Expert Opinion on the Appropriate Classification of Clothianidin for Reproductive Toxicity

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1 EXECUTIVE SUMMARY

At the request [redacted], the final report on harmonized classification on labelling and the study reports on Clothianidin were reviewed to provide an expert opinion on the appropriate classification of Clothianidin, specifically for reproductive and developmental toxicity. Based on hazard assessment only, the proposed classification for reproductive toxicity is is Repr. 2; H361fd Suspected of damaging fertility. Suspected of damaging the unborn child is proposed.

The classification was based on the results of a complete set of studies (See Section 7 REFERENCES for specific references) of the potential reproductive and developmental toxicity of Clothianidin conducted in the rat (multigenerational and developmental toxicity studies) and rabbit (developmental toxicity studies only).

The potential reproductive and developmental toxicity of Clothianidin has been adequately evaluated in a series of studies including developmental toxicity studies in two species, rats and rabbits and a multigenerational study in the rat. Appropriate dosage-range studies to select doses and exposure levels for the definitive studies were conducted and two additional studies, a developmental immunotoxicity and developmental neurotoxicity study were also conducted. These last two studies while not included in the classification process did provide relevant data in determining reproductive hazard.

The final proposal for harmonization and labelling report noted: "In rats, no findings in offspring relevant for a possible classification for developmental effects were reported. In rabbits, the fetal findings occurred at maternally toxic doses and therefore were not considered to justify classification for reproductive toxicity." Although this opinion report disagrees with the relevance of the fetal findings, we do agree that the findings occurred at maternally toxic levels and therefore would not be considered in the labelling process.

In the multigenerational study, the final report on harmonization and labelling noted several effects including: P and F1 maternal reduced body weight gain during pre-mating, pregnancy and lactation; and reduced thymus weights. In the paternal animals a decrease in sperm (progressive) motility occurred at highest dose level of 2500 ppm. The final report noted "The effects did not influence the fertility of these animals. However, because of differences in sperm parameters between rodents and humans, the effect is considered to be test substance-related and potentially adverse for species with lower sperm production than the rat (e.g. humans). No effects relevant for adverse effects on fertility were reported in repeat-dose studies at dose levels not leading to severe deterioration of animals."

Additional effects noted included "The reproducible increase in stillbirths and decreased perinatal viability observed in both F1 and F2 offspring at 2500 ppm is considered to be test substance related. In the offspring, sexual maturation of males was delayed at a dose level of 500 ppm in the F1 pups, which was not evaluated in the F2 generation. Female sexual maturation was affected at highest dose level, only. The anogenital distance in F2 pups was not affected. Significant delay in sexual maturation (preputial separation) was present despite normal post-weaning growth in F1 pups at 500 ppm."

The final report on harmonization and labelling concluded:

> In summary, classification for developmental toxicity is warranted as Repr. 2, H361fd (H361fd Suspected of damaging fertility. Suspected of damaging the unborn child) based on the findings in the multi-generation study. It is considered not necessary to derive a SCL for Repr. 2, H361fd, because the relevant effect dose levels were within the medium potency group.

This reviewer does not agree that the conclusion of the final report that lead to the proposed classification based on the conclusion that the toxicity caused by Clothianidin either occurred only at maternally toxic doses, were direct effects of general toxicity, or the effect was not due to Clothianidin.

Specifically:

The effect on sperm motility may be indicative of general systemic toxicity and should not be considered a unique effect on reproduction. The effect was only evident at 2500 ppm in the presence of reduced terminal body weights sufficient to cause changes in sperm motility in the absence of any effect on functional fertility.

No statistically significant differences in the percentage of stillborn pups in the F0 or F1 generation occurred. The incidences (%) were increased across exposure groups but the values were reported to be within the historical control range for the Testing Facility. Stillborn pups are expected to occur at a low incidence in rodent litters. Two additional studies with comparable levels of exposure, a developmental immunotoxicity study and a developmental neurotoxicity study each included an evaluation of stillborn pups from litters exposed to similar concentrations of Clothianidin in the diet demonstrated no increase in stillbirths. Considering the data from all three studies in which litters following dietary exposure to Clothianidin were evaluated, the incidence of stillborn pups across all three studies demonstrates the within study and between Testing Facility variation for this parameter.

The viability percentages for surviving pups were in correctly calculated. The apparent reduction of 92.4% was incorrectly calculated in the final report because the calculation included 0% for the litter from a dam that was found dead prior to PND 4. A recalculation of the data set results in a viability index for the 2500 ppm F1 generation exposure group of 95.7%. It should also be noted that the value for the F2 generation was 97.0%. All values when properly calculated were within the historical control data range for the Testing Facility.

A delay in F1 preputial separation at 500 ppm and 2500 ppm. Recalculation of the preputial separation data from the multigenerational study indicated a significant interaction between dose and the PND 21 male pup body. The output of this analysis resulted in no statistically significant difference at the 500 ppm exposure level but the difference between the 2500 ppm exposure group and the control group was still present. The effect at the high dose on the time to preputial separation could be independent of any effect from in utero exposure and an effect on postnatal growth. It is however probable that the effect was due to direct toxicity caused by the high

exposure that occurred in the 2500 ppm group pups when they started to consume the test diets and being exposed to dosages on a mg/kg of body weight basis as high as 600 mg/kg/day of the Clothianidin. These high dose levels of Clothianidin are known to cause general toxicity.

A delay in F1 generation vaginal patency at 2500 ppm. This delay appeared to correlate with a reduction with body weight on the day of vaginal patency. Analysis of the data using a covariant of the PND 21 body weight resulted in the delay in vaginal opening at the 2500 ppm exposure level no longer being statistically significant.

Decreases in birth weight and postnatal body weight gain at 2500 ppm in the F1 and F2 pups. The 2500 ppm exposure was clearly toxic to the dams and the actual dose of Clothianidin received by the F1 generation pups prior to weaning was probably almost double that noted for the adult females and may have been as high as 600 mg/kg/day. This high dose may have resulted in direct systemic toxicity including delays in maturation as previously noted and in delays in growth. The dose of 500 mg/kg in the dosage-range developmental toxicity stud in rats did produce significant general toxicity.

Decreased thymus weights in the offspring at ≥500 ppm in males and at 2500 ppm in females. The ratio of the thymus weight to the final body weights were not statistically significantly different from control values. There were no dose-response relationships were evident in male or female pups of either generation. Noting again that the pups start consuming the feed on or about PND 14 and that the organ weights for the offspring were assessed in PND 21 pups, it is highly likely the thymus weights reflect direct systemic toxicity.

Based on the review by this expert, it is clear that no classification for reproductive toxicity for this chemical is appropriate, and the proposed classification Repr. 2; H361d Suspected of damaging the unborn child is not warranted.

2 STUDIES AND DOCUMENTS REVIEWED

Developmental Toxicity Studies

A series of dosage-range and full developmental toxicity studies were conducted in the rat and rabbit. Doses of Clothianidin as high as 1000 mg/kg/day were tested in the dosage-range study in rats and 500 mg/kg/day in the rabbit.

[redacted] Study 1120-001P. Study Title: Oral (Gavage) Dosage-Range Developmental Toxicity Study of TI-435 in Rats. [redacted] November 11, 1999.

