Thiamethoxam

Thiamethoxam – Assessment of Testis Toxicity in the Dog

Supporting Document for Submission

DATA REQUIREMENT(S): Not Applicable

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COMPLETION DATE: 19 March 2019

PERFORMING LABORATORY: Syngenta Ltd [redacted]

LABORATORY PROJECT ID: Report Number: TMX-0003

SPONSOR(S): Syngenta Ltd [redacted]

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1.0 BACKGROUND

In January 2018 France submitted a CLH dossier to ECHA for the substance thiamethoxam (TMX). In the dossier France proposed a classification of H361 Reproductive Toxicity Category 2 based on effects in the testes in the rat multigeneration studies and repeat dose dog studies as well as increased qualitative susceptibility of offspring in the developmental neurotoxicity (DNT) study (CLH report, 2018 and Annex to CLH report Thiamethoxam dRAR Volume 3 –B6 (AS)).

This response document will address the comments relating to the effects in the dog testes, and the effects on the rat testes and the comments relating to the developmental neurotoxicity study will be addressed in separate documents ([redacted] *et al*. 2019 and [redacted] *et al.* 2019).

2.0 SUBMITTER INTERPRETATIONS AND OVERALL SYNGENTA RESPONSE

Conclusions related to effects in the dog testes in the CLH proposal for thiamethoxam (CLH report (2018) were as follows:

"Testicular effects in dogs: effects are observed in the 90-d and 1-year studies. While, decreased bodyweight gain was observed at the same dose level in the 90-d study, final body weight was slightly affected. In the 1-year dog study increased incidence of tubular atrophy was observed from the mid-dose onwards and reduced body weight gain was observed only at the high dose levels with no impact on final body weight. Therefore those effects cannot be ruled out as secondary to severe general toxicity."

Syngenta's position on these findings is as follows;

- Lower testicular weight and immature histopathological appearance were observed only in conjunction with evidence of systemic toxicity in both 90 day and 1 dog year studies.
- This is a non-specific secondary consequence of systemic toxicity to the immature dog.
- **Therefore thiamethoxam does not meet the criteria for classification with Repro Category 2 (H361).**

3.0 DETAILED COMMENTS

3.1 Testicular Immaturity in Beagle Dogs Dosed with Thiamethoxam for 13 Weeks and 1 Year

In a 13 week dietary toxicity study in beagle dogs with thiamethoxam ([redacted] 1996b) the top dose level (2500/2000 ppm) was associated with an increase in the incidence of testes with histological features of immaturity or peri-pubescent testes in males. A slight increase

in the incidence of background histological findings associated with peri-pubescent testes was also observed in males in the mid and high dose (750 and 1500 ppm respectively) treatment groups of a 1 year dietary toxicity study in beagle dogs with thiamethoxam ([redacted] 1998). In both studies these testicular effects in dogs occurred only in the presence of severe systemic toxicity. In the 90 day dog study ([redacted] 1996b) a dose of 2500/2000 ppm was associated with a marked reduction in body weight gain relative to control animals in males. Similarly, in the 1 year dog study ([redacted] 1998) dose levels of 750 and 1500 ppm were associated with delayed body weight gain. Since dogs are generally sexually immature at the start of studies (regulatory guidelines recommend that dogs are 4-6 month old at the start of the study), factors that affect the normal progression of the testes from sexual immaturity to prepubescent, peri-pubescent and sexual maturity (such as nutritional status and body weight gain) may cause an apparent difference in the morphological appearance of the testes of adolescent animals. As testicular immaturity, and a higher incidences of findings associated with peri-pubescence were observed only in conjunction with evidence of systemic toxicity in both 90 day and 1 year studies this effect is likely to reflect delayed maturation due to systemic toxicity. Consequently, there is insufficient evidence for Reproductive Toxicity Category 2 *'suspected of damaging fertility'* (H361f) and thiamethoxam does not meet the criteria for classification.

13-week study

Thiamethoxam was administered orally for 13 weeks to groups of 4 male and 4 female beagle dogs, by admixture in the diet, at concentrations of 0, 50, 250, 1000 and 2500/2000 ppm ([redacted] 1996b).

