SUPPLEMENTAL MATERIAL

A Critical Review of Neonicotinoid Insecticides for Developmental Neurotoxicity

by

LP Sheets, AA Li, DJ Minnema, RH Collier, MR Creek and RC Peffer

Critical Reviews in Toxicology, 2015

Appendix I: Neuropathology and Morphometry Procedures for the guideline DNT study with Imidacloprid

Neuropathology and Morphometry – PND 11

Brains were divided into eight coronal blocks (including forebrain, cerebrum, midbrain, cerebellum, pons and medulla oblongata) for microscopic examination (Fig. 1). Each block was processed according to standard procedures for paraffin embedding and sectioned at 5 μm, with slides prepared with hematoxylin and eosin (H&E), luxol fast blue/cresyl violet and Sevier-Munger stains.

Seven linear measurements were taken from brain sections (levels 4, 5 and 7), as follows:
(1) **Frontal cortex thickness** (forebrain); the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm;
(2) **Parietal cortex thickness** (forebrain); the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm;
(3) **Caudate putamen horizontal width** (forebrain; maximum cross-sectional width) - performed on the coronal section, taken at the level of the optic chiasm;
(4) **Corpus callosum thickness** (forebrain) - at the mid-point, from the section taken at the level of the optic chiasm;
(5) **Hippocampal gyrus thickness** (midbrain) - the full width of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter;
(6) **Cerebellum height** (cerebellum/pons) - from the roof of the fourth ventricle to the dorsal surface; and
(7) **External germinal layer** (cerebellum) thickness (cerebellum/pons; PND 11 only) - due to considerable regional differences in thickness, multiple areas were measured over the dorsum of the cerebellum.

Neuropathology and Morphometry – PND 75

The brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected from each animal and post-fixed in 10% buffered formalin. The fixed brain was weighed upon removal from the skull, before placement into formalin.

Vernier calipers were used to obtain two linear measurements of the brain, as described for the PND 11 pups. The following tissues from control and high-dose perfused animals, and any gross lesions collected at necropsy, were routinely processed for microscopic examination:

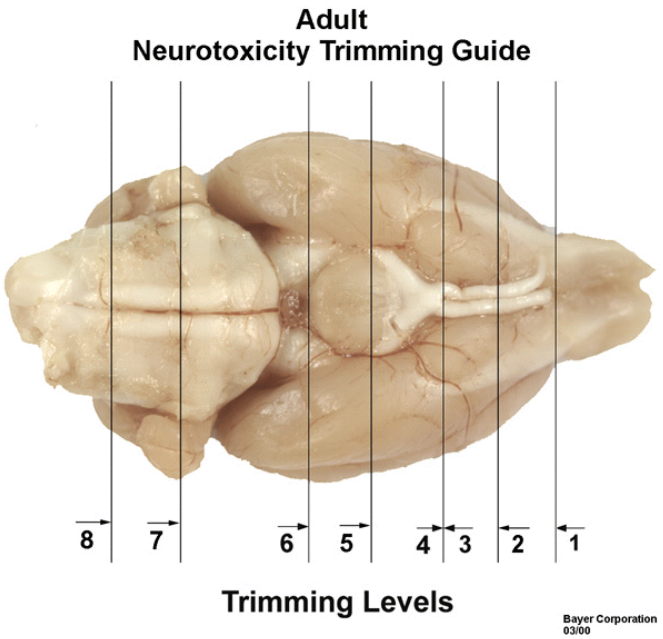
- 8 coronal sections of the brain, including forebrain, center of cerebrum, midbrain, cerebellum, pons, and medulla oblongata;
- 4 sections from 3 levels of the spinal cord (cervical, thoracic, and lumbar) and the cauda equina were embedded in paraffin and stained with H&E, luxol fast blue/cresyl violet, and Sevier-Munger stains;
- Eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin and stained with H&E;
- Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings, gasserian ganglia, and peripheral nerve tissues (sciatic, tibial, and sural) were embedded in glycol methacrylate resin (GMA), sectioned at 2-3 μm, and stained with a modified Lee's stain.

Microscopic evaluation and measurements of brain tissue were performed as described for the PND 11 animals, with modifications appropriate for the adult.