[redacted] Study 1120-001. THT-061. Study title: Oral (Stomach Tube) Developmental Toxicity Study of TI-435 in Rats. [redacted] April 14, 1998

[redacted] Study 1120-002P. Study Title: Oral (Gavage) Dosage-Range Developmental Toxicity Study of TI-435 in Rabbits. [redacted] November 11, 1999.

Table 1: Teratogenicity range-finding study of clothianidin in rabbits
(Source: Table B.6.6-10; York, 1999b.)

Dose (mg/kg bw/d)	**0**	**62.5**	**125**	**250**	**500**
MATERNAL DATA					
Mortality found dead sacrificed moribund	0 0	0 1 d29	5 d16,18,19,24 1 d21	5 d13,14,15,16,17 0	4 d14,16 1 d15
Clinical signs		yes	yes	yes	yes
Feed consumption		-	↓	↓	↓
Body weight d13 d29		- -	↓10 % n.a.	↓17% n.a.	↓13% n.a.
Body weight gain		-	-	-	-
Gravid uterine weight		↓26%[a]	n.a.	n.a.	n.a.
CAESARIAN SECTION DATA					
Number of pregnant ♀ Aborted/premature litters Surviving litters	5 0 5	5 1 4	5 1 0	5 0 0	5 0 0
Corpora lutea	11.2	11.0	-	-	-
Implantations	9.8	7.0	-	-	-
Resorptions early/late	0.2 / 0.2	0.0 / 0.0	-	-	-
live foetuses	9.6	7.0	-	-	-
live ♂ foetuses (%)	59.7	42.0	-	-	-
foetal weight (g) ♂/♀	36.97 / 36.37	40.66 / 38.25	-	-	-
foetal external anomalies	-	-	-	-	-

Maternal data are calculated for the gestational period d6-28.
a. excludes decedents, premature or abortive deliveries

[redacted] Study 1120-002. THT-059. Study title: Oral (Gavage) Developmental Toxicity Study of TI-435 in Rabbits. [redacted] April 16, 1998

Table 2: Teratogenicity main study of Clothianidin in rabbits (maternal data)
(Source: Table 22; CLH July, 2020.)

Dose (mg/kg bw/d)	**0**	**10**	**25**	**75**	**100**
N° rabbits examined	23	23	23	23	23
Mortality found dead sacrificed moribund %	 0 0 0	 0 0 0	 0 0 0	 2 d25,27 0 8.7	 2 d17,20 1 d19 13.0
Clinical signs[a]					
localised alopecia	4(45)	2(18)	3(18)	3(41)	8(61)
scant faeces	1(1)	3(4)	4(10)	10(40) **	16(79) **
no faeces				1 (1)	11(22) **
soft/liquid faeces					1 (5)
orange urine			2(4)	9(38)**	9(64) **
red substance in pan			1(1)	0	4(8) **
decreased motor activity loss righting reflex					1 (1)

Feed consumption (g/d)					
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

Maternal data are calculated for the gestational period d6-28; Caesarian section data are expressed in mean nbr of foetuses per litter; [a]: litter incidence, and (..) =daily incidence, on maximal possible incidence during observation period [i.e. maximally 23 rabbits*29d=667]; [b]: corrected body weight=gestation bw minus gravid uterine weight; n.d.: dose level tested but not displayed in CLH report
Statistical significance: *p<0.05, **p<0.01, Continuous data: Dunnett's test (homogeneous variances); Kruskall-Wallis/Dunn's test (nonhomogeneous variances, ≤ 75% ties) or Fisher's Exact test (>75% ties). Discontinuous data: Kruskall-Wallis procedure.

Table 3: Teratogenicity main study of Clothianidin in rabbits (caeserean section)
(Source: Table 23; CLH, July 2020.)

Dose (mg/kg bw/d)	**0**	**10**	**25**	**75**	**100**
Number of surviving pregnant ♀	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 ♂ foetuses (%)	51.8	47.2	50.2	48.8	39.6
foetal weight ♂/♀ (g)	44.2 / 43.0	43.2 / 42.2	40.7 / 40.0	40.7 / 40.2	37.7 / 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

Statistical significance: *p<0.05, **p<0.01, Continuous data: Dunnett's test (homogeneous variances); Kruskall-Wallis/Dunn's test (nonhomogeneous variances, ≤ 75% ties) or Fisher's Exact test (>75% ties). Discontinuous data: Kruskall-Wallis procedure.

Table 4: Summary of foetal alterations in rabbits
(Source: Table 24; CLH, July 2020.)

			Dose (mg/kg bw/d)				
location	parameter	l/f	**0**	**10**	**25**	**75**	**100**
external	medially rotated hindlimbs [m]	l f	0	0	1 (4.5) 3 (1.7**)	0	0
visceral	intermediate lung lobe absent [v]	l f	0 0	0 0	0 0	3 (17.6)** 3 (2.2)	5 (45.5)** 8 (9.4)**
	small kidney [m]	l f	0 0	0 0	0 0	0 0	1 (9.1) 3 (3.5)**
skeletal	fused caudal vertebrae m	l f	0 0	0 0	1 (4.5) 2 (1.1)	0 0	2 (18.2)** 2 (2.4)**
	incompletely ossified sternal centra [v]	l f	0 0	0 0	0 0	0 0	2 (18.2)** 2 (2.4)
	absent hindpaw phalanges [m]	l f	0 0	0 0	0 0	0 0	2 (18.2)** 2 (2.4)**
Ossification sites: number per foetus/litter							
sternal centers			3.99 / 0.03	3.91 / 0.14	3.94 / 0.13	3.83 / 0.2**	3.76 / 0.25**
hindlimbs phalanx			12.00 / 0.00	12.00 / 0.00	12.00 / 0.00	12.00 / 0.00	11.78 / 0.63**

[m]: malformation; [v]: variation; values referring to number of foetuses; litter (l) or foetal (f) incidence; (..): incidence in %;
Statistical significance: *p<0.05, **p<0.01 (see above).
Historical control incidences:
intermediate lung lobe absent: (i) litter inc.: n=0-5, 0-27.8% (m=10.1%); (ii) foetal inc.: n=0-7, 0-4.4% (m=1.54%)
small kidney: no reported data
fused caudal vertebrae: (i) litter inc. n=0-1, 0-5.9% (m=1.4%); (ii) foetal inc.: n=0-1, 0-1.9% (m=0.21%)
incompletely/not ossified sternebrae: (i) litter inc. n=0-10.5, 0-5.9% (m=1.8%); (ii) foetal inc.: n=0-2, 0-1.2% (m=0.21%)
absent hindpaw phalanx: (i) litter inc. n=0-1, 0-5.9% (m=0.2%); (ii) foetal inc.: n=0-1, 0-0.6% (m=0.02%)
number of sternal center ossification sites: 3.81-3.97 (m=3.91)
number of hindlimb phalanx ossification sites: 11.99-12.00 (m=12)

Opinion of this Expert Reviewer on the Developmental Toxicity of Clothianidin

Two developmental toxicity studies each compliant with OECD guidelines, were conducted. Dosage-range studies, in limited number of animals, were conducted prior to each of these studies to ensure that appropriate doses of Clothianidin that would allow a sufficient number of litters to be produced were used in the full studies. All four studies were considered acceptable for review. There was general agreement between the opinion noted in the renewal assessment and the submitted reports on the maternal toxicity that was observed. High doses of 125 mg/kg/day in the rat and 100 mg/kg/day in the rabbit were selected for the full developmental toxicity studies. The higher dose levels used in the dosage-range study caused excessive maternal toxicity for evaluation in the full studies, but they were also generally also developmentally toxic doses. All three manifestations of developmental toxicity that can be evaluated in this study design occurred; embryo/fetal death, fetal anomalies and growth retardation.

