Response of PPR Panel to Bayer Crop Science (BCS) report from June 2014 on the potential DNT of imidacloprid

The major point of disagreement between BCS and the PPR Panel is the interpretation of morphometric data. BCS contends that imidacloprid caused no morphometric effects in their developmental neurotoxicity (DNT) study; meanwhile BCS recognizes that their morphometric investigations were limited to the high dose and control groups. By contrast, the PPR Panel considered the morphometric data a source of concern and the lack of intermediate and low dose data as important missing information. Using the available data it is impossible to assess a dose-response relationship for morphometric changes. The level of uncertainty identified in brain morphometry precludes a robust characterization of the DNT potential of imidacloprid.

The BCS report (page 5) states that differences in brain morphometry at the highest dose level were spurious and unrelated to treatment. The PPR Panel disagrees with this interpretation and requested more data (particularly regarding the intermediate dose level). However, this relevant information was not provided and hence the concerns raised by the Panel as to a potential DNT effect of imidacloprid cannot be ruled out.

BCS states that complex biological multi-parameter experiments such as the DNT study show a number of differences between treated groups and controls that could either be spurious or caused by the test substance. The statement could overinterpret true differences as potential false positive results due to variation in the test system might be further used to exclude any potential finding from *in vivo* DNT studies, making them unfit for regulatory purposes. By finally bringing this unwarranted flexibility to the interpretation of DNT studies, many statistically significant findings observed were challenged without foundation. On this basis, the claim could be advanced that it would not make sense to perform such studies. EFSA has recommended the correct use and understanding of the concepts of biological relevance and statistical significance when conducting risk assessments as both terms are often confused. A particular outcome could be statistically significant, but without any biological relevance, and vice versa. The concept of biological relevance refers to an effect of interest or to the size of an effect that is considered

important and biologically meaningful and which, in risk assessment, may have consequences for human health. In addition, the biological relevance of any change found should be of primary importance in the assessment rather than the specific level of statistical significance.[1]

The PPR Panel notes that great variation in data collected from DNT studies is a limitation to identification of dose-related and statistically significant associations. Variation of this extent can erroneously lead to firm negative conclusions concerning DNT, as some treatment-related effects in these studies could be masked and go unrecognised. The same applies when data are compared to historical controls, as the range of historical variation may be larger than the magnitude of effects observed in treated animals.

Brain morphometry

The BCS report provides the following table of brain morphometric measurements:

	PND 11		PND 75	
	Male	Female	Male	Female
Frontal Cortex	-3.4 %	-4.1 %	+1.2 %	-1.7 %
Parietal Cortex	+0.5 %	-0.8 %	+1.8 %	-1.7 %
Hyppocampal gyrus	+0.2 %	-4.1 %	-8.1 %	-1.1 %
Cerebellum	+5.5 %	+1.3 %	-3.2 %	-0.3 %
Caudate-Putamen	+0.3 %	**-5.5 %**	+1.0 %	**-1.9 %**
Corpus Callosum	-0.6 %	**-27.6 %**	+6.0 %	**-7.1 %**

Percent change in different rat brain structures stratified by sex between the highest dose tested and concurrent controls a two different time periods.

The table shows a consistent pattern of decreased morphometric measurements in females at both ages for all measurements with the exception of cerebellum at PND11 (cerebellum is a structurally unusual and primitive part of the CNS situated at the junction of the brain and brain stem concerned with co-ordination of movement. Although only 10% of the skull contents by weight, this part contains approximately 50% of the neurons). The PPR Panel was concerned about this pattern and although it does not directly demonstrate neurotoxicity, it cannot be dismissed, especially considering the magnitude of decrease in corpus callosum dimension. The fact that a similar trend in brain morphometry was not observed in male rats does not necessarily

[1] Scientific Opinion. Statistical Significance and Biological Relevance. EFSA Journal 2011;9(9):2372 http://www.efsa.europa.eu/en/efsajournal/doc/2372.pdf

means that the effect is irrelevant as it is well known that some adverse effects are gender-dependent (*vide infra*). The data evaluation record (DER) prepared for US-EPA[2] also considered changes in one sex as toxicologically plausible, thus gender-dependency should not be the basis for rejecting their relevance. In addition, epidemiological studies on birth cohorts evaluating neurodevelopmental effects have found adverse effects in only one sex[3,4].

