1	3	0	1	2	0	1	2
total	1	4	3	4	4	5	6	5
Viable pups								
d0	14	14	14	14	14	13	13	12
d4	13	13	14	13	14	13	12	12
Birth index	.898	.918	.905	.934	.945	.874	.940	.878
Live birth index	1.00	.998	.993	.991	.984	.974	.979	.965
Viability index	.992	.980	.994	.981	.987	.988	.924	.970
Lactation index	.995	.995	.996	.985	.978	.974	.960	.960
Body weight (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		

d0							↓8*	↓3
d4							↓16**	↓9
d7							↓18**	↓1**
d14							↓22**	↓16**
d21							↓26**	↓21**
Organ weights								
Thymus weight (males)	n.d.	n.d.	n.d.	n.d.				
Absolute (%)					↓13*	-	↓29**	↓25**
Relative (%)					↓10	-	↓5	↓8
Thymus weight (females)	n.d.	n.d.	n.d.	n.d.				
Absolute (%)					↓10	-	↓28**	↓24**
Relative (%)					↓8	-	↓7	↓9
Spleen weight (males)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Absolute (%)							↓30**	↓31**
Relative (%)							↓7	↓16**
Spleen weight (females)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Absolute (%)							↓35**	↓30**
Relative (%)							↓16**	↓17**

Conclusions of this study

At the highest dose level of 2500 ppm (approximately 160 mg/kg bw/day), effects on parental generations (P, F1) included reduced body weight gain during pre-mating, pregnancy and lactation and reduced thymus weights. Decreased sperm (progressive) motility was also noted at 2500 ppm. At 500 ppm, reduced body weight gain was restricted to day 14 of the lactation period, only. However, the substance intake at this dose level was much higher in comparison to pre-mating and gestation period.

Increase in stillbirths observed in F1 offspring at 2500 ppm was reproducible in F2 offspring and is therefore considered a test substance-related effect.

Sexual maturation of males was delayed at a dose level of > 500 ppm in the F1 pups; this endpoint has not been evaluated in the F2 generation. At 150 ppm, the slight delay is considered not to be biologically relevant. In addition, this delay of preputial separation was statistically not significant. Sexual maturation of females was affected only at highest dose level. As the significant delay in preputial separation was present despite normal post-weaning growth in F1 pups of the 500 ppm group it was considered treatment-related.

Developmental toxicity study in rats

The study was performed following OECD TG 414 and observing GLP. Twenty-five naturally mated female Sprague-Dawley rats per group were orally exposed via diet to 0, 10, 40 and 125 mg/kg bw/day of clothianidin (95.2% purity) during gestation days 6-19.

Pregnant females

No moralities and no clinical signs were reported at any dose. At the top dose, a slight decrease of body weight was observed from day 8, the largest body weight gain reduction (152%) was noted between gestation days 6-9, being the body weight reduction between days 6-20 of 17% and the corrected body weight reduction of 45% (table below). Body weight gain in the period between gestation days 6-9 was reduced by 42% at 40 mg/kg bw/day; while at this dose level the corrected body weight gain was also reduced by 19%. The body weight change was decreased at the top dose group and was essentially attributable to effects during days 6-9. At 40 mg/kg bw/day, the body weight change was altered during period days 6-9 (table below). No modification of gravid uterine weight was observable when compared to the control dams. Caesarean sectioning observations revealed no remarkable differences between treated and control animals (table below).

Table: Developmental toxicity study of clothianidin in rats. * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$. [a] corrected body weight = gestation bw minus gravid uterine weight. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw/day)	0	10	40	125
MATERNAL DATA				
Feed consumption				
day 6-9	20.9 g/day	20.5 g/day	19 g/day* (↓9%)	11.1 g/day** (↓47%)
day 6-20	23.6 g/day	23.6 g/day	22.7g/day (↓3.8%)	19.5 g/day** (↓17%)
Body weight	n.d.	n.d.	n.d.	
day 20				↓5.3%**
day 20[a]				↓6.8%**
Body weight gain				
day 6-7	1.9	0.7	-1.1*	-8.4**
day 6-8	7.3	5.6	0.5**	-11.1**
day 6-9	11.8	10.2	6.8** (↓42%)	-6.1** (↓152%)
day 6-20	120.9	122.6	117.0	100.8**(↓17%)
day 6-20[a]	46.7	46.4	37.7 (19%)**	25.8** (↓45%)
FOETAL DATA				
Nº pregnant females	23	22	24	25
Corpora lutea/ dam	15.9	15.8	15.9	16.0
Implantations/ dam	13.6	14.3	14.2	14.3
Resorptions early/late/ dam	0.6 / 0.0	0.9 / 0.0	0.4 / 0.0	0.6 / 0.1
Live foetuses / litter	13.0	13.4	13.8	13.6
Live males foetuses (%)	49	49	52	48
Foetal weight (g) male/female	3.60/3.43	3.57/3.44	3.63/3.46	3.40/3.28
Foetal variations (%)	34.8	40.9	37.5	40.0
Foetal malformations (%)	8.7	4.5	4.2	4.0

Foetal data

Foetal alterations occurred at similar incidences in litters of all dosage groups or were comparable to recent historical controls or showed no consistency with dose (see table below).

Table: Summary of foetal alterations in the developmental toxicity study of clothianidin in rats. [m] malformation; [v] variation. Values referring to number of foetuses (litter incidence in %). Historical control litter incidence: microphthalmia=0.59%, fused ribs=0.46%, small eye socket/depressed eye bulge: not reported).

		Dose (mg/kg bw/day)			
Location	**Parameter**	**0**	**10**	**40**	**125**
	N° of litters evaluated	23	22	24	25
External	No remarkable findings	23	22	24	25
Visceral	Depressed eye bulge[m]	0 (0.0)	1 (4.5)	1 (4.2)	0 (0.0)
	Microphthalmia[m]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
	Innominate artery absent[v]	0 (0.0)	0 (0.0)	1 (4.2)	1 (4.0)
	Aortic arche dorsal to trachea/oesophagus[v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	Carotid (l) arises right of subclavian (r)[v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Skeletal	Small eye socket[m]	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
	Fused ribs[m]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	Bifid centrum in thoracic vertebra[v]	1 (4.3)	0 (0.0)	1 (4.2)	2 (8.0)
	Cervical rib present at 7th cervical vertebra[v]	0 (0.0)	1 (4.5)	1 (4.2)	2 (8.0)
	Incompletely/not ossified sternal centra[v]	5 (21.7)	4 (18.2)	6 (25.0)	5 (20.0)
	Incompletely/not ossified pelvis[v]	1 (4.3)	3 (13.6)	3 (12.5)	2 (8.0)

Conclusions of this study

No remarkable developmental toxicity were observed up to the highest dose (125 mg/kg bw/day) tested. This dose caused negative body weight gain between days 6-9 and a reduction of corrected body weight of 6.8% and of corrected body weight gain of 45% during days 6-20.

