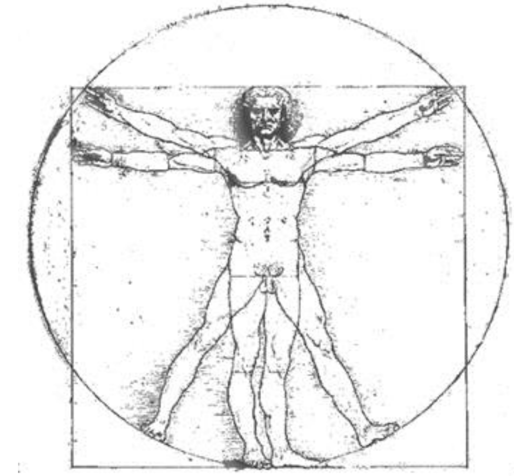
Henk Tennekes
Experimental Toxicology Services Nederland



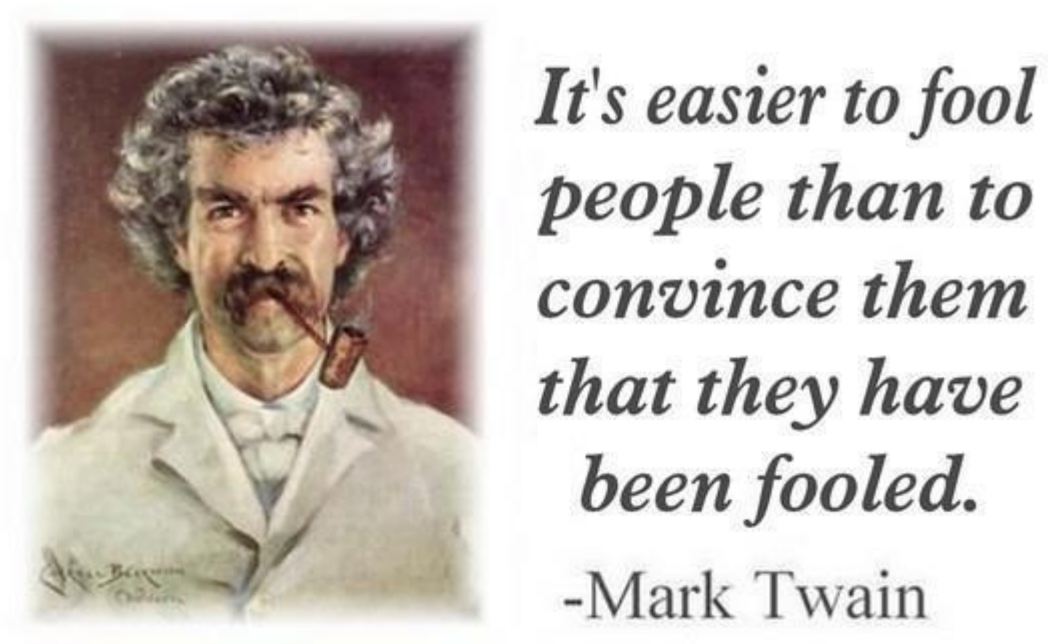
New Approaches to Pesticide Risk Assessment

Dr. Henk Tennekes

Workshop on CCD and Neonicotinoid Insecticides

The Radcliffe Institute at Harvard University
Cambridge, Massachusetts
February 11, 2015

