Toxicology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

News and views

The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time

Henk A. Tennekes*

Experimental Toxicology Services (ETS) Nederland bv, Frankensteeg 4, 7201KN Zutphen, The Netherlands

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Insecticides
Carcinogens
Arthropods
Toxicity

ABSTRACT

The essence of the Druckrey–Küpfmüller equation dt^n = constant (where d = daily dose and t = exposure time-to-effect, with $n > 1$) for chemical carcinogens is 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. Druckrey and Küpfmüller inferred that if both receptor binding and the effect are irreversible, exposure time would reinforce the effect. The Druckrey–Küpfmüller equation explains why toxicity may occur after prolonged exposure to very low toxicant levels. Recently, similar dose–response characteristics have been established for the toxicity of the neonicotinoid insecticides imidacloprid and thiacloprid to arthropods. This observation is highly relevant for environmental risk assessment. Traditional approaches that consider toxic effects at fixed exposure times are unable to allow extrapolation from measured endpoints to effects that may occur at other times of exposure. Time-to-effect approaches that provide information on the doses and exposure times needed to produce toxic effects on tested organisms are required for prediction of toxic effects for any combination of concentration and time in the environment.

1. A brief history of the Druckrey–Küpfmüller equation

The first benchmark study of dose–response relationships in chemical carcinogenesis was reported by Druckrey with 4-dimethylaminoazobenzene (4-DAB), also known as "butter yellow", in BDIII rats (Druckrey, 1943). Within the range of daily dosages from 3 to 30 mg per rat the time up to the appearance of liver cancer (t) was found to be inversely proportional to the daily dose (d). The product of the daily dosage and the median tumor induction time, which corresponds to the sum of all daily doses, i.e. to the total dose (D), was found to be practically constant (Table 1)

$$dt = D \sim 1000\,\text{mg} = \text{constant} \tag{1}$$

Assuming proportionality between the daily dosage (d) and the 4-DAB concentration (c) at the site of carcinogenic action, Eq. (1) would read as:

$$ct = \text{constant} \tag{2}$$

Eq. (2), that the product of exposure concentration and duration produces a constant toxic effect, is known as Haber's rule (Haber, 1924), after the German chemist Fritz Haber who in the early 1900s characterized the acute toxicity of gases used in chemical warfare (for a review, see Witschi, 1999). Druckrey and Küpfmüller (1948) provided a theoretical explanation for Haber's rule, 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:

$$\frac{dC_R}{dt} = K(R - C_R)C - \frac{C_R}{T_R} \tag{3}$$

where K is the reaction constant for association and T_R the time constant for dissociation. Druckrey and Küpfmüller then inferred that their experiment had shown that the carcinogenic action of 4-DAB was *irreversible*, and that as $T_R \to \infty$

$$\frac{dC_R}{dt} = K(R - C_R)C \tag{4}$$

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

$$\frac{dC_R}{dt} = KRC \tag{5}$$

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

$$C_R = KRCt \tag{6}$$

* Tel.: +31 575 545 500; fax: +31 575 516 717.
E-mail address: info@toxicology.nl.

0300-483X/$ – see front matter
doi:10.1016/j.tox.2010.07.005

Table 1
Induction of liver cancer in BDIII rats by 4-DAB (Druckrey, 1943).

Daily dose, d (mg/rat)	Median tumor induction time, t (days)	Total dose, D (mg/rat)
30	34	1020
20	52	1040
10	95	950
5	190	950
3	350	1050

which is Haber's rule. Eq. (6) provided a theoretical explanation for Haber's rule, but it assumed proportionality between the concentration of occupied receptors and the *effect*. This may not always be the case. The reversibility of an effect can have the same significance for dose–response characteristics as the reversibility of receptor binding. Denoting the time constant for the reversibility of the effect as T_r, three types of dose–response characteristics were identified by Druckrey and Küpfmüller (1949) when the time constants T_R and T_r approach either zero or infinity (Table 2).