In the full developmental toxicity study in rats, the renewal assessment and the conclusion in the final study report were again in general agreement. Both found no developmental toxicity up to the highest dose tested even though small reduction (5%) in fetal weight (not statistically significant) appears to have occurred in the 125 mg/kg/day dose group. This small change is

noted here only because the general trend of developmental toxicity being observed only in the presences of maternal toxicity did occur in the rats.

In the full developmental toxicity study in rabbits, the renewal assessment based the developmental NOAEL at 25 mg/kg/day verses 75 mg/kg/day in the final report, based on a different interpretation of the absence of the intermediate lobe of the lung and reduced ossification of sternal centers at 75 mg/kg/day.

> Based on individual data of the study report a reduced number of lung lobes in 3 foetuses out of 3 litters at 75 mg/kg bw/ and 8 foetuses (9 %) out of 5 litters (45.5 %) at 100 mg/kg bw/d were observed. The missing lung lobes appear to indicate a dose-related effect on lung branching morphogenesis at high, maternally toxic doses. Furthermore, ossification was delayed at ≥75 mg/kg bw/d (sternal centers), but within HCD and at the top dose (hindlimb phalanges). At the top dose of 100 mg/kg bw/d some malformations were slightly, but significantly increased on litter incidence base like small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hindpaw phalanges.

This reviewer believes that the apparent increase in these two common variations in this strain of rabbit were incorrectly associated as an effect on the fetuses at the severely maternally toxic doses of 75 mg/kg/day and 100 mg/kg/day. The incidence of incompletely ossified sternal centra was already noted in the report as within the historical control range for the Testing Facility. This finding is neither a malformation nor variation. It is a developmental delay common in this strain of rabbit.

The incidence of missing intermediate lobe of the lung appeared to be occurring in a dosage-related pattern and the renewal assessment report incorrectly noted that there may have been an effect on lung branching. However, if an effect on lung branching had been occurring, it would have made more sense embryologically that all lobes of the lung would have been affected and the lungs would have generally been small. In addition, this reviewer has provided additional historical control data from the same Testing Facility (see Appendix B) that demonstrates the high variability in the incidence of this genetic variation. Based on this additional data it should be clearer that an apparent dose related response probably occurred by chance and such a distribution across dose groups would not be unexpected. See Appendix C for publication regarding the incidence of missing lung lobe in rabbits (Christian et al., 1987).

Therefore, this reviewer agrees with the reported developmental NOAEL of 75 mg/kg/day, and a classification of Category 2 based on the results of these developmental toxicity studies would not be appropriate. The reported criteria for a Category 2 reproduction effect which is "shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects"

Reproductive Toxicity Studies

A dosage-range and full multigenerational study with Clothianidin administered in the diet was conducted. Both studies were considered acceptable for review.

[redacted] Study Number 97-972-LJ [redacted] Report Number 108035. Study Title: A Pilot Reproductive Toxicity Study with TI-435 in the Sprague-Dawley Rat. [redacted] February 15, 2000.

In the dosage-range study doses of 0, 50, 100, 500 and 1000 ppm were evaluated. The reported mg/kg doses ranged from 2.9 to 58.9 mg/kg for male rats and 3.4 to 68.6 mg/kg for females. Only minimal toxicity occurred in the adults; initial decreases in food consumption and body weight during week one of administration. These reductions were less than 10% and may have represented an initial taste aversion. No reproductive effects or litter abnormalities occurred.

[redacted] Study Number 98-672-PF [redacted] 109282. Study Title: A Two Generation Reproductive Toxicity Study with TI-435 in the Sprague-Dawley Rat. [redacted] March 27, 2000.

[redacted] Study Number 98-672-PF [redacted] Report Number 109282-1. Supplemental Submission to [redacted] Report No. 109282. Study Title: A Two Generation Reproductive Toxicity Study with TI-435 in the Sprague-Dawley Rat. [redacted] Original Report: March 27, 2000. Addendum: April 6, 2001.

In the full multigenerational study, groups of 30 rats/sex of SD strain were exposed to 0, 150, 500 and 2500 ppm of Clothianidin in the diet. Parameters evaluated in the two generation following at 16 to 24 weeks of exposure included clinical observations, body weight, body weight gain, food consumption, estrous cycle, anogenital distance, sexual maturation, fertility, gross necropsy, sperm morphology, concentration and motility, ovarian follicle histopathology, reproductive organ weights, reproductive organ histopathology.

Exposure in the male rats ranged from 9.8 mg/kg/day to 195.7 mg/kg/day and in the female rats from 9.7 mg/kg/day to 318.3 mg/kg/day (note: the values reported for the second week of lactation were excluded from consideration as the pups were eating the test diets by then).

The actual dose received by the F1 generation pups prior to weaning was probably almost double that noted for the adult females and may have been as high as 600 mg/kg/day. This high dose may have many have resulted in direct systemic toxicity including delays in maturation. The dose of 500 mg/kg in the dosage-range developmental toxicity stud in rats did produce significant general toxicity.

Statistically significant reductions in female body weights; 8 – 15% during the premating period for the P-generation and 16-20% for the F1-generation occurred at 2500 ppm.

Reproductive endpoints including estrous cycling and litter parameters were unaffected by Clothianidin treatment for both generations.

Illustrative of the in utero and direct exposure Clothianidin to the F1 generation, F1 pup weights at 2500 ppm at birth were significantly reduced compared to control. By PND 21, F1 pups at 2500 ppm weighed 74% of the control pups. A similar pattern was observed in the F2-generation, pups. At 2500 ppm F2 pup weights were lower than control weights at birth; and continued to decline especially after pups began eating diet during lactation. On PND 21 pup weights were 79.5% of controls.

Motile sperm (%) was significantly reduced at 2500 ppm in the F1 males. In both the P and F1 males progressively motile sperm (%) was significantly reduced. Overall sperm count and morphology were not affected in either generation.

Delayed preputial separation occurred in the F1 males at 500 and 2500 ppm with no difference in the body weight when sexual maturation was achieved. Vaginal opening was significantly delayed at 2500 ppm in the F1 females.

The NOAEL for paternal toxicity was reported as 150 ppm based on reduced in body weights 500 and 2500 ppm during lactation. This very conservative estimate by the study authors was not reflected in the regulatory review as these reviewers considered the NOAEL for paternal toxicity to be 500 ppm considering the reduced body weight gains and reduced thymus weights in both sexes at 2500 ppm.

The NOAEL for reproductive toxicity was considered 2500 ppm by the study investigators based on an absence of reproductive effects. Regulators determined the reproductive NOAEL to be 500 ppm based on effects at 2500 ppm including increased stillbirths, reduced early postnatal viability, and reduced sperm motility.

The NOAEL for growth and viability of the offspring was 150 based on reduced pup weights and subsequent effects on preputial separation. The RMS agreed with this offspring NOAEL due to delayed sexual maturation at ≥500 ppm; they also noted increased perinatal deaths at 2500 ppm.

Table 5: Two generation reproduction toxicity in rats: body and organ weight data
(Source: Table 15; CLH, July 2020).

