DRIPPING POISON: AN ANALYSIS OF NEONICOTINOID INSECTICIDES IN THE GUTTATION FLUID OF GROWING MAIZE PLANTS

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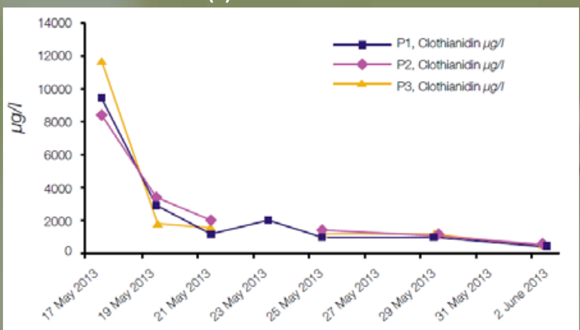
Introduction: For the neonicotinoid pesticides restricted in Europe, a key uncertainty identified by the European Food Safety Authority (EFSA) is the significance of 'guttation fluid' (xylem sap exuded from the tips or edges of leaves) as a source of exposure to bees. Transfer from seed coating to guttation fluid is established, but data on concentrations in droplets forming under field conditions remain limited. Greenpeace therefore sampled guttation droplets from maize plants emerging from seed known to have been treated either with Poncho® (active ingredient clothianidin) or Cruiser® (thiamethoxam) and growing under field conditions in Pest County, central Hungary, in May and June 2013.

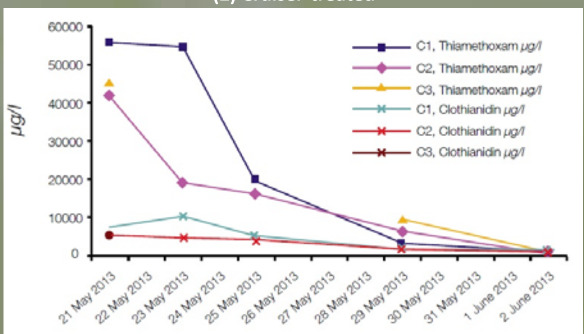
Materials & Methods: Sampling began approximately 2-3 weeks after seed had been sown, corresponding to emergence of first three leaves (approx. 8-12 cm seedling height) and continued approximately every two days for around 2 weeks (until seedlings were between 25 and 35 cm in height). All samples were collected at dawn from the tips or edges of the leaves of plants in three parallel planting rows from the centre of each of two fields, using Gilson micropipettes fitted with 10 or 50 µl tips to collect a total of 1.5-2.5 ml of composite sample per row. Samples were filtered through 0.45 µm Teflon syringe filters and stored in the dark at 3-4°C. Analysis for pesticide active ingredients was carried out by an independent laboratory using LC-MS/MS[1]. Limits of detection (LOD) and limits of quantitation (LOQ) were 5 µg/l and 10 µg/l respectively for both clothianidin and thiamethoxam.

Seed treatment	Poncho® (clothianidin)	Cruiser® (thiamethoxam)
Sowing date	24th April 2013	1st May 2013
	Numbers of samples collected	
17th May	3	-
19th May	3	-
21st May	3	3
23rd May	1	2
25th May	3	2
29th May	3	3
2nd June	3	3

(1) Poncho-treated



(2) Cruiser-treated



Figures 1 & 2: concentrations of (1) clothianidin and of (2) thiamethoxam and clothianidin (as a metabolite) in guttation fluid collected from maize plants grown from Poncho- and Cruiser-treated seed respectively in central Hungary, May-June 2013

Results: Concentrations of the two neonicotinoid pesticides in the guttation fluid fell progressively over the duration of the sampling (Figures 1 & 2), from initial concentrations averaging 9.6 mg/l for clothianidin and in excess of 50 mg/l for thiamethoxam, equalling or exceeding concentrations recommended for these active ingredients when prepared as pesticide sprays[2]. By the end of the sampling period, concentrations of both had fallen to around 0.5 – 1.0 mg/l fluid in all cases. In the case of the plants growing from Cruiser-treated seed, significant concentrations of clothianidin were also detected in the guttation fluid, presumably as a metabolite of the active agent thiamethoxam.

Implications for honeybees: based on model calculations provided by EFSA[3]:-

- acute oral LD_{50} of thiamethoxam to honeybees is 0.005 µg active substance per bee, and for clothianidin is 0.00379 µg/bee
- honeybees estimated to make an average of 46 foraging trips for water per day, ingesting between 30-58 µl fluid to the crop each time (equivalent to 1.4-2.7 ml fluid per bee per day)
- at the two highest concentrations recorded (11.7 mg/l for clothianidin and 55.26 mg/l for thiamethoxam), an individual bee would need to consume only 0.324 µl guttation fluid as part of its forage in order to ingest a dose equivalent to the acute oral LD_{50} for clothianidin and as little as 0.09 µl in the case of thiamethoxam
- approximately 1 month after sowing, guttation fluid produced by the maize crops could still deliver an equivalent lethal dose of thiamethoxam in as little as 6.04 µl and of clothianidin in only 3.61 µl

Conclusions: Neonicotinoid insecticides applied as seed treatments to commercially available maize seed can be found in the guttation fluid of plants grown in a conventional agricultural system. The concentrations found suggest that guttation fluid could pose a serious toxic hazard to bees if used as a contributory source of fluid by bees foraging for water. Although concentrations of both active ingredients declined significantly over the sampling period, even a single foraging event occurring at any time throughout that period could result in a bee ingesting into its crop doses far higher than the published acute oral LD_{50} for both clothianidin and thiamethoxam. There is an urgent need to address key outstanding questions regarding (1) the extent to which guttation fluid is collected by foraging bees under field conditions, (2) what proportion of that fluid is retained by individual bees and (3) the significance and impacts of the transfer of pesticide-contaminated guttation fluid to the colony for purposes of evaporative cooling and dilution of honey for brood feeding.

[1]Waters Acquity UPLC coupled to Xevo TQS MS operated in electrospray ionisation positive mode; mobile phase A - 95% H2O and 5% methanol in 0.25 mM ammonium acetate & 0.01% acetic acid; mobile phase B - 100% methanol in 0.25 mM ammonium acetate; column - 50 mm Kinetex, 2.6 µm particle size, C8 phase, 100A pore size x 2.1 mm internal diameter reverse phase (Phenomenex); flow rate 0.4 ml/min.; run time 5 min.

[2]For example, for spraying on paprika, tomato and lettuce, Syngenta recommends dilutions of 10–40 g Actara® per 100 l water, which is equivalent to between 25-100 mg of the active ingredient thiamethoxam/l of water.

[3]European Food Safety Authority (2013b). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid EFSA Journal 11(1): 3068 [55 pp.] http://www.efsa.europa.eu/en/efsajournal/doc/3068.pdf

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