Developmental toxicity studies in rabbits

The study was performed following OECD TG 414 and observing GLP. Twenty-three naturally mated female New Zealand White rabbits per group were orally exposed via diet to 0, 10, 25, 75 and 100 mg/kg bw/day of clothianidin (95.2% purity) during gestation days 6-28.

Pregnant females

Two animals were found dead at both 75 and 100 mg/kg bw/day, and one animal was sacrificed moribund at the high dose (table below). Both decreased faecal output and orange/red urine was observed at 25 mg/kg bw/day and above. No relevant findings were noted in the necropsy. Feed consumption was significantly decreased at 75 mg/kg bw/day and above, while body weight and body weight gain was only affected at the top dose. However, no effect on corrected body weight was noted (table below). The reductions of uterine weight at 75 mg/kg bw/day and above were considered biologically significant.

Table: Maternal effects of clothianidin noted in the developmental toxicity study in rabbits. Corrected body weight = gestation bw minus gravid uterine weight; * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw/day)	0	10	25	75	100
N° rabbits examined	23	23	23	23	23
Mortality					
found dead	0	0	0	2[d25,27]	2[d17,20]
sacrificed moribund	0	0	0	0	1[d19]
%	0	0	0	8.7	13.0
Clinical signs					
localized alopecia	4	2	3	3	8
scant faeces	1	3	4	10	16
no faeces	n.d.	n.d.	n.d.	1	11
soft/liquid faeces	n.d.	n.d.	n.d.	n.d.	1
orange urine	n.d.	n.d.	2	9	9
red substance in pan	n.d.	n.d.	1	0	4
decreased motor activity, loss righting reflex	n.d.	n.d.	n.d.	n.d.	1
Feed consumption (g/day)					
Day 6-9	141.3	154.2	168.7	142.9	90.9*
Day 9-15	135.4	160.5	149.4	119.0	60.3**
Day 15-21	153.8	167.0	159.3	123.5	86.4**
Day 21-24	143.5	143.1	137.4	101.2*	104.7*
Day 24-29	90.0	94.2	85.4	89.4	64.2
Day 6-29	142.8	145.2	139.5	118.8*	89.6*
Body weight (kg)					
Day 0	4.02	4.01	4.00	3.98	4.01
Day 29	4.36	4.40	4.37	4.22	3.98**
Corrected body weight (kg)	3.84	3.88	3.86	3.83	3.67
Body weight gain (g)					
Day 0-6	50	70	80	80	60
Day 6-9	10	10	50	20	-40**
Day 9-15	90	120	90	30	-160
Day 15-21	90	110	90	30	60
Day 21-29	50	90	60	30	-70
Day 6-29	320	320	300	170	-200**
Day 0-29	390	390	380	260	50**
Gravid uterine weight (g)	517.8	524.5	516.5	461.6	420.1

Foetal data

A statistical significant decrease in female foetus weight was observed at the top dose (table above). The decrease of the proportion of male life foetuses (24%) and the increase (4-times) of % resorbed foetuses/litter at the top dose could be biologically relevant despite did not attain statistical significance at the top dose (table below). There was a dose dependent increase of animals showing absence of intermediate lung lobe. Despite the relatively high spontaneous incidence of the variation in this strain, the effect is probably also of toxicological significance since the incidence was not covered by the historical control data (table: 'Historical control data incidences for alterations…'). Other effects, including small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hind paw phalanges are minimally but significantly increased on litter incidence base at the top dose. Historical control data were also exceeded for these effects (table: 'Historical control data incidences for alterations…'). A delay in ossification was observed at 75 mg/kg bw/day and above (sternal centres) and at the top dose (hind limb phalanges, see table below), although these effects were covered by historical control data (table: 'Historical control data incidences for alterations…').

Table: Main developmental toxicity study of clothianidin in rabbits. [m] malformation; [v] variation; * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$.

Dose (mg/kg bw/d)	0	10	25	75	100
Number of surviving pregnant females	21	23	22	20	20
Aborted/premature litters (%)	3 (13.0)	0	0	1.2 (13.0)	6.2 (34.8*)
Litters with 3 live foetuses	0	0	2	0	1
Included in analysis	18	23	20	17	11
Corpora lutea	9.4	9.6	9.9	8.7	9.8
Implantations	8.6	8.9	9.2	8.0	8.8
Resorptions early/late	0.1/0.2	0.0/0.1	0.1/0.2	0.0/0.2	0.9/0.4
% Resorptions	3.1	1.4	3.6	3.4	12.7
Live foetuses	8.3	8.7	8.8	7.8	8.2
Live male foetuses (%)	51.8	47.2	50.2	48.8	39.6
Foetal weight (g)					
male	44.2	43.2	40.7	40.7	37.7
female	43.0	42.2	40.0	40.2	36.1**
Variations (%)					
litter incidence	22.2	30.4	45.4	29.4	45.4
foetal incidence	2.7	3.5	9.5**	4.5	8.2**
% malformations					
litter incidence	0.0	21.7	9.1	11.8	27.3
foetal incidence	0.0	3.0**	1.7	1.5	5.9**
Litters with ≥ 1 variation (%)	2.6	3.8	13.6	5.0	11.5
Litters with ≥ 1 malformation (%)	0.0	2.9	1.5	1.6	9.0
External medially rotated hind limbs[m]					
litter	0	0	1 (4.5)	0	0
foetus			3 (1.7**)		
Visceral intermediate lung lobe absent [v]					
litter	0	0	0	3 (17.6)**	5 (45.5)**
foetus	0	0	0	3 (2.2)	8 (9.4)**
Visceral small kidney[m]					
litter	0	0	0	0	1 (9.1)
foetus	0	0	0	0	3 (3.5)**
Skeletal fused caudal vertebrae[m]					
litter	0	0	1 (4.5)	0	2 (18.2)**
foetus	0	0	2 (1.1)	0	2 (2.4)**

Skeletal incompletely ossified sternal centra[v]					
litter	0	0	0	0	2 (18.2)**
foetus	0	0	0	0	2 (2.4)
Skeletal absent hind paw phalanges[m]					
litter	0	0	0	0	2 (18.2)**
foetus	0	0	0	0	2 (2.4)**
Ossification sites: number per foetus/litter					
Sternal centres					
foetus (HCD: 3.81-3.97, mean: 3.91)	3.99	3.91	3.94	3.83	3.76
litter	0.03	0.14	0.13	0.2**	0.25**
Hindlimbs phalanx					
foetus (HCD: 11.99-12.00, mean: 12.0)	12.00	12.00	12.00	12.00	11.78
litter	0.00	0.00	0.00	0.00	0.63**

Table: Historical control data incidences for alterations reported in table: 'Maternal effects of clothianidin noted in the developmental toxicity study in rabbits'.

