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Letter to the Editor

The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: Responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG

In 1948, Druckrey and Küpfmüller provided a theoretical explanation for Haber's rule (Haber, 1924). Haber had noted that exposure to a low concentration of a poisonous gas for a long time often had the same effect (death) as exposure to a high concentration for a short time. Haber's rule (for a review, see Witschi, 1999) states that the product of exposure concentration (c) and exposure duration (t) produces a constant toxic effect (ct = constant). The results of Druckrey's ground-breaking study on the carcinogenicity of 4-dimethylaminoazobenzene (4-DAB) in BDIII rats (Druckrey, 1943) were strikingly similar to Haber's rule: doubling the daily 4-DAB dose (d), and thereby presumably doubling the concentration of the carcinogen at the site of action (c), halved the time up to the appearance of liver cancer (t). However, the extended latency periods at lower 4-DAB dose levels had never before been observed in an experimental study, and were highly intriguing. As described in detail in my paper (Tennekes, 2010a), Druckrey and Küpfmüller reasoned that Haber's rule was, in theory, consistent with *irreversible receptor binding*. Many years later Warwick and Roberts confirmed the theorem of Druckrey and Küpfmüller by demonstrating covalent binding of a 4-DAB metabolite to DNA (Warwick and Roberts, 1967). Haber's rule was recently shown to describe the toxicity of the neonicotinoid insecticide imidacloprid to midges *Chironomus tentans* (Stoughton et al., 2008). The product of exposure concentration (c) and exposure duration to 50% mortality (t) for *C. tentans* was very similar under acute and chronic exposure conditions. Drs. Maus and Nauen of Bayer CropScience argue that, in this particular case, Haber's rule cannot be explained by irreversible receptor binding. They retract a certification made by their colleague Abbink in 1991 that "*imidacloprid is the first highly effective insecticide whose mode of action has been found to derive from almost complete and virtually irreversible blockage of postsynaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects*" (Abbink, 1991). Instead, Maus and Nauen state that "*all commercial neonicotinoid insecticides bind to insect nAChRs and cause the same effect as the natural neurotransmitter acetylcholine (ACh), i.e., agonistically activating the receptors resulting in a transient inward-current leading to the generation of action potentials. Similar to ACh, a neonicotinoid is binding to the nAChRs, and the binding of neonicotinoid insecticides is reversible. The synaptic action of ACh 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*".

In essence, what Maus and Nauen are inferring is that while the binding of neonicotinoid insecticides to nAChRs should be considered to be reversible, in principle, neonicotinoids do persist at the binding sites because the enzyme acetylcholinesterase cannot remove these compounds from the binding sites. Persistent receptor binding was considered by Druckrey and Küpfmüller in mathematical terms in their book *Dosis und Wirkung* (dose and response), and their reasoning was as follows (Druckrey and Küpfmüller, 1949): denoting the concentration of specific receptors that a poison reacts with as R, the concentration of receptors that a poison has reacted with as C_R, and the poison concentration at the site of action as C, the reaction kinetics in the case of a bimolecular reaction are:

$$\frac{dC_R}{dt} = K(R - C_R)C - \frac{C_R}{T_R} \quad (1)$$

where K is the reaction constant for association and T_R the time constant for dissociation.

If the effect occurs under circumstances where $C_R \ll R$, *i.e.*, with first order kinetics, then R remains practically constant, in which case

$$K\,(R - C_R) = \frac{1}{T_A} \quad (2)$$

where T_A can be regarded as the time constant for association. Eq. (1) then simplifies to

$$\frac{dC_R}{dt} = \frac{C}{T_A} - \frac{C_R}{T_R} \quad (3)$$

Denoting the initial concentration of receptors R_0, and replacing the concentration of bound receptors C_R by the relative concentration of bound receptors C_R/R_0, we obtain

$$\frac{dC_R/R_0}{dt} = \frac{C}{R_0 T_A} - \frac{C_R/R_0}{T_R} \quad (4)$$

In equilibrium, where $(dC_R/\,R_0)/dt = 0$, Eq. (4) simplifies to

$$\frac{C_R}{R_0} = \frac{1}{R_0}\frac{T_R}{T_A}\mathrm{C} \quad (5)$$

Based on this reasoning, Druckrey and Küpfmüller (1949) drew fundamentally important conclusions for all poisons that interact with specific receptors in a first order bimolecular reaction where the (toxic) effect is determined by the relative concentration of bound receptors C_R/R_0:

- *The effect is proportional to the concentration of the poison at the site of action C* (Paracelsus).
- *The effect is inversely proportional to* R_0. If the concentration of specific receptors R_0 is low, the poison may induce pronounced toxicity at very low concentrations at the site of action C. In insects, ACh is the most abundant neurotransmitter in the CNS

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and insect nervous tissue is one of the richest sources of neuronal nAChRs (Gauthier, 2010).

- *The effect is proportional to* T_R/T_A, *i.e.*, to the quotient of the time constant for dissociation T_R and the time constant for association T_A. If T_R/T_A is high, the poison may induce pronounced toxicity at very low concentrations at the site of action *C*.
- If both time constants (T_R and T_A) are low, equilibrium will be established quickly but the toxic effect will also regress quickly.
- If T_R is low, the time course of the effect will be the same as the time course of the concentration of the poison at the site of action *C* and the maximum effect will occur when *C* is at its maximum, while T_A will determine the fraction of the poison that reacts with the specific receptors *R*.
- If the time constant for dissociation T_R is quite high (which must be the case with neonicotinoids bound to insect nAChRs because the enzyme acetylcholinesterase cannot remove these compounds from the binding sites), the time to maximum effect will be delayed, and the (toxic) effect will also be slowly reversible. As a result, there will be a latency period up to a defined effect, and the only way to shorten this latency period is to increase the concentration of the poison at the site of action *C* (which is the essence of Haber's rule).