An effect may be dose-dependent when the time constants for receptor binding (T_R) and the effect (T_r) are quite small. Haber's Rule (ct = constant) may be obtained when either receptor binding or the effect is irreversible. If, however, both receptor binding and the effect are irreversible, the effect would be proportional to the double integral of compound concentration over time. The implication would be that exposure time would *reinforce the effect.* The first indication that exposure time may reinforce the action of a chemical carcinogen was a significantly smaller total carcinogenic dose (700 mg instead of 1000 mg) at a 4-DAB dose of 1 mg/day with a median liver tumor induction time of 700 days, but Druckrey and co-workers reported more pertinent evidence in 1963 with benchmark studies of the production of ear-duct and liver carcinomas by 4-dimethylaminostilbene (4-DAST) (Druckrey and Dischler, 1963) and diethylnitrosamine (DENA) (Druckrey et al., 1963), respectively, in BDII rats (Tables 3 and 4).

In contrast to the 4-DAB carcinogenicity study, where the total carcinogenic dose remained practically constant at daily dosages from 3 to 30 mg, the total carcinogenic dose *decreased* with decreasing daily 4-DAST or DENA dose levels, even though the median tumor induction times *increased* with decreasing daily dose levels. In a logarithmic system of coordinates, there was a linear relationship between the median tumor induction time (t) and the daily dosage (d):

$$\ln d = \ln k - n \ln t \tag{7}$$

or

$$dt^n = \text{constant} \tag{8}$$

where the time exponent n was 3.0 and 2.3 for 4-DAST and DENA, respectively (Fig. 1).

Table 3
Induction of ear-duct carcinomas in BDII rats by 4-DAST (Druckrey and Dischler, 1963).

Daily dose, d (mg/kg)	Median tumor induction time, t (days)	Total dose, D (mg/kg)
3.4	250	850
2.0	340	680
1.0	407	407
0.5	550	275
0.28	607	170
0.2	700	140
0.1	900	90

Table 4
Induction of liver carcinomas in BDII rats by DENA (Druckrey et al., 1963).

Daily dose, d (mg/kg)	Median tumor induction time, t (days)	Total dose, D (mg/kg)
9.6	101	963
4.8	137	660
2.4	192	460
1.2	238	285
0.6	355	213
0.3	457	137
0.15	609	91
0.075	840	64

2. The significance of the Druckrey–Küpfmüller equation for risk assessment

Dose and time dependencies in the form of Druckrey–Küpfmüller equation (8) were confirmed in the ED_{01} study, the largest toxicology experiment ever conducted, examining the carcinogenicity of 2-acetylaminofluorene in about 25,000 mice (Littlefield et al., 1980) and in the BIBRA study with 4000 rats to investigate the carcinogenicity of nitrosamines (Peto et al., 1991). The Druckrey–Küpfmüller equation (8) was also found to

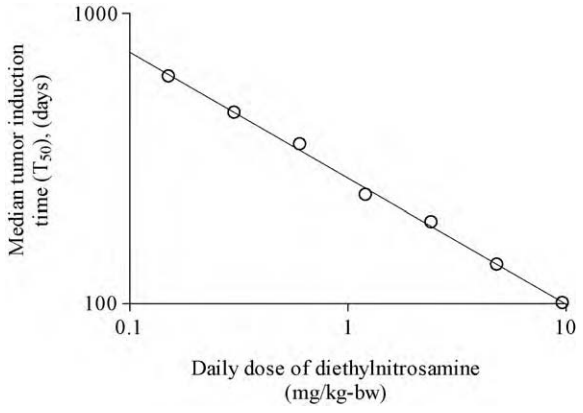

Fig. 1. A linear relationship in a logarithmic system of coordinates between the median liver tumor induction time (T_{50}) in BD II rats and the daily dosage of diethylnitrosamine. Linearity leads to Eq. (7).

Table 2
Dose–response characteristics according to Druckrey and Küpfmüller (1949).

Reversibility of receptor binding	Receptor binding in relation to compound concentration	Reversibility of the effect	Effect in relation to receptor binding	Effect in relation to compound concentration	Dose–response characteristics
$T_R \to 0$	$C_R \sim C$	$T_r \to 0$	$E \sim C_R$	$E \sim C$	Dose-dependent
		$T_r \to \infty$	$E \sim \int C_R\,dt$	$E \sim \int C\,dt$	ct = constant
$T_R \to \infty$	$C_R \sim \int C\,dt$	$T_r \to 0$	$E \sim C_R$	$E \sim \int C\,dt$	ct = constant
		$T_r \to \infty$	$E \sim \int C_R\,dt$	$E \sim \iint C\,dt$	Reinforced by time

Table 5
Mortality of arthropods induced by neonicotinoid insecticides (Sanchez-Bayo, 2009).[a].

