Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

acetamiprid (ISO); (1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N'*-cyano-*N*-methylethanimidamide; (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine

EC Number: -

CAS Number: 135410-20-7; 160430-64-8

Index Number: 608-032-00-2

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Version number: 3 **Date: October 2018**

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1.1 Reproductive toxicity

1.1.1 Animal data

1.1.1.1 Evaluation of the oral developmental neurotoxicity study of acetamiprid in rats

Reference:	CA 5.7.1/03, Anonymous. (2008)
Title:	An oral developmental neurotoxicity study of acetamiprid in rats
Report number:	Report No. WIL-21193, Document No. RD-01310
Guidelines:	OPPTS 870.6300
GLP/QA:	Yes/Yes

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Light brown, crystalline powder
Lot/Batch:	NNI-03
Purity:	>99%
Stability:	Expiry date: 16 April 2001

2. Vehicle and/or positive control: 5% gum arabic with 0.01% Tween 80/ no positive control

3. Test Animals

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD(SD)IGS BR)
Age:	10-11 weeks
Weight at dosing:	Females: 226 to 265 g
Source:	Charles River Laboratories, Canada
Acclimation period:	10 days

B. STUDY DESIGN AND METHODS

1. In-life dates

2 November 1999 (receipt of female animals) to 21 October 2000 (Last F1 PND 72 neuropathologic examination)

2. Animal assignment and treatment

At the conclusion of the acclimation period, all available females were weighed and examined in detail for physical abnormalities. Animals judged to be in good health and meeting acceptable body weight requirements (210-270 g) were placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilised exclusively for breeding. These rats were maintained under similar laboratory conditions as the females. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed day 0 of gestation, and the animals were separated. Following the completion of the mating period, the bred females were assigned to groups containing 25 rats each using a computer program which

randomized the animals based on body weight stratification in a block design. The dosing preparations were administered orally using 16-gauge stainless steel gavage cannulas, once daily from gestation day 6 through lactation day 21, with the following exception. If parturition was occurring for a given animal during dose administration, that animal was not dosed on that day. A dosage volume of 5 mL/kg was used. Individual dosages were based on the most recently recorded body weight to provide the correct mg/kg/day dose. All animals were dosed at approximately the same time each day.

3. Body weight and food consumption

Individual F0 female body weights were recorded on gestation days 0, 3, 6, 9, 12, 15 and 20, and on lactation days 1, 4, 7, 10, 16 and 21. Mean body weights were calculated for each of these days, and mean body weight changes were calculated for each corresponding gestation or lactation interval and for gestation days 6-20 and lactation days 1-21. Food consumption was measured on the same days, and food intake calculated as g/animal/day and g/kg/day for the corresponding body weight change intervals.

4. Clinical observations and detailed clinical observations

The animals were observed twice daily, for moribundity and mortality. Detailed physical examinations were recorded daily from receipt through lactation day 21 (prior to test article administration during the dosing period). Animals were also observed daily for signs of toxicity approximately one hour following dosing throughout the treatment period. Females that delivered were also observed twice daily during the period of expected parturition and at parturition for dystocia, prolonged labour, delayed labour or other difficulties. Ten randomly selected dams per group were observed outside of the home cage (for 1 minute) on gestation days 6 and 12 and on lactation days 4 and 7. The following parameters were evaluated:

Ease of removal from cage

Lacrimation/Chromodacryorrhea; Piloerection; Palpebral closure; Red/crusty deposits; Eye prominence; Mobility; Convulsions/Tremors; Grooming; Bizarre/Stereotypic behaviour; Ease of handling animal; Salivation; Fur appearance; Respiratory rate/character; Mucous membranes/eye/skin colour; Muscle tone; Gait; Arousal; Urination/Defecation; Backing.

Females that were selected for detailed clinical observations but failed to deliver were arbitrarily replaced by females that delivered. Testing was performed by the same trained technicians when possible, who did not know the group assignment of the animals.

5. Parturition

All females from each dose group were allowed to deliver naturally and rear their young to weaning (PND 21). During the period of expected parturition (beginning on the first gestation day 21 and continuing until the last post-mating day 25), the females were observed three times daily (at 8:00 a.m., 12:00 p.m. and 4:00 p.m.) for initiation and completion of parturition and for signs of dystocia. The day on which delivery was complete was designated lactation day 0. When parturition was judged complete, litters were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. The duration of gestation was calculated for each animal using the date delivery initiated.

6. Pathology

Unscheduled deaths: The female that died was subjected to a gross necropsy. The number and location of implantation sites and corpora lutea were recorded. Recognisable foetuses were examined externally and discarded.

Females that failed to deliver: F0 females that did not deliver within 25 days following mating were euthanized by carbon dioxide inhalation and examined macroscopically. The number of implantation sites was recorded. Uteri with no evidence of implantation were opened and placed in 10% ammonium sulfide solution as described by Salewski for the detection of implantation sites.

Females with total litter loss: Females with total litter loss were euthanized within 24 hours by carbon dioxide inhalation and subjected to a gross necropsy. The number of former implantation sites was recorded.

Females with litters that failed to meet sex ratio criteria: Females with litters that failed to meet the sex ratio criteria (4:4, 3:5 or 5:3 male:female) were euthanized on lactation day 4 and subjected to a gross necropsy. The number of implantation sites was recorded.

Females that delivered: On lactation day 21, each F0 female was euthanized by carbon dioxide inhalation and subjected to a complete gross necropsy. The number of implantation sites was recorded.

In all cases tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. The carcasses were then discarded.

7. F1 generation

7.1 Litter viability and deaths

Each litter was examined twice daily for survival, and all deaths were recorded. All pups were individually identified by the application of tattoo markings on the digits on PND 0. A daily record of litter size was maintained. Offspring found dead, euthanized *in extremis* or euthanized due to failure to meet sex ratio criteria (by an intrathoracic injection of sodium pentobarbital) between PND 0 and PND 4 were examined externally and sexed. The sex of each pup was confirmed by internal examination. The stomachs were examined for the presence of milk. The carcasses were discarded. Pups with external abnormalities were preserved in 10% neutral-buffered formalin for possible future examination. A detailed gross necropsy was performed on any pup dying after PND 4; tissues were saved for possible histopathological examination only as deemed necessary by the gross findings.

