The potential toxicity to insects and other arthropods of Dutch surface water contaminated with imidacloprid

A 12 µL intake of Noordwijkerhout water excessively contaminated with imidacloprid at 320,000 ng/L (Table 2) would expose a honey bee to the acutely lethal dose (3.84 nanogram ~ acute (48h) LD50 for a honey bee, see Table 1). However, Suchail et al. (2001) reported that much lower imidacloprid concentrations (≥ 1,000 ng/L) killed honey bees within 8 days[1].

The 96-h lethal imidacloprid concentration (LC50) value for for midges (*Chironomus tentans*) was determined to be 5,750 ng/L, but when the animals were continuously exposed for 28 days the LC50 value was much lower: 910 ng/L[2]. The product of exposure concentration (c) and exposure time (t) remained approximately constant, i.e. c . t = constant, indicating that the total lethal imidacloprid dose remained virtually the same under acute and chronic exposure conditions.

Such dose : response relationships are known as Haber's rule (the product of exposure concentration and duration produces a constant toxic effect), after the German chemist Fritz Haber who in the early 1900s characterized the acute toxicity of nerve gases used in chemical warfare[3]. Haber's rule was subsequently (in the 1940s) shown to apply to the carcinogenicity of 4-dimethylaminoazobenzene (4-DAB) in rats[4] (the time up to the appearance of liver cancer was found to be inversely proportional to the daily dose), which led to an important theoretical explanation of Haber's rule[5], as follows:

denoting the initial concentration of specific receptors that 4-DAB reacts with as R, the concentration of receptors that 4-DAB has reacted with as C_R, and the mean 4-DAB concentration at the site of action as C, the reaction kinetics in the case of a bimolecular reaction are:

$$dC_R / dt = K (R - C_R) C - C_R / T_R \hspace{4cm} (1)$$

where K is the reaction constant for association and T_R the time constant for dissociation. Since the carcinogenic action of 4-DAB was *irreversible,* and $T_R \rightarrow \infty$, we obtain

$$dC_R / dt = K (R - C_R) C \hspace{5cm} (2)$$

[1] Suchail S et al. (2001). Environmental Toxicology and Chemistry 20, 2482–2486
[2] Stoughton SJ et al. (2008) Arch Environ Contam Toxicol 54:662-673
[3] Haber F (1924) Zur Geschichte des Gaskrieges. In Fünf Vorträge aus den Jahren 1920-1923, pp. 76-92. Julius Springer, Berlin
[4] Druckrey H (1943). Quantitative Grundlagen der Krebserzeugung. Klinische Wochenschriften 22: 532.
[5] Druckrey H and Küpfmüller K (1948) Quantitative Analyse der Krebsentstehung. Z Naturforschung 3b: 254-266

Now, assuming that up to the time of action $C_R \ll R$, which appears reasonable, then R remains practically constant, therefore

$$dC_R / dt = K\ R\ C \qquad\qquad\qquad\qquad\qquad\qquad (3)$$

Since the dose level was kept constant throughout the study, C probably remained constant as well. Integration yields

$$C_R = K\ R\ C\ t \qquad\qquad\qquad\qquad\qquad\qquad (4)$$

which is Haber's Rule.

Thus, Haber's Rule points to *cumulative blockage of critical receptors* [6]. Indeed, 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 nicotinergic acetylcholine receptors in the central nervous system of insects [7,8].

The British pharmacologist AJ Clark further expanded Haber's rule to characterise the action of a number of drugs, and pointed to an important additional aspect of Haber's Rule[9]:

$$(c - c_m)\ (t - t_m) = \text{constant} \qquad\qquad\qquad\qquad\qquad (5)$$

where c_m = a threshold concentration, and t_m = a minimum time of response. Clark commented at the time

"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".