Species	Chemical	Concentration (C) in $\mu g\,L^{-1}$	Time to 50% mortality (T) in days	CT 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
		1000	2.0	2000
		4000	0.9	3600
Daphnia magna	Imidacloprid	250	384.7	96,175
		750	69.7	52,275
		2220	18.6	41,292
		6700	15.0	100,500
		20,000	18.4	368,000
		60,000	3.0	180,000
Gammarus pulex	Thiacloprid	99	63.6	6296.4
		364	16.7	6078.8
		988	6.5	6422
		3100	3.2	9920
		9520	0.9	8568
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

[a] 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 (12).

apply to non-melanoma skin cancer induced by solar ultraviolet radiation (UVR). UVR is usually subdivided into Ultraviolet A (UVA) wavebands (UVA2: 315–340 nm, and UVA1: 340–400 nm) and the ultraviolet B (UVB) waveband (280–315 nm). The relationship between the daily dose (d) and the median non-melanoma skin tumor induction time (t) in hairless mice for both UVA1 (de Laat et al., 1997) and UVB (Sterenborg et al., 1988) has been demonstrated to be:

$$d^r t = \text{constant} \tag{9}$$

or

$$dt^{1/r} = \text{constant} \tag{10}$$

where $r = 0.62$ for UVB and 0.35 for UVA1 and $1/r = 1.6$ for UVB and 2.9 for UVA1.

The Druckrey–Küpfmüller equation (8) was recently shown to be of great significance for environmental risk assessment as well. For the neonicotinoid insecticides imidacloprid and thiacloprid, Sanchez-Bayo (2009) demonstrated that the relationship between toxicant concentration in a medium (C) and time to 50% mortality (T) of exposed arthropods followed a hyperbolic curve described by the equation

$$T = a \times C^{-b} \tag{11}$$

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

$$\ln T = a' - b \ln C \tag{12}$$

where a' is the intercept and b is the slope. Eq. (12) can be transformed to

$$C^b T = \text{constant} \tag{13}$$

or

$$CT^{1/b} = \text{constant} \tag{14}$$

which is the Druckrey–Küpfmüller equation (8). Similar to the dose–response characteristics of chemical carcinogens, exposure time was found to reinforce the toxicity of imidacloprid and thiacloprid to various arthropod species (Sanchez-Bayo, 2009). The CT product, which reflects the total dose required for a lethal effect, decreased with decreasing toxicant concentration C (Table 5) even though the times to 50% mortality T *increased* with decreasing toxicant concentration C.

The value of the time exponent ($1/b$) in Eq. (14) 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).[1] 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 60–6000 times lower than the doses needed to produce the same effect in acute intoxication tests. Stoughton et al. (2008) determined the 96-h lethal imidacloprid concentration (LC50) value for midges (*Chironomus tentans*) to be 5.75 μg/L, but when the animals were continuously exposed for 28 days the LC50 value was much lower: 0.91 μg/L. The CT product for *C. tentans* was very similar under acute and chronic exposure conditions.

3. Discussion

Druckrey and Küpfmüller (1949) explained the dose–response characteristics of chemical carcinogens by pointing out that if both receptor binding and the effect are irreversible, exposure time would reinforce the effect. The Druckrey–Küpfmüller equation (8) established in the 1960s in experiments in rats with 4-DAST or DENA has subsequently been confirmed in large scale carcinogenicity studies in mice and rats and was also found to apply to non-melanoma skin cancer induced in hairless mice by solar ultraviolet radiation. The essential feature of the Druckrey–Küpfmüller equation (8) is 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. Recently, similar dose–response characteristics have been established for the toxicity of the neonicotinoid insecticides imidacloprid and thiacloprid to arthropods, which confirm the theorem of Druckrey and Küpfmüller (Table 2). Imidacloprid was the first highly effective insecticide whose mode

[1] The effects of thiacloprid on *Simulium latigonium* reported by Sanchez-Bayo (2009) were not considered in view of a poor fit to the regression Eq. (12).