7.2 Litter reduction and sex determination

To reduce variability among the litters, eight pups per litter were randomly selected on PND 4. The remaining offspring were weighed, euthanized and discarded on PND 4. If a litter did not meet the sex ratio criteria (4:4, 3:5 or 5:3, male:female), the pups from the litter were not used for neurobehavioural or neuropathological evaluations and were euthanized and necropsied on PND 4. Pups were individually sexed on PND 0, 4 (prior to culling), 11 and 21.

7.3 Clinical observations and detailed clinical observations

All pups were examined twice daily for moribundity and mortality from the day of parturition through euthanasia. Individual clinical observations regarding general appearance, behaviour, and all signs of overt toxicity were recorded on postnatal days 1, 4, 7, 11, 14, 17 and 21 and at weekly intervals thereafter until euthanasia. Ten pups/sex/group were observed outside of the home cage on PND 4, 11, 21, 34, 45 and 60. Body weights were collected for these animals on each of these days. Parameters evaluated were identical to those evaluated for the F0 with the following exceptions. On PND 4, pups were not observed for piloerection or fur appearance since the hair coat is not yet present at this age. Eye colour, palpebral closure and eye prominence were not recorded on PND 4 and 10 since eye opening had not occurred prior to these time points.

7.4 Body weight

Pups were individually weighed on PND 1, 4, 7, 11, 14, 17, 21 and at weekly intervals therefore until euthanasia. The F1 offspring were also weighed whenever they were removed from their cages for behavioural testing.

7.5 F1 post-weaning developmental landmarks and neurobehavioural testing

Each male pup was observed for balanopreputial separation beginning on PND 35. The day on which balanopreputial separation was first observed was recorded for each pup. Examination of the

pups continued daily until balanopreputial separation was present. The body weight of each male was recorded on the day of acquisition of balanopreputial separation. Each female pup was observed for vaginal patency beginning on PND 25. The day on which the vaginal lumen was first observed to open was recorded for each pup. Examination of the females was continued daily until vaginal opening was present. The body weight of each female was recorded on the day of acquisition of vaginal patency.

Motor activity observations were made on 10 rats/sex/group on PND 13, 17, 21 and 61. The same animals were monitored at each interval, when possible. One control pup died on PND 16 and was replaced. Motor activity was measured automatically using the SDI Photobeam Activity System; this personal computer-controlled system utilizes a series of infrared photobeams surrounding a clear, plastic rectangular cage to quantify animal's motor activity. The testing of treatment groups was done according to replicate sequence. Each animal was tested separately. Data were collected in five-minute epochs (print intervals), and the test session duration was 60 minutes. Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming; interruption of a single photobeam) and ambulatory motor activity (interruption of two or more consecutive photobeams).

The acoustic startle response was assessed for 10 rats/sex/group on PND 20 and 60 using the SR-Lab Startle Response System. The same animals were tested at each interval. Each isolation cabinet was composed of a wood core covered with a laboratory-grade plastic laminate and measured 15 x 16 x 23 inches. Each cabinet was equipped with an internal light, a fan, two viewing lenses and a complete white-noise generation system. The animal was placed in a cylindrical enclosure of appropriate size, which was then placed into the isolation cabinet. Each enclosure was equipped with a motion sensor. Acoustic startle response testing was performed in a room equipped with a white-noise generation system set to operate at 70 decibels (db). Each test session consisted of a five-minute acclimation period with a 65-db broadband background white noise. The startle stimulus for each trial was a 115-db mixed-frequency noise burst stimulus, approximately 20 milliseconds in duration. Responses were recorded during the first 100 milliseconds following onset of the startle stimulus for each trial. Each test session consisted of 50 trials, with an eight-second interval. Startle response data were analysed in five blocks of 10 trials each. Startle response measurements obtained were maximum response amplitude (V_{max}), average response amplitude (V_{ave}) and latency to V_{max} (T_{max}).

Swimming ability and learning and memory were assessed for 10 rats per sex per group using a water-filled, eight-unit T-maze. Animals were placed in the maze and were required to traverse the maze and escape by locating a platform that was hidden beneath the surface of the water. The amount of time required to transverse the maze and the number of errors were recorded, with an error defined as any instance when an animal deviates from the correct channel with all four feet. The first testing interval initiated for each animal on PND 22; the second testing interval initiated for each animal on PND 62. Animals tested on PND 22 were not used for the second testing interval. Each testing interval consisted of three phases that were conducted over seven consecutive days. Phase one was an evaluation of swimming ability and motivation to escape from the maze and was performed on day one of the Biel maze procedure. For this evaluation, animals were placed in a straight channel opposite the escape platform, and the time required for each animal to escape was recorded. Each animal was allowed four trials to evaluate swimming ability and motivation. Phase two of the Biel maze procedure evaluated sequential learning. This evaluation was conducted on days 2-6 of the Biel maze procedure. Animals were then allowed two trials per day for two consecutive days to solve the maze in path B (reverse of path A). For each trial, animals were allowed three minutes to solve the maze. If an animal did not escape the maze within the allotted three minutes, the animal was removed from the maze. The minimum intertrial interval was one hour. Phase three of the Biel maze procedure tested memory by challenging the animal to solve the maze in path A. This evaluation was conducted on day 7 of the Biel maze procedure. Each animal was allowed two trials to solve the maze in path A. Biel maze data were evaluated as the mean time to escape over all trials for each of the three phases (i.e., swimming ability and

motivation, sequential learning and memory) of the Biel maze procedure. Also, the numbers of errors committed were evaluated for phases two and three.

7.6 Macroscopic examination

Offspring not selected for behavioural evaluation: On PND 28, all F1 offspring not selected for behavioural evaluations were euthanized by carbon dioxide inhalation and subjected to gross pathology examinations.

Offspring euthanized at study termination: Offspring scheduled for euthanasia on PND 72 but not allocated for neuropathology/brain weight measurements were euthanized by carbon dioxide inhalation and subjected to a gross pathology examination.

In both cases Organs and tissue samples were preserved in 10% neutral-buffered formalin as deemed necessary by the gross findings. The carcass of each animal was then discarded.