Effect	Mean (%)		Range (%)	
	Foetal	Litter	Foetal	Litter
Intermediate lung lobe absent	1.54	10.1	0-4.4	0-27.8
Small kidney	no reported data			
Fused caudal vertebrae	0.21	1.4	0-1.9	0-5.9
Incompletely/not ossified sternebrae	0.21	1.8	0-1.2	0-5.9
Absent hind paw phalanx	0.02	0.2	0-0.6	0-5.9

Conclusions of this study

At doses of 75 mg/kg bw/day and higher 2 deaths and at top dose 1 sacrifice occurred, reduced food intake and faecal output, but no significant decreased body weight gain. Additionally, at 100 mg/kg bw/day an increase of abortions or prematurely deliveries were noted. A reduced number of lung lobes in 3 foetuses out of 3 litters at 75 mg/kg bw/day and 8 foetuses (9.4%) out of 5 litters (45.5%) at 100 mg/kg bw/day were observed. The missing lung lobes appear to indicate a dose-related effect on lung branching morphogenesis at maternally toxic doses. At the top dose of 100 mg/kg bw/day some malformations were slightly, but significantly increased on litter incidence base like small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hind paw phalanges.

Comparison with the criteria

Fertility

The 2-generation reproduction toxicity study with clothianidin reported a reduction in sperm motility (see table: 'Gross pathology and histopathology in adult animals…'). RAC notes that there are notable differences in sperm parameters between rats and humans since the volume of sperm in humans is lower than in rodents. Thus, this reduction in sperm motility caused by clothianidin is an issue of potential concern in humans.

This same 2-generation reproduction toxicity study reported minor (2.3 days) but statistically significant delays in vaginal opening of F1 litters (the effect was not assessed in F2) at the top dose (see table: 'Gross pathology and histopathology in adult animals …'). During the public consultation the applicant submitted a position paper entitled *"Evaluation of the DART Database for Clothianidin"* with a covariate analysis between the day of vaginal opening and pup body weight at post-natal day 21. When this interaction is used as a covariate in the statistical analysis, the delay in vaginal opening at the high dose is no longer statistically significant (table below). Thus, RAC notes that clothianidin did not significantly affect pubertal development in female rats and therefore this effect does not support a classification.

Table: Age at puberty and weight at post-natal day 21 in F1 generation pups reported at the rat reproductive study on clothianidin. ** = Statistically different from control for p ≤ 0.01. [a] Mean ± SE. [b] Mean ± SD.

ppm:	0	150	500	2000
Mean age of preputial separation (days)[a]	41.2 ± 0.27	41.9 ± 0.25	42.5** ± 0.30	47.9** ± 0.53
Mean body weight at preputial separation (g)[b]	190.1 ± 17.6	198.7 ± 12.8	191.9 ± 19.4	189.7 ± 15.4
Mean age of vaginal opening (days)[a]	32.4 ± 0.31	32.2 ± 0.32	32.1 ± 0.46	34.7** ± 0.45
Mean body weight at vaginal opening (g)[b]	104.6 ± 13.0	104.4 ± 10.6	98.8 ± 10.6	90.6 ± 14.3

A more severe and statistically significant delay in male sexual maturation was noted in F1 litters (effect was not assessed in F2) exposed to 500 ppm (1.3 days) and to 2500 ppm (6.7 days) (tables 'Gross pathology and histopathology in adult animals…' , 'F1 male sexual maturation in the 2-generation…' and 'Age at puberty and weight at post-natal day 21…'). On the contrary to the case of vaginal opening delay, the pup weight at post-natal day 21 did not significantly variate with the dose ('Age at puberty and weight at post-natal day 21…'). It suggests that the delay in preputial separation is indeed a clothianidin-related effect. The applicant plotted in the position paper the day of preputial separation versus post-natal day 21 pup weight (see the figure below). As can be seen from the graph, the lines for the control and mid-dose groups have similar slopes and overlap somewhat in the distribution of their data points. In contrast, the slope of the line and distribution of data points for the high dose group differ substantially from that of the control group. The results of this analysis indicate that 2500 ppm clothianidin had a significant effect on preputial separation that was independent of its effect on pup growth in general.

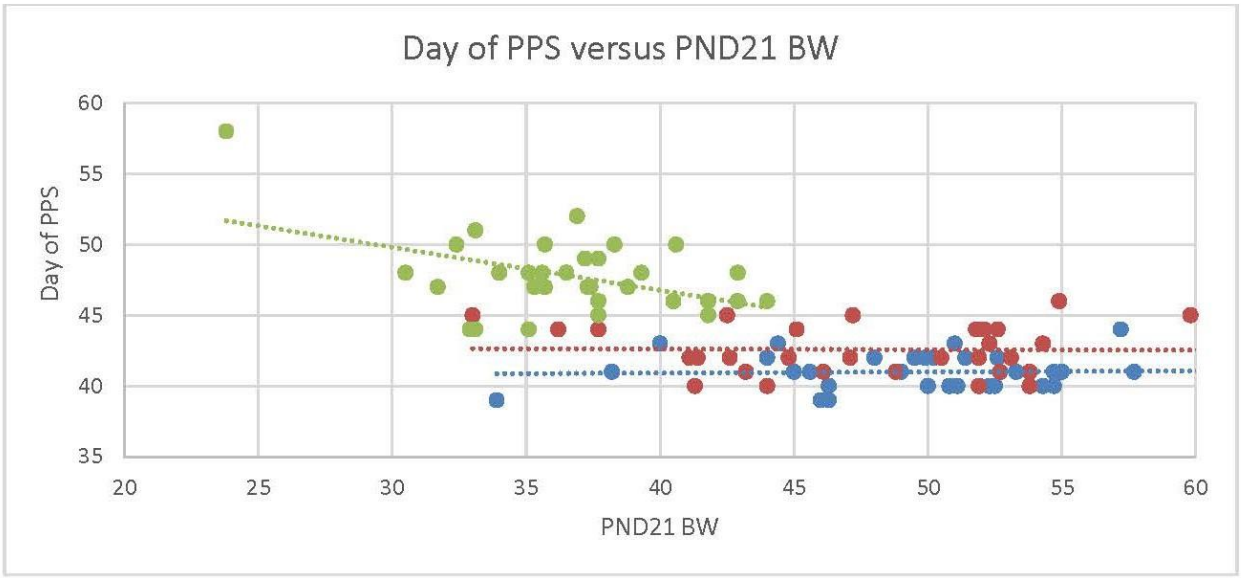


Figure 1. Day of F1 preputial separation (PPS) as a function of the PND 21 body weight (BW) in grams in the rat reproductive study of clothianidin (Freshwater and Astroff, 2000). Blue = control group; red = mid-dose group; green = high dose group.

The applicant considered that the effect on preputial separation at 2500 ppm is secondary to general systemic toxicity derived from undernutrition. However, it is unclear to RAC that general toxicity could justify such a delay since a clear dose-response is observed (1.3 days of delay at

500 ppm and 6.7 days of delay at 2000 ppm). The applicant also argued that no effect on male and female sexual maturation was noted in a developmental neurotoxicity study (see details below in the section "*Supplemental information*") and therefore the effect noted in the 2-generation study would be also incidental. However, RAC notes that in the developmental neurotoxicity study, the top dose was slightly lower than in the 2-generation study and therefore the results of this study do not allow ruling out that the effect of clothianidin on male sexual maturation.