Dose (ppm)	0		150		500		2500	
ADULTS	P	F1	P	F1	P	F1	P	F1
BW♂	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	↓8-10%**	↓16-20%**
BW ♀ (d7-70) [pm] (d0,6,13,20)[ge] (d0,4,7,14,21)[la] (d14) [la]	n.d.	n.d.	n.d	n.d.	n.d. ↓6.5%*	n.d.	 ↓9-15%** ↓12-14%** ↓11-18%**	 ↓16-19%** ↓13-16%** ↓13-15%**
BW gain ♀ (d0,6,13,20) [ge]						n.d.	 ↓17%**	 ↓8%

Feed consumption ♂	n.d.	n.d.	n.d.	n.d.	↓4%*wk1 pm	-	↓18% **wk1 pm ↑8% *wk3;**wk4,5,8 pm	↑16%**wk1-10 pm
♀	n.d.	n.d.	n.d.	n.d.	↑7.6%*wk10 pm	-	↓18% **wk1 pm ↑16% **wk8-10pm ↑11%* d6-13ge ↑13%* d14-21la	↑21%*wk1,5-6 pm **wk2-4,7-10 pm ↑11%* d6-13 ge ↑13%* d7-21 la
Organ weight (a:absolute, r:relative)								
Thymus ♂ a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓16%* ↓8%	↓29%* ↓13%*
♀ a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓35%* ↓32%*	↓41%* ↓32%*
Ovary a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓19%* ↓16%*	↓11% -
Uterus a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓25%* ↓20%*	↓21% ↓10%

n.d. Dose level tested but not displayed in CLH report, pm premating; ge gestation; la lactation
Statistically significant modifications *p<0.05,**p<0.01 (parametric data: Anova+Dunnett's, nonparametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight).

Table 6: Two generation reproduction toxicity: gross pathology and histopathology
(Source: Table 16; CLH, July 2020.)

Dose (ppm)	**0**		**150**		**500**		**2500**	
ADULTS	P	F1	P	F1	P	F1	P	F1
Gross pathology								
Preputial separation (day n)	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.		133.7
epididymis	146.1	149.5					141.6	103.8
testis	129.9	105.7					129.5	
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**
Estrus 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
Estrus stage (sacrifice) (n)								
diestrus	18	19	19	18	25	16	24	25
proestrus	2	0	0	3	1	0	1	1
estrus	9	11	10	9	3	14	2	4
Ovarial follicle count (mean /ovary)								
'non-anthral' follicles	12.17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12.22
'anthral' 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

*p<0.05 **p<0.01

n.a.: dose level not tested; n.d.: dose level tested but not displayed in CLH report; Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight, histopathology), nonparametric dichotomous: χ+Fisher Exact Test/Bonferroni adjustment.

Table 7: Two generation reproduction toxicity in rats: pup data
(Source: Table 17; CLH, July 2020.)

Dose (ppm)	0		150		500		2500	
	F1	F2	F1	F2	F1	F2	F1	F2
Litter data								
No. observed (d21)	24	23	29	25	28	20	29	28
No. pups missing[a]	2	1	2	4	1	4	4	6
No. stillborn pups[a]								
early[b]	0	1	3	3	3	5	6	4
late[c]	1	3	0	1	2	0	1	2
total[d]	1	4	3	4	4	5	6	5
No. viable pups[a]								
d0	14	14	14	14	14	13	13	12
d4	13	13	14	13	14	13	12	12
Birth index[d]	.898	. 918	.905	.934	.945	.874	.940	.878
Live birth index[e]	1.00	.998	.993	.991	.984	.974	.979	.965
Viability index[f]	.992	.980	.994	.981	.987	.988	.924	.970
Lactation Index[g]	.995	.995	.996	.985	.978	.974	.960	.960
Pup weight at birth [g]	6.5/6.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BW♂+♀								
d0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓8%*	↓3%
d4							↓16%**	↓9%
d7							↓18%**	↓13%**
d14							↓22%**	↓16%**
d21							↓26%**	↓21%**
BW gain F1 ♂+♀ day 4-21cumulative bw gain % of control	100 ♂	100♀	99♂	96♀	94♂	93♀	71♂	71♀
BW gain F2 ♂+♀ day 4-21 cumulative bw % of control	100♂	100♀	100♂	102♀	100♂	101♀	75♂	77♀
Organ Weights								
Thymus weight ♂	n.d.	n.d.	n.d.	n.d.				
absolute					↓13%*	-	↓29%**	↓25%**
relative					↓10%	-	↓5%	↓8%
Thymus weight ♀	n.d.	n.d.	n.d.	n.d.				
absolute					↓10%	-	↓28%**	↓24%**
relative					↓8%	-	↓7%	↓9%
Spleen weight ♂	n.d.	n.d.	n.d.	n.d.				
absolute							↓30%**	↓31%**
relative							↓7%	↓16%**
Spleen weight ♀	n.d.	n.d.	n.d.	n.d.				
absolute							↓35%**	↓30%**
relative							↓16%**	↓17%**

n.a.: dose level not tested; n.d.: dose level tested but not displayed in CLH report; [a] data expressed as litter incidence; Early and late stillborn determined by observation of [b] non-floating, or [c] floating lung;
Indices (data expressed as #pups/litter) defined as [d],pups born/implantation sites, [e] pups liveborn/implantation sites, [f] pups alive (d4)/pups born, [g] pups alive (d21)/pups post-culling (d4).
Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight, histopathology), nonparametric dichotomous: χ+Fisher Exact Test/Bonferroni adjustment.

Table 8: Litter distribution of perinatal deaths
(Source: Table 18; CLH, July 2020.)

Parameter	Number of dead pups in litter	0 ppm		150 ppm		500 ppm		2500 ppm	
		F1	F2	F1	F2	F1	F2	F1	F2
Litters evaluated		24	23	29	25	28	21	29	28
Total litter loss at birth		0	0	0	0	0	1	0	0
Litters with any live pups		24	23	29	25	28	20	29	28
Litters with stillborn pups	0	24	22	26	22	25	15	23	24
	1	-	1	3	3	2	4	3	-
	2	-	-	-	-	-	-	3	-
	3	-	-	-	-	-	-	-	3
	4	-	-	-	-	1	1	-	1
Litters with early postnatal deaths (postnatal day 0-4)	0	21	18	25	20	25	17	23	19
	1	3	3	-	3	2	3	4	8
	2	-	2	1	1	1	-	-	1
	3	-	-	-	1	-	-	-	-
	12	-	-	-	-	-	-	1	-

Table 9: F1 male sexual maturation
Source: Table 19; CLH, July 2020.

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

Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight, histopathology), nonparametric dichotomous: χ+Fisher Exact Test/Bonferroni adjustment

Comparison with criteria

Adverse effects on sexual function and fertility:

Table 10: Criteria concerning classification for adverse effects on sexual function and fertility
(Source: Table 25; CLH, July 2020.)