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[6] Tennekes H et al. (2010) Hazard and Risk Assessment of Chemical Carcinogenicity Within a Regulatory Context. In: Cancer Risk Assessment, edited by Ching-Hung Hsu and Todd Stedeford, John Wiley & Sons, Inc.
[7] Buckingham SD et al. (1997) The Journal of Experimental Biology 200: 2685-2692
[8] Abbink, J. (1991). The biochemistry of imidacloprid. Pflanzenschutz-Nachrichten Bayer (Germany, F.R.) Serial ID – ISSN: 0340-1723
[9] Clark, A. J. (1937). General pharmacology. In Handbuch der Experimentellen Pharmakologie (W. Heubner and J. Schuller, eds.), Vol. 4, pp. 123-142. Springer Verlag, Berlin/New York

So, an approximate fit of Haber's rule to the action of a compound indicates not only cumulative blockage of critical receptors but also that *the threshold concentration (c_m) is very small*. Haber's rule is characterised by a linear relationship (on logarithmic coordinates) between exposure concentration and median time to effect, i.e. mortality. Similar relationships have now also been demonstrated for the toxicity of imidacloprid to the freshwater ostracod *Cypridopsis vidua* and to *Daphnia magna* as well as for the toxicity of thiacloprid to *Gammarus* and, *Sympetrum*[10]. Sanchez-Bayo (2009) demonstrated that the relationship between the concentration of the neonicotinoid insecticides imidacloprid and thiacloprid in a medium (C) and the time to 50% mortality (T) of these exposed arthropods followed a hyperbolic curve described by the equation

$$T = a * C^{-b} \tag{6}$$

Accordingly, there was a linear relationship when the logarithms of the variables C and T were used

$$\ln T = a' - b \ln C \tag{7}$$

where a' is the intercept and b is the slope. Equation (7) can be transformed to

$$C^{b} T = \text{constant} \tag{8}$$

or

$$C T^{1/b} = \text{constant} \tag{9}$$

Equation (9) is very similar to the Druckrey-Küpfmüller equation for the action of chemical carcinogens such as diethylnitrosamine (DENA)

$$d\, t^{n} = \text{constant} \tag{10}$$

where d= daily dose and t = exposure time to effect (cancer), and n = 2.3 in the case of DENA.

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[10] Sánchez-Bayo F (2009) Ecotoxicology 18:343-354

Similar to the dose-response characteristics of DENA, exposure time was found to reinforce the toxicity of imidacloprid and thiacloprid to the tested arthropod species. The C T product, which reflects the total dose required for a lethal effect, decreased with decreasing toxicant concentration C (Table 3). even though the times to 50% mortality T *increased* with decreasing toxicant concentration C. The value of the time exponent (1/b) in equation (9) was shown to be 4.65 and 1.31 for the toxicity of imidacloprid to *Cypridopsis vidua* and *Daphnia magna*, respectively, and 1.10 and 1.53 for the toxicity of thiacloprid to *Gammarus pulex* and *Sympetrum striolatum*, respectively (Sanchez-Bayo, 2009)[11,12] Suchail et al. (2001) also noted that at concentrations of 0.1, 1, and 10 µg of imidacloprid per liter, the total cumulated dose ingested by honeybees in chronic intoxication was about 60 to 6,000 times lower than the doses needed to produce the same effect in acute intoxication tests. Thus, low environmental concentrations of these insecticides (that may not be acutely toxic) could be detrimental to many invertebrate species in the long term, in particular because these compounds are persistent in soil and stable to breakdown by water (Table 1) and their toxicity to invertebrates may be reinforced by exposure time.

Nicotinic acetylcholine receptors (nAChRs) play roles in many cognitive processes. At sub-lethal doses imidacloprid can alter honey bee foraging and learning[13,14,15,16]. Imidacloprid has been detected at levels of 5.7 µg/kg in pollen from French hives[17] and foraging honey bees reduced their visits to a syrup feeder when it was contaminated with 3 µg/kg of imidacloprid[18]. Mayflies of the genera *Baetis* and *Epeorus* showed a reduction in reproductive success when exposed to concentrations of imidacloprid as low as 100 ng/L[19].