In the last position paper submitted by the applicant in August 2021, it was estimated that the actual dose received by F1 pups at weaning time was indeed 3.3 times higher than the P animals fed at the same dose level. Applicant speculated with the possibility that this high dose could have been responsible of a notable bodyweight reduction that could justify the delay in preputial separation. RAC notes that, as the same position paper states, in this multigenerational study, the weekly body weights and food consumption measurements for the F1 generation did not start to be collected until all litters had been weaned. It means that the values presented post-weaning are not accurate compared to body weight and food consumption values collected for the P generation or other general toxicity studies. Therefore, the systemic toxicity is not well documented and it does not allow RAC to diminish the relevance of the 7 days delay in the preputial separation, especially considering the above described dose-response response.

RAC concludes that the delay in preputial separation noted in the 2-generation toxicity study was indeed a treatment-related effect. Therefore, RAC proposes the **classification of clothianidin for fertility and sexual function as Repr. 2 (H361f)** mainly based in the delayed male sexual maturation and supported by alterations in sperm motility.

Development

The developmental toxicity study in rats showed no remarkable developmental toxicity at the top dose (causing negative body weight gain between days 6-9 and a reduction of corrected body weight gain of 45% during days 6-20). The results of this study do not support a potential classification of clothianidin for developmental toxicity.

The developmental toxicity study in rabbits showed statistically significant increments in the incidences of visceral intermediate lung lobe absent, visceral small kidney, skeletal fused caudal vertebrae, skeletal incompletely ossified sternal centra, skeletal absent hind paw phalanges and in the ossification sites (sternal centers and hind limbs phalanx) in rabbits fed with 2000 ppm clothianidin (table: 'Main developmental toxicity study of clothianidin in rabbits'). The incidences of visceral intermediate lung lobe absent was also statistically increased in rabbits fed with 500 ppm clothianidin (table: 'Main developmental toxicity study of clothianidin in rabbits'). However, RAC notes that in most of the cases, these increased incidences are still within the historical control data and moreover, the top dose of 2000 ppm caused 13% mortality (includes 2 dead and 1 sacrificed animal out of 23 animals); which is above the limit value of 10% of mortality established in the CLP Regulation to be considered as excessive maternal toxicity. Therefore, the data for 2000 ppm dose level shall not be considered by RAC for further evaluation.

The incidence of visceral intermediate lung lobe absent in rabbits fed with 500 ppm clothianidin is statistically significantly higher than the concurrent control and above the historical control data. The applicant argued in the position paper that lobation of the lung in rabbits is similar to that for humans and absence of the intermediate lung lobe occurs due to failure in formation of a lung fissure and do not involve the absence of lung tissue or alter lung functionality. Moreover, RAC notes that these effects appear at dose causing 9% mortality (borderline with the 10% limit that would avoid considering this effect as supportive of developmental toxicity). Overall, RAC

considers that the absence of visceral intermediate lung lobe does not warrant a classification for developmental toxicity.

The CLH report noted an increase in early stillborn pups at 500 ppm (F2 generation) and at 2500 ppm (F1, F2 generations) (table: 'Pup data in the 2-generation reproduction toxicity study…'). However, applicant noted in the released comments that according to the original study report the percent of stillborn pups in both generations did not differ statistically across groups. Although the incidences were increased with treatment (table below), they also were reported to be within the laboratory's historical control range of 0-3.9% (mean 1.9%). Further, there was no significant effect on the live-birth index.

Table: Stillborn pup incidence (% stillborn) and live-birth index reported in the rat reproductive study of clothianidin according to the applicant's comments. [a] No. live pups per litter/total pups per litter x 100

ppm:	0	150	500	2500
		Stillborn pups – # (pup %)		
P generation	0 (0)	3 (0.7)	6 (1.5)	9 (2.3)
F1 generation	1 (0.3)	3 (0.9)	8 (3.1)	13 (3.7)
		Live-birth index[a]		
P generation	100	99	98	98
F1 generation	100	99	97	97

Further support to the lack of effect of clothianidin on stillborn incidence was found in the neurotoxicity and immunotoxicity developmental studies where such effect was not reported (see below supplemental information for details). Overall, RAC notes that the alterations in stillborn incidence reported in the CLH report are not enough for supporting classification as developmental toxicity.

According to the CLH report, the viability index in F1 rats exposed to 2000 ppm clothianidin was reduced to 92.4% (table: 'Pup data in the 2-generation reproduction toxicity study…'). The applicant released comments clarifying that the viability index for the F1 generation at 2500 ppm was miscalculated since included in this calculation a value of 0% viability for a dam (PF3108) that died prior to post-natal day 4. The fact that all of her pups died prior to PND 4 is a function of her death; thus, the value derived from this animal should have been excluded from the calculations. When calculated correctly, the viability index for the F1 generation animals at 2500 ppm is 95.7% (table below); which is a value that RAC does not consider of concern for supporting a classification for developmental toxicity.

Table: Corrected viability index (%) reported in the rat reproductive study of clothianidin. [a] The value provided in the study reported (92.4%) was miscalculated due to inclusion of dam PF3108 that died prior to PND 4. The value given herein has been calculated based on exclusion of this dam from the calculation.

ppm:	0	150	500	2500
F1 generation	99.2	99.4	98.7	95.7[a]
F2 generation	98.0	98.1	98.8	97.0

Other effects noted in the 2-generation toxicity study was reductions in body, thymus and spleen weights in pups exposed to 2000 ppm clothianidin (table: 'Pup data in the 2-generation reproduction toxicity study…'). However, RAC also notes that the reductions in body weights and in thymus weight was also reported in adult animals and therefore could be interpreted as systemic rather than developmental effects. In the same way, the reductions in spleen weight in pups could also be secondary to reductions in body weight.

In conclusion, RAC notes that none of the developmental effects reported in the CLH report is strong and robust enough for supporting a classification of clothianidin for developmental toxicity.

Lactation

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child. The available reproductive study does not provide evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk. Toxicokinetic studies do not indicate the likelihood that the substance can be potentially present in breast milk. Thus, there were no effects to warrant classification of clothianidin for effects on or via lactation and RAC proposes no classification for this category hazard.

Overall, RAC considers that **clothianidin should be classified for fertility and sexual function as Repr. 2 (H361f).**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Clothianidin has an existing entry for environmental hazard classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with a generic M-factor of 10 in Annex VI of CLP.

During the re-evaluation procedure of clothianidin under the PPP regulation, a new chronic toxicity study became available for classification and a potential update of the current classification (mainly the derivation of new M-factors) was proposed. Based on the new data, retention of the existing hazard categories and new M-factors for Aquatic Acute and Aquatic Chronic have been proposed by the DS.