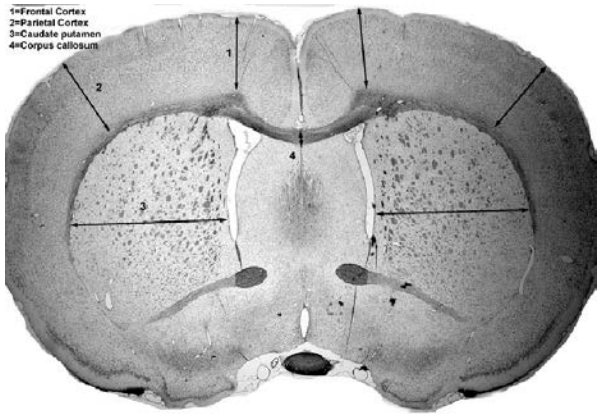
The CHECKED (X) tissues will be evaluated for adult offspring.

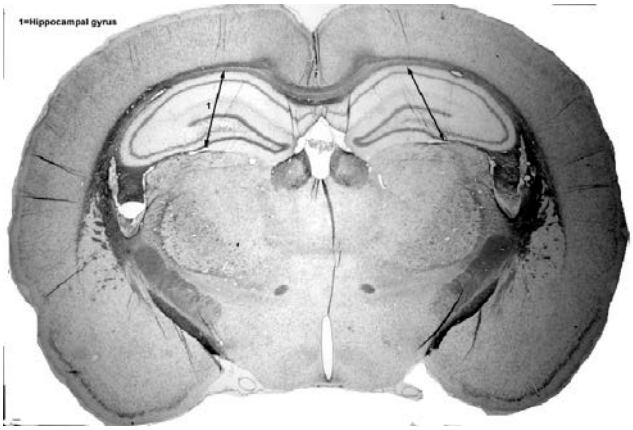
CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM	
	BRAIN (8 levels, including)		**PERIPHERAL NERVES**
x	Forebrain	x	Sciatic
x	Center of cerebrum	x	Tibial
x	Midbrain	x	Sural
x	Cerebellum		
x	Pons		
x	Medulla oblongata		
	SPINAL CORD		**OTHER**
x	Cervical swelling	x	Lumbar dorsal root ganglion
x	Thoracic	x	Lumbar dorsal root fibers[①]
x	Lumbar swelling	x	Lumbar ventral root fibers[①]
	OTHER	x	Cervical dorsal root ganglion
x	Gasserian Ganglion	x	Cervical dorsal root fibers[①]
x	Optic nerves	x	Cervical ventral root fibers[①]
x	Eyes	x	Gastrocnemius muscle
x	Cauda equina		

[①] Dorsal and ventral root fibers were evaluated as they were generally included with the ganglion.

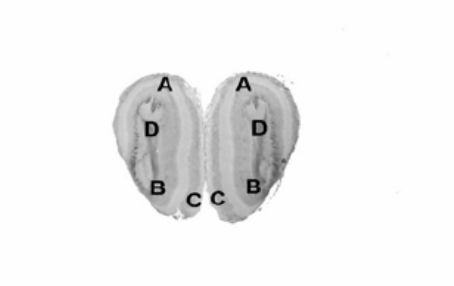


Levels 4 and 5 (Level 7 is not shown)