Offspring selected for neuropathology/brain weight measurements: On PND 11, one male and one female pup were removed from each litter and macroscopically examined for neuropathology. The pups were euthanized by carbon dioxide inhalation and perfused *in situ*. At the termination of the study (PND 72), 10 F1 animals/sex/group were randomly selected from those pups dedicated to locomotor activity, auditory startle and learning and memory tests (minimum of 10 rats/sex/group). The animals were euthanized by carbon dioxide inhalation and perfused *in situ*. The central nervous system and other appropriate tissues were dissected and preserved.

The brains (including olfactory bulbs) were removed from the offspring selected for neuropathology (brain weight measurements on PND 11 and PND 72). The brains were weighed, and the size (length and width) was recorded. Any abnormal coloration or lesions of the brain and spinal cord were recorded.

A tiered approach to neuropathologic examinations and a detailed neuropathologic evaluation were relied upon for evaluation of the brains for this study.

PND 11: Of the pups perfused *in situ* for macroscopic examination on PND 11, 10 randomly selected pups/sex/group from all dose groups were prepared for microscopic neuropathologic examination (only the control and high dose groups were evaluated). The brains were prepared for qualitative histopathologic examination by embedding in paraffin, sectioning and staining with hematoxylin and eosin. Sections from all major brain regions (olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum) were examined.

PND 72: Of the offspring perfused in situ at study termination (PND 72), 10 randomly selected rats/sex/group from all dose groups were prepared for neuropathologic evaluation. The following tissues were microscopically examined from the control and high dose group animals:
Brain-olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum; Spinal cord -at cervical swellings C3 - C7 and at lumbar swellings T13 -L4; Gasserian ganglion/trigeminal nerves (2); Lumbar dorsal root ganglion at T13 - T4*; Lumbar dorsal root fibers at T13 - T4*; Lumbar ventral root fibers at T13 - T4*; Cervical dorsal root ganglion at C3 - C7*; Cervical ventral root fibers at C3 - C7*; Sciatic nerves (2); Sural nerves (2); Tibial nerves (2); Peroneal nerves (2); Optic nerves; Eyes; Skeletal muscle (gastrocnemius)
* = 4-6 tissues were collected at necropsy; 2 tissues were evaluated microscopically
The central and peripheral nervous system tissues listed above were dissected and preserved. The central nervous system tissues were embedded in paraffin. The peripheral nervous system tissues were embedded in plastic. Tissues were prepared for histopathologic evaluation by sectioning and staining with hematoxylin and eosin. The neuropathologic examination of tissues was conducted outside the testing facility (Karen S. Regan, D.V.M., D.A.C.V.P., D.A.B.T., Regan

Pathology/Toxicology Services, Inc.); the morphometric analysis was performed at the testing facility.

Quantitative examinations of the brains from PND 11 and PND 72 offspring was done on images digitized from the glass slides using Pax-It™ computer software. Linear measurements were made bilaterally on each hemisphere. Specific levels analysed, designated as Levels 1, 3 and 5, were defined (with the tabular presentation referenced in parentheses) as follows. Level 1 was a coronal section that corresponded to Plate 11 in the Paxinos and Watson rat brain atlas. The two measurements taken bilaterally on Level 1 consisted of the height of a hemisphere (Ht Hemisphere) measured just at the beginning of the lateral ventricle, and the vertical cortical thickness (V Thickness Cortex) measured at the apex of the corpus callosum (the 12 o'clock position) and parallel to the height. Level 3 was a coronal section that corresponded to Plate 217 and the four measurements on Level 3 included the radial thickness (Radial Thickness Cortex) of the frontoparietal cortex (at the 2 o'clock position on the hemisphere on the right in the image, and at the 10 o'clock position of the hemisphere on the left), the vertical height between the layers of the hippocampal pyramidal neurons (V Ht Btw Hippocampal Pyramidal Neuron Layers) measured along a line that passed through the termination of the dorsal limb of the medial dentate hilus, the vertical height of the dentate hilus (V Ht Dentate Hilus) measured at the termination of the ventral limb, and the length of the ventral limb of the dentate hilus (Length Ventral Limb Dentate Hilus). Level 5 measurements were single from only one midsagittal section. Level 5 corresponded to Plate 447 and measurements consisted of the thickness of the caudal pons (V Thickness of Pons) toward lobule No. 9, and the distance across the base of cerebellar lobule No. 9 (Base of Lobule 9) measured perpendicular to the white matter tract. Measurements were made on homologous sections to ensure that the dimensions of the regions were comparable. Pair-wise matching between measured groups was performed, as needed, to achieve comparable homology. In some cases, there was a deformity or irregularity of the desired region. As a result, morphometric analysis could not be made for some regions in a few animals.

8. Statistics

All analyses were conducted for a minimum significance level of 5% comparing each treated group to the vehicle control group; all means were presented with standard deviations (SD). All tests for significance at the 5% probability level were two-tailed for the group comparisons. The litter was used as the experimental unit. The numbers of animals (N) used to calculate the means are provided on the individual data tables. Statistical analyses were not performed if the number of animals was two or less. Statistical analyses were performed using appropriate computing devices or programs and referenced on the report tables.

ANOVA (two-tailed) with Dunnett's test was used for analysing F0 gestation and lactation body weights and weight gains, maternal food consumption, mean litter weights, length of gestation, numbers of pups born, live litter sizes, organ weights, locomotor activity, age at balanopreputial separation or vaginal patency, Biel maze, morphometric analyses. Kruskal-Wallis test with Mann-Whitney U test was used for pup sexes at birth (% males per litter) and postnatal survival. Kolmogorov-Smirnov test was used for histopathologic findings. Repeated measures ANOVA was used for analysing startle response.

