Thiacloprid.
Information delivered by Greenpeace, Ctgb, RIVM, 5.1.2.e 5.1.2.e

(Goulson 2013; Van der Sluijs, Simon-Delso et al. 2013)
Van der Sluijs et al (2013) and Goulson (2013) present an overview of data on environmental fate and ecotoxicity of neonicotinoids. No new information is presented.

(Mota-Sanchez, Hollingworth et al. 2006)
Mota-Sanches et al. tested toxicity of **thiacloprid** (99.7% analytical grade) to susceptible and imidacloprid-resistant Colorado potatoe beetles *Leptinotarsa decemlineata* in contact bioassays. Other compounds tested are not reported here. At least five test concentrations, 10 beetles (150-200 mg/beetle) were treated with a 1 µg/L solution on the ventral area of the abdomen. Controls were treated with acetone. Mortality was assessed after 10 days. LD50 calculated with probit analysis.
Results
Control mortality typically <3%. 10d LD50 0.032 µg/beetle for the susceptible beetles; and 0.80 µg/beetle for the resistant ones.
Remarks
Presumably more replicates were tested, since the number of tested beetles was >60.

(Jeyalakshmi, Shanmugasundaram et al. 2011)
Jeyalakshmi et al. (2011) investigated the toxicity of neonicotinoids to *Apis cerana indica*. Thiacloprid and acetamiprid were not tested.

(Miao, Du et al. 2013)
Miao et al. tested toxicity of **thiacloprid** (in Calypso 70WG) as seed treatment (winter wheat) on aphid *Sitobion avenae*. Five test concentrations, 6.5-1000 mg as/kg seed, and uncoated seeds as control. Seeds were grown in plastic cups (5 cm width) and 7-d old plants were infested with 50 adult apterous aphids per cup for 24h.
Next, the same set-up was used with seeds treated at LC10 and LC50 concentrations. Ten adults were used, and when >30 offspring were produced, all adults were removed and 30 1st instar nymphs were kept to initiate a cohort. Each treatment in five replicates. Thereafter, development and reproduction were recorded daily. Newborns were counted and removed. Test was continued until all adults of this cohort had died.
Effects on feeding behavior were determined with LC10 and LC50 concentrations. Probing behavior was recorded using a DC EPG amplifier after 2h starvation in at least 15 adults. Plants were 7d old. LC50 determined with probit analysis, other effects with one-way ANOVA.
Results
24h LC50 48 mg as/kg seed (Y=5.2+0.69x).
At LC10 and LC50, sub-lethal effects (p=0.05) on probing behavior were found: Salivary excretion into the sieve element was reduced (respectively 35% and 78% reduction); phloem sap ingestion was reduced (54% and 72%), and non-probing was increased (156% and 370%). Ingestion in xylem was about 1/3 of control at LC10 (non-significant) and was zero at LC50.
At LC50, average generation life span, finite of natural increase, were not different from control, and net reproductive rate (0.073x), rate of natural increase (0.1x), and doubling time (13x) were different from control. At LC10 there were no significant differences (p=0.05), although net reproductive rate was down 23% and doubling time was up 13%.

(Vojoudi and Saber 2013)
Vojoudi et al tested the toxicity of thiacloprid (as Calypso 480SC) to larvae of the moth *Helicoverpa armigera* in the laboratory. First instar larvae were exposed via the diet. Twenty larvae were individually placed into 40ml vials containing a 2 cm^3 cube of thiacloprid infused diet. Six

concentrations plus control in triplicate. Mortality was recorded after 24h. LC30 and LC50 calculated with probit analysis. Sublethal parameters analysed with ANOVA (p=0.05).

Results

24h LC50 329 µg as/ml diet. 24h LC30 226 µg as/ml diet. Compared to control, the effects at LC30 level on duration of larval stage (up 18%), pupal weight (down 21%), and duration of pupal stage (up 17%) were significant. Also the number of eggs per female was reduced (not significantly) with 21%; and the longevity was significantly reduced with 19%.