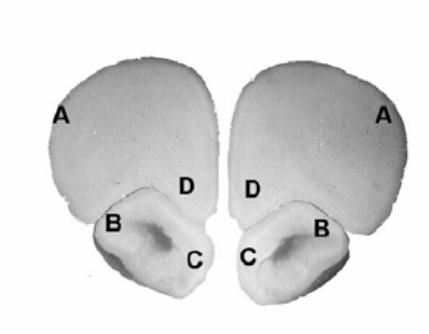




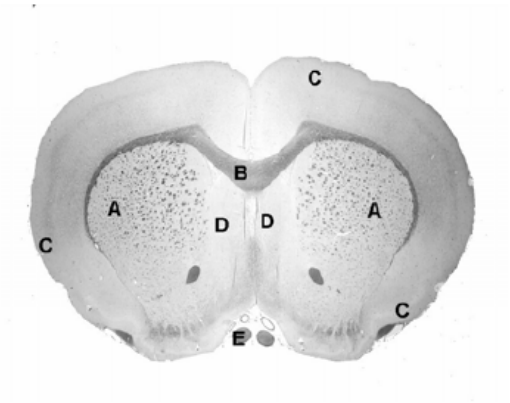
RAT BRAIN LEVEL SECTIONS 1 - 8



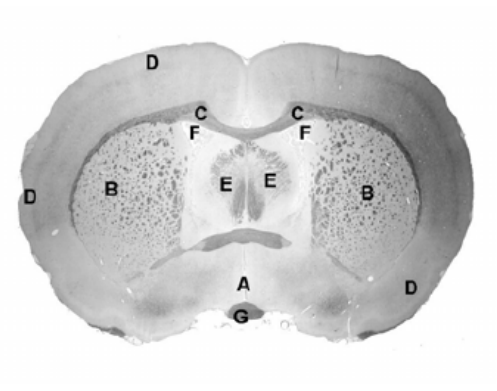
OLFACTORY BULB SECTION
Figure 1. Rat Brain Level 1
A = External Plexiform Level
B = Granular Cell Layer
C = Olfactory Nerve Layer
D = Olfactory Ventricle



OLFACTORY REGION SECTION
Figure 2. Rat Brain Level 2
A = Motor Cortex
B = Olfactory Nucleus
C = Olfactory Ventricle
D = Orbital Cortex

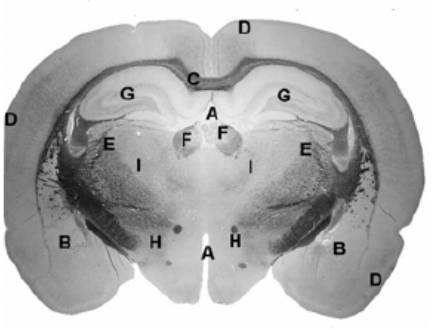


FOREBRAIN (optic nerve) SECTION
Figure 3. Rat Brain Level 3
A = Caudate Putamen
B = Corpus Callosum
C = Cortex
D = Lateral Septal Nucleus
E = Optic Nerves

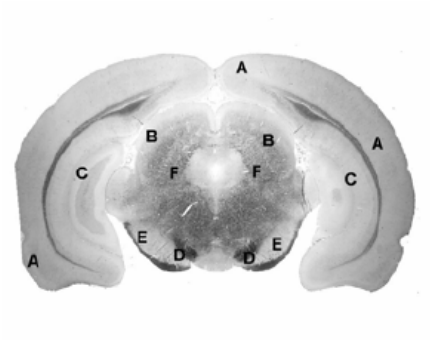


FOREBRAIN (Optic Chiasma) SECTION
Figure 4. Rat Brain Level 4
A = 3rd Ventricle
B = Caudate Putamen
C = Corpus Callosum
D = Cortex
E = Lateral Septal Nucleus
F = Lateral Ventricle
G = Optic Chiasma

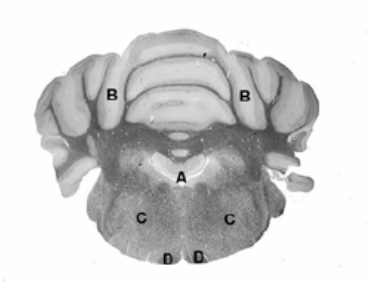
RAT BRAIN LEVEL SECTIONS 1 - 8



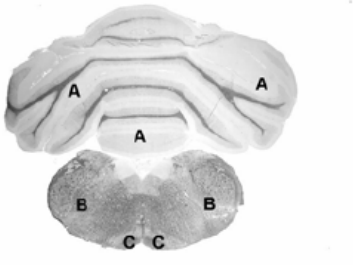
MIDBRAIN SECTION
Figure 5. Rat Brain Level 5
A = 3rd Ventricle
B = Amygdaloid Nucleus
C = Corpus Callosum
D = Cortex
E = Geniculate Nucleus
F = Habenular Nucleus
G = Hippocampus
H = Hypothalamus
I = Thalamic Nucleus



MESENCEPHALON SECTION
Figure 6. Rat Brain Level 6
A = Cortex
B = Geniculate Nucleus
C = Hippocampus
D = Mammillary Nucleus
E = Substantia Nigra
F = Thalamus



CEREBELLUM/PONS SECTION
Figure 7. Rat Brain Level 7
A = 4th Ventricle
B = Cerebellum
C = Medulla Oblongata
D = Pyramidal Tract



CEREBELLUM/MEDULLA OBLONGATA SECTION
Figure 8. Rat Brain Level 8
A = Cerebellum
B = Medulla Oblongata
C = Pyramidal Tract

Appendix II: Occupational and Residential Exposure and Risk Assessment for Imidacloprid and Flumethrin Formulated Flea Collars (from Lunchick, 2010).