II. RESULTS AND DISCUSSION

F0 maternal generation

Maternal toxicity was observed only at 45 mg/kg/day. One female died during parturition on gestation day 23, following parturition of one foetus, and without showing any clinical sign prior to death. 16 foetuses with apparent no malformation were found in the uterus. A mean body weight loss of 3 g was observed during gestation days 6-9, compared to a gain (13 g) in the control group; the difference was statistically significant ($p<0.01$). When the entire gestation treatment period (gestation days 6-20) was evaluated, mean body weight gain was statistically significantly reduced compared to the control group value. Mean gestation body

weights in this group were lower than the control group values on gestation days 9, 12, 15, and 20. Food consumption mirrored the effects on body weight/body weight gains, with statistically significant decreases in the 45 mg/kg bw/day group recorded on days 6-9 and 9-12, and for the entire gestation period. During the lactation period, mean body weight gain was statistically significantly increased in the 45 mg/kg bw/day group compared to the control group value during lactation days 1-4 and when the entire lactation treatment period (lactation days 1-21) was evaluated. The increases in mean body weight gains in this group were considered to be compensatory following the decrements in body weight gain during gestation (mean body weight in this group was statistically significantly reduced compared to the control group value on lactation day 1). In this case food consumption was not statistically significantly increased, with the exception of the period lactation days 4-7, where the increase was attributed to the reduction in mean body weight since the g/animal/day value during this interval was similar to that in the control group.

There were no significant differences among groups for pregnancy rates, gestation length and parturition, the only exception being the high dose female which died because of dystocia. Two F0 females in each of the control, 2.5 and 10 mg/kg/day groups failed to deliver and were euthanized 25 days following mating; all of these females were non-gravid. In addition, one female in each of the control, 2.5 and 10 mg/kg/day groups were euthanized with litters that failed to meet the sex ratio criteria. Three females in the 45 mg/kg/day group had total litter loss by lactation day 1. No abnormalities were noted at necropsy of all these females. At the scheduled necropsy of F0 females on lactation day 21, no internal findings were noted. The mean numbers of implantation sites, numbers of pups born and numbers of unaccounted sites recorded at the scheduled necropsy were unaffected by treatment. A summary of reproductive performance is presented in Table 52.

Table 52: Summary of reproductive performance

Parameter	Control		2.5 mg/kg/day		10 mg/kg/day		45 mg/kg/day	
	No.	%	No.	%	No.	%	No.	%
Females on study	25		25		25		25	
Females that died	-		-		-		1	
Females allowed to deliver	25		25		25		25	
Non-gravid	2	8	2	8	2	8	-	-
Gravid	23	92	23	92	23	92	25	100
Females with total litter loss	-		-		-		3	12
Females with viable pups	23	100	23	100	23	100	21	84
Gestation length (days – mean value)	21.5		21.5		21.5		21.7	
Implantation sites (mean value)	15.4		15.7		16.2		15.9	
Number born (mean value)	15.1		15.3		15.5		15.5	
Unaccounted sites (mean value)	0.4		0.3		0.7		0.5	

F1 generation

1. PND 0 litter data, postnatal survival and detailed observations

The mean numbers of pups born, mean live litter size and percentage of males per litter at birth were unaffected by treatment. Postnatal survival in the 45 mg/kg/day group was reduced on PND 0 and PND 0-1, attaining statistical significance during PND 0-1. Three females had total litter loss on PND 1 and the only pup born was found dead together with the mother on PND 0. From birth to PND 4, postnatal survival in this group was slightly reduced compared to that in the control group. Throughout the

remainder of the pre-weaning postnatal period (PND 1-4, 4-7, 7-14 and 14-21), survival was similar to that in the control group. During the pre-weaning period, pups that were found dead or euthanized *in extremis* or due to the death of the dam were 7, 14, 3 and 39 in the control, 2.5, 10 and 45 mg/kg/day groups, respectively. The numbers of pups missing and presumed cannibalized were 2, 5, 3 and 22 in the same respective groups. The general physical condition of the F1 pups was similar in all groups, including the control group. During the post-weaning period all F1 animals survived to their respective scheduled necropsies. No remarkable differences were apparent between the control and treated groups when the detailed clinical observation data were evaluated for PND 4, 11, 21, 35, 45 and 60.

2. F1 development and neurobehaviour

Mean offspring body weight gains in the treated groups were similar to the control group values during the pre-weaning period (PND 1-4, 4-7, 7-11, 11-14, 14-17 and 17-21). Mean body weights in the 45 mg/kg/day group males and females were slightly reduced on PND 1, with the difference attaining statistical significance ($p<0.05$) only in the females. Mean body weights in the 45 mg/kg/day group males and females were generally lower than the control group values throughout the post-weaning period. When the entire post-weaning period (PND 21-72) was evaluated, the mean body weight gain was statistically significantly reduced in offspring of both sexes ($p<0.01$) compared to the control group values. The differences from the control group values were statistically significant on PND 28 ($p<0.05$, males), PND 35 and 42 ($p<0.01$, females) and from PND 49 through PND 72 ($p<0.01$, males and females). Some reductions in mean body weights and body weight gains were recorded for offspring in the 2.5 mg/kg/day group, attaining statistical significance on a single sex and/or on few occasions however these were not attributed to test article administration to the F0 females since similar reductions were not observed in the 10 mg/kg/day group males and females. Balanopreputial separation was not affected by the test article at any dose level. All male pups were observed to have balanopreputial separation by PND 50. The mean days of acquisition were PND 43.6, 43.7, 43.1 and 43.9 for the control, 2.5, 10 and 45 mg/kg/day groups, respectively, without any statistically significant differences from the control group, and with similar to control mean body weights in the treated groups at the time of acquisition. Vaginal patency was not affected by the test article at any dose level. All female pups were observed to have vaginal patency by PND 37. The mean days of acquisition were PND 32.2, 32.3, 32.7 and 32.5 for the control, 2.5, 10 and 45 mg/kg/day groups, respectively.

Mean body weight on the day of vaginal patency was statistically significantly reduced in the 45 mg/kg/day group.

Test article-related reductions in startle response were observed in the 45 mg/kg/day group males.

The maximum response amplitudes (V_{max}) were reduced in males on PND 20 and 60 compared to the control group values, attaining statistical significance in the 45 mg/kg bw/day group on both days ($p<0.05$).

The average response amplitudes (V_{ave}) in these males were also statistically significantly reduced compared to the control group values on PND 20 and 60, while latencies to maximum response amplitude (T_{max}) were similar to those in the control group on PND 20 and 60.

On PND 20, V_{max} and V_{ave} were significantly reduced in females in the 45 mg/kg bw/day group in comparison to the control group values, while T_{max} was significantly increased in comparison to the control group at this time point.

No test article-related effects on auditory startle response were observed in females on PND 60.