Wise words from the author of Huckleberry Finn and Tom Sawyer

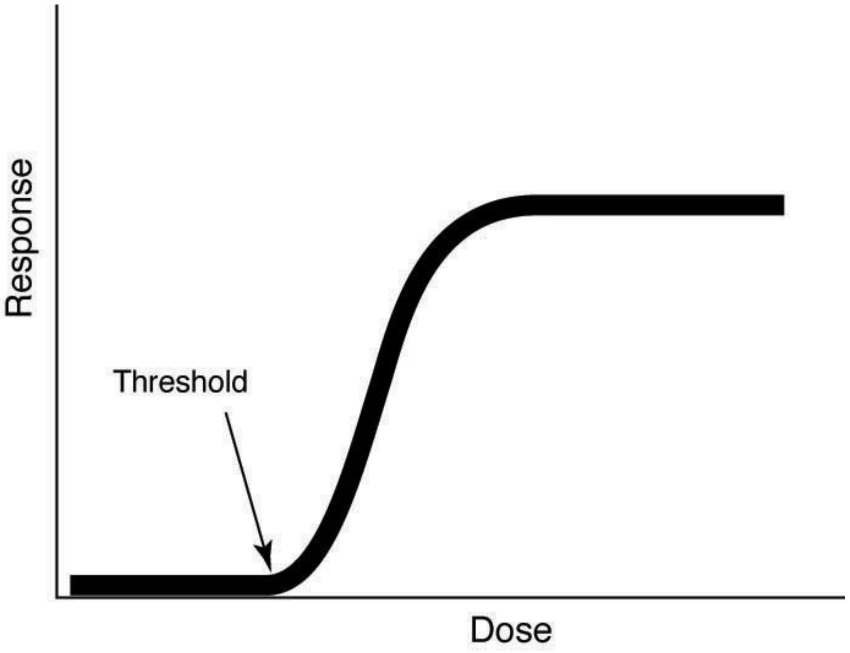


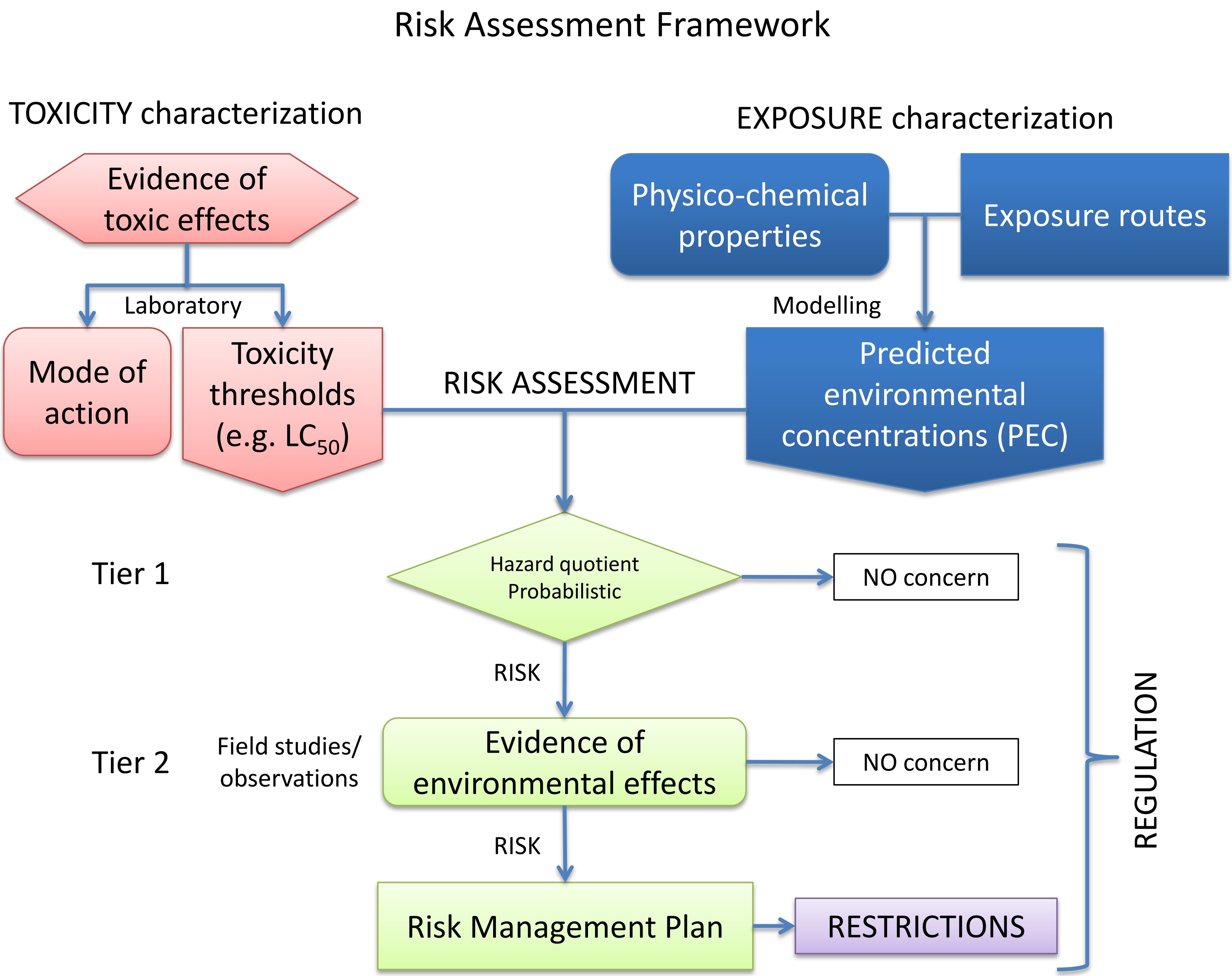
Dosis facit venenum - The dose makes the poison