Overall, the DS concluded that clothianidin is 'not rapidly degradable', has a low potential for bioaccumulation and proposed classification based on aquatic acute and chronic toxicity in aquatic insects:

Aquatic Acute 1 with an M-factor of 10, based on the lowest measured 48-hour EC_{50} value of 0.029 mg/L for *Chironomus riparius*; and

Aquatic Chronic 1 with an M-factor of 100, based on the lowest measured 28-d EC_{10} of 0.0004 mg/L for *C. riparius*.

Degradation

A ready biodegradability test (Carbon Dioxide Evolution Test - Directive 92/69/EEC C.4-C) showed 1.5% biodegradation of clothianidin after 28 days (Bealing and Watson, 1999). Therefore, clothianidin was considered as "not readily biodegradable" by the DS.

The results of a hydrolysis study (92/69/EEC, C.7; US EPA, Subdivision N, Section 161-1) showed that clothianidin is stable in sterile buffer solutions at pH 4, 5, and 7, but degrades at pH 9 (Lewis, 2000a). Nevertheless, relevant amounts of metabolites (CTNU (N-(2-chlorothiazol-5-ylmethyl)-N'-nitrourea), TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea), and ACT•HCl (2-chlorothiazol-5-ylmethylamine hydrochloride)) were formed in pH 9 only at elevated temperatures (50°C). At an environmentally relevant temperature (20°C) the calculated half-live

was 1401 days. Therefore, the DS considered that hydrolysis is not a significant degradation route for clothianidin at environmentally relevant temperature and pH.

Regarding photolysis in pure water under the experimental conditions used, clothianidin degraded rapidly with an experimental half-life (DT_{50}) of 3.3 hours (SETAC and US EPA 161-2). The experimental half-life corresponds to a calculated environmental half-life of < 0.6 day under midsummer solar light conditions in Phoenix, USA (Babczinski and Bornatsch, 2000). However, assessing environmental half-lives by means of an arithmetical model (Hellpointer, 1999a), with additional parameters, besides the laboratory data, resulted in half-lives up to 23.4 days for the 50^{th} degree of latitude. Also, with another arithmetic model (Hellpointer, 1999a), which takes cloudiness in Central Europe as an additional parameter into consideration, there were estimated high values for the environmental half-lives in autumn and winter (over 1 year). Therefore, the DS considered that although there is no doubt that photolysis in water will contribute to the degradation of clothianidin in water. However, assuming photo transformation in the whole water body would highly overestimate the degradation potential.

Photolysis in air (estimation method by AOPWIN) showed a half-life of 2.81 hours indicating photodegradation in air (Hellpointer, 1998; Extended by Hellpointer, 2005).

Regarding the water/sediment system, one study following BBA part IV (Gilges and Brumhard, 2000) was provided. The dissipation behaviour of clothianidin applied at a concentration of 50 µg a.s./L water was studied in two German water/sediment systems incubated in the dark at 20°C over a period of 100 days. Primary degradation (dissipation) of clothianidin in the water phase and in the entire systems was slow. For systems I and II, first order DT_{50} values of 30.8 and 49.8 days at 20°C (58.4 and 91.0 days at 12°C), respectively, were determined in the water phase and 48.0 and 64.8 days (94.4 and 122.9 days at 12°C) for the entire systems. Max mineralisation of 3.2% (100 d) was at system I and 4.4% at system II. Therefore, metabolism of clothianidin in the water phase of the aerobic water/sediment study is insignificant. Only the parent compound was identified in relevant amounts in both systems.

Overall, due to the results summarised above, the DS concluded that the available degradation information does not provide sufficient evidence to show that clothianidin is ultimately degraded to above 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, clothianidin was considered to be not rapidly degradable by the DS, according to the CLP criteria.

Aquatic Bioaccumulation

No measured BCF data are available for clothianidin, although a determined Log K_{OW} of 0.7 is available. However, an approximate estimation of the bioconcentration factor BCF_{fish} of 0.78 was performed using the standard equation given in the Guidance on the BPR. Although according to the EU Guidance (2015) the linear relationship for estimating the bioconcentration factor based on Log K_{OW} is not applicable to clothianidin as its Log K_{OW} is below the QSAR validity range (2 < Log K_{OW} < 6), the calculated BCF was accepted by the DS as it describes the order of magnitude in which the BCF is expected. Therefore, the DS concluded that clothianidin has a low potential for bioaccumulation.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies for all trophic levels of clothianidin are summarised in the following table and sections. Only the valid acute and chronic studies on clothianidin which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below. The most sensitive trophic group for acute and chronic toxicity are insects (Midge larvae: *C. riparius)*.

Table: Aquatic Acute toxicity mm: mean measured concentration, nom: nominal concentration

Test organism	Guideline, test method	Short-term result (endpoint)	Reference
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 203	96h LC_{50} > 100 mg/L (nom. confirmed by analytical monitoring)	Anonymous 22, 1998a
Bluegill sunfish *(Lepomis macrochirus)*	OECD TG 203	96h LC_{50} > 120 mg/L (nom)	Anonymous 23, 2000a
Sheepshead minnow *(Cyprinodon variegates)*	US EPA OPPTS Draft Guideline 850.1075	96h LC_{50} > 100 mg/L (nom. confirmed by analytical monitoring)	Anonymous 24, 1999a
Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 202	48h EC_{50} > 120 mg/L (nom. confirmed by analytical monitoring)	Palmer *et al*., 2000b
Water flea *(Daphnia magna)*	OECD TG 202	48h EC_{50} = 26 mg/L (nom. confirmed by analytical monitoring)	Noack and Geffke, 1997
Salt-water mysids *(Americamysis bahia)*	US EPA Guideline OPPTS No. 850.1035 and ASTM Standard E729-88a	48h EC_{50} = 0.082 mg/L (mm) 96h EC_{50} = 0.053 mg/L (mm)	Drottar *et al*., 2000a
Algae / other aquatic plants			
Green algae *(Selenastrum capricornutum)*	FIFRA Subdivision J Series 123-2	72h E_bC_{50} = 70 mg/L 72h E_rC_{50} > 120 mg/L (nom. confirmed by analytical monitoring)	Sutherland *et al*., 2000
Green algae *(Scenedesmus subspicatus)*	OECD TG 201	120h E_bC_{50} = 228 mg/L 120h E_rC_{50} > 270 mg/L (mm)	Wilhelmy and Geffke, 1998b
Saltwater diatom *(Skeletonema costatum)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h E_rC_{50} = 33.2 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012b
Freshwater diatom *(Navicula pelliculosa)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h E_rC_{50} = 67.2 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012c
Sediment dwelling organisms			
Midge larvae *(Chironomus riparius)*	No recommended guideline	**48h EC_{50} = 0.029 mg/L** (nominal concentration)	Mattock, 2001

Three studies have been submitted on the acute toxicity of clothianidin to fish. The reported 96-hour LC_{50} values of clothianidin in all studies with fish were above 100 mg/L based on nominal test concentration confirmed by analytical monitoring.