Toxicological result	CLP Criteria
	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
2-generation reproduction study in Sprague-Dawley rats (Anonymous (2), 2000), **Delayed sexual maturation in males despite normal (post-weaning) growth.** **Delayed vaginal opening associated with growth retardation.** Decreased sperm motility (and not significant morphology effects) at the highest dose (2500 ppm). No adverse effects on conception rate and fertility indices.	Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Adverse effects on development:

Table 11: Criteria concerning classification for adverse effects development (Source: Table 26; CLH, July 2020.)	
Toxicological result	**CLP Criteria**
	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
2-generation reproduction study in Sprague-Dawley rats (Anonymous (2); 2000), Higher incidence of stillbirth, decreased perinatal viability in the absence of excessive parental toxicity.	Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Preliminary teratogenicity study, rat (Anonymous (16), 1998a): Limited evaluation of fetuses **Teratogenicity study, rat** (Anonymous (19), 1998): No effects on foetuses reported up to highest dose tested (125 mg/kg bw/d) **Preliminary teratogenicity study, rabbit** (Anonymous (17), 1998): Limited evaluation of foetuses; loss of all litters at doses ≥ 125 mg/kg bw/d **Teratogenicity study, rabbit** (Anonymous (18), 1999b): 75 and 100 mg/kg bw/d: intermediate lung lobe absent ↑	**No classification**

Conclusions on classification and labelling

In summary, classification for developmental toxicity is warranted as Repr. 2, H361fd (H361fd Suspected of damaging fertility. Suspected of damaging the unborn child) based on the findings in the multi-generation study. It is considered not necessary to

derive a SCL for Repr. 2, H361fd, because the relevant effect dose levels were within the medium potency group

3. OPINION ON CONCLUSIONS ON CLASSIFICATION AND LABELLING

Regulatory review determined that no classification based on the results of the developmental toxicity studies was warranted. Classification was considered warranted based on findings in the multigenerational study. Each of the findings will be discussed below.

Potential Effects in Males (Sperm Motility and Morphology)

Sperm count and morphology were unaffected by Clothianidin treatment in both the F0 and F1 generations. There were also no effects on fertility in either generation or on the histopathology of the testes. Although there was no effect on functional fertility and the number of sperm and structure of the sperm were apparently normal, in the 2500 ppm exposure group, the percentage (%) of motile sperm and % of progressive sperm were slightly reduced and/or significantly reduced ($p \leq 0.01$) F0 and P1 generations. As the rodent is known for its excess capacity in sperm compared to humans, it can be speculated that the reductions in motile and progressive sperm may have an effect on functional fertility in humans.

In rodents, the reductions in motility and the related parameter of progressive motility occurred only at the highest dose of 2500 ppm. This exposure significantly reduced ($p \leq 0.05$) the terminal body weight in the F0 generation males by 9% and in the F1 generation by 19%. It has been shown in the literature (Chapin, 1997) that reductions in body weight of 90% or greater relative to control can affect sperm motility in the absence of any effect on other sperm parameters. Therefore, the effect on sperm motility may be indicative of general systemic toxicity and should not be considered a unique effect on reproduction. No effect on these parameters occurred at the low or mid exposure levels where not systemic toxicity was occurring.

Increased Stillborn Pups and Decreased Pup Viability

The authors of the multigenerational study noted no statistically significant differences in the percent of stillborn pups in the F0 or F1 generations. The incidences (%) were increased across exposure groups the values were reported to be within the historical control range for the Testing Facility. Stillborn pups are expected to occur at a low incidence in rodent litters.

The final proposal for classification and labelling considered the increase in percent stillborn pups to be an effect as increases were observed in both the F1 and F2 generations.

However, two additional studies, a developmental immunotoxicity study and a developmental neurotoxicity study each which included an evaluation of stillborn pups from litters exposed to similar concentrations of Clothianidin in the diet are available to compare to the multigenerational study.

In the developmental immunotoxicity study ([redacted] 2008), Clothianidin was administered in the diet at concentrations 0, 150, 500 or 2000 ppm from GD 6 through weaning and until sacrifice of the F1 generation pups for a functional assessment of the immune system. Illustrative of the normal variation in the incidence of stillborn pups both within a study and from Testing Facility to Testing Facility 0, 0, 1 and 3 stillborn pups were pups observed across the 0 150, 500 and 2000 ppm exposure groups. Although a dose response appeared to be present for the mid and high dose, no statistically significant effect on the incidence of stillborn pups was observed and the incidence was within historical control range for the Testing Facility. The exposure levels were at or near the exposure in the mid and high dose for the multigenerational study.

In the developmental neurotoxicity (DNT) study of Clothianidin [redacted] 2000), dietary concentrations of 0, 150, 500 and 1750 ppm were administered to groups of pregnant rats from GD 0 through to PND 22. A total of 1, 1, 6 and 3 stillborn pups were observed in the four respective exposure groups. Again the normal variation in the incidence for stillborn pups was observed.

Considering the data from all three studies in which litters following dietary exposure to Clothianidin were evaluated, the incidence of stillborn pups across all three studies demonstrates the within study and between Testing Facility variation for this parameter. Based on these data, we agree with the authors of the two-generation reproductive study in rats that there was no effect of Clothianidin treatment on stillborn pup incidences. Therefore, stillborn pups should not be considered to have been affected and should not be considered in the classification of Clothianidin.

The final proposal for harmonized classification and labelling also noted that the pup viability appeared to be affected at the 2500 ppm exposure level and that this finding correlated with the increase in stillborn pups. The apparent reduction of 92.4% was incorrectly calculated in the final report because the calculation included 0% for the litter from a dam (PF3108) that was found dead prior to postnatal day (PND) 4. A recalculation of the data set without PF3108 results in a viability index for the 2500 ppm F1 generation exposure group of 95.7%. It should also be noted that the value for the F2 generation was 97.0%. All values when properly calculated were within the historical control data range for the Testing Facility.

With the recalculation of the pup viability index this parameter should not be included in assessment of classification and the lack of an effect in viability further supports the lack of any effect on pups, supporting the exclusion of stillbirths from the assessment of classification.

Delayed Preputial Separation

The final proposal for harmonization and labelling noted a delay in F1 preputial separation at 500 ppm and 2500 ppm. Based on an analysis of variance, there was a statistically significant ($p \leq 0.01$) exposure dependent delay in the day of preputial separation but interestingly no statistical difference in the body weight on the day of preputial separation. At the time the study was conducted and reported, it was not common practice to use the covariant of the body weight on PND 21 in the analysis of variance statistic. It has been demonstrated (Melching-Kollmuss et

al., 2014) that pup body weight reductions due to maternal toxicity during gestation can lead to significant delays in preputial separation. Melching-Kollmuss et al. also identified that the pup body weights on PND 21 should be considered as a covariate in the statistical analysis.

Recalculation of the preputial separation data from the multigenerational study indicated a significant interaction between dose and the PND 21 male pup body weight. The output of this analysis resulted in no statistically significant difference at the 500 ppm exposure level but the difference between the 2500 ppm exposure group and the control group was still present. The effect at the high dose on the time to preputial separation could be independent of the any effect from in utero exposure and an effect on postnatal growth. It is however probable that the effect was due to direct toxicity caused by the high exposure that occurred in the 2500 ppm group pups when they started to consume the test diets and being exposed to dosages on a mg/kg of body weight basis as high as 600 mg/kg/day of the Clothianidin. These high dose levels of Clothianidin are known to cause general toxicity. Therefore, the effect on preputial separation at 2500 ppm should be considered a toxic effect but it should not be considered a reproductive effect for purposes of classification of labelling.