The evidence indicates that, in any case in the western part of the Netherlands (Table 2), high concentrations of imidacloprid are diffused through the environment, which may kill or debilitate insects and possibly other arthropods. There is supporting evidence. The number of butterflies in the Netherlands is presently at the lowest point ever recorded (Figure 1)[20], and the lowest numbers of butterflies are being recorded in the western part of the country (apart from the coastal dunes)[21]. The water beetle *Graphoderus bilineatus*, widely recorded in the Netherlands up to the 1980s, is now nearly extinct in the western province of South-Holland[22]. In a comprehensive appraisal of the impact of neonicotinoid insecticides

[11] The effects of thiacloprid on Simulium latigonium reported by Sanchez-Bayo (2009) were not considered in view of a poor fit to the regression equation (7)

[12] Sánchez-Bayo F (2009) Ecotoxicology 18:343-354

[13] Guez D et al. (2001) Neurobiol. Learn. Mem. 76: 183-191

[14] Lambin M et al. (2001). Arch. Insect Biochem. Physiol. 48: 129-134

[15] Decourtye A et al (2004) Ecotoxicol. Environ. Saf. 57: 410-419

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[17] Chauzat MP et al. (2006) Apiculture and Social Insects 99(2): 253-262

[18] Yang EC et al (2008) Journal of Economic Entomology 101(6): 1743-1748

[19] Alexander AC et al. (2008) Freshwater Biology 53: 171-180

[20] van Swaaij CAM et al. (2009). Vlinders en libellen geteld. Jaarverslag 2008, Vlinderstichting (Butterfly Foundation), Wageningen & the Dutch Central Statistics Office (CBS), The Hague. Report No. VS2009.007

[21] van Swaay CAM et al (2006) Hotspots Dagvlinder Biodiversiteit, Rapport VS2006.016, De Vlinderstichting, Wageningen

[22] Cuppen JGM (2005) De gestreepte waterroofkever Graphoderus bilineatus in Zuid-Holland. Stichting European Invertebrate Survey-Nederland, Leiden

on bumblebees, honey bees and other non-target invertebrates, Kindemba also concluded that significant negative impacts of imidacloprid on bees and other non-target insects occur at levels predicted to be present in the UK countryside (based on imidacloprid application rates approved for use in the UK)[23].

Aantalsontwikkeling vlinders
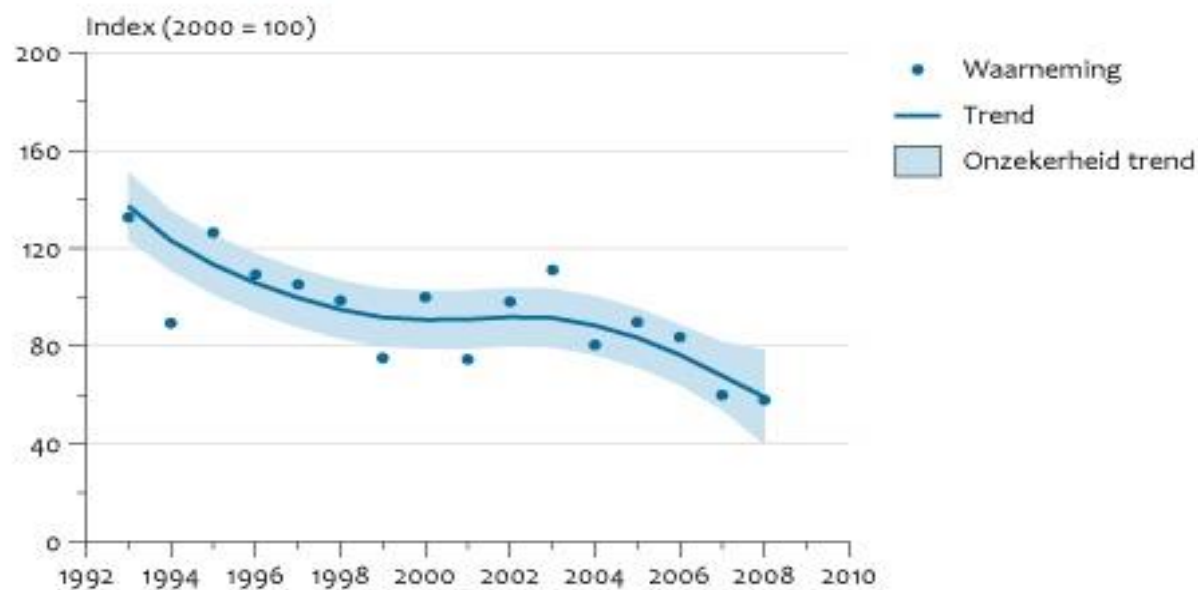
Bron: NEM (Vlinderstichting, CBS).