The 5.5% decrease in caudate-putamen at PND 11 was borderline statistically significant (p= 0.07), among other reasons because the sample size was not large enough to detect the effect. This finding persisted at PND 75 (study termination, corresponding stage to adult human) where a statistically significant 1.9% decrease was observed compared to concurrent controls. At PND11 the main change was the markedly and significantly reduced thickness of corpus callosum (27.6%) in female pups; this reduction persisted until study termination (7.1%) albeit without reaching statistical significance, and it was considered by the PPR Panel as a biologically relevant finding because of its magnitude. These findings did not correspond to changes in mean brain weights, suggesting a possible effect on the development of specific brain components. This consistency over time was also considered by the evaluation record prepared for US-EPA as treatment-related; accordingly, the record recommended that the registrant should examine intermediate doses using brain morphometry. The PPR is in agreement with the DER in regretting the lack of morphometric data at the lower dose levels. Its absence precludes any proper dose-response assessment.

Crofton et al (2011)[5] assessed variability of morphometric assessments of *in vivo* DNT studies and concluded that "variability in the most common morphometric measures is modest, less than that associated with body weights, and not significantly greater at PND 11 than in young adults". However, these authors assessed morphometric data for cortex, cerebellum and hippocampus (but not for putamen-caudate and corpus callosum). Cortical width is thinner than caudate-putamen width as can be seen in the original DNT study on imidacloprid performed by BCS. Owing to gaps in the available data, the PPR Panel could only propose health reference values (as requested by the terms of reference) taking into account the uncertainties identified as regards brain morphometry, and considered that more data are needed. Accordingly, as new and more data are made available any dose-response relationship present can be evaluated and the PPR recommendation of modifying the current health-based reference values can be reconsidered.

[2] http://www.epa.gov/opp00001/chem_search/cleared_reviews/csr_PC-129099_undated_a.pdf

[3] Horton MK, Kahn LG, Perera F, Barr DB, Rauh V. Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? Neurotoxicol Teratol 2012; 34: 534–541

[4] Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ Health Perspect 2010; 118: 1768–1774

[5] Crofton KM, Sutton JL, Makris SL, Raffaele K, Sette WF. Developmental neurotoxicity testing guidelines: variability in morphometric assessments of neuropathology Toxicologist 2001; 60: 113.

BCS suggests that these findings are spurious and invoked some of the differences at study termination as a result of the unusually high mean value for the controls in this study. The values were close to the upper-limit for the 19 DNT studies that constitute their historical control. However, if the variation within historical controls is invoked as a rationale for discounting the statistical or biological relevance of findings, *in vivo* DNT studies would not be useful for regulatory purposes. This follows because the magnitude of changes observed in a given test should be high enough to exceed the full range of historical control variability in order to be considered as a positive finding. A substantial level of uncertainty would remain and potential harm to consumers' health could not be properly guarded against. The PPR considers that the historical values may be of use when the relationship of a given effect with treatment is doubtful; conversely, historical values should not be used to discount findings that are dose-related, statistically significant and biologically relevant. In the specific case of imidacloprid, it would have been useful to have morphometric data from intermediate doses as previously requested. The aforementioned data evaluation record prepared for US-EPA also identified this data gap as a study deficiency and asked the registrant to submit more data, at least for an intermediate dose.

The PPR Panel considered the relevance of morphometric analyses as endpoint for hazard characterization. Tsuji and Crofton (2012)[6] have reported that morphometric analyses of brain regions, properly performed, can supply valuable data for regulatory authorities. In a review of DNT studies (Raffaele et al., 2010)[7] it was reported that the Office on Pesticide Programs (OPP) in US-EPA identified major issues in some of the submitted DNT studies that complicated both the interpretation of study results and the possible use of the studies as a point of departure for risk assessment. One of these issues was failure to evaluate brain morphometric measurements at low and mid doses, when significant differences in morphometry were seen between high dose and control animals. The same paper (Raffaele et al., 2010) indicates that in contrast to the lack of qualitative neuropathological findings, changes in quantitative neuropathological findings (i.e. linear morphometric measurements) were seen in a substantial number of studies. Hence, it is possible that a more detailed and extensive quantitative neuropathological examination might identify more treatment-related changes or changes at lower dose levels. This possibility is supported by the frequent detection of effects on brain weight and quantitative (linear morphometric) neuropathology, where effects at LOAEL were detected in 14/69 and 22/69 studies submitted to OPP, respectively.