(Yáñez, Bernal et al. 2013)

Authors developed a LC-MS method to analyse neonicotinoids in bees wax.

Thiacloprid: LOD and LOQ were established at 1.2 and 4.0 µg/kg. Correlation coefficient for determination over 4-1000 µg/kg was 0.992. Acetamiprid: LOD and LOQ were established at 0.6 and 2.0 µg/kg. Correlation coefficient for determination over 2-1000 µg/kg was 0.998.

Thirty beeswax samples were collected in Murcia, Spain (2012) from different apiaries located close to fruit orchards. No further details on these sampling sites. Samples were analysed in triplicate. Thiacloprid was not detected. Acetamiprid was detected in four samples, at 11, 23, 36, and 61 µg/kg.

(Wu, Anelli et al. 2011)

Wu et al investigated the toxicity of brood combs containing pesticides on the develepment of worker bees. Brood comb blocks from migratory beekeeping operations that used miticides, and from the USDA honey bee laboratory (suspect of CCD), were analysed for residues. In the experiment, a section was cut out of a frame, and filled with two blocks, one of a 'treated' block, and one control. Laying sister queens were caegd for 24h over the frames. Only frames with 224 eggs on control and treatment blocks were selected for the experiment. Egg patches were monitored for larval mortality on days 4, 8, 12, and 19 of development, and photographs of larvae were mapped. On day 19, frames with pupae were incubated. Treatment and control blocks were isolated. Emergence of adults was recorded daily. Adults were tagged, and treatment and control bees were kept in the same cage with free access to water, syrup and pollen. Some frames were re-used up to 3 times. Total amount of replicates was 28.

Combs were analysed for 171 pesticides in the wax in all combs before the experiment. Five paired treatment and control combs were also analysed after the experiment. LOD was reported to be in the low ng/g.

Statistical analysis of sub-lethal effects was done with ANOVA.

Results

Residues: Number of pesticides identified in combs ranged between 4 and 17, averaging 10. In total 39 substances were identified. Most frequently (=100%), and highest, detected were fluvalinate, coumaphos (and -oxon): averages were 6712, 8079 and 596 ng/g. Average individual concentrations of other pesticides were below or equal to 283 ng/g.

Comparison of analysis before and after the experiment, showed that residues are transferred. Four new pesticides were found in the control combs, while three disappeared from the treatment comb. Quantities also increased in the control, and decreased in the treatments.

Thiacloprid was detected in 1 out of 13 (total) combs, at a level of 113 ng/g. After treatment, is was below the LOD of 8 ng/g.

Brood effects: total larval mortality control and treatment was 26 and 33%, respectively. At p=0.059 this is significant. Delayed development in treatments with high residue level (from CCD-suspected hives). In control combs, larval mortality increased from 1st, 2nd, to 3rd repeated use (13-28-39%). In treatment combs, mortaltiy increased from 1st to 2nd repeat (17 to 37%), but decreased again at 3rd (22%). Authors suggest that transfer of pesticide residues from treatment to control may play a role. Worker bees in control lived an average 4 days longer (p=0.005). Emergence time was also affected by contamination of control comb after repeated use. The distribution over days 20-21-22 shifted away from 20-21 days towards 21 and 22 days.

Remarks

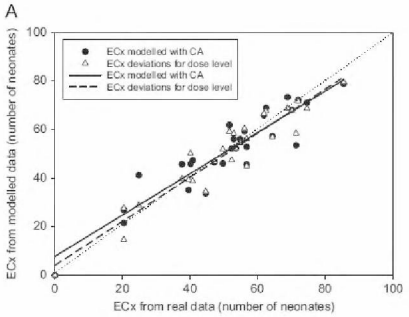
No data on bee diseases.
General conclusions on the impact of pesticide load on brood development and worker bee longevity (both are negatively impacted, but a dose-response curve has not been established) are clearly not related to the presence of thiacloprid.