In the 10 mg/kg/day group males, V_{max} and V_{ave} were reduced on PND 20 and on PND 60, without attaining statistical significance. The differences were not considered to be toxicologically significant in the study report since 1) the values were within the expected ranges (within the 25th and 75th percentiles of the WIL startle response historical control data), 2) none of the findings were statistically significantly different from the control group values, and 3) there were no other concomitant effects in other functional endpoints or neuropathology. T_{max} values in males were similar to those in the control group on PND 20 and 60. No test article-related effects on auditory startle response were noted in the 10 mg/kg/day group females. No test article-related effects on locomotor activity were observed in males and females at any dose level on PND 13, 17, 21 and 61. Evaluation of Biel maze data demonstrated no

adverse effects on swimming ability or motivation and showed no impairments in learning and memory ability.

Note RMS: RMS does not fully agree with the arguments given on why the differences in V_{max} and V_{ave} in the 10 mg/kg bw/day group were not considered toxicologically significant. 1) For males in the 10 mg/kg bw/day group, V_{max} on PND60 was outside the HCD interquartile range. Furthermore, no HCD for V_{ave} were provided in the study report. In addition, some significant changes in the 45 mg/kg bw/day group were withing the HCD and some data from the control group were outside the HCD. 2) this argument is correct, however, there are large differences even though they are not significant (decrease of 26-40% in the 10 mg/kg bw/day group in comparison to the control group). 3) For the 10 mg/kg bw/day females, V_{max} decreased 11% on PND20 (not significant) and in the 45 mg/kg bw/day group, a significant decrease of 29% was seen. This is comparable to the males, where a decrease was already seen in the 10 mg/kg bw/day group, which was not significant (yet) and did get significant in the higher dose group.

Table 53: Maximum response amplitudes (V_{max}) found in this study and given HCD

PND20													
		1-10		**11-20**		**21-30**		**31-40**		**41-50**		**All trials**	
	Dose (mg/kg bw/day) / sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mean	0	248	231	232	187	181	172	204	150	207	167	214	181
	2.5	204	199	201	165	157	170	176	182	175	197	183	182
	10	185	194	169	162	165	157	137	162	131	128	157	161
	45	143	168	118	133	121	137	117	103	118	103	123*	129*
HCD[a]	-	171-214	164-205	147-172	139-179	133-164	119-166	129-151	112-170	123-148	117-161	140-188	127-175
HCD[b]	-	127.7-264.5	135.4-241.9	98.7-232.7	117.3-204.6	90.9-206.9	105.8-191.3	99.0-189.2	103.5-188.8	97.8-200.2	103.0-194.7	102.8-210.9	117.6-200.4
PND60													
Mean	0	330	117	237	81	176	72	176	62	130	59	210	78
	2.5	284	115	230	89	172	66	130	49	132	75	189	79
	10	205	124	140	88	102	58	104	65	79	63	126	80
	45	157	124	100	86	85	67	75	57	76	67	99*	80
HCD[a]	-	179-281	108-159	138-204	79-119	125-164	69-100	94-152	64-98	91-154	66-85	132-185	80-109
HCD[b]	-	126.7-341.0	72.4-188.4	95.2-272.6	51.2-176.7	58.6-253.2	39.7-146.6	59.2-214.7	39.5-137.6	57.3-188.3	37.1-116.0	87.2-247.1	48.6-147.5

*: $p<0.05$

[a]: $25^{th} – 75^{th}$ percentiles

[b]: minimum-maximum values

Table 54: Average response amplitudes (V_{ave}) found in this study

PND20													
		1-10		**11-20**		**21-30**		**31-40**		**41-50**		**All trials**	
	Dose												

	(mg/kg bw/day) / sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mean	0	56	47	48	39	38	38	43	34	43	39	46	40
	2.5	44	41	42	33	34	35	38	39	37	41	39	38
	10	39	41	36	35	35	34	30	35	30	27	34	34
	45	30	36	25	29	26	29	25	22	26	22	27*	27*
PND60													
Mean	0	75	25	55	18	39	16	36	13	27	12	47	17
	2.5	61	25	47	20	36	14	28	11	29	16	40	17
	10	51	26	31	18	22	12	23	13	18	12	29	16
	45	34	27	22	18	19	14	16	11	16	13	22*	16

*: $p<0.05$

3. F1 necropsy and neuropathology

Necropsy findings for F1 pups that were found dead or euthanized *in extremis* during the pre-weaning period were not suggestive of any correlation with maternal treatment. At the necropsy of pups from litters that failed to meet the sex ratio criteria (PND 4), pups that were not selected for behavioural evaluations (PND 28), offspring not selected for neuropathology and brain weight measurements (PND 72) and offspring not selected for neuropathology and brain weight measurements, (PND 11 and PND 72) no internal findings were observed that could be attributed to maternal test article administration.

Mean brain weights and brain length and width measurements were not affected by the test article at any dose level on PND 72. The only statistically significant difference from the control group values was a 4.6% reduction ($p<0.01$) in the brain width in the 45 mg/kg bw/day group males. The brain length in this group was similar to that in the control group. In addition, no test article-related microscopic findings were observed at any dose level. Therefore, the reduction in brain width was not considered to be test article-related.

There were no test article-related microscopic findings in the central and peripheral nervous system tissues examined from the selected PND 11 and PND 72 pups. Mean absolute brain weights, brain weights relative to final body weights, brain lengths, and F1 brain morphometry at PND 11 and PND 72 in the treated group males and females were not affected by F0 maternal test article administration.

III. CONCLUSIONS

In conclusion, F0 maternal toxicity was expressed at the dose level of 45 mg/kg/day by a single mortality and reductions in body weight gain and food consumption. F1 developmental toxicity was expressed at the dose level of 45 mg/kg/day by early postnatal mortality and reduced post-weaning body weights. Deficits in auditory startle response occurred in the 45 mg/kg/day group F1 males and females without concomitant effects in other functional endpoints (FOB), neuropathology or brain morphometry.

Based on the results of this study, the NOAEL (no-observed-adverse-effect level) for maternal toxicity, developmental toxicity and developmental neurotoxicity was considered to be 10 mg/kg/day.