SUMMARY
An assessment of the potential exposure resulting from the use of PNR 1427 flea collars containing imidacloprid as an active ingredient was calculated for post application contact with treated cats, small dogs, and large dogs. The small flea collars weigh 12.5 grams and contain 1.25 grams imidacloprid and are intended for use on cats or small dogs up to 8 kg in weight. The large flea collar is intended for dogs weighing over 8 kg and weighs 45.0 grams, with 4.5 grams of imidacloprid.

The daily application rate of imidacloprid from the collars to the pets was determined based on release rate kinetic studies. Approximately 40% of the imidacloprid is released over an eight month period at a rate that slightly decreases over the period. During the first month of use when the release rate is slightly greater, the daily rate of release of imidacloprid from the small collar is 8.35 mg/day and from the large collar it is 22.7 mg/day. Fur kinetic data supported the release rate kinetics.

The revised HED guidelines for pet exposure and risk assessments were used with the release rate data to estimate the exposure and risks to both adults and small children contacting pets wearing the collars. Margins of Exposure (MOEs) for adult post application contact with the treated pets ranged from 2,200 to 4,400. The aggregate oral and dermal MOEs for small children ranged from 1,300 to 2,600.

INTRODUCTION
PNR 1427T and PNR 1427G are antiparasitic collars for flea and tick control for cats and dogs, respectively. Both collars contain 10% imidacloprid as an active ingredient. The collars will be used for an eight month period and therefore would be used once per year in many regions of the United States and no more than twice per year in the warmer regions of the country. The purpose of this exposure and risk assessment is to support the registration application for PNR 1427T and PNR 1427G.

PNR 1427 Release Kinetics
The flea collar contains a patented Bayer polymer matrix system that ensures the active ingredients are slowly and continuously released in low concentrations from the collar towards the animal. This minimizes peak concentrations and ensures that acaricidal / insecticidal concentrations are present in the dog or cat's haircoat during the entire eight month efficacy period. The active substances spread from the site of direct contact over the entire skin surface. Bayer conducted several studies to quantify the release rate of imidacloprid from the collar and also to measure residues on the animal's fur over time.

Toxicology (imidacloprid)
HED selected a NOEL of 10 mg/kg/day from the rat developmental toxicity study as the most appropriate endpoint for short-term oral, dermal, and inhalation exposure risk assessments. A NOEL of 9.3 mg/kg/day from the rat subchronic neurotoxicity toxicity study was selected as the most appropriate endpoint for intermediate-term oral, dermal, and inhalation exposure risk assessments. For the purpose of this assessment the intermediate-term NOEL of 9.3 mg/kg/day is used to address both short-term and intermediate-term exposures. Based on the results of the dermal absorption study a dermal absorption adjustment of 4.2% is used for the dermal exposure risk assessment.

POST-APPLICATION EXPOSURE AND RISK

Post-application exposure to imidacloprid contained in the flea collars is estimated based on the guidance provided in the "Current Guidance for Residential Exposure Risk Assessment for Pet Insecticide Treatments" of 14 January 2009. Post application exposure occurs from pet owner contact with the dog or cat wearing the flea collar. The guidance for pet flea collar assessments assumes that the daily dose on the day of application is based on 20% of the maximum application rate being available on the pet's body and transferred to adults and children as a dislodgeable residue.

As previously stated, the cat/small dog flea collar contains 1.25 grams of imidacloprid and the large dog flea collar contains 4.50 grams of imidacloprid. Based on the kinetic data, the daily release rates are 8.35 mg/day and 22.7 mg/day of imidacloprid from the small and large collars, respectively.

The small flea collar will be used to assess exposure to a 9 lb cat and the large flea collar will be used to assess exposure to a 30 lb dog. The HED defaults for the surface area of the cat and dog are 2737 cm^2 for the cat and 5986 cm^2 for the dog. The Agency uses the single hug method to assess post application dermal exposure with a 5625 cm^2 surface area for 70 kg adults and 1875 cm^2 for a 15 kg 3 year old child.