Delayed Vaginal Opening at Highest Dose

The final proposal for harmonization and labelling noted a delay in F1 generation vaginal patency at 2500 ppm. This delay appeared to correlate with a reduction with body weight on the day of vaginal patency. Analysis of the data using a covariant of the PND 21 body weight resulted in the delay in vaginal opening at the 2500 ppm exposure level no longer being statistically significant. Therefore, this difference from control should not be considered in harmonization process for labelling as a reproductive toxicant.

Reduced Birth Weight and Postnatal Body Weight Gain

The final proposal for harmonization and labelling noted decreases in birth weight and postnatal body weight gain at 2500 ppm in the F1 and F2 pups. The 2500 ppm exposure was clearly toxic to the dams and the actual dose of Clothianidin received by the F1 generation pups prior to weaning was probably almost double that noted for the adult females and may have been as high as 600 mg/kg/day. This high dose may have resulted in direct systemic toxicity including delays in maturation as previously noted and in delays in growth. The dose of 500 mg/kg in the dosage-range developmental toxicity stud in rats did produce significant general toxicity. Therefore this toxicity that occurred at maternally toxic doses and was exacerbated by direct exposure should not be considered for labelling as a reproductive toxicant.

Reduced Thymus Weights

The final proposal for harmonization and labelling noted decreased thymus weights in the offspring at ≥500 ppm in males and at 2500 ppm in females.

The ratio of the thymus weight to the final body weights were not statistically significantly different from control values. There were no dose-response relationships were evident in male or female pups of either generation.

Noting again that the pups start consuming the feed on or about PND 14 and that the organ weights for the offspring were assessed in PND 21 pups, it is highly likely the thymus weights reflect direct systemic toxicity. Therefore, the reduction in thymus should not be considered for the classification of Clothianidin as a reproductive toxicant.

4. CONCLUSIONS

The no-observable- adverse -effect levels (NOAELs) from the final proposal for harmonization and labelling report are summarized below.

Table 12: Summary table of relevant reproductive toxicity studies
(Source: Table 14; CLH, July 2020.)

Type of study doses (reference)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects	
Pilot Reproductive Toxicity Study with TI-435 in the Sprague-Dawley Rat (Anonymous (1), 2000) 0, 50, 100, 500, 1000 ppm	>1000 ppm (59-69 mg/kg bw/d)	-	Parents, offspring, reproduction: No adverse effects	
2-generation reproduction study in Sprague-Dawley rats, 30M, 30F, route: oral/diet (Anonymous (2), 2000), 0, 150, 500, 2500 ppm (approx. (♂): 0, 10; 31; 163 mg/kg bw/d (♀): 0, 10; 31; 161 mg/kg bw/d OECD TG 416 Key study	500 ppm (31 mg/kg bw/d)	2500 ppm (163 mg/kg bw/d)	Parents	Reduced body weight gain; thymus wt, decreased sperm motility (and not significant morphology effects)
	150 ppm (10 mg/kg bw/d)	500 ppm (31 mg/kg bw/d)	Offspring	Delayed preputial separation without decreased body weight At 2500 ppm additionally, decreased bw (gain), thymus & spleen weights, delayed vaginal opening
	500 ppm (31 mg/kg bw/d)	2500 ppm (163 mg/kg bw/d)	Reproduction	No adverse effects on conception rate and fertility indices. Increased number of stillborn pups, decreased perinatal survival, decreased sperm motility (and not significant morphology effects) at 2500 ppm.
Note: [redacted] batch no. 30037120 (purity 95.2-96.0 % w/w) was used in both studies				
Developmental toxicity dose range finding study in Sprague-Dawley rats (Anonymous (16), 1998a), 0, 125, 250, 500, 1000 mg/kg bw/d	Not established	125	Maternal	Mortality at 1000 mg/kg bw/d, clinical signs, reduced body weight and food consumption, reduced spleen weight
	Not established	125	Developmental	Only limited parameters tested, reduced litter size, reduced foetal weight and foetal anomalies at maternal toxicity
Developmental toxicity in Sprague-Dawley rats, Crl:CD [BR] VAF/Plus SD,	10	40	Maternal	Decreased body weight and food consumption
	125	-	Developmental	No adverse effects

25F/group Oral, pregnancy day 6-190, 10, 40, 125 mg/kg bw/d (Anonymous (19), 1998), OECD 414 Key study				
Developmental toxicity dose range finding study in New Zealand White Rabbits (Anonymous (17), 1998), 0, 62.5, 125, 250, 500 mg/kg bw/day	Not established	62.5	Maternal	Mortality, abortions, clinical signs of toxicity, decreased body weight and food consumption
	Not established	-	Developmental	No surviving litters at ≥ 125 mg/kg bw/d
Developmental toxicity in New Zealand White rabbits, Hra:[NZW] SPF 23F Oral, pregnancy day 6-28 (Anonymous (19), 1998), 0, 10, 25, 75, 100 mg/kg bw/d OECD 414 Key study	10	25	Maternal	Clinical signs
	25	75	Developmental	Absence of intermediate lung lobe, decreased ossification sternal centres, increased embryolethality, decreased fetal wt
Note: [redacted] batch no. 30034708 (purity 96.0 % w/w) was used in both dose range finding studies. [redacted] batch 30037120 (purity 95.2% w/w) was used in both main studies.				

The potential reproductive and developmental toxicity of Clothianidin has been adequately evaluated in a series of studies including developmental toxicity studies in two species, rats and rabbits and a multigenerational study in the rat. Appropriate dosage-range studies to select doses and exposure levels for the definitive studies were conducted and two additional studies, a developmental immunotoxicity and developmental neurotoxicity study were also conducted. These last two studies while not included in the classification process did provide relevant data in determining reproductive hazard.

The final proposal for harmonization and labelling report noted:

> In rats, no findings in offspring relevant for a possible classification for developmental effects were reported.
>
> In the teratology study in rabbits no treatment-related differences in reproductive parameters in females, including the mean number of corpora lutea, implantations losses and the numbers of live or dead fetuses were observed. The number of resorptions was increased in top dose and fetal birth weights were reduced.
>
> At 75 and 100 mg/kg bw/d, significantly increased incidences of absent intermediate lung lobes were observed. According to modern nomenclature, this finding is considered

> a malformation. In top dose group, further malformations were seen during skeletal examination (fused caudal vertebrae, absent hind paw phalanges). The reported findings are severe and irreversible.
>
> Yet, maternal toxicity was observed in this rabbit study at doses of 25 mg/kg bw/d and above. At 75 and 100 mg/kg bw/d, two and three does were found dead or in moribund condition, respectively. The occurrence of severe findings in rabbit offspring needs to be balanced against the increased incidences of mortalities and moribund condition seen at the same dose levels. Thus, these findings are not considered to justify classification for reproductive toxicity.

Although this opinion report disagrees with the relevance of the fetal findings, we do agree that the findings occurred at maternally toxic levels and therefore would not be considered in the labelling process.

In the multigenerational study, the final report on harmonization and labelling noted several effects including: P and F1 maternal reduced body weight gain during pre-mating, pregnancy and lactation; and reduced thymus weights. In the paternal animals a decrease in sperm (progressive) motility occurred at highest dose level of 2500 ppm. The final report noted "The effects did not influence the fertility of these animals. However, because of differences in sperm parameters between rodents and humans, the effect is considered to be test substance-related and potentially adverse for species with lower sperm production than the rat (e.g. humans). No effects relevant for adverse effects on fertility were reported in repeat-dose studies at dose levels not leading to severe deterioration of animals."