To address acute toxicity of clothianidin to invertebrates two studies with *Daphnia magna* and one study with *Americamysis bahia* were submitted. The reported 48-hour EC_{50} values of clothianidin in studies with *D. magna* were 26 mg/L and > 120 mg/L, respectively, based on nominal test concentrations confirmed by analytical monitoring. The reported 48-hour and 96-hour EC_{50} values of clothianidin in the study with *A. bahia* were 0.082 and 0.053 mg/L, respectively, based on mean measured concentrations.

Two studies on the green algae, one study on saltwater diatom and one study on freshwater diatom were submitted to address acute toxicity of clothianidin to algae. The reported 72-hour and 120-hours E_rC_{50} values in green alga study were above 100 mg/L. The reported E_rC_{50} values with salt and fresh-water diatoms were in the range between > 10 – < 100 mg/L based on nominal test concentrations confirmed by analytical monitoring.

One 48-hour static toxicity test without sediment with larvae of the midge *C. riparius* has been submitted. The results indicate a high toxicity of the clothianidin to insects with the test parameter being immobility. The larvae were considered immobile if they did not move during a 15 second period of observation. After 24-hours and 48-hours, the numbers of immobilized midges were recorded. Based on the measured immobilisation, the 24-hour EC_{50} of 0.072 mg/L and 48-hour EC_{50} of 0.029 mg/l were calculated based on nominal concentrations.

Overall, the DS proposed to classify clothianidin as Aquatic Acute 1 based on the 48-hour EC_{50} for *C. riparius* of 0.029 mg/L based on nominal concentrations for the endpoint mortality/immobilisation. As this acute toxicity value falls within the $0.01 < L(E)C_{50} \leq 0.1$ mg/L range, the acute M-factor proposed by the DS was 10.

Table: Aquatic Chronic toxicity mm: mean measured concentration, nom: nominal concentration

Test organism	Guideline, test method	Long-term result (endpoint)	Reference / Test item
Fish			
Fathead minnow *(Pimephales promelas)*	US EPA OPPTS Draft Guideline No. 850.1400	33d NOEC ≥ 20 mg/L (nom. confirmed by analytical monitoring)	Anonymous, 2000
Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 211	21d NOEC = 0.12 mg/L (nom. confirmed by analytical monitoring)	Noack and Geffke, 1998
Salt-water mysids *(Americamysis bahia)*	US EPA Guideline OPPTS No 850.1350 and ASTM Standard E1191-90	39d NOEC = 0.0097 mg/L (mm)	Drottar *et al*., 2000b
Algae /other aquatic plants			
Green algae *(Selenastrum capricornutum)*	FIFRA Subdivision J Series 123-2	72 / 96h NOE_rC = 15 mg/L 120h NOE_rC = 30 mg/L (nom. confirmed by analytical monitoring)	Sutherland *et al*., 2000
Green algae *(Scenedesmus subspicatus)*	OECD TG 201	120h NOE_rC = 180 mg/L (mm)	Wilhelmy and Geffke, 1998b
Freshwater diatom *(Navicula pelliculosa)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h NOE_rC = 16 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012c
Sediment dwelling organisms			
Midge larvae *(Chironomus riparius)*	BBA Guideline proposal (1995)	28d EC_{10} = 0.0004 (recalculated to mm)	Heimbach, 1999

The toxicity of clothianidin to early life-stages of Fathead minnow (*Pimephales promelas*) was performed according to US EPA OPPTS draft guideline No. 850.1400 (1996), US EPA-FIFRA Subdivision E, Series 72-4 (1982) and ASTM Standard E1241-88 (1988) in compliance with OECD TG 210. Exposure to clothianidin at the concentrations tested showed no statistically significant effects on hatching success, larval survival, total length, or growth when compared to the controls. The reported 33-days NOEC of clothianidin was ≥ 20 mg/L based on nominal test concentrations confirmed by analytical monitoring.

Two studies have been submitted on the chronic toxicity of clothianidin to invertebrates. The reported chronic toxicity values were a 21-day NOEC of 0.12 mg/L for *D. magna* based on nominal test concentrations confirmed by analytical monitoring and a 39-day NOEC of 0.0097 mg/L for *A. bahia*, based on mean measured concentration.

Two studies on the green algae and one study with a freshwater diatom were submitted to address chronic toxicity of clothianidin to algae. The reported 72-hours, 96-hours and/or 120-hours chronic toxicity values were in range of NOE_rC > 10 – < 100 mg/L for *Selenastrum capricornutum* and *Navicula pelliculosa* based on nominal test concentrations confirmed by analytical monitoring. The reported 120-hours chronic toxicity value for *Scenedesmus subspicatus* was above 100 mg/L based on mean measured concentration.

One 28-day static toxicity test in a water-sediment system was determined with larvae of the midge *C. riparius* with the observed endpoints being emergence and development of midges after 28 days. However, no measurement of clothianidin concentration in the sediment was performed and it was assumed that the decrease in test substance concentration in the water phase is mainly due to degradation than due to adsorption onto the sediment. Therefore, use of nominal concentrations underestimates the toxicity of clothianidin to *Chironomus*. To consider this decline in test substance concentration, the geometric mean of the measured concentrations for the time 0, day 7 and day 28 for the nominal concentration was calculated. This resulted in a recalculated mean measured concentration of EC_{10} = 0.0004 mg/L for the emergence endpoint.

Overall, as clothianidin is considered not rapidly degradable the DS proposed to classify clothianidin as Aquatic Chronic 1 based on the 28-day EC_{10} for *C. riparius* of 0.0004 mg/L, based on recalculated mean measured concentration for the emergence endpoint. As chronic toxicity falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the chronic M-factor proposed by the DS was 100.

Comments received during consultation

One MSCA and one National Authority (NA) provided comments on the proposal. The MSCA agreed with proposed classification and made only one editorial remark.

The NA in principle agreed with the proposed classification but raised questions in order to confirm the proposed classification. The first question raised by the NA was whether the DS considered research of literature data on the aquatic toxicity of clothianidin to mayfly, as the harmonised classification of other neonicotinoids are based on data for mayfly, which is very sensitive to these substances.

In addition, the NA pointed out that the acute key endpoint for *C. riparius* is based on initial measured concentrations. Test concentrations were not measured at the end of the 48-hour exposure period to verify that these remained within 80-120% of the nominal. In parallel, the acute mysid (*A. bahia*) study used similar test concentrations and mean measured concentrations are provided in the CLH report. Therefore, the NA asked for information on the actual concentrations for this mysid study in order to support that the test substance was stable from 0 – 48 or 96 hours and support the *C. riparius* endpoints.

The last question raised by the NA was related to the proposed Aquatic Chronic classification as is it based on a *C. 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. Therefore, the NA asked for further information to help understand test substance concentrations in the sediment phase during the course of the experiment in order to help consider if the aquatic phase endpoint is reliable for hazard classification.