CBS/jul09/1386
www.compendiumvoordeleefomgeving.nl

Figure 1. The decline of day butterflies in the Netherlands since 1992. Source: Netwerk Ecologische Monitoring (NEM) [CBS (Dutch Central Statistics Office), Vlinderstichting (Butterfly Foundation)]

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[23] Kindemba V (2009) The impact of neonicotinoid insecticides on bumblebees, honey bees and other non-target invertebrates. Buglife Invertebrate Conservation Trust, UK

Table 3: Mortality of Arthropods Induced by Neonicotinoid Insecticides (Sanchez-Bayo, 2009)*

Species	Chemical	Concentration (C) in $\mu g.L^{-1}$	Time to 50% mortality (T) in days	C x T product in $\mu g.L^{-1}$.days
Cypridopsis vidua	Imidacloprid	4	…5.2	20.8
		16	3.0	48
		64	3.3	211.2
		250	2.3	575
		1,000	2.0	2,000
		4,000	0.9	3,600
Daphnia magna	Imidacloprid	250	384.7	96,175
		750	69.7	52,275
		2,220	18.6	41,292
		6,700	15.0	100,500
		20,000	18.4	368,000
		60,000	3.0	180,000
Gammarus pulex	Thiacloprid	99	63.6	6,296.4
		364	16.7	6,078.8
		988	6.5	6,422
		3,100	3.2	9,920
		9,520	0.9	8,568
Sympetrum striolatum	Thiacloprid	7.2	20.6	148.3
		8.0	17.2	137.6
		12.7	13.0	165.1
		113.3	3.2	362.6

* The effects of thiacloprid on *Simulium latigonium* reported by Sanchez-Bayo (2009) were not considered in view of a poor fit to the regression equation (7)

General environmental studies with imidacloprid
Neonicotinoid insecticides differ from conventional spray products in that they can be used as seed dressings or as soil treatments. When used as a seed dressing, imidacloprid will migrate from the stem to the leaf tips and eventually into flowers and pollen[24,25,26]. Imidacloprid has been detected on vegetation near corn fields sown with imidacloprid-dressed seeds[27].

Suppression of non-target arthropods by neonicotinoid insecticides under field conditions
A study conducted over 3 years on an experimental home lawn[28] revealed that three consecutive yearly imidacloprid applications to the same field plots suppressed numbers of total hexapods, *Collembola*, *Thysanoptera* and *Coleoptera* adults by 54-62%. Turfgrass-residue exposure of the carabid *Harpalus pennsylvanicus* to imidacloprid has caused neurotoxic effects, making them highly vulnerable to predation[29].When applied directly to aquatic microcosms to simulate leaching from soils, imidacloprid was shown to be highly toxic to aquatic insects[30]. A single pulse contamination of mesocosms (designed to realistically mimic communities in small streams within the agricultural landscape) with the neonicotinoid insecticide thiacloprid to resulted in long-term alteration of the overall invertebrate community structure[31]. One species, the stonefly *Nemoura cinerea*, was affected at the lowest tested concentration, 70 times below the lowest known median lethal concentration (LC50). Nearly 5,000 historical and contemporary specimen records of stoneflies (*Plecoptera*) from Illinois demonstrated that this fauna is highly imperilled, boding poorly for aquatic insect communities in North America and elsewhere[32]. When applied as a systemic insecticide to trees by direct stem injections or by soil injections and drenches, imidacloprid may be indirectly introduced to aquatic systems via leaf fall or leaching. Imidacloprid at realistic concentrations in leaves can inhibit leaf litter breakdown through adverse effects on decomposer invertebrates[33]. When imidacloprid is applied as a systemic insecticide to the soil around trees it may cause adverse effects on earthworms[34]. Imidacloprid at realistic field concentrations in maple leaves had adverse effects on aquatic insects and earthworms[35]. A recent study indicates high toxicity of imidacloprid to the non-target terrestrial arthropod *Porcellio scaber*[36]. Imidacloprid affects isopods at similar exposure concentrations as insects.

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