Recent data indicate the relevance for humans of brain morphometry changes. Raugh et al (2012)[8] have reported significant associations of prenatal exposure to standard use levels of

[6] Tsuji R, Crofton KM. Developmental neurotoxicity guideline study: issues with methodology, evaluation and regulation. Congenit Anom (Kyoto) 2012; 52: 122-128

[7] Raffaele KC, Rowland J, May B, Makris SL, Schumacher K, Scarano LJ. The use of developmental neurotoxicity data in pesticide risk assessments. Neurotoxicol Teratol 2010; 32: 563-572

[8] Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Peterson BS. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc Natl Acad Sci USA 2012; 109: 7871-7876

chlorpyrifos with structural changes in the developing human brain. In particular, upper tertile levels of this organophosphate in cord blood had a measureable effect on brain structure in a sample of 40 children 6–11 years of age, who showed frontal and parietal cortical thinning. Thus, an inverse dose–response relationship between chlorpyrifos exposure and cortical thickness was concluded, further supporting the need for a careful consideration of brain morphometry in DNT studies.

Motor activity

The BCS report reviews the main results obtained in the DNT study on imidacloprid performed in 1999 (page 6) but fails to mention the magnitude of the decrease in motor activity, which ranged from 31 to 38% (in females and males, respectively) at PND 17 in high-dose animals and then a 37% decrease in females at PND 21. Although differences were not statistically significant, the effects on motor activity were deemed to be treatment related and taken into account because of its biological relevance. The apparent reversibility does not necessarily means that the effect is not adverse, as the decrement in motor activity in pre-weaning rats might have an impact in later life on different domains.

The apparent reversibility of a functional impairment and the persistence of a morphological change are not necessarily contradictory findings. Regardless of any functional compensation or adaptive response of the developing brain, these adverse effects were considered of concern by the PPR Panel as indicators of potential developmental neurotoxicity. Similarly, in spite of the fact that reversible inhibition of brain acetylcholinesterase induced by N-methylcarbamates can be recovered over time, this finding is considered as an adverse one. For regulatory purposes, transient effects are also relevant and cannot be dismissed because otherwise a full protection of human health would not be afforded.

The BCS report states that the decreased motor activity occurred in association with the period of higher exposure to pups, and no residual effect on PND 60 was observed, leading to the conclusion that this finding is consistent with results of acute neurotoxicity studies also performed in the same laboratory. However, based on the above considerations, the PPR Panel notes that, in spite of being a transient finding, its toxicological relevance has to be taken into account. In addition, there is increasing evidence that exposures occurring during the prenatal or early postnatal periods may lead to delayed adverse effects later in life. In this regard, it has been suggested that subclinical chemical injury may kill silently a fraction of the cells required to sustain brain function in later life. While these latent impairments cause no symptoms in childhood, they may be unmasked during the neuronal attrition associated with aging because of an age-related decline in functional reserve capacity of the brain[9]. In particular, there is uncertainty whether the reduced motor activity clearly observed in pups and apparently reversed at study termination (young adulthood) might signal toxicity mechanisms with an impact on later

[9] Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet 2006; 368: 2167-2178

life, e.g., on the aging brain. Accordingly, the precautionary principle has to be applied to this case until there is sufficient evidence to the contrary.

The BCS report states that "a transient decrease in activity is a non-specific effect that can result from such diverse causes as hypoglycaemia, hypothermia, gastric disturbance or toxicity to various systems, including the central nervous system. Due to this lack of specificity it is rarely appropriate to associate a transient decrease in motor activity with a particular mode of action". However, in the 2013 Scientific Opinion on cumulative assessment groups (CAGs)[10], the PPR Panel considered "decreased motor activity" as a relevant and specific effect and BCS did not challenge this during the public consultation of the opinion. On the other hand, BCS acknowledges that the reduced motor activity reflects "acute toxicity rather than DNT". In doing so, BCS is explicitly considering this effect as an adverse outcome: all the possible alternative causes invoked by BCS (e.g., hypoglycaemia, hypothermia) are, indeed, substance-induced adverse effects.

BCS summarizes the position of the PPR Panel by stating in page 5 of the BCS report "Differences in brain measurements in rats at the highest dietary level were associated with exposure to imidacloprid during development and evidence of impaired motor control (decreased motor activity); this indicates a possible DNT potential of imidacloprid". However, the Scientific Opinion did not draw this conclusion, rather it observed consistency between those two findings: morphometric and functional. The PPR Panel, in its 2013 DNT scientific opinion[11] offered a plausible explanation for the link between caudate-putamen width and motor activity: "Since putamen is involved in movement regulation and influences various types of learning, a decrease in thickness of this structure could be due to decreased number of neurons/glia ultimately leading to decreased motor activity". The lack of morphometric data for at least intermediate doses, together with the reduced motor activity observed in pups at the highest dose, prevented the Panel from *ruling out* any potential adverse effect on the developing brain in the lower dose ranges.