Post Application Dermal Exposure to Cats

Adult: 8.35 mg a.i. x 0.2 ÷ 2737 cm^2 x 5625 cm^2 x 0.042 ÷ 70 kg = 2.1 x 10^{-3} mg/kg/day

Child: 8.35 mg a.i. x 0.2 ÷ 2737 cm^2 x 1875 cm^2 x 0.042 ÷ 15 kg = 3.2 x 10^{-3} mg/kg/day

Post Application Dermal Risk to Cats

Adult: 9.3 mg/kg/day ÷ 2.1 x 10^{-3} mg/kg/day = 4,429

Child: 9.3 mg/kg/day ÷ 3.2 x 10^{-3} mg/kg/day = 2,906

Post Application Dermal Exposure to Dogs

Adult: 22.7 mg a.i. x 0.2 ÷ 5986 cm^2 x 5625 cm^2 x 0.042 ÷ 70 kg = 2.6 x 10^{-3} mg/kg/day

Child: 22.7 mg a.i. x 0.2 ÷ 5986 cm^2 x 1875 cm^2 x 0.042 ÷ 15 kg = 4.0 x 10^{-3} mg/kg/day

Post Application Dermal Risk to Dogs

Adult: 9.3 mg/kg/day ÷ 2.6 x 10^{-3} mg/kg/day = 3,577

Child: 9.3 mg/kg/day ÷ 4.0 x 10^{-3} mg/kg/day = 2,325

Post application incidental oral exposure is only calculated for the three year old child. An equilibrium approach is taken by HED and assumes that 20% of the available residues are available for transfer to the hands of the child. The surface area of the hands that provide oral contact is 20 cm^2 and that the saliva removes 50% of the residue that is transferred to the hands. The oral NOAEL of 9.3 mg/kg/day is used to calculate the MOEs for imidacloprid. The oral incidental exposure to the child is calculated as follows:

Post Application Incidental Oral Exposure to Cats

Child: 8.35 mg a.i. x 0.2 ÷ 2737 cm^2 x 20 cm^2 x 0.5 ÷ 15 kg = 4.07 x 10^{-4} mg/kg/day

Post Application Incidental Oral Risk to Cats

Child: 9.3 mg/kg/day ÷ 4.07 x 10^{-4} mg/kg/day = 22,850

Post Application Incidental Oral Exposure to Dogs
Child: 22.7 mg a.i. x 0.2 ÷ 5986 cm^2 x 20 cm^2 x 0.5 ÷ 15 kg = 5.06 x 10^{-4} mg/kg/day

Post Application Incidental Oral Risk to Dogs
Child: 9.3 mg/kg/day ÷ 5.06 x 10^{-4} mg/kg/day = 18,380

Post Application Exposure and Risk Summary
Adult post application exposure resulting from contact with the treated animal is by the dermal route of exposure resulting from the transfer of imidacloprid residues from the animal's fur to the adult's skin. The imidacloprid exposures were calculated to be 2.1 x 10^{-3} mg/kg/day for animals wearing the small collar and 2.6 x 10^{-3} mg/kg/day for animals wearing the large collar. The resultant MOEs were 4,429 and 3,577 for the small and large collars, respectively.

The total post application exposure and risk to small children contacting pets wearing the flea collars results from both the dermal contact and the incidental ingestion of imidacloprid residues from the hands. For children contacting pets wearing the small collars, the aggregate post application exposure to imidacloprid is 3.61 x 10^{-3} mg/kg/day and 4.51 x 10^{-3} mg/kg/day with the large collar. The dermal and oral NOAEL for imidacloprid is 9.3 mg/kg/day and the aggregate MOEs for the small child are 2,576 with the small collar and 2,062 with the large collars.

CONCLUSIONS
An assessment of the potential exposure resulting from the use of PNR 1427 flea collars containing imidacloprid as an active ingredient was calculated for post application contact with treated cats, small dogs, and large dogs. The small flea collars weigh 12.5 grams and contain 1.25 grams imidacloprid and are intended for use on cats or small dogs up to 8 kg in weight. The large flea collar is intended for dogs weighing over 8 kg and weighs 45.0 grams and contains 4.5 grams of imidacloprid.