The reasoning by Druckrey and Küpfmüller (1949) thus shows that the mechanism of action of neonicotinoids put forward by Maus and Nauen is not inconsistent with Haber's rule. In fact, their definition of imidacloprid's insecticidal action strikes me as being very similar to Abbink's conclusion that "*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), because that is exactly what neonicotinoids will ultimately do. Like nicotine, imidacloprid mimics the action of acetylcholine, which is the major neurotransmitter in the insect nervous system, but nicotine and imidacloprid are not deactivated by acetylcholinesterase and thus persistently activate nAChRs (Thany, 2010). Chronic exposure of insects to neonicotinoids therefore leads to *cumulative* and virtually irreversible blockage of nAChRs in their central nervous system. Maus and Nauen then infer that "*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*". I agree that pharmacokinetics determine the time course of the concentration of the poison at the site of action *C*. Upon continuous exposure to a poison, *C* is the only variable determining the effect (in equilibrium), as shown in Eq. (5). Foraging as well as hive worker bees and brood are likely to be continuously exposed to imidacloprid when contaminated food is collected and stored inside the hive (Decourtye and Devillers, 2010). Moreover, as a result of ground- and surface water contamination with imidacloprid, as recorded in western provinces of the Netherlands, which exposes wild plants to imidacloprid, many other *non-target* insect species may also face chronic exposure to imidacloprid (Tennekes, 2010b). The inference made by Maus and Nauen that, under discontinuous exposure conditions, imidacloprid will only be available at the site of action for a limited period of time, is certainly true, although they do not mention that imidacloprid metabolism in honey bees generates two metabolites (olefine- and 5-OH-imidacloprid) with very high binding affinity for nAChRs (Nauen et al., 2001). However, even after short-term exposure, blockage of nAChRs by imidacloprid and its metabolites may persist long after these poisons have been eliminated from the body, because dissociation from these receptors will be a very slow process (T_R is high). Persistent blockage of nAChRs explains impaired honey bee foraging and learning, as induced by imidacloprid at sub-lethal doses (Guez et al., 2001; Decourtye et al., 2004; Colin et al., 2004). A honey bee during a foraging flight must learn and recall many complex visual patterns (Menzel et al., 1998; Capaldi and Dyer, 1999). These cognitive functions may be perturbed when nAChRs, necessary for the formation of long-term memory and involved in acquisition and retrieval processes, are persistently blocked (Gauthier, 2010). These observations are entirely consistent with the theorem of Druckrey and Küpfmüller (1949). Both receptor binding and the effect of receptor binding are virtually irreversible, and exposure time will therefore reinforce the effect. This is why the Druckrey–Küpfmüller equation dt^n = constant (where d = daily dose and t = exposure time to effect, with $n \geq 1$), indicating that 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* with decreasing exposure levels, characterises not only chemical carcinogenesis (Druckrey and Dischler, 1963; Druckrey et al., 1963; Littlefield et al., 1980; Peto et al., 1991) and photocarcinogenesis (Sterenborg et al., 1988; de Laat et al., 1997) but also the toxicity of neonicotinoid insecticides to arthropods (Sanchez-Bayo, 2009). Therefore I consider the claim made by Maus and Nauen that "*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*" to be unfounded.

The British pharmacologist Clark unwittingly pointed to a crucial additional aspect of Haber's rule when he expanded Haber's rule to characterise the action of a number of drugs (Clark, 1937):

$$(c - c_m)(t - t_m) = \text{constant} \tag{6}$$

where c_m = *a* threshold concentration, and t_m = *a* minimum time of response. Clark commented at the time (Clark, 1937):

> "*The formula ct = constant is indeed an impossible one in the case of drugs acting on biological material because it implies that an infinitely small concentration of a drug will produce the selected action in infinite time, and conversely that a sufficiently high concentration will produce an instantaneous effect. In some cases ct = constant gives an approximate fit, but this merely implies that* ***c_m and t_m are so small as not to produce a measurable error***".

So, an approximate fit of Haber's rule to the action of a poison indicates not only cumulative blockage of critical receptors but also that **the threshold concentration (c_m) is very small**. For genotoxic carcinogens it is now commonly accepted to apply the regulatory default based on the assumption that if "one hit" could cause a mutation and eventually result in cancer, then any exposure level could be associated with a finite cancer probability. With this in mind, the U.S. EPA evaluates carcinogens using a low-dose, linear model (EPA, 2005). In stark contrast, Maus and Nauen assert "*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*". They refer to numerous studies providing evidence "*that there is under realistic conditions no correlation between exposure of honey bees to imidacloprid-treated crops and increased colony mortality*", but they discredit the results of a study conducted by Suchail et al. (2001), which are consistent with the theorem of Druckrey and Küpfmüller (1949), and completely ignore the authoritative French STC (Scientific and Technical Committee for the Multifactor Study of the Honeybee Apiaries Decline) report as well as a significant number of other studies showing harmful impacts to both honey bees and bumblebees at environmentally relevant levels, mainly in studies of chronic toxicity and sub-lethal impacts of imidacloprid, as recently reviewed by Kindemba (2009). I could not disagree more with Maus and Nauen. In my view, neonicotinoids are destroying the web of life and should be banned (Tennekes, 2010b)

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