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Letter to the editor

Response to the publication: Tennekes, H.A. (2010): The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time

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In his paper "The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time" (Tennekes, 2010), the author refers to the Druckrey–Küpfmüller equation and postulates its relevance for honeybee risk assessments. The Druckrey–Küpfmüller equation was established to explain the chronic effect of low concentrations of chemical carcinogens to mammals. Its essence is that for these substances the total dose required to produce the same effect decreases with decreasing exposure levels, even though the exposure times required to produce the same effect increase as the exposure decreases. Therefore, when both the receptor binding and the effect are irreversible, increasing the exposure time would enhance the effect. The author claims likewise, that recently similar dose–response characteristics have been established for the toxicity of neonicotinoid insecticides to arthropods. His conclusion is that the equation would explain patterns of chronic effects of imidacloprid to honeybees and other arthropods and that this phenomenon, so far, has not been sufficiently considered in the risk assessment.

The approach to extrapolate the pattern of long-term effects of carcinogenic substances to the effects of pesticides to arthropods has a certain degree of novelty. However, the concerns of the author about potentially underestimated long-term toxicity of imidacloprid to honeybees are unfounded as the approach chosen cannot be applied to evaluate neonicotinoid chronic toxicity to insects (which is based on reversible receptor binding), and moreover, as the risk assessment of imidacloprid to honey bees is based on data in which a chronic exposure of bees to imidacloprid is already fully considered. This is outlined in detail below:

1. All commercial neonicotinoid insecticides bind to insect nicotinic acetylcholine receptors and cause the same effect as the natural neurotransmitter acetylcholine, i.e. agonistically activating the receptors resulting in a transient inward-current leading to the generation of action potentials. Similar to acetylcholine, a neonicotinoid is binding to the nicotinic acetylcholine receptors, and the binding of neonicotinoid insecticides is reversible as shown by their rapid desensitization/recovery during short-term exposure in electrophysiological whole-cell voltage clamp assays on isolated insect neurons (Nauen et al., 2001; Tomizawa and Casida, 2003; Jeschke and Nauen, 2005). Radio-ligand binding studies conducted with tritiated imidacloprid also revealed saturatable, specific and reversible binding with fast kinetics (Liu and Casida, 1993). The synaptic action of acetylcholine under normal physiological conditions is terminated by acetylcholinesterase, which hydrolyzes the transmitter. Neonicotinoids cannot be hydrolyzed by the enzyme, i.e. they persist at the binding sites leading to over-stimulation of cholinergic synapses, resulting in hyperexcitation and paralysis of the insect (Yu, 2008). However, due to the reversible nature of binding of neonicotinoids, their toxic action strongly depends on the pharmacokinetics including the rate of metabolic detoxification as shown in aphids recovering from imidacloprid intoxication under discontinuous exposure conditions (Nauen, 1995). Therefore, the basic conditions for the applicability of the Druckrey–Küpfmüller equation (i.e. both receptor binding and the effect are irreversible) are not fulfilled in this case.
2. The author's conclusions regarding an underestimated risk caused by chronic exposure to imidacloprid are based on the assumption that the assessment of risk employs an extrapolation from short-time to long-time toxicity of a compound. In the EU, the risk assessment for honeybees has for many years been conducted according to a tiered, hierarchical system (outlined by Alix and Lewis, 2010). In the framework of this system, each compound that displays a potential for intrinsic toxicity to honeybees in an acute toxicity test in the laboratory, will automatically be tested in additional studies with a more realistic design, (so-called higher-tier studies) which also includes longer testing periods. Therefore the key hypothesis of the author that "Traditional approaches that consider toxic effects at fixed exposure times are unable to allow extrapolate from the measured endpoints to effects that may occur at other times of exposure", is unfounded and does not reflect the facts of regulatory practice.
3. In particular, there are extensive data available on the chronic toxicity of imidacloprid under laboratory conditions. These data were summarized by Schmuck (2004). In these studies, the chronic effects of imidacloprid were directly measured over longer times (e.g. in 10-day feeding studies) and not extrapolated from short-time exposure studies. This therefore excludes an underestimation of chronic toxicity based on the phenomena described by the author.
4. There are studies available where whole bee colonies have been chronically fed with diet spiked with imidacloprid at practically relevant exposure levels under realistic conditions (e.g. the study of Faucon et al., 2004), where bee hives were exposed to field-relevant concentrations of imidacloprid over 34 days (which covers very well the normal lifespan of an adult worker bee which is two to four, at maximum six weeks (e.g. Free and Spencer-Booth, 1959; Lie big, 2002; Amdam and Omholt, 2002;

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Dukas, 2008) and were monitored for several months. No effects on mortality or adverse effects on other endpoints were seen in these studies.

5. There have been numerous higher-tier (tunnel and field) studies conducted where honeybee colonies were exposed to imidacloprid-treated crops under realistic conditions (see for instance Schmuck, 1999; Curé et al., 2001; Maus et al., 2003; Schmuck et al., 2005). These studies, likewise, include the observation of chronic effects, as their duration is normally covering several generations of honeybees. In none of these studies, increased chronic or acute mortalities were seen with longer term exposure.
6. As evidence for his hypothesis of an underestimated chronic toxicity of imidacloprid, the author cites the study of Suchail et al. (2001), which claimed to have found a chronic toxicity of imidacloprid to bees which is, by far, in excess to the measured acute toxicity. The results of this study, however, were found to be flawed, and could not be reproduced by several independent research institutions (Schmuck, 2004).