The daily application rate of imidacloprid from the collars to the pets was determined based on release rate kinetic studies. Approximately 40% of the imidacloprid is released over an eight month period at a rate that slightly decreases over the period. During the first month of use when the release rate is slightly higher the daily rate of release of imidacloprid from the small collar is 8.35 mg/day and from the large collar it is 22.7 mg/day. Fur kinetic data supported the release rate kinetics.

The revised HED guidelines for pet exposure and risk assessments were used with the release rate data to estimate the exposure and risks to adults applying the collars and to both adults and small children contacting pets wearing the collars. MOEs for adult post application contact with the treated pets ranged from 2,200 to 4,400. The aggregate oral and dermal MOEs for small children ranged from 1,300 to 2,600.

Lunchick C (2010). Occupational and residential exposure and risk assessment for PNR 1427 dog and cat collars formulated with imidacloprid and flumethrin. Bayer HealthCare Report No. 33861.

Suppl. Table 1. Coefficients of Variation (CV) for Total Session Average Peak Startle Amplitude from DNT-like studies published by EPA scientists

DNT Study	CV Weaning (Habituation[a]) (percent)	CV Adult (Blank Prepulse) (percent)	Reference
Maternal Separation (n=8)		42	Stanton et al. (1992)
IDPN control (n=12)	39	56	Crofton et al. (1993)
MAM control (n=8)		42	Goldey et al. (1994)
Methylmercury control (n=8)		58	Goldey et al. (1994)
Methanol control (n=6)		58	Stanton et al. (1995)
Propylthiouracil control (n=6)	27	58	Goldey et al. (1995a)
Aroclor 1254 (n=8)	27	55	Goldey et al. (1995b)
Aroclor 1254 & thyroxine replacement (n=8)	27	27	Goldey and Crofton (1998)
Iodine levels (assume n=12 for males based on figure legend for cognitive test)		60	Gilbert et al. (2013)
Average	30	51	

Note: DNT - developmental neurotoxicity
IDPN - 3,3'-iminodiproprionitrile (a within-litter study design)
MAM - methylazoxymethanol
PND - post-natal day

[a] The startle reflex with blank prepulse in the reflex modification design is comparable to the startle reflex in the habituation design. The CVs were estimated from the figures which reported total session peak amplitude and the SEM. Sample size (n) was used to calculate SD from SEM [SD = SEM * SQRT(n)]. CV=SD x 100/mean

REFERENCES

Crofton KM, Peele DB, Stanton ME. Developmental neurotoxicity following neonatal exposure to 3,3'-iminodipropionitrile in the rat. Neurotoxicol Teratol 1993; 15:117−129.

Gilbert ME, Hedge JM, Valentín-Blasini L, Blount BC, Kannan K, Tietge J, Zoeller RT, Crofton KM, Jarrett JM, Fisher JW. An animal model of marginal iodinedeficiency during development: the thyroid axis and neurodevelopmental outcome. Toxicol Sci. 2013; 132(1):177-195.

Goldey ES, O'Callaghan JP, Stanton ME, Barone S, Crofton KM. Developmental neurotoxicity: Evaluation of testing procedures with methylazoxymethanol and methylmercury. Fundam Appl Toxicol 1994; 23:447−464.

Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. Toxicol Appl Pharmacol 1995a; 135: 67-76.

Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduced circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 1995b; 135: 77-88.

Goldey ES and Crofton KM. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. Toxicol Sci 1998; 45(1): 94-105.

Stanton ME, Crofton KM, Gray LE, Gordon CJ, Boyes WK, Mole ML, Peele DB, Bushnell PJ. 1995. Assessment of offspring development and behavior following gestational exposure to inhaled methanol in the rat. Fund. Appl. Toxicol. 1995; 28:100−110.

Stanton ME, Crofton KM, Lau C. Behavioral development following daily episodes of mother-infant separation in the rat. Fundam Appl Toxicol. 1992;19(3):474-477.