Additional effects noted included "The reproducible increase in stillbirths and decreased perinatal viability observed in both F1 and F2 offspring at 2500 ppm is considered to be test substance related. In the offspring sexual maturation of males was delayed at a dose level of ≥ 500 ppm in the F1 pups, which was not evaluated in the F2 generation. Female sexual maturation was affected at highest dose level, only. The anogenital distance in F2 pups was not affected. Significant delay in sexual maturation (preputial separation) was present despite normal post-weaning growth in F1 pups at 500 ppm."

The final report on harmonization classification and labelling (CLH) concluded:

> In summary, classification for developmental toxicity is warranted as Repr. 2, H361fd (H361fd Suspected of damaging fertility. Suspected of damaging the unborn child) based on the findings in the multi-generation study. It is considered not necessary to derive a SCL for Repr. 2, H361fd, because the relevant effect dose levels were within the medium potency group.

This reviewer does not agree that the conclusion of the final report that lead to proposed classification based on the conclusion that the toxicity caused by Clothianidin either occurred only at maternally toxic doses, were direct effects of general toxicity or the effect was not due to Clothianidin.

Specifically:

The effect on sperm motility may be indicative of general systemic toxicity and should not be considered a unique effect on reproduction. The effect was only evident at 2500 ppm in the presence of reduced terminal body weights sufficient to cause changes in sperm motility in the absence of any effect on functional fertility.

No statistically significant differences in the percent of stillborn pups in the F0 or F1 generation occurred. The incidences (%) were increased across exposure groups but the values were reported to be within the historical control range for the Testing Facility. Stillborn pups are expected to occur at a low incidence in rodent litters. Two additional studies with comparable levels of exposure, a developmental immunotoxicity study and a developmental neurotoxicity study each included an evaluation of stillborn pups from litters exposed to similar concentrations of Clothianidin in the diet demonstrated no increase in stillbirths. Considering the data from all three studies in which litters following dietary exposure to Clothianidin were evaluated, the incidence of stillborn pups across all three studies was the variation observed within a study at each Testing Facility and within the historical control range for each Testing Facility.

The viability percentages for surviving pups were in correctly calculated. The apparent reduction of 92.4% was incorrectly calculated in the final report because the calculation included 0% for the litter from a dam that was found dead prior to PND 4. A recalculation of the data set results in a viability index for the 2500 ppm F1 generation exposure group of 95.7%. It should also be noted that the value for the F2 generation was 97.0%. All values when properly calculated were within the historical control data range for the Testing Facility.

A delay in F1 preputial separation at 500 ppm and 2500 ppm. Recalculation of the preputial separation data from the multigenerational study indicated a significant interaction between dose and the PND 21 male pup body weight. The output of this analysis resulted in no statistically significant difference at the 500 ppm exposure level but the difference between the 2500 ppm exposure group and the control group was still present. The effect at the high dose on the time to preputial separation could be independent of the any effect from in utero exposure and an effect on postnatal growth. It is however probable that the effect was due to direct toxicity caused by the high exposure that occurred in the 2500 ppm group pups when they started to consume the test diets and being exposed to dosages on a mg/kg of body weight basis as high as 600 mg/kg/day of the Clothianidin. These high dose levels of Clothianidin are known to cause general toxicity.

A delay in F1 generation vaginal patency at 2500 ppm. This delay appeared to correlate with a reduction with body weight on the day of vaginal patency. Analysis of the data using a covariant of the PND 21 body weight resulted in the delay in vaginal opening at the 2500 ppm exposure level no longer being statistically significant.

Decreases in birth weight and postnatal body weight gain at 2500 ppm in the F1 and F2 pups. The 2500 ppm exposure was clearly toxic to the dams and the actual dose of Clothianidin received by the F1 generation pups prior to weaning was probably almost double that noted for the adult females and may have been as high as 600 mg/kg/day. This high dose may have resulted in direct systemic toxicity including delays in maturation as previously noted and in

delays in growth. The dose of 500 mg/kg in the dosage-range developmental toxicity stud in rats did produce significant general toxicity.

Decreased thymus weights in the offspring at ≥500 ppm in males and at 2500ppm in females. The ratio of the thymus weight to the final body weights were not statistically significantly different from control values. There were no dose-response relationships were evident in male or female pups of either generation. Noting again that the pups start consuming the feed on or about PND 14 and that the organ weights for the offspring were assessed in PND 21 pups, it is highly likely the thymus weights reflect direct systemic toxicity.

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APPENDIX A - SUMMARY OF EXPERIENCE

[redacted]

Experience

[redacted]

- [redacted]

Publications and Professional Activities (see Curriculum Vitae for details)

- [redacted]

Education

[redacted]

Additional Certification

[redacted]

Corporate Affiliations (see Curriculum Vitae for details)

- [redacted]

APPENDIX B - HISTORICAL CONTROL DATA FOR ABSENT LUNG LOBE

Year or		Total		Fetuses affected			Litters affected		
Date-of	Study	Fetuses	Litters			% Range			% Range
C-Sect	Code	Evaluated	Evaluated	Number	%	per Study	Number	%	per Study
1987 - 1988									
1987	1	138	18	2	1.4	0 - 1.4	2	11.1	0 - 11.1
1987	2	122	16	3	2.5	0 - 2.5	3	18.8	0 - 18.8
1987	3	84	12	1	1.2	0 - 1.2	1	8.3	0 - 8.3
1987	18	128	16	0	0.0	0	0	0.0	0
1987	25	87	12	4	4.6	0 - 4.6	2	16.7	0 - 16.7
1987	27	129	16	0	0.0	0	0	0.0	0
1987	30	120	13	1	0.8	0 - 0.8	1	7.7	0 - 7.7
1987	31	109	14	2	1.8	0 - 1.8	1	7.1	0 - 7.1
1987	33	109	16	1	0.9	0 - 0.9	1	6.3	0 - 6.3
1987	35	87	10	1	1.1	0 - 1.1	1	10.0	0 - 10.0
1988	36	137	16	7	5.1	0 - 5.1	5	31.2	0 - 31.2
1987	38	91	13	3	3.3	0 - 3.3	2	15.4	0 - 15.4
1988	41	130	18	3	2.3	0 - 2.3	1	5.6	0 - 5.6
1987	42	104	14	2	2.2	0 - 2.2	2	14.3	0 - 14.3
1988	45	98	17	0	0.0	0	0	0.0	0
June 1992 - June 6 1995									
5/92	181	202	28	13	6.4	0 - 6.4	5	17.8	0 - 17.8
5/92	182	123	18	2	1.6	0 - 1.6	2	11.1	0 - 11.1
9/92	186	87	12	0	0.0	0	0	0.0	0
9/92	187	93	16	1	1.1	0 - 1.1	1	6.2	0 - 6.2
9/92	188	109	16	4	3.7	0 - 3.7	2	12.5	0 - 12.5
11/92	189	96	16	0	0.0	0	0	0.0	0
2/93	195	97	14	0	0.0	0	0	0.0	0
2/93	196	129	17	0	0.0	0	0	0.0	0
5/93	201	122	16	3	2.5	0 - 2.4	3	18.8	0 - 18.8
6/93	203	125	17	1	0.8	0 - 0.8	1	5.9	0 - 5.9
6/93	204	96	13	4	4.2	0 - 4.2	2	15.4	0 - 15.4
6/93	206	89	12	3	3.4	0 - 3.4	2	16.7	0 - 16.7
7/93	207	147	18	3	2.0	0 - 2.0	3	16.7	0 - 16.7
8/93	209	143	17	1	0.7	0 - 0.7	1	5.9	0 - 5.9
8/93	210	89	14	1	1.1	0 - 1.1	1	7.1	0 - 7.1
9/93	212	142	19	4	2.8	0 - 2.8	2	10.5	0 - 10.5
9/93	213	117	15	3	2.6	0 - 2.6	2	13.3	0 - 13.3
9/93	214	117	16	1	0.9	0 - 0.8	1	6.2	0 - 6.2
10/93	216	112	14	3	2.7	0 - 2.7	2	14.3	0 - 14.3