The draft document "Retrospective performance assessment of the test guideline 426 on DNT" (2005)[12] cited the criticism that variability of some endpoints (e.g., motor activity, morphometrics) was too great to be useful. More recently (January 2014), the International STakeholder NETwork (ISTNET) held a meeting in Zurich to build consensus on development and use of *in vitro* methods to deliver useful data for regulatory decisions. There was consensus that animal-based test methods currently used for developmental neurotoxicity evaluation for regulatory purposes were not being routinely used owing to high costs, use of large numbers of animals and limited confidence in the use of results for regulatory purposes (Crofton et al., 2014) [13]. These controversial opinions do not invalidate the *in vivo* DNT study for hazard identification

[10] http://www.efsa.europa.eu/en/efsajournal/pub/3293.htm

[11] http://www.efsa.europa.eu/en/efsajournal/doc/3471.pdf

[12] http://www.oecd.org/dataoecd/57/7/39824463.doc

[13] Crofton K, Fritsche E, Ylikomi T, Bal-Price A. International Stakeholder NETwork (ISTNET) for creating a developmental neurotoxicity testing (DNT) roadmap for regulatory purposes. ALTEX 2014; 31: 223-224

and risk assessment, but rather they calls for the need of incorporating improvements in this important protocol.

The PPR Panel interpreted motor activity and morphometry findings into a broader context by taking into account other studies (Abou-Donia et al, 2008 and Kimura-Kuroda et al., 2012). Whilst these were not considered useful for regulation, they do inform about the mechanisms and the potential for damage to the CNS. In the study of Abou-Donia et al (2008)[14] a single large non-lethal dose of imidacloprid administered on gestational day 9 produced significant deficits in sensorimotor performance (inclined plane, beam-walking and forepaw grip) and an increased expression of GFAP (glial fibrillary acidic protein) in motor cortex and the dentate gyrus of the hippocampus of the offspring on PND 30. Authors concluded that these changes may have long-term adverse health effects in the offspring as neurobehavioral deficits may reflect dysfunction at multiple anatomical areas in the nervous system, and accumulation of GFAP is a characteristic response of both the immature and adult brain to a variety of neurotoxic insults.

Kimura-Kuroda et al (2012)[15] carried out an *in vitro* study and suggested that excitation and/or desensitisation of nicotinic acetylcholine receptors (nAChRs) by imidacloprid might affect the developing mammalian nervous system as it exerts excitatory effects on nAChRs of cerebellar granular cells at low concentrations (1 µM and above). The PPR Panel estimated a NOAEL for DNT of imidacloprid of 5.5 mg/kg bw/day. This figure corresponds to approximately a T_{max} of 15 µM (as reported in the PPR Panel scientific opinion) and the Panel notes that such a T_{max} falls within the dose range assayed by Kimura-Kuroda et al. (2012) that induced adverse effects on cerebellar granular cells (1-100 µM). That figure is also in the range of concentrations reported to change the membrane properties and function of neurons having nAChRs in cholinergic synapses of the stellate cells of the mouse cochlear nucleus (≥ 10 µM for < 1 min)[16]. From these results, it can be inferred that a clearly effective *in vitro* concentration corresponds to a dose level *in vivo* devoid of any neurotoxic effects in the available DNT studies as stated in the scientific opinion (2013); however, there would only be cause for concern if the T_{max} is achieved at neuronal level.

[14] Abou-Donia MB, Goldstein LB, Bullman S, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA. Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following *in utero* exposure. J Toxicol Environ Health A 2008; 71: 119-130

[15] Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M and Kawano H, 2012. Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats. PLoS One 2012; 7(2): e32432

[16] Bal R, Erdogan S, Theophilidis G, Baydas G, Naziroglu M. Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording. Neurotoxicology 2010; 31: 113-120

More recently, Keil et al (2014)[17] have reported a weak positive association between autism spectrum disorder and maternally-reported use of imidacloprid during pregnancy in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case–control study.

Considering the available evidence, the PPR Panel concluded that imidacloprid may have potential DNT effects. Therefore, taking a precautionary approach, health-based reference values should be revised accordingly to conservatively protect human health as indicated in the DNT Scientific Opinion (2013).