Suppl. Table 2. Brain Morphometry Measurements Adjusted for Terminal Body Weight – Thiamethoxam DNT Study

	Day 11 – Adjusted for Body Weight				Day 62 – Adjusted for Body Weight			
	0 ppm Males	**4000 ppm Males**	**0 ppm Females**	**4000 ppm Females**	**0 ppm Males**	**4000 ppm Males**	**0 ppm Females**	**4000 ppm Females**
Level 2 – Frontal Cortex – Height	5.50	5.83	5.67	6.04	6.68	6.51	6.41	6.47
Level 2 – Frontal Cortex – Width	4.29	4.53	4.44	4.74	4.84	5.00	4.72	4.79
Level 3 – Dorsal Cortex 1- Thickness	1.29	1.38	1.32	1.39	1.58	**1.40****	1.54	**1.41***
Level 3 – Dorsal Cortex 2- Thickness	1.43	1.48	1.46	1.47	1.89	1.73	1.79	1.70
Level 3 – Piriform Cortex – Thickness	1.02	1.12	1.03	1.07	1.51	1.38	1.40	1.42
Level 3 – Hippocampus – Length from Midline	2.64	2.62	2.58	2.46	2.50	2.45	2.50	2.64
Level 4 – Dorsal Cortex – Thickness	1.19	1.18	1.21	1.19	1.54	1.35	1.40	1.30
Level 4 – Piriform Cortex – Thickness	0.96	1.02	0.95	1.01	1.29	1.33	1.28	1.38
Level 4 – Corpus Callosum – Thickness	0.61	0.52	0.57	0.50	0.42	0.40	0.39	0.42
Level 4 – Thalamus – Height	4.91	4.94	5.05	4.97	5.41	5.26	5.18	5.26
Level 4 – Thalamus – Width	7.43	7.46	7.58	7.30	8.83	8.53	8.44	**8.04****
Level 4 – Thalamus/Cortex – Overall Width	12.31	12.21	12.43	12.08	14.79	14.12	14.44	**13.55****
Level 4 - Hippocampus – Length from Midline	3.89	3.91	4.00	3.81	4.13	4.04	4.04	3.91
Level 4 – Hippocampus – Width Dentate Gyrus	0.48	0.46	0.47	0.48	0.62	0.60	0.60	0.60
Level 4 – Hippocampus – Length Dentate Gyrus	1.53	1.44	1.41	1.43	1.63	1.71	1.70	1.64

	Day 11 – Adjusted for Body Weight				Day 62 – Adjusted for Body Weight			
	0 ppm Males	**4000 ppm Males**	**0 ppm Females**	**4000 ppm Females**	**0 ppm Males**	**4000 ppm Males**	**0 ppm Females**	**4000 ppm Females**
Level 5 – Dorsal Cortex – Thickness	1.08	1.09	1.10	1.11	1.41	1.31	1.40	1.33
Level 5 – Piriform Cortex – Thickness	1.05	1.10	1.08	1.10	1.24	1.23	1.23	1.20
Level 5 – Thalamus Width	6.77	6.50	6.59	7.04*	8.10	7.51**	7.86	7.31**
Level 5 – Hippocampus Width Dentate Gyrus	0.61	0.64	0.61	0.66	0.76	0.69	0.74	0.75
Level 5 – Hippocampus Width Overall	1.19	1.23	1.26	1.29	1.56	1.45*	1.53	1.49
Cerebellum – Height	3.68	3.93	3.82	4.01	5.59	5.36	5.48	5.37
Cerebellum – Length	4.26	4.24	4.11	4.36	7.18	6.94	6.75	6.75
Cerebellum – Preculminate Fissure – Thickness of Outer Granular Layer	40.1	41.5	42.1	39.3	ND	ND	ND	ND
Cerebellum – Preculminate Fissure – Thickness of Molecular Layer	68.6	73.7	73.1	73.0	213.8	202.1	214.1	196.4
Cerebellum - Preculminate Fissure – Thickness of Inner Granular Layer	126	141	138	141	179	161	185	189
Cerebellum – Prepyramidal Fissure – Thickness of Outer Granular Layer	47.7	48.4	45.9	45.0	ND	ND	ND	ND
Cerebellum – Prepyramidal Fissure – Thickness of Molecular Layer	59.8	59.1	59.2	60.5	218.7	198.9	211.5	196.2
Cerebellum – Prepyramidal Fissure – Thickness of Inner Granular Layer	128	133	122	137	170	156	151	158