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; Buckingham et al., 1997; Zhang et al., 2000). Imidacloprid has a much higher affinity for insect nAChRs than for vertebrate nAChRs (Liu and Casida, 1993) and is therefore less toxic to mammals and birds. Nicotinic acetylcholine receptors (nAChRs) mediate fast cholinergic synaptic transmission and play roles in many cognitive processes. Honey bee foraging and learning may be impaired by imidacloprid at sub-lethal doses (Guez et al., 2001; Decourtye et al., 2004; Colin et al., 2004), which may in the course of time be detrimental to the bee colony, and ultimately cause colony collapse. The Druckrey–Küpfmüller equation (8) explains why such effects may occur at very low exposure levels and is therefore highly relevant for environmental risk assessment. Traditional approaches that consider toxic effects at fixed exposure times are unable to allow extrapolation from the measured endpoints to effects that may occur at other times of exposure. Time-to-effect approaches that provide information on the doses and exposure times needed to produce toxic effects on the tested organisms are required for prediction of toxic effects for any combination of concentration and time in the environment.

Conflict of interest statement

None.

References

Abbink, J., 1991. The Biochemistry of Imidacloprid. Pflanzenschutz–Nachrichten Bayer, Germany, F.R, Serial ID, ISSN:0340-1723.

Buckingham, S.D., Lapied, B., Le Corronic, H., et al., 1997. Imidacloprid actions on insect neuronal acetylcholine receptors. J. Exp. Biol. 200, 2685–2692.

Colin, M.E., Bonmatin, J.M., Moineau, I., et al., 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to sub-lethal effects induced by systemic insecticides. Arch. Environ. Contam. Toxicol. 47 (3), 387–395.

Decourtye, A., Devillers, J., Cluzeau, S., et al., 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol. Environ. Saf. 57, 410–419.

de Laat, A., van der Leun, J.C., de Gruijl, F.R., 1997. Carcinogenesis induced by UVA (365-nm) radiation: the dose–time dependence of tumour formation in hairless mice. Carcinogenesis 18, 1013–1020.

Druckrey, H., 1943. Quantitative Grundlagen der Krebserzeugung. Klinische Wochenschriften 22, 532.

Druckrey, H., Küpfmüller, K., 1948. Quantitative Analyse der Krebsentstehung. Z. Naturforsch 3b, 254–266.

Druckrey, H., Küpfmüller, K., 1949. Dosis und Wirkung. Beiträge zur theoretischen Pharmakologie. Editio Cantor GmbH, Freiburg im Breisgau, Germany.

Druckrey, H., Dischler, W., 1963. Dosis-Wirkungsbeziehungen bei der Krebserzeugung durch 4-dimethylaminostilben bei Ratten. Z. Krebsforsch. 65, 272.

Druckrey, H., Schildbach, A., Schmaehl, D., Preussmann, R., Ivankovic, S., 1963. Quantitative analysis of the carcinogenic effect of diethylnitrosamine. Arzneimittelforschung 13, 841–851.

Guez, D., Suchail, S., Gauthier, M., et al., 2001. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). Neurobiol. Learn. Mem. 76, 183–191.

Haber, F., 1924. Zur Geschichte des Gaskrieges. In: Fünf Vorträge aus den Jahren 1920–1923. Julius Springer, Berlin, pp. 76–92.

Littlefield, N.A., Farmer, J.H., Gaylor, D.W., Sheldon, W.G., 1980. Effects of dose and time in a long-term, low-dose carcinogenic study. J. Environ. Pathol. Toxicol. 3, 17–34.

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.

Peto, R., Gray, R., Brantom, P., Grasso, P., 1991. Effects on 4080 rats of chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine: a detailed dose–response study. Cancer Res. 51, 6415–6451.

Sanchez-Bayo, F., 2009. From simple toxicological models to prediction of toxic effects in time. Ecotoxicology 18, 343–354.

Sterenborg, H.J., van Weelden, H., van der Leun, J.C., 1988. The dose–response relationship for tumourigenesis by UV radiation in the region 311–312 nm. J. Photochem. Photobiol. B 2, 179–194.

Stoughton, S.J., Liber, K., Culp, J., Cessna, A., 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates Chironomus tentans and Hyalella azteca under constant- and pulse-exposure conditions. Arch. Environ. Contam. Toxicol. 54, 662–673.

Suchail, S., Guez, D., Belzunces, 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ. Toxicol. Chem. 20, 2482–2486.

Witschi, H., 1999. Some notes on the history of Haber's law. Toxicol. Sci. 50, 164–168.

Zhang, A., Kayser, H., Maienfisch, P., Casida, J.E., 2000. Insect nicotinic acetylcholine receptor: conserved neonicotinoid specificity of [^{3}H] imidacloprid binding site. J. Neurochem. 75, 1294–1303.