Gender dependent effects in DNT studies

In the BCS report attention is drawn to gender specific effects and the suggestion is made that these are probably spurious on the grounds that toxic effects are an unlikely occurrence. This criticism of the EFSA opinion is misguided and ill informed as numerous studies have shown such effects. They include:

1. Special issue of "Toxicology" on Gender development in Neurotoxicity. Volume 311, Issues 1–2, Pages 1-86 (6 September 2013) http://www.sciencedirect.com/science/journal/0300483X/311/1-2
2. Beronius A, Johansson N, Rudén C, Hanberg A. The influence of study design and sex-differences on results from developmental neurotoxicity studies of bisphenol A: implications for toxicity testing. Toxicology 2013; 311: 13-26. *[This study suggests that DNT studies conducted according to the standardized OECD TG 426 may overlook sensitive effects of bisphenol A, and possibly other potential endocrine disruptors, especially in female offspring]*.
3. Levin ED, Addy N, Baruah A, Elias A, Christopher NC, Seidler FJ, Slotkin TA. Prenatal chlorpyrifos exposure in rats causes persistent behavioural alterations. Neurotoxicol Teratol 2002; 24: 733-741. *[This study indicates that late prenatal exposure to chlorpyrifos induces long-term changes in cognitive performance that are distinctly gender-selective]*.
4. Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioural consequences of neonatal chlorpyrifos exposure in rats. Dev Brain Res 2001; 130: 83–89. *[Sex-selective developmental effects have been seen in animal models exposed to organophosphates. Chlorpyrifos exposure (1 mg/kg/day) in rats during postnatal days 1–4 decreased the number of errors in working and reference memory made by females, but increased the number of such errors made by males. These effects*

[17] Keil AP, Daniels JL, Hertz-Picciotto I. Autism spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Environ Health 2014; 13(1): 3

persisted into adolescence and adulthood, indicating a long-term consequence of exposure].

5. Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT, Wrench N, et al. Early postnatal parathion exposure in rats causes sexselective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res 2009; 208: 319–327. *[Rat developmental exposures to low doses of the organophosphate parathion induced greater developmental deficits in spatial navigation and working memory among males than females]*.
6. Dam K, Seidler FJ, Slotkin TA. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. Brain Res Dev Brain Res 2000; 121: 179-187. *[Chlorpyrifos given during a critical neonatal period, even at levels below the threshold for overt toxicity, can elicit both immediate and delayed gender-selective behavioural abnormalities]*.
7. Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Peterson BS. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. PNAS 2012; 109: 7871-7876. *[Early life chlorpyrifos exposure interferes with normal sexual differentiation of the brain, reducing or reversing the normal sex differences in cognitive and emotion-related behaviours and correlating with sex-specific effects on the neurotransmitter systems that support those behaviours... The high-chlorpyrifos group also displayed disruption of normal sexual dimorphisms in brain structure. These morphological findings are consistent with those from animal models showing that early chlorpyrifos exposure obtunds or reverses normal sex differences in learning, memory, and emotional behaviours]*.
8. Jedrychowski W, Perera F, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, Edwards S Skarupa A, Lisowska-Miszczyk I. Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: The prospective cohort study in three-year olds. Early Hum Dev 2009; 85: 503–510. *[This study provides evidence that 3-year old boys are more susceptible than girls to prenatal very low lead exposure. The results of this study should persuade policy makers to consider gender-related susceptibility to lead and possibly to other toxic hazards in setting environmental protection guidelines]*.
9. Venerosi A, Ricceri L, Tait S, Calamandrei G. Sex dimorphic behaviours as markers of neuroendocrine disruption by environmental chemicals: The case of chlorpyrifos. NeuroToxicology 2012; 33: 1420–1426. *[In mice exposed to chlorpyrifos in utero and/or in early development social/emotional responses are differently affected in the two sexes… Chlorpyrifos interferes with maturation of neuroendocrine pathways in rodents and support the hypothesis that in utero or neonatal exposure to low dosages of chlorpyrifos influences the maturation of sex-dimorphic clusters of behavioural items relevant for proper expression of social responses]*.

10. Tait S, Ricceri L, Venerosi A, Maranghi F, Mantovani A, Calamandrei G. Long-term effects on hypothalamic neuropeptides after developmental exposure to chlorpyrifos in mice. Environ Health Perspect 2009; 117: 112-116. *[Developmental exposure to chlorpyrifos may permanently interfere with specific key signalling proteins of the hypothalamic peptidergic system, with time-, dose-, and sex-related effects still evident at adulthood]*.