Three out of the four males at 2500ppm lost weight during the first 2 weeks necessitating a dose reduction to 2000ppm. Subsequent weight gain at this dose level remained lower than control, being 72% lower than control after 4 weeks, and 36% lower than control at 13 weeks.

Body Weight Effects in Male Dogs in 13 Week Study

Dose level [ppm]	0	50	250	1000	2500/2000
Cumulative body weight gain [kg]					
Week 4	1.075	1.125	1.050	1.175	0.300
% weight change compared to control over 4 weeks	-	+ 4.7%	- 2.3%	+ 9.3%	- 72.1%
Week 8	1.975	2.100	1.900	2.125	1.075
% weight change compared to control over 8 weeks	-	+ 6.3%	- 3.8%	+ 7.6%	- 45.6%
Week 13	2.950	3.275	2.750	2.925	1.875
% weight change compared to control over 13 weeks	-	+ 11.0%	- 6.8%	- 0.8%	- 36.4%

* $P \leq 0.05$ (Wilcoxon)

At termination, mean testis weight was lower than controls at 2000ppm. Several dogs in this group had testis weights which were below the control range for the 13 week study; testis

weights were within the control range for the 28 day study and therefore were similar to those of younger animals.

Testis Findings in 13 Week Study

Dose [ppm]	0	50	250	1000	2000
number of animals in study	4	4	4	4	4
Testis weight					
absolute (g)	16.54	14.76	14.60	15.59	9.38*
absolute range (g)	12.22-19.98	12.78-17.48	12.26-17.16	14.52-17.59	5.54-13.91
relative (g/kg bodyweight)	1.545	1.336	1.384	1.439	0.929*-
Histopathology (number of animals affected, with mean severity grade in brackets)					
Tubular atrophy [a]	0	0	0	0	1(2)
Spermatogenesis reduced	0	1(3)	0	0	4(2)
Spermatic giant cells	1(1)	1(2)	0	1(1)	4(1)

$p \leq 0.05$ (Dunnett's t-Test, two tailed)
- negative trend (Jonckheere)
a May be more accurately descibed as immature testis

Some differences in testis histopathology were noted in the 2000ppm group compared to the control, with a minimal to marked reduction in spermatogenesis and an increased incidence of spermatic giant cells being recorded in the testes of all males at 2000ppm. This histological appearance is typical of testes from peripubertal animals. One male also showed what was termed "moderate tubular atrophy"; the recording of atrophy may be a misnomer in this instance, as the appearance is very similar to that of an immature testis. This particular dog was the most severely affected in terms of body weight, showing an actual weight loss of 0.5 kg at the end of the study.

1-year study

Thiamethoxam was administered orally for 52 weeks to groups of 4 male and 4 female beagle dogs, by admixture in the diet, at concentrations of 0, 25, 150, 750 and 1500ppm ([redacted] 1998).

Body Weight Effects in Male Dogs during 1 Year Study (kg)

Dose level [ppm]	0	25	150	750	1500
Weight week –1	11.30	11.28	11.63	11.18	11.68
Gain at week 13	0.350	0.225	0.175	0.175	0.150
% weight change compared to control over 13 weeks	-	- 36%	- 50%	- 50%	-57%
Gain at week 26	0.575	0.575	0.400	0.575	0.325
Gain at week 39	0.975	1.050	0.950	0.875	0.300
Gain at week 52	0.950	1.125	1.125	1.000	0.700
% weight change compared to control over 52 weeks	-	+ 18%	+ 18%	+ 5%	- 26%

The overall body weight gain of males at 1500ppm was reduced by 26% compared with controls during the study, with the reduction in weight gain being more marked early on in the study.

The mean absolute and relative testis weights in the 1500ppm animals were slightly, but not statistically significantly, lower than the control, due mainly to slightly lower than control range testis weights from two animals. Although some were slightly below the control range for the 1 year study, the testis weights of dogs at 1500ppm were within the control range for the 13 week study, and thus similar to those of younger animals.