The Threshold of Exposure Concept

Dybing E et al. 2002. Food Chem. Toxicol. 40, 237-282

- For most toxic processes, it is assumed that there is a threshold of exposure below which no biologically significant effect will be induced
- A 100-fold uncertainty factor is routinely applied to the No-Observed-Adverse-Effect-Level (NOAEL) from an animal study to derive a limit value, e.g. ADI (acceptable daily intake)




Risk Assessment Framework
TOXICITY characterization
EXPOSURE characterization
Evidence of toxic effects
Laboratory
Mode of action
Toxicity thresholds (e.g. LC_{50})
Physico-chemical properties
Exposure routes
Modelling
Predicted environmental concentrations (PEC)
RISK ASSESSMENT
Tier 1
Hazard quotient
Probabilistic
NO concern
RISK
Tier 2
Field studies/ observations
Evidence of environmental effects
NO concern
RISK
Risk Management Plan
RESTRICTIONS
REGULATION

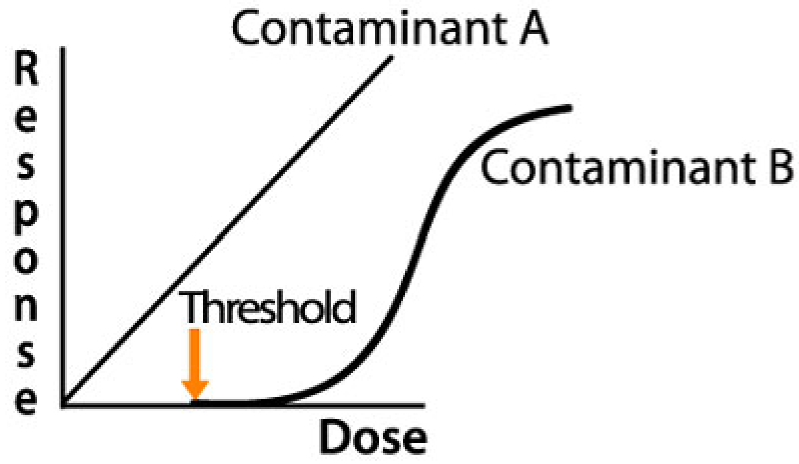
Genotoxic Carcinogens Are The Exception To The Rule

S. Barlow et al. (2006) Risk assessment of substances that are both genotoxic and carcinogenic. Food and Chemical Toxicology 44, 1636-1650

Linear Dose-Response

- covalent binding to DNA
- *a linear dose – response relationship in the low-dose range*
- no indication of a threshold

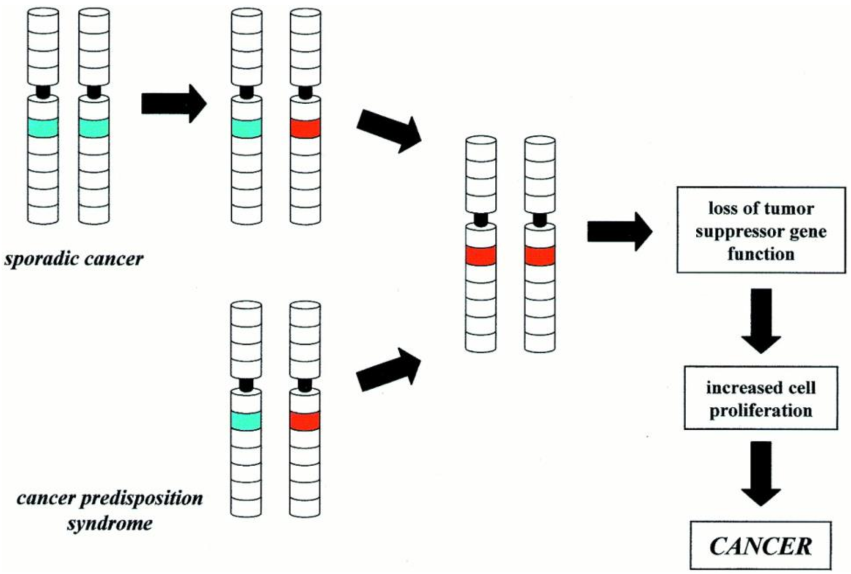
No Threshold Dose



Risk Analysis of Genotoxic Carcinogens

EPA, 2005. Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, pp. 1–166

- 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



Dose-Response Relationship of a Genotoxic Carcinogen

Liver Cancer Induction in Rats by Diethylnitrosamine

Druckrey, H., Schildbach, A., Schmaehl, D., Preussmann, R., Ivankovic, S., 1963. Arzneimittelforsch. 13, 841–851

- The carcinogenic dose decreases with exposure time
- The Dose : Response Relationship is described by what is now known as the Druckrey-Küpfmüller Equation