Irreversible binding of neonicotinoids to insect nAChRs

The DNT Scientific Opinion (2013) states that "the binding of neonicotinoids to insect nAChRs is virtually irreversible" and the BCS report challenges this statement. While the binding is not a covalent one, and therefore can be theoretically reversible, the produced effect is irreversible. However, the available literature supports the PPR comment:

- Imidacloprid is the first highly effective insecticide whose mode of action has been found to derive from almost complete and virtually irreversible blockage of nAChRs in the central nervous system of insects (Abbink, 1991).[18]
- Neonicotinoids have partially positive charge and can irreversibly bind to nAChRs (Mattiazzi Ušaj et al., 2014).[19]
- Binding of neonicotinoids to these receptors is irreversible in arthropods (Sánchez-Bayo et al., 2013).[20]

Besides, Dr. Tennekes has reported that neonicotinoid insecticides cause irreversible and cumulative damage to the central nervous system of insects. The Druckrey-Küpfmüller equation states that if both receptor binding and the effect are irreversible, exposure time would reinforce the hazardous effect (Tennekes 2010a)[21]. Tennekes (2010b)[22] also claims that there is no safe level of exposure, as even tiny amounts of systemic insecticides can have deleterious effects in the long term. This is attributed to the fact that the damage neonicotinoids cause to the central

[18] Abbink J. The biochemistry of Imidacloprid. Pflanzenschutz-Nachrichten Bayer, Germany, 1991, F.R, Serial ID – ISSN: 0340-1723

[19] Mattiazzi Ušaj M, Kaferle P, Toplak A, Trebše P, Petrovič U. Determination of toxicity of neonicotinoids on the genome level using chemogenomics in yeast. Chemosphere 2014; 104: 91-96

[20] Sánchez-Bayo F, Tennekes HA, Goka K. Impact of systemic insecticides on organisms and ecosystems. Insecticides - Development of Safer and More Effective Technologies. Trdan S (ed.), ISBN: 978-953-51-0958-7, InTech, 2013. DOI: 10.5772/52831. Available from: http://www.intechopen.com/books/insecticides-development-of-safer-and-more-effective-technologies/impact-of-systemic-insecticides-on-organisms-and-ecosystems.

[21] Tennekes HA. The significance of the Druckrey-Küpfmüller equation for risk assessment--the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. Toxicology 2010a; 276: 1-4.

[22] Tennekes HA. Systemic Insecticides: A disaster in the making. ETS Nederland BV, Zutphen, The Netherlands, 2010b

nervous system of insects is both irreversible and cumulative. Tennekes et al. (2011)[23] also demonstrated that chemicals that bind irreversibly to specific receptors (including neonicotinoid insecticides) will produce toxic effects in a time-dependent manner, no matter how low the level of exposure. This position has been considered by the Directorate General for internal policies, environment, public health and food safety of the European Parliament.[24]

Overall conclusion

With the data available and the new and limited data presented by the BCS and Exponent, there is insufficient evidence to discard the indications of potential developmental neurotoxicity provided by the regulatory DNT studies performed on imidacloprid and acetamiprid. Accordingly, the recommendation of modifying health-bases reference levels made by the PPR Panel in its DNT Scientific Opinion (2013) continues to be supported by the panel. The PPR Panel notes that until more consistent evidence is provided, uncertainties still persist and hence, human health cannot be guaranteed with the current (unrevised) levels.

The PPR Panel has taken a precautionary approach in its scientific opinion by also considering recent evidence on potential delayed neurological disturbances following prenatal exposure to neurotoxicants. This type of exposure, which may adversely impair the nervous system, has been suggested to be associated with a number of developmental disabilities, such as learning disabilities, attention-deficit hyperactivity disorder, dyslexia, sensory deficits, mental retardation and autism spectrum disorders.[25]

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.

[23] Tennekes HA, Sánchez-Bayo F. Time-dependent toxicity of neonicotinoids and other toxicants: Implications for a new approach to risk assessment. J Environment Analytic Toxicol 2011, S:4

[24] Existing scientific evidence of the effects of the neonicotinoid pesticides on bees. Directorate General for internal policies. Enrironment, public health and food safety. European Parlament, IP/A/ENVI/NT/2012-09, December 2012 (http://www.europarl.europa.eu/RegData/etudes/note/join/2012/492465/IPOL-ENVI_NT(2012)492465_EN.pdf)

[25] Giordano G, Costa LG. Developmental Neurotoxicity_Some old and new issues. ISRN Toxicology. Volume 2012, Article ID 814795, 12 pages