Testis Findings in 1 Year Study

Dose level (ppm)	0	25	150	750	1500
animals on study	4	4	4	4	4
Testis weight					
absolute (g)	19.07	20.48	19.83	20.65	16.06[a]
absolute range (g)	15.24-23.65	17.17-22.92	17.71-21.01	18.23-23.15	12.93-18.87
relative (g/kg body weight)	1.66	1.79	1.69	1.89	1.44
Testis histopathology (number of animals affected, with mean severity grade in brackets)					
tubular atrophy [b]	1(1)	1(1)	1(3)	2(2)	2(2)[c]
spermatic giant cells	2(1)	1(1)	1(1)	-	1(3)
inflammatory cell infiltration	-	-	1(2)	-	-

a The testis weight for 2 males in this group was below the control range (14.57 and 12.93g).
b May be more accurately described as immature testis.
c Bilateral in both the affected males in this group, and in one male in the 750-ppm group, as opposed to unilateral in all other affected animals. There was no correlation between testis weight & histopathological change (the testis weights of the dogs with histopathological change at 1500ppm were 17.86 & 12.93g).

Histopathological examination showed the presence of what was described as “tubular atrophy” in one or two dogs in all groups, including the control. Many animals also showed the presence of spermatic giant cells, thus, as in the 13-week study, this histological appearance is consistent with an observation of immature testis, rather than tubular atrophy.

Background incidence of testicular effects and the impact of retarded body weight gain on testicular maturation

Testis weights in young beagle dogs (up to 24 months of age) are typically variable and are highly dependent upon the stage of sexual maturation of the animal (Goedken, 2008). Testicular tubular atrophy, also referred to as segmental hypoplasia (Rehm, 2000), and reduced spermatogenesis, also referred to as hypospermatogenesis (Rehm, 2000), are also common background findings in beagle dogs (Rehm, 2000; Goedken, 2008). In control animals these histological findings are often found in conjunction with reduced testis weight and are considered to indicate delays to sexual maturation (Goedken, 2008).

Histological evidence of sexual maturity in the testes is generally achieved between 8 and 9 months in most dogs but background histological findings consistent with peri-pubescent testes such as hypoplasia/atrophy, hypospermatogenesis/reduced spermatogenesis and/or sperm giant cells often occur at relatively high incidences (40-75 %) until the animals are over a year old, when the incidences of these age-related spontaneous findings drop to about

half these incidences. There is therefore a considerable variation in the histological appearance of the peri-pubertal testes in dogs which is in part due to variations in the age at the onset of sexual maturation. The histological variation seen in peripubertal dogs makes the assessment of potential testicular toxicity especially difficult when this age of animals is included in study designs, particularly where small group sizes are utilised.

Nutritional deficiency and stress are known to result in effects on the testis, including the observation of reduced spermatogenesis, particularly in immature animals (Garside and Harvey, 1992; Gopinath et al, 1987).

In both the 90 day and 1 year studies, a general delay in growth and maturation manifest as retarded body weight gain was seen at high dose levels of 1500 & 2000ppm. At these high dose levels there was also evidence of a delay in reaching full testicular maturity in some dogs, in terms of lower testis weight when compared to controls. This correlated with histological evidence of testis immaturity (recorded as tubular atrophy, reduced spermatogenesis, and increased spermatic giant cells) and/or an increase in the incidence of background findings associated with peri-pubescent testes. This appearance is consistent with a delay in testicular maturation, secondary to a general delay in body weight development in these animals.

4.0 CONCLUSIONS

It is concluded that the lower testis weight and immature histological appearance seen in a small number of dogs treated with high doses of thiamethoxam indicate a delay in maturation of the testis. This is a non-specific secondary consequence of generalised toxicity producing an overall delay in both growth and maturation resulting from exposure to high levels of thiamethoxam in the diet. Thus it is not a specific effect of thiamethoxam on the testis. Consequently, there is insufficient evidence for Category 2 ‘*suspected of damaging fertility*’ (H361f) and thiamethoxam does not meet the criteria for classification.

5.0 REFERENCES

NOTE: Only external literature references are listed here.

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