In answer, the DS confirmed that there are no indications that mayflies are more sensitive to clothianidin than *Chironomus*. The DS provided the study of Macaulay *et al*. (2019) which investigated the chronic toxicity of several neonicotinoids (among them clothianidin and imidacloprid) to nymphs of the mayfly *Deleatidium spp*., which indicated:

- The 28-day LC_{50} for clothianidin was 0.00136 mg/L for mayfly. This is in the same range as the 28-day acute toxicity value of EC_{50} value of 0.00106 mg/L for *Chironomus*.
- The 28-day LC_{50} for imidacloprid was 0.00028 mg/L, indicating a higher toxicity of imidacloprid compared to clothianidin. The study authors also concluded from the available literature, that mayflies and midges showed similar sensitivity to clothianidin.

With regards to providing further information on acute mysid test actual concentrations and *C. riparius* chronic test substance concentrations in the sediment phase during the course of the experiment, the DS did not reflect.

Assessment and comparison with the classification criteria

Degradation

A ready biodegradation study with clothianidin indicated 1.5% degradation after 28 days, indicating that clothianidin is not readily biodegradable.

Clothianidin is stable in sterile buffer solutions at pH 4, 5 and 7, but degrades at pH 9. However, at relevant temperatures of 20°C the degradation is very slow with calculated half-life of 1401 days.

Under photolysis in pure water under the experimental conditions used, clothianidin degraded rapidly with an experimental half-life of 3.3 hours. The experimental half-life corresponds to a calculated environmental half-life of < 0.6 day under midsummer solar light conditions at Phoenix / USA. However, assessing environmental half-life by means of an arithmetical model (with additional parameters), besides the laboratory data, half-lives resulted in up to 23.4 days for the 50th degree of latitude. As well with another arithmetic model, which takes cloudiness in central Europe as an additional parameter into consideration, estimated half-life was over 1 year (especially in autumn and winter).

In water/sediment systems, primary degradation of clothianidin in the water phase and in the entire systems was slow. For systems I and II, first order DT_{50} values of 30.8 and 49.8 days at 20°C (58.4 and 91.0 days at 12°C), respectively, were determined in the water phase and 48.0 and 64.8 days (94.4 and 122.9 days at 12°C) for the entire systems. Max mineralisation of 3.2% (100 d) was at system I and 4.4% at system II.

Overall, due to the results summarised above, RAC agrees with the assessment of the DS that clothianidin is not ultimately degraded to > 70% within 28 days (equivalent to a half-life < 16 days), or rapidly degraded via primary degradation routes to non-classifiable products. Consequently, RAC agrees that clothianidin should be considered as not rapidly degradable under the CLP regulation.

Aquatic Bioaccumulation

No measured data on bioaccumulation (BCF_{fish}) are available for clothianidin. However the derived Log K_{OW} value of 0.7 is well below the CLP trigger value for indication of bioaccumulation (Log K_{OW} < 4). In addition, the calculated BCF_{fish} of 0.78 was performed using standard equations given in the Guidance of BPR. Although according to the EU Guidance (2015) the linear relationship for estimating the bioconcentration factor is not applicable to clothianidin as derived Log K_{OW} of 0.7 is below the QSAR validity range (2 < Log K_{OW} < 6), still calculated BCF_{fish} of 0.78

could be used as supportive information as it describes the order of magnitude in which the BCF is expected.

Therefore, based on the derived Log K_{OW} that is well below 4 and a calculated BCF_{fish} well below 500 used as supportive information, RAC agrees with the DS that clothianidin has a low potential for bioaccumulation according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. RAC agrees that based on provided data in the CLH dossier the most acutely and chronically sensitive trophic group is aquatic insects. RAC recognises that based on comments and answers in the RCOM there are no indications that mayflies will be more sensitive to clothianidin than *Chironomus* according to the results from study of Macaulay *et al*. (2019). However, RAC notes that this particular study was not part of the CLH dossier and has not been evaluated by RAC. Furthermore, RAC also notes that although the salt-water mysid (*A. bahia*) study (Drottar *et al*., 2000a) summary has been included in the CLH report, the robust study summary was not available to the RAC.

Regarding the 48-hour static toxicity test without sediment using larvae of the midge *C. riparius*, RAC acknowledges that nominal test concentrations of clothianidin were 0.007, 0.013, 0.025, 0.050 and 0.10 mg/L. The concentration ranges selected for the definite tests were based on range-finding tests. The range-finding tests were not reported, although the results of the range-finding tests were consistent with those of the definite tests. After 24 and 48 hours, the numbers of immobilised *C. riparius* were recorded. Individual animals were considered immobile if they did not move during a 15 second period of observation. Analysis of test substance concentrations was performed at the start of each of the definite tests. The pH, temperature and concentration of dissolved oxygen were determined in the control and test treatments at the start and end of the exposure period. Total hardness and alkalinity of the control medium was determined at the start and end of the exposure period. The 24-hour and 48-hour EC_{50} values (with 95% confidence limits) were determined by Probit analysis of the data. The highest test concentration causing no immobility and the lowest test concentration causing 100% immobility was based on observation rather than on calculation. The measured concentrations of the test substances were 0.0068, 0.0115, 0.0211, 0.0412 and 0.0816 mg/L which was close to the nominal concentrations. Analytical results indicate that measured concentrations were within 20% of nominal so the toxicity of clothianidin is based on the nominal exposure concentrations throughout the test. Based on immobilisation, a 24-hour EC_{50} of 0.072 mg/L (95% confidence limit) and a 48-hour EC_{50} of 0.029 mg/L (95% confidence limit) were calculated, based on nominal concentration. Based on the measured immobilisation, the highest concentrations causing no immobility of < 0.007 mg/L and lowest concentrations causing 100% immobility of 0.050 mg/L were determined based on nominal concentration.

Regarding the 28-day static toxicity test in a water-sediment system RAC acknowledges that the adsorption coefficient based on organic carbon content (K_{OC}) varies between 84 - 345 mL/g (depending on soil type and concentration) with an arithmetic mean value of 160 mL/g. Therefore, the decrease in test substance concentration in the water phase is assumed to be mainly due to degradation rather than adsorption onto the sediment due to the low Log K_{OW} and adsorption coefficient. Hence, clothianidin will not partition to sediment to a great extent indicating that the test is valid for classification. RAC finds this approach acceptable and a that it provides a suitable worst-case value for chronic toxicity in larvae of the midge *C. riparius*.

RAC also acknowledges that no measurement of clothianidin concentration in the sediment was performed. However adopting a conservative approach, it was assumed that the decrease in the test substance concentration in the water phase was mainly due to degradation rather than due

to adsorption onto the sediment. Nevertheless, that means that the use of nominal concentrations underestimates the toxicity of clothianidin to *Chironomus*. To consider this decline in test substance concentration, the geometric mean of the measured concentrations for the time 0 (0.000472 mg/L), day 7 (0.000322 mg/L) and day 28 (<0.0003 mg/L) for the nominal concentration of 0.00056 mg/L (concentration near to EC_{10}) was calculated. This results in a mean measured concentration of 0.000357 mg/L, corresponding to a recovery of 63%. Applying this recovery to the nominal EC_{10} of 0.00065 mg/L results in a concentration of 0.0004 mg/L.