Year or		Total		Fetuses affected			Litters affected		
Date-of	Study	Fetuses	Litters			% Range			% Range
C-Sect	Code	Evaluated	Evaluated	Number	%	per Study	Number	%	per Study
10/93	217a	147	17	4	2.7	0 - 2.7	3	17.6	0 - 17.6
10/93	217b	119	14	2	1.7	0 - 1.7	2	14.3	0 - 14.3
11/93	218	160	20	1	0.6	0 - 0.6	1	5.0	0 - 5.0
12/93	221	88	14	2	2.3	0 - 2.3	1	7.1	0 - 7.1
1/94	223	165	20	0	0.0	0	0	0.0	0
3/94	228	184	23	1	0.5	0 - 0.5	1	4.3	0 - 4.3
4/94	229	136	17	2	1.5	0 - 1.5	2	11.8	0 - 11.8
11/93	230	123	15	2	1.6	0 - 1.6	2	13.3	0 - 13.3
7/94	235	127	18	2	1.6	0 - 1.6	2	11.1	0 - 11.1
9/94	236	130	17	9	6.9	0 - 6.9	5	29.4	0 - 29.4
12/94	238	132	17	1	0.8	0 - 0.8	1	5.9	0 - 5.9
3/95	240	139	18	1	0.7	0 - 0.7	1	5.6	0 - 5.6
1/95	243	121	16	0	0.0	0	0	0.0	0
8/95	246	156	19	5	3.2	0 - 3.2	2	10.5	0 - 10.5
8/95	249	166	19	3	1.8	0 - 1.8	3	15.8	0 - 15.8
10/95	255	161	17	0	0.0	0	0	0.0	0
12/95	257	154	19	1	0.6	0 - 0.6	1	5.3	0 - 5.3
12/95	258	157	17	0	0.0	0	0	0.0	0
1/96	260	175	20	4	2.3	0 - 2.3	4	20.0	0 - 20.0
4/96	265	158	18	7	4.4	0 - 4.4	5	27.8	0 - 27.8
4/96	266	139	18	2	1.4	0 - 1.4	2	11.1	0 - 11.1
4/96	268	160	19	2	1.3	0 - 1.2	2	10.5	0 - 10.5
4/96	269	153	19	4	2.6	0 - 2.6	2	10.5	0 - 10.5
5/96	270	162	19	3	1.8	0 - 1.8	3	15.8	0 - 15.8
6/96	271	160	19	3	1.9	0 - 1.9	2	10.5	0 - 10.5
7/96	273	181	20	7	3.9	0 - 3.9	3	15.0	0 - 15.0
8/96	276	149	17	2	1.3	0 - 1.3	2	11.8	0 - 11.8
8/96	277a	161	20	3	1.9	0 - 1.9	3	15.0	0 - 15.0
8/96	277b	166	20	5	3.0	0 - 3.0	4	20.0	0 - 20.0
9/96	281	187	19	1	0.5	0 - 0.5	1	5.3	0 - 5.3
9/96	282	147	18	1	0.7	0 - 0.7	1	5.6	0 - 5.6
10/96	283	181	19	5	2.8	0 - 2.8	3	15.8	0 - 15.8
4/96	285	169	18	1	0.6	0 - 0.6	1	5.6	0 - 5.6
10/96	288	154	17	1	0.6	0 - 0.6	1	5.9	0 - 5.9
11/96	290	174	19	3	1.7	0 - 1.7	3	15.8	0 - 15.8
11/96	293a	107	14	0	0.0	0	0	0.0	0
11/96	294	170	18	4	2.4	0 - 2.4	4	22.2	0 - 22.2
12/96	295	202	23	1	0.5	0 - 0.5	1	4.3	0 - 4.3

Year or		Total		Fetuses affected			Litters affected		
Date-of	Study	Fetuses	Litters			% Range			% Range
C-Sect	Code	Evaluated	Evaluated	Number	%	per Study	Number	%	per Study
1/97	298	190	23	2	1.1	0 - 1.1	1	4.3	0 - 4.3
1/97	299	152	18	1	0.6	0 - 0.6	1	5.6	0 - 5.6
2/97	300	169	19	2	1.2	0 - 1.2	2	10.5	0 - 10.5
3/96	301a	128	20	2	1.6	0 - 1.6	2	10.0	0 - 10.0
3/97	301b	163	21	4	2.5	0 - 2.5	4	19.0	0 - 19.0
3/97	303	168	19	2	1.2	0 - 1.2	2	10.5	0 - 10.5
4/97	306	173	20	0	0.0	0	0	0.0	0
TOTAL		10871	1360	185			138		
AVERAGE		137.6	17.2	2.3	1.7		1.7	10.1	

APPENDIX C – LUNG PUBLICATION

CHRISTIAN, M.S., MC CARTY. R.J., COX-SICA, D.K. (1987). Recent Increases in the Incidences of Skull. Lung and Rib Alterations in Vehicle Control New Zealand White Rabbits. Journal of the American College of Toxicology. Special Issue 6:3 A 126. Presented at Eighth Annual Meeting of American College of Toxicology, Baltimore, MD December 7-9, 1987.

126 Recent Increases in the Incidences of Skull. Lung and Rib Alterations in Vehicle Control New Zealand White Rabbits. CHRISTIAN, M.S., MC CARTY. R.J., COX-SICA, D.K., Argus Research Labs. Inc., Horsham, PA 19044

The evaluation of historical control data for fetal alteration incidences in common laboratory species has become increasingly important. Unfortunately, these data are often tabulated without reference to animal source, age, genetic background and health. In many cases, data are collected by multiple investigators, and, as a result, inconsistent terminology may be used. During the past seven years, all rabbits used at our laboratory were obtained from the same source. The scientific staff of the laboratory and the criteria used for fetal evaluation have remained constant during this period. However, the incidences of several alterations has fluctuated remarkably for some shipments of rabbits. It was presumed that such changes in background incidences reflected genetic variation in the test population. Data from developmental toxicity evaluations conducted in 1985 to 1987 were summarized and compared with the incidences for the same values obtained in 38 studies tabulated during the preceding five years (1983 to 1987). This comparison demonstrated that the incidences of minor alterations in skull ossifications (irregular suture lines) and the presence of intersutural bones (internasals, etc.) and small holes has increased in New Zeeland White rabbits [Hra: (NZW) SPF] over two years. The incidence of agenesis of the intermediate lobe of the lungs has also increased. Increases of rib variations remain sporadic. These skull, lung and rib alterations also occur in the does. Incidences of spontaneous fetal skull, lung and rib alterations noted in this strain of rabbit during the past two years will be presented and compared with the incidences previously noted, as well as with the incidences of the same alterations in adult rabbits.