(Tennekes 2010; Tennekes and Sanchez-Bayo 2013)
Tennekes (2010) introduces the Druckrey–Küpfmüller equation to account for irreversible binding to receptors and demonstrates its applicability using (a.o.) toxicity of thiacloprid to arthropods. Tennekes and Sanchez-Bayo (2013) place the concept in a broader perspective touching upon other time-to-effect toxicity models. Tennekes (2010) provides examples with thiacloprid. Time to 50% effect were derived from (Sanches-Bayo 2009) who based these on the original data are from (Beketov and Liess 2008). The time-exponent for *G. pulex* and *S. striolatum* are >1 (1.11 and 1.53). For S. latigonium it was not calculated. The toxicity pattern of thiacloprid to dragonfly nymphs (*S. striolatum*) suggests an irreversible binding to their nicotinic acetylcholine receptors (nAChRs) in arthropods (Tennekes and Sanchez-Bayo 2013), but for *G. pulex* is could be considered to follow Haber's law (time exponent = 1). Note that these organisms were placed in clean water after 1d exposure.
Remarks
Implications for risk assessment are:

- In principle, environmental risk assessment should target population endpoints that are relevant proxys for the protection goal and the desired level of protection. The dynamics of time-to-effect, and the nature of that effect, should be part of these considerations.
- The EFSA Guidance on bee risk assessment (2013) follows the PPR Opinion in the science behind bee risk assessment and calls for a check on the cumulative effect of exposure over time, where short term exposure endpoints are used for assessment. However, if this is the case, the Guidance indicates that a higher tier assessment is needed. Higher tier assessments seem to be used to refine the assessment rather than overrule the outcome of the previous assessment. How to assess with sufficient certainty that no unacceptable effects occur, this is not furter guided. Routes of exposure and other relevant aspects will have to be considered on a case-by-case basis. Thus, in the event a TER based on 'routine' testing was not violated, but the accumulative toxicity test does indicate that accumulative toxicity is in play, the guidance gives basically no guidance.
- Co-exposure to other pesticides with concentration-addition interaction, over the relevant time periods, needs to be considered.

(Mason, Tennekes et al. 2013)
Mason et al (2013) summarize information on world-wide species declines (bees, bats, amphibians, birds, fish), use and persistence, and distribution of neonicotinoids. Authors consider the impact of neonicotinoids on the immune system as demonstrated in bees (neonicotinoid exposure results in higher infection rates with Nosema; co-exposure increases mortality) and fish (imidacloprid exposed zebra fish had higher infection rates with the ectoparasite Cyclochaeta (=Trichodina) domerguei). Authors hypothesize that exposure to neonicotinoids underlays all described phenomena through immunesuppression mediated pathways of toxicity.

(Pavlaki, Pereira et al. 2011)
Pavlaki et al assessed the impact of joint exposure of Daphnia magna to thiacloprid and imidacloprid. Impact on body length was best explained with concentration-addition. Impact on number of neonates was best explained with a synergistic model. However, the numerical differences are very small (see figure). When combination toxicity is assessed in risk assessment, concentration addition is the default approach.



(Beketov and Liess 2008; Beketov, Schäfer et al. 2008; Liess and Beketov 2011; Liess and Beketov 2012; Van den Brink and Ter Braak 2012)
Beketov and Liess (2008) demonstrated different sensitivities among freshwater arthropods to thiacloprid. Also very relevant was the observation that pulse exposure resulted in long-term sublethal effects. Beketov et al (2008) show that concentrations of pesticides at which majority of the species is affected can be predicted by acute organism level toxicity tests with sensitive species. However, tests with longer observation periods, as well as consideration of environmental factors and inter-taxon variability in sensitivity are required to predict effects on all species comprising a community. Realistic prediction of community recovery dynamics requires consideration of the species' life-cycle traits. Liess and Beketov (2011) demonstrate the use of a priori ecotoxicological knowledge, individuals of species were aggregated into trait-based groups that reflected stressor-specific vulnerability of populations to toxicant exposure. This should reduce inter-replicate variation that is not related to toxicant effects and enables to better link exposure and effect. This approach is compared to the Principal Response Curves, using aggregations of individuals around species and taxa, claiming that the SPEAR analysis is more relevant, also because it identifies no-effect concentrations at much lower levels. In a response to this paper, Van den Brink and Ter Braak (2012) question (amongst others) the a priori classification and the added value of the approach compared to the standing practice of PCR complemented with univariate tests at the taxon level. Liess and Beketov (2012) then reply (a.o.) highlighting that PRC identifies the dominant response to the treatment, which is not necessarily what the risk assessment is concerned about, and that SPEAR and PRC analysis jointly would provide a basis for risk assessment.