Therefore, it can be concluded that potential chronic effects of imidacloprid to honeybees are appropriately covered by the studies that have been conducted and the ecotoxicological risk assessment on which the registration of the respective products are based and that there is no substantiation for concerns that effects like described by the Druckrey–Küpfmüller equation might entail a higher chronic toxicity than currently determined. In contrast, recent studies provide evidence that there is under realistic conditions no correlation between exposure of honeybees to imidacloprid-treated crops and increased colony mortality (e.g. Nguyen et al., 2009, 2010; Chauzat et al., 2009; Genersch et al., 2010).

Conflict of interest

There are none.

References

Alix, A., Lewis, G., 2010. Guidance for the assessment of risks to bees from the use of plant protection products under the framework of Council Directive 91/414 and Regulation 1107/2009. Bull. OEPP 40, 196–203.
Amdam, G.V., Omholt, S.W., 2002. The regulatory anatomy of honeybee lifespan. J. Theor. Biol. 216, 209–228.
Chauzat, M.-P., Carpentier, P., Martel, A.C., Bourgeard, S., Cougoule, N., Porta, Ph., Lachaize, J., Madec, F., Aubert, M., Faucon, J.P., 2009. Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ. Entomol. 38, 514–523.
Curé, G., Schmidt, H.W., Schmuck, R., 2001. Results of a comprehensive field research programme with the systemic insecticide imidacloprid (Gaucho®). In: Proceedings of the Symposium Hazard of Pesticides to Bees, vol. 98, Avignon (France), September 7–9, 1999, INRA (Paris) (Les Colloques), pp. 49–59.
Dukas, R., 2008. Mortality rates of honey bees in the wild. Insect. Soc. 55, 252–255.
Faucon, J.P., Aurieres, C., Drajnudel, P., Ribiere, M., Martel, A.C., Zeggance, S., Chauzat, M.P., Aubert, M., 2004. Experimental study of the toxicity of imidacloprid distributed in feeder syrup to bee colonies (*Apis mellifera*). AFSSA, Laboratory for studies and research into the pathology of small ruminants and bees, Sophia Antipolis, 32 pp.
Free, J.B., Spencer-Booth, Y., 1959. The longevity of worker honey bees (*Apis mellifera*). Proc. R. Entomol. Soc. Lond. 34A, 141–150.
Genersch, E., von der Hoe, W., Katz, H., Schroeder, A., Often, Ch., Buhler, R., Berg, S., Ritter, W., Mullen, W., Girder, S., Mainer, M., Lie big, G., Rosenkranz, P., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332–352.
Jeschke, P., Nauen, R., 2005. Neonicotinoid insecticides. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science. Elsevier B.V., Oxford, pp. 53–105.
Lie big, G., 2002. Einfach imkern, second ed. Aichtal.
Liu, M.Y., Casida, J.E., 1993. High-affinity binding of [^{3}H]imidacloprid in the insect acetylcholine receptor. Pestic. Biochem. Physiol. 46, 40–46.
Maus, Ch., Curée, G., Schmuck, R., 2003. Safety of imidacloprid seed dressings to honey bees: a comprehensive overview and compilation of the current state of knowledge. Bull. Insectol. 56, 51–57.
Nauen, R., 1995. Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. Pestic. Sci. 44, 145–153.
Nauen, R., Ebbinghaus-Kintscher, U., Elbert, A., Jeschke, P., Tietjen, K., 2001. Acetylcholine receptors as sites for developing neonicotinoid insecticides. In: Ishaava, I. (Ed.), Biochemical Sites of Insecticide Action and Resistance. Springer, Berlin, Heidelberg, pp. 77–105.
Nguyen, B.K., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Thironet, B., Verheggen, F.J., Berkvens, D., De Pauw, E., Haubruge, E., 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality? J. Econ. Entomol. 102, 616–623.
Nguyen, B.K., Mignon, J., Laget, D., de Graaf, D.D., Jacobs, F.J., van Engelsdorp, D., Brostaux, Y., Saegerman, C., Haubruge, E., 2010. Honey bee colony losses in Belgium during the 2008–9 winter. J. Apic. Res. 49, 337–339.
Schmuck, R., 2004. Effects of a chronic dietary exposure of the honeybee *Apis mellifera* (Hymenoptera: Apidae) to imidacloprid. Arch. Environ. Contam. Toxicol. 47, 471–478.
Schmuck, R., 1999. No causal relationship between Gaucho® seed dressing in sunflowers and the French bee syndrome. Pflanzenschutz-Nachrichten Bayer 52, 257–299.
Schmuck, R., Schöning, R., Sur, R., 2005. Studies on the effects of plant protection products containing imidacloprid on the honeybee, *Apis mellifera* L. In: Forster, R., Bode, E., Brasse, D. (Eds.), Das "Bienensterben" im Winter 2002/2003 in Deutschland – Zum Stand der wissenschaftlichen Erkenntnisse. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Braunschweig, pp. 68–92.
Suchail, S., Guez, D., Belzunces, L.P., 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ. Toxicol. Chem. 20, 2482–2486.
Tennekes, H.A., 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, 1–4.
Tomizawa, M., Casida, J.E., 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48, 339–364.
Yu, S.J., 2008. The Toxicology and Biochemistry of Insecticides. CRC Press, Taylor & Francis Group, Boca Raton, pp. 115–142.

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