Note RMS:

The conclusion as stated above was the conclusion of the study report and of the applicant. RMS proposes to lower the NOAEL at 2.5 mg/kg bw/day; detailed explanation is given below.

Note RMS on the developmental neurotoxicity of acetamiprid:

Discussions have been ongoing regarding the developmental neurotoxicity of acetamiprid. In 2013, EFSA published a scientific opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid (EFSA Journal 2013;11(12):3471). In this scientific opinion, EFSA proposed to lower the NOAEL to 2.5 mg/kg bw/day. The PPR panel noted that the data available did not allow any firm conclusion to be drawn since important endpoints such as motor activity, learning and memory evaluation could not be properly assessed and that insufficient arguments support the straight conclusion that reduced auditory startle

responses in offspring first noted at 10 mg/kg bw/day was not related to treatment. Therefore, they recommended to lower the NOAEL until such time as new and scientifically sound evidence is provided.

The applicant did not agree with the conclusions from the EFSA scientific opinion, and has provided statements, new statistical analyses of the data and historical control data. The applicant remains of the opinion that the reduction in startle response at 10 mg/kg bw/day is not toxicologically relevant and that the NOAEL should remain 10 mg/kg bw/day.

In the 10 mg/kg/day group males, V_{max} and V_{ave} were somewhat reduced on PND 20 and on PND 60, without attaining statistical significance. but the differences were not considered to be toxicologically significant since 1) the values were within the expected ranges (within the 25th and 75th percentiles of the WIL startle response historical control data), 2) none of the findings were statistically significantly different from the control group values, and 3) there were no other concomitant effects in other functional endpoints or neuropathology. T_{max} values in males were similar to those in the control group on PND 20 and 60. No test article-related effects on auditory startle response were noted in the 10 mg/kg/day group females. No test article-related effects on locomotor activity were observed in males and females at any dose level on PND 13, 17, 21 and 61. Evaluation of Biel maze data demonstrated no adverse effects on swimming ability or motivation and showed no impairments in learning and memory ability.

Overview of data submitted by the notifier:

Rebuttal of data evaluation record for Acetamiprid – Anonymous, M.D. 2006

This document was submitted to provide a response to the US EPA evaluation and conclusions on the developmental neurotoxicity test with acetamiprid. In addition, the document addresses specific questions raised by the EPA during a meeting in 2005. The US EPA had previously concluded the following: i) the offspring LOAEL is 10 mg/kg bw/day based on decreased maximum auditory startle response in males at PND20 and 60. The offspring NOAEL is 2.5 mg/kg bw/day; ii) in the dams no systemic toxicity was seen in the doses tested. The maternal NOAEL is 45 mg/kg bw/day, the highest dose tested. The maternal LOAEL is not established; iii) the study is classified Acceptable/Non-guideline and may be used for regulatory purposes, but does not satisfy the guideline requirement for a DNT in rats due to inadequacies in the assessment of motor activity and learning and memory in the offspring, and lack of positive control data. Specifically the normal developmental pattern in motor activity was not seen in male control animals and there was high variability in the learning and memory data.

The following responses were given in this rebuttal report:

- *Positive control data*: There are two positive control studies being submitted that demonstrate proficiency of the laboratory personnel and sensitivity of the methods used to detect behavioural and morphological changes in the developmental nervous system.
- *LOAEL for maternal toxicity is 45 mg/kg bw/day*: There were signs of maternal toxicity (decreased food intake and body weight) during gestation at the highest dose level (45 mg/kg bw/day). Other studies provide additional support for this conclusion.
- *NOAEL for auditory startle habituation test is 10 mg/kg bw/day*: Additional statistical analysis using a repeated measures analysis model supports the conclusion that effects were observed at 45 mg/kg bw/day, but not at 10 mg/kg bw/day. There were no effects on the intrasession pattern of auditory startle habituation at any dose level. At the 10 mg/kg bw/day dose level, the mean Vmax on each block of 10 trials was within the historical control range of the laboratory and close to or within the middle 50th percentile (i.e. 25-75%) range of the WIL historical control data. Thus, the apparent decrease in the overall mean 50-trial Vmax at 10 mg/kg bw/day should not be considered an adverse effect.
- *NOAEL for motor activity is greater than 45 mg*/kg bw/day: The motor activity data is interpretable and indicates that there are no adverse effects at any dose level. The EPA DER states that there is low confidence in the motor activity data because the control male animals do not demonstrate the normal ontogenic pattern, but dismisses the control female animals which show the “normal ontogenic pattern”, because the female values are much higher than the males at PND17. While there is evidence that daily testing of motor activity might result in an inverted U-shaped pattern of activity prior to weaning, this “normal ontogenic pattern” has not been consistently observed when

rat pups are tested every 4th day as required by EPA DNT Guidelines. EPA should be open to accepting data based on criteria relevant to EPA Guidelines for DNT studies.

- *NOAEL for learning and memory is greater than 45 mg/kg bw/day:* The learning and memory test used by WIL indicates no treatment-related effects on learning task, reversal learning task, and memory probe at any dose level. Compared to learning and memory tests commonly used by other laboratories (e.g. passive avoidance), the Biel maze paradigm used by WIL is much more challenging learning task. Although this leads to increased variability in the data, the overall pattern of the data can be evaluated and indicates no treatment-related effects on offspring.
- *DNT test results should be evaluated against criteria that are comparable and relevant to test guideline requirements:* EPA Guidelines for DNT studies require a minimum of 20 litters/dose group ad 4 dose groups resulting in a minimum of 640 pups born over a span of time. Thus, neurobehavioural testing for any one time point 9e.g. PND13) can occur across multiple days and over several hours within any one day. These experimental conditions will increase the variability of behavioural data.
- *Weight of evidence:* EPA OMB proposed guidance on risk assessment that emphasizes the importance of determining whether or not effects are adverse. Based on a weight-of-evidence approach to evaluating the DNT data in terms of overall pattern of effects and comparison against the middle 50% range of historical control data, the mid-dose (10 mg/kg bw/day) should be considered a NOAEL.