- There is consensus that a trait-based assessment is a way forward to inform risk assessment for pesticides. On the issue what instruments can be used, alone or in concert, the make sure that no unacceptable treatment-related effects are overlooked, the EFSA guidance document (2013) provides some guidance. The EFSA Guidance already acknowledges the careful consideration of the relevant exposure profile.
- The fact that this research was performed with thiacloprid, generating useful data, can be taken on board in the aquatic risk assessment.

References

Beketov, M. A. and M. Liess (2008). "Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthopods." Environmental Toxicology and Chemistry **27**(2): 461-470.

Beketov, M. A., R. B. Schäfer, et al. (2008). "Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics." Science of the total environment **405**: 96-108.

Goulson, D. (2013). "An overview of the environmental risks posed by neonicotinoid insecticides." Journal of Applied Ecology **50**: 977-987.

Jeyalakshmi, T., R. Shanmugasundaram, et al. (2011). "Comparative toxicity of certain insecticides against apis cerana indica under semi field and laboratory conditions." Pestology **35**(12): 23-26.

Liess, M. and M. Beketov (2011). "Traits and stress: keys to identify community effects of low levels of toxicants in test systems." Ecotoxicology **20**: 1328–1340.

Liess, M. and M. A. Beketov (2012). "Rebuttal related to ‘‘Traits and Stress: Keys to identify community effects of low levels of toxicants in test systems’’ by Liess and Beketov (2011)." Ecotoxicology **21**: 300-303.

Mason, R., H. Tennekes, et al. (2013). "Immune Suppression by Neonicotinoid Insecticides at the Root of Global Wildlife Declines." Journal of Environmental Immunology and Toxicology **1**(1): 3-12.

Miao, J., Z.-B. Du, et al. (2013). "Sub-lethal effects of four neonicotinoid seed treatments on the demography and feeding behaviour of the wheat aphid *Sitobion avenae*." Pest Management Science **DOI 10.1002/ps.3523**.

Mota-Sanchez, D., R. M. Hollingworth, et al. (2006). "Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae)." Pest Management Science **62**(1): 30-37.

Pavlaki, M. D., R. Pereira, et al. (2011). "Effects ofbinary mixtures on the life traits of *Daphnia magna*." Ecotoxicology and Environmental Safety **74**: 99-110.

Sanches-Bayo, F. (2009). "From simple toxicological models to prediction of toxic effects in time." Ecotoxicology **18**: 343-354.

Tennekes, H. (2010). "The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time." Toxicology **276**(1): 1-4.

Tennekes, H. and F. Sanchez-Bayo (2013). "The molecular basis of simple relationships between exposure concentration and toxic effects with time." Toxicology **309**: 39-51.

Van den Brink, P. J. and C. J. F. Ter Braak (2012). "Response to ‘‘traits and stress: keys to identify community effects of low levels of toxicants in test systems’’ by Liess and Beketov (2011)." Ecotoxicology **21**: 297–299.

Van der Sluijs, J. P., N. Simon-Delso, et al. (2013). "Neonicotinoids, bee disorders and the sustainability of pollinator services." Current Opinion in Environmental Sustainability **5**: Corrected proof.

Vojoudi, S. and M. Saber (2013). "Lethal and sublethal effects of thiacloprid on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae)." Archives Of Phytopathology And Plant Protection.

Wu, J. Y., C. M. Anelli, et al. (2011). "Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity." PlosOne **6**(2): 1-11.

Yáñez, K. P., J. L. Bernal, et al. (2013). "Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electronspray-mass spectrometry using a fused-core column." Journal of Chromatography A **1285**: 110-117.