Consequently, RAC agrees that 48-hour static toxicity test without sediment and 28-day static toxicity test in a water-sediment system with larvae of the midge *C. riparius* are reliable and acceptable for classification. RAC agrees that the lowest acute endpoint for aquatic acute classification is the 48-hour EC_{50} for *C. riparius* of 0.029 mg/L based on nominal concentration for the endpoint mortality/immobilisation. The lowest chronic endpoint for aquatic chronic classification is the 28-day EC_{10} for *C. riparius* of 0.0004 mg/L, based on recalculated mean measured concentrations for the emergence endpoint.

Conclusion on classification

Clothianidin is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that **clothianidin warrants classification as**:

Aquatic Acute 1 based on EC_{50} = 0.029 mg/L for *C. riparius.* As this acute toxicity value falls within the 0.01 < $L(E)C_{50}$ ≤ 0.1 mg/L range, the **acute M-factor is 10**.

Aquatic Chronic 1 based on EC_{10} = 0.0004 mg/L for *C. riparius*. As this chronic toxicity value falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the **chronic M-factor is 100.**

ANNEXES:

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

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

Annex 3 Summary of comments about toxicity to reproduction received during Consultation and compiled in RCOM.

Annex 3: Summary of comments about toxicity to reproduction received during Consultation and compiled in RCOM

Comment number in RCOM	Commenter	Comment	DS reply
2	Company manufacturer from United Kingdom	• Severe effects in developmental study in rabbits are not considered appropriate to justify classification for developmental toxicity.	• Agreed. However, the proposal of classification was based on developmental effects observed in the 2-generation study in rats.
3	Individual from United Kingdom	• Given the size of the body weight reductions in adults and offspring at 2500 ppm in the 2-gen study, 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. • It is plausible that the sperm effects are attributable to the observed reductions in adult body weight, as shown by Chapin et al. (1993). • An analysis of covariance for the day of attainment of vaginal opening versus female pup body weight on PND 21 (BioSTAT, 2018) indicated that the slight delay in vaginal opening at 2500 ppm is attributable to the reduction in female offspring body weight in this group. • An analysis of covariance for the day of attainment of preputial separation (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. • It is unlikely vaginal opening and PPS are specific developmental effect due to lack of any delay in the developmental neurotoxicity study (Argus Research Laboratories, 2000) • Clothianidin is unlikely to be anti-androgenic (or oestrogenic) substance. 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. • Category 2 is not warranted.	• Disagreed with the conclusions. • In the 2-gen study in rats, no clinical signs were observed up to 2500 ppm. Effects were restricted to body weight (gain), despite increased food consumption in P and F1 adults at top dose level of 2500 ppm. • Uterus, ovaries and thymus weight were decreased. • At mid dose, level of 500 ppm preputial separation (PPS) was delayed despite normal post-weaning growth of F1-pups. • Recalculation of PPS with body weight measurement on post-natal day (PND) 21 is considered of questionable relevance. • At 500 ppm sperm motility was also affected, but without statistical significance. • No effects on epididymides weight were observed, although according to the present comment a decrease in epididymides weight should be considered as the most plausible explanation for reduced sperm motility. • Although the effects on sperm did not result in any impairment of fertility in the 2-gen study, it is considered potentially adverse for species with lower sperm production than the rat (e.g. humans). • In the DNT-study (Argus Research Laboratories, 2000) the treatment duration was shorter and the highest dose level was 1750 ppm. • No mechanistic data was provided to assess possible endocrine disrupting properties of clothianidin

			• Increased stillbirths observed in F1 offspring at 2500 ppm was reproducible in F2 offspring and is therefore considered a test substance-related effect.
4	Company manufacturer from United States	• Disagreement with the criteria for classification for sexual function and fertility and development Category 2. • The delay in F1 PPS 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. • The effect on PPS 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 because concentrations greater than 500 mg/kg bw/day is known to cause general toxicity in rodents. • The leading cause for this slight decrease is likely attributable to general toxicity. 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. • Disagree that clothianidin has evidence of a higher incidence of stillbirth and decreased perinatal viability in the absence of excessive parental toxicity. 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.	• Disagreement with the conclusions. • See answer to comment three as regard PPS. • The two-generation study in males did not reveal evidence, that the effects on sperms observed in P and F1-males should be considered as a secondary non-specific consequence of the other toxic effects. • The effects are test substance-related and potentially adverse for species with differences in sperm parameter than the rat (e.g. humans). • Even if the decreased viability index was a result of a calculation error and is now - after recalculation - of no concern, the perinatal deaths are still increased at highest dose level as shown in Table 18 of CLH report.
5	Company manufacturer from United Kingdom	• There are no adverse findings in the rat embryo-foetal developmental toxicity study. • In the developmental rabbit study, the severe maternal toxicity that occurred at these dose levels precluded a meaningful interpretation of these data. • Findings are not considered appropriate to justify classification for developmental toxicity.	• Agreed.
6	Member State	• Agreed with the new classification H361fd Repr. 2.	• Noted.
7	Company manufacturer from United Kingdom	• The effects observed in the multigenerational study can all be attributed to systemic toxicity and are not a direct effect on	• See answers to comments 3 and 4.

		reproduction or an effect on embryo-foetal or postnatal development.	
8	Company manufacturer from United Kingdom	• 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.	• See answers to comments 3 and 4.
9	Individual from United States	• The absence of the intermediate lung lobe and reduced ossification of sternal centres are minor variations occurring in the presence of significant maternal toxicity. • The incidence of these two findings are within the expected normal variation for New Zealand rabbits. • It is likely that the sperm motility findings are indicative of a delay or alteration in sperm development secondary to general systemic toxicity. • No effect of clothianidin treatment on the incidence of stillborn rat pups. The slightly lower value reported for the F1 generation at 2500 ppm is due to miscalculation of the value. • 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.	• See answers to comments 3 and 4. • Historical control data (HCD) are only available for the past three years before the present multi-generation study was conducted, which is considered not sufficient according to current data requirements.
10	An individual reproductive toxicologist from United Kingdom	A small number of findings in the high dose group (2500 ppm) of the 2-generation reproductive toxicity study do not constitute a basis for classification of clothianidin as toxic to reproduction (Repr. 2) for the following reasons: • Chapin *et al.* (1993) 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	• See answers to comments 3 and 4.

		agreement with the findings of Chapin *et al.* (1993). • When compared with the historical control data for this strain of rat, the concurrent control values for number of stillborn pups 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. • 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. • One dam in the 2500 ppm 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%). • References in literature demonstrate that reduced offspring body weight ay result in PPS.	
11	Company manufacturer from United Kingdom	• A number of the findings noted in the RAR list of endpoints (LoEP) 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.	• The LoEP is considered not subject in the current procedure. • See answers to comments 3 and 4.
12	Company manufacturer from United Kingdom	• 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 is not met based	• Refer to answers to comments 3 and 4.