Acetamiprid DNT study: Statistical analysis for auditory startle response – Nguyen, J. 2007

A critical review was conducted by Chemistry and Exposure Brach (CEB) on the statistical analyses performed for the DNT study with acetamiprid (as performed in the rebuttal, Anonymous 2006). CEB was able to reproduce most of the results from the BioSTAT analysis (submitted as part of the rebuttal). The BioSTAT analyses included a multilevel analysis which incorporated random effects to model the repeated measured design of the DNT study. There were four separate analyses for four subsets of maximum auditory startle response data: PND20 males, PND60 males, PND20 females and PND60 females. For each of the four separate analyses, Dunnett's test was used to determine of there were significant differences between the group means of the treatment groups compared to the control group mean.

In general, CEB had concerns with the incorrect reporting of the results of some of the significance tests, as well as the selected model used to analyse the data which did not allow the statistical power of the DNT study design to be optimised.

In addition, CEB looked at the statistical analyses performed by Exponent (Li and Lau 2007). CEB had some statistical concern with these analyses: the Tukey sequential linear trend test was not correct. The p-values of the trend test were not associated with the Tukey linear sequential trend test; the p-values were from the global F-test in ANOVE which is used to determine if there is *at least one* group mean different from the other group means. Another problem was the incorrect elimination of the high dose data to produce separate subsets of data for testing the C-L-M sequence. CEB also believes that the multilevel model used is not the most appropriate since it did not combine the two PND periods.

CEB provided a statistical analysis which utilised in their opinion a more appropriate model given the structure of the data and also increased the power of the statistical tests to detect significant differences and trends. CEB used a mixed-effect model to perform an analysis of the combined data from males and females on PND20 and PND60. This analysis indicates that for male rats the mid-dose and high-dose group means were significantly different from the control group mean ($p=0.0321$ and $p=0.0015$, respectively), but the low-dose group mean was not significantly different from the control group mean. For female rats, there was no difference for any treatment group compared to the control group.

Acetamiprid DNT study: Response to EPA CEB Statistical analyses and weight of evidence supporting NOAEL of 10 mg/kg bw/day – Li, A.A., Lau, E. 2007b

In this document, a response is given to the CEB report (Nguyen 2007). The following points are made:

- There is no mistake in the Exponent statistical analyses: The Exponent analyses intended to provide the p-values for the F-statistics from the RANOVA and appropriate multiple comparison tests, because this is the approach typically employed by academic neurobehavioural scientists in their publications. This approach is identical to the approach taken in the paper published by the

International Life Science Institute (ILSI) expert panel on statistical analyses of DNT endpoints (Holson et al., 2007).
- Startle data from PND20 and PND60 cannot be combined in a comprehensive RANOVA because the scale of measurement is different at PND20 compared to PND60.
- Using the same statistical approach recommended by ILSI expert paper on statistical analyses for DNT studies (Holson et al. 2007), there were no statistically significant effects at 10 mg/kg bw/day dose level when genders are combined at each age group.
- EPA CEB's statistical analysis for the sequential linear trend test was not applied the same way for the mid-dose (3-dose sequential analyses) as for the high-dose (4-dose) or low-dose (2-dose).

The following weight of evidence is provided to support a NOAEL of 10 mg/kg bw/day for auditory startle response:
- The average Vmax auditory startle response for the 10 mg/kg bw/day dose level are near or within the narrower interquartile range of historical control study mean data at PND20 and PND60.
- The PND20 and PND60 male control startle levels are at or above the upper 75th percentile range of mean control values from 19-22 historical control studies.
- The percent decrease from control in startle amplitude at the 10 mg/kg bw/day dose group is within control variability for similar DNT studies conducted by EPA scientists around the time of the acetamiprid study.
- There are no effects of acetamiprid on the pattern of startle habituation at any dose level.
- There are no effects on FOB, motor activity, and neuropathology measured that are related to startle, such as reactivity to handling and sensory stimuli or histopathology findings in brain areas relevant to the 5-neuron startle circuit.

Therefore, the weight of evidence support the setting of the NOAEL at 10 mg/kg bw/day.

Characterization of critical toxicology endpoints for acetamiprid risk assessment: auditory startle habituation and maternal toxicity from the developmental neurotoxicology study – Li, A.A., Lau, E. 2007a

In this document it is stated that the EPA's response to the rebuttal and the revised DER did not discuss several principlepoints presented in the Nisso rebuttal. For example, comparison of the auditory startle intersession data from the 10 mg/kg/day group with a refined set of historical control data was not mentioned in the Agency's response. (There were 19-22 control studies with study start dates provided in Appendix 2 of the rebuttal). An apparent decrease can be achieved by an attenuated response from the dosed animals and/or a higher startled response from the controls. In this regard, evaluation of the historical controls is relevant. The historical control data along with the lack of statistical significance following multiple analyses by EPA and by Nisso; the relatively high variability associated with auditory startle amplitude in this study and in the literature; the lack of effect on the pattern of auditory startle habituation (i.e. no significant dose x time bin interaction); the lack of effects on females at the mid and high dose, and the absence of evidence of increased sensitivity in female offspring to acetamiprid; and the lack of related findings in FOB, neuropathology, and motor activity especially at the 45 mg/kg/day dose level; leads us to conclude that the 10 mg/kg/day is a no-observed-*adverse*-effect level.

In addition, we believe that the substantial decreases in food consumption together with the smaller but significant decreases in body weight and body weight gain during gestation at the 45 mg/kg/day dose level is clear evidence of maternal toxicity following gavage doses of acetamiprid. These effects are consistent with other developmental and reproduction studies evaluating maternal toxicity as well as with the results of an acute neurotoxicity study in adults that resulted in a LOAEL of 30 mg/kg/day based on
decreased motor activity following a single gavage dose.