ND = not determined. Values in mm, except for thickness of cerebellum inner granular layer, outer granular layer and molecular layer (μm)
*p<0.05; **p<0.01 (ANCOVA on terminal body weight + Student's t-test)

Suppl. Table 3. Historical Control Data – Brain Morphometry Measurements at Day 62 – Thiamethoxam DNT Study

	Males – Range	Females – Range
Level 3 – Dorsal Cortex 1 – Thickness	1.25 – 1.58	1.31 – 1.51
0 ppm – Mean	1.58	1.51
4000 ppm – Mean	1.40	1.46
Level 4 – Thalamus – Width	NA	7.6 – 8.46
0 ppm – Mean	NA	8.46
4000 ppm – Mean	NA	8.01
Level 4 - Thalamus/Cortex – Overall Width	NA	13 – 14.5
0 ppm – Mean	NA	14.5
4000 ppm – Mean	NA	13.5
Level 5 – Thalamus Width	7.33 – 8.11	7 – 7.88
0 ppm – Mean	8.11	7.88
4000 ppm – Mean	7.49	7.28
Level 5 – Hippocampus – Width Overall	1.34 – 1.55	NA
0 ppm – Mean	1.55	NA
4000 ppm – Mean	1.45	NA

Values (mm) are not adjusted for terminal body weight. Range of historic controls = means from 5 studies that were initiated in 2000 – 2002, including the control group from the thiamethoxam study. Mean values from control (0 ppm) and high-dose (4000 ppm) groups in the thiamethoxam study (unadjusted) are also shown, for comparison. NA = not applicable.

Suppl. Table 4. Selected literature studies for the effects of postnatal or gestational nicotine exposure in rats on motor activity

	Exposure	Dose	Route	Weight loss?	Age of testing	Activity Test	Motor activity results		
							Horizontal activity	Habituation	Rearing
Huang et al. 2007	PND 1-7	6 mg/kg/day (2 mg/kg 3x/day)	Gavage	Yes	PND 35 and 70	10 min open field	↓ (35) or Ɵ (70)	N.M.	↓
Thomas 2000	PND 4-9	6 mg/kg/day (1.5 mg//kg 4x/day)	Gastronomy tube	No[1]	PND 18-19	1 hr open field	↑	Ɵ	N.M.
Peters et al. 1979	2 mo prior to mating until birth (cross fostered) or weaning (not cross fostered)	6 mg/kg/day	Drinking water	Yes (males only birth weight reported)	PND 60-80	24-hr home cage	*Not cross-fostered:* ↑ day; ↓ night; *cross-fostered* ↑ day	N.M.[2]	N.M.
Newman et al. 1999	Gestation (19 days) until PND 16	0.75, 1.5, 3.0 mg/kg/day	Osmotic minipumps in dams	Yes at birth (↑ PND 14 due to ↓ litter size)	PND 14 and 21	40 min open field	↑ 0.75 and 3.0 at PND 14 but not PND 21	No habituation in controls or treated	N.M.
LeSage 2006	GD 4 -delivery	2 mg/kg/day (0.03 mg/kg every 14 min 16 hr/day)	i.v. cannula to jugular vein	Yes	PND 19-21	30 min open field	↓ (n.s.)	↓ activity first 5-min	↓ (n.s.)
Schneider 2012	Gestation: 3 weeks before mating until delivery	4.2 mg/kg	Drinking Water	Yes, and pair-fed control group included	PND 25-40 (testing on 9 consecutive days)	1 hr open field	↑ (4^{th} to 9^{th} day of testing	Less habituation after first 15 min period	N.M.
Tizabi 2000[3]	GD 4 to delivery	9 mg/kg/day (previous study no effects 3 & 6 mg/kg/day)	s.c. via Osmotic mini pump	Yes	PND 20-24 (daily for 4 consecutive days)	1 hr open field	Ɵ [4]	N.M.	↑

n.s. not statistically significant; N.M. not measured

Ɵ signifies endpoint was measured but no effects were reported

[1] Some adjustments to diet were made

[2] Day and night pattern within 24-hr session was measured so "habituation", as defined by EPA guidelines within a shorter session, was measured

[3] Litter was not used as the experimental unit.

[4] The authors state there was a numerical increase in horizontal activity that was not statistically significant, but based on inspection of figure and final conclusions there are no overall effects.