Acetamiprid: Data evaluation record for acetamiprid developmental neurotoxicity study and EPA response to rebuttals submitted by Nisso America – US EPA 2008

EPA concluded the following:
- Auditory startle response: Considering the statistical analyses, the historical control data and the acetamiprid toxicity profile, HED determined to establish the offspring LOAEL at 45 mg/kg bw/day based on decreased body weight and body weight gains in males and females, decreased pre-weaning survival (PND 0-1) and decreased maximum auditory startle response in males on PND20 and PND60. The offspring NOAEL is 10 mg/kg bw/day. The following conisderations were made:

  - Statistical evaluation of the mid-dose response was dependent upon combining age data; without combining the age data, only the high-dose group emerges as statistically different from the controls at PND20 and PND60. The equivocal nature of the statistical analysis includes a lack of internal consensus on whether data from different times should be combined. Taken together, these points weaken the statistical relevance of the mid-dose response.
  - Review of HCD (in relation to the concurrent controls and the mid-dose group) call into question the effect seen in the mid-dose group. Graphic representation of the study response means alongside the range of HCD means and standard deviations shows an effect at the high dose only in males at PND20 and PND60.
  - Establishement of the LOAEL at 45 mg/kg bw/day is substantiated by other adverse effects seen in the data base (i.e. decreased body weight and body weight gains and decreased preweaning survival (PND 0-1) in pups).
  - There was no alteration in necropsy, brain weight, or brain morphometrics at the mid-dose.
- Maternal toxicity: HED now agrees with the registrants conclusion that 45 mg/kg bw/day is the maternal LOAEL, as decreases in food consumption and body weight were seen at this dose level.

<u>Response to EFSA PPR Scientific Opinion on the Acetamiprid DNT study - Li 2014a, Addendum to response to EFSA PPR – Li 2014b</u>

The following key observations and conclusions are discussed in this report:

- EFSA PPR's review was based on incomplete information of the ACE DNT.
- The EFSA PPR and EPA's Data Evaluation Record (DER) statements on variability do not reflect the current understanding based on greater experience, as well as retrospective analysis of DNT studies.
- The ACE DNT study is a well-conducted study that appears to be considered a poor quality study based on variability that is comparable to, if not better than, other DNT guideline studies.
- The data are fully interpretable based on robust repeated measures analyses of variance and visual comparison with the interquartile range of the historical control data (middle 50%).
- The auditory startle, motor activity, and learning and memory data indicate no effects of ACE at 2.5 and 10 mg/kg bw/day. 6. Benchmark dose (BMD) analysis of the startle data results in reference points (equivalent to no-observed-adverse-effect level [NOAELs]) of 12.6 and 13.1 which further supports the NOAEL of 10 mg/kg bw/day selected by both EPA and the Joint Meeting on Pesticide Residues (JMPR).
- There is no justification for additional conservatism in evaluating the ACE DNT study due to high variability and lack of normal developmental pattern in motor activity of controls because:
  - The variability in the ACE DNT study is comparable if not better than other submitted DNT studies
  - The scientific evidence for a consistent developmental pattern in motor activity is weak due, in part, to high inherent variability at PND 13 and 17.
- Repeating the DNT study is unlikely to produce data with less variability and would be a very poor use of a large number of animals.

Conclusion:

The ACE DNT study is a well-conducted and interpretable study that satisfies DNT guideline requirements. The variability of this study is comparable to, if not better than, other DNT guideline studies based on retrospective analyses of DNT studies. Repeating the DNT study is unlikely to produce data with less variability and would be a very poor use of a large number of animals. The data have been analyzed rigorously and in comparison with historical control data from the same laboratory. The new statistical analyses combine males and females together, which increases the sample size from 10 to 20 and takes into account repeated measures of each individual animal. The NOAEL for auditory startle and learning and memory is 10 mg/kg bw/day. This NOAEL is further supported by BMD analysis. There were no effects at any dose level for motor activity. The overall NOAEL for offspring toxicity in males and females is 10 mg/kg bw/day.

<u>EFSA PPR 2015: response of PPR panel to Nippon Soda report on potential DNT ACE 2015</u>

In this document, the EFSA PPR Panel responds to Li et al (2014a,b) and the arguments given to set the NOAEL at 10 mg/kg bw/day. The Panel still notes that the data available at this point do not allow any firm conclusion to be drawn since important endpoints such as motor activity, learning and memory evaluation could not be properly assessed and that insufficient arguments support the straight conclusion that reduced auditory startle responses in offspring first noted at 10 mg/kg bw/day was not related to treatment. In consequence the PPR Panel recommended the lower NOAEL (2.5 mg/kg bw/day) until such time as new and scientifically sound evidence is provided. The PPR Panel admits to have taken a precautionary approach. The current in vivo DNT studies may not be sensitive enough to detect subtle effects, such as those on cognition, behaviour or brain morphometry, and might lead to false negatives. Therefore, the scientific assessment of in vivo DNT studies should be conservative in their application.

RMS:
Different statistical analyses were performed after the initial DNT study report had been finished. RMS, in line with EFSA, does not support these new data as they represent a post hoc analysis. When writing a study plan, a hypothesis and matching statistical analysis method should be chosen. Performing different statistical tests afterwards to change to a different hypothesis does not seem justified.
In addition, in the new statistical analyses, male and female rats were combined to increase statistical power. Male and female rats are tested separately for a reason and should not be so easily combined as it is unclear if a sex-dependent effect can be excluded. Furthermore, female rats showed less effect on Vmax, therefore, by combining the results for male rats with female rats, the effect seen in male rats will be diminished..
As for the historical control data. On PND20, the male rats control group has a Vmax value of 214 which is above the historical control data (min-max:103-211). Therefore, these data are difficult to interpret. The statistical significant effect seen in the 45 mg/kg bw/day group could be just attributed to the high value in the control group.
However, for PND60, the male rats control group has a Vmax value of 210 which is within the HCD (min-max: 87-247). A statistically significant reduction ($p<0.05$) was seen in the 45 mg/kg bw/day; the Vmax value was decreased by 53%. For the 10 mg/kg bw/day male rats, a reduction of 40% in Vmax was seen compared to the control, however, this did was not a statistically significant decrease. However, the conclusion of the study report / notifier that this reduction in the 10 mg/kg bw/day group should be regarded as non-treatment related is not supported by RMS.
RMS is in agreement with the EFSA PPR panel conclusions (See appendix 1). Uncertainties are still present regarding this toxicological endpoint and RMS proposes to lower the NOAEL to 2.5 mg/kg bw/day. RMS also proposes to discuss this issue during an expert meeting.

In the Peer review of the pesticide risk assessment of the active substance acetamiprid report by EFSA (2016) (http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4610/full) it is stated that it could not be concluded that reduced auditory startle responses in offspring at 10 mg/kg bw were not related to treatment, resulting in a NOAEL of 2.5 mg/kg bw per day.