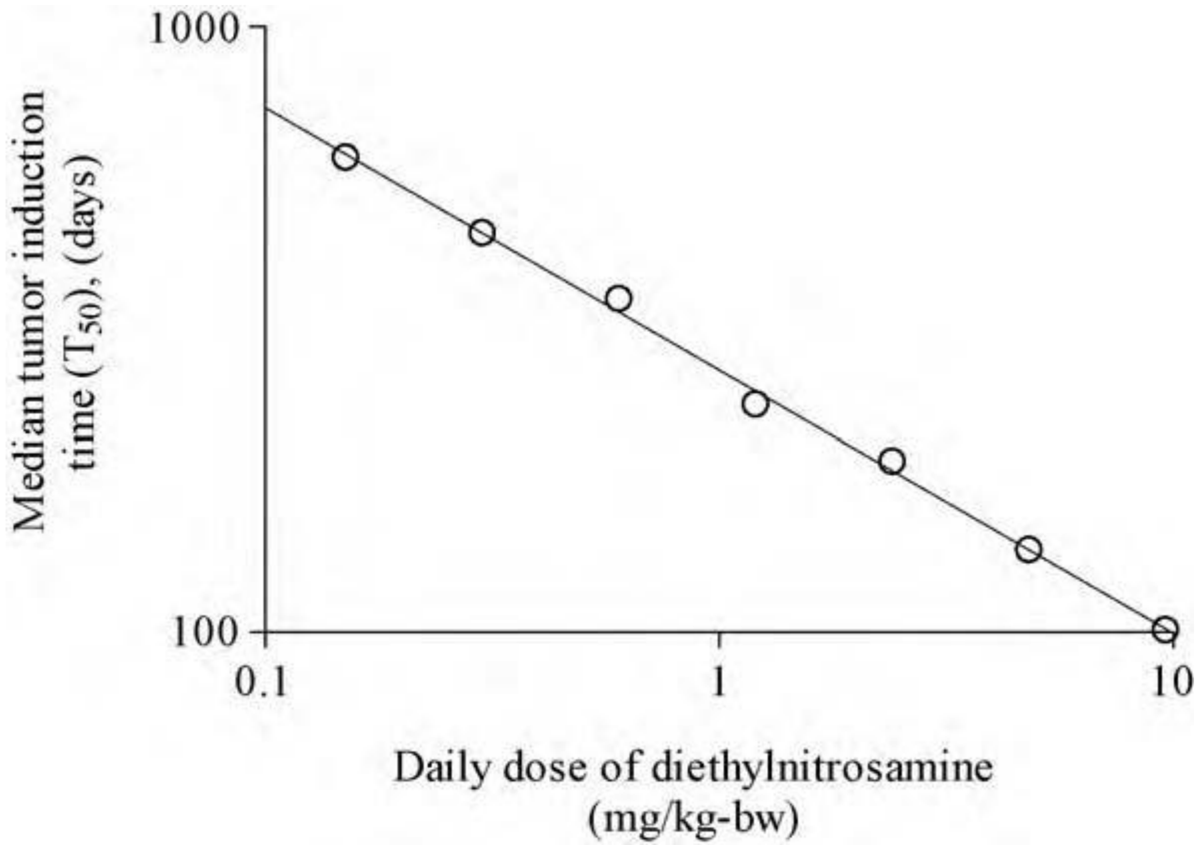
$$D \times T50^{2.3} = \text{constant}$$

Daily Dose D (mg/kg)	Median Tumor Induction Time T50 (Days)	Carcinogenic Dose (mg/kg)
9,6	101	**963**
1,2	238	**285**
0,3	457	**137**
0,075	840	**64**

Dose-Response Relationship of a Genotoxic Carcinogen

Liver Cancer Induction in Rats by Diethylnitrosamine

Druckrey, H., Schildbach, A., Schmaehl, D., Preussmann, R., Ivankovic, S., 1963. Arzneimittelforsch. 13, 841–851



Dose Response Relationship of Lethal Effects of Imidacloprid on Honey Bees

Suchail S, Guez D, Belzunces LP, 2001. Environ. Toxicol. Chem. 20: 2482-2486
Tennekes HA, Sánchez-Bayo F, 2012. J. Environment. Analytic Toxicol. S4- 001

- The lethal dose decreases with exposure time
- The Dose : Response Relationship can also be described as a Druckrey-Küpfmüller Equation

$C \times T50^{5.9} = \text{constant}$

Concentration **C** (µg/L)	Median Time to Lethal Effect **T50** (hours)	Lethal Dose (µg/L x hours)
57	48	**2,736**
37	72	**2,664**
10	173	**1,730**
1	162	**162**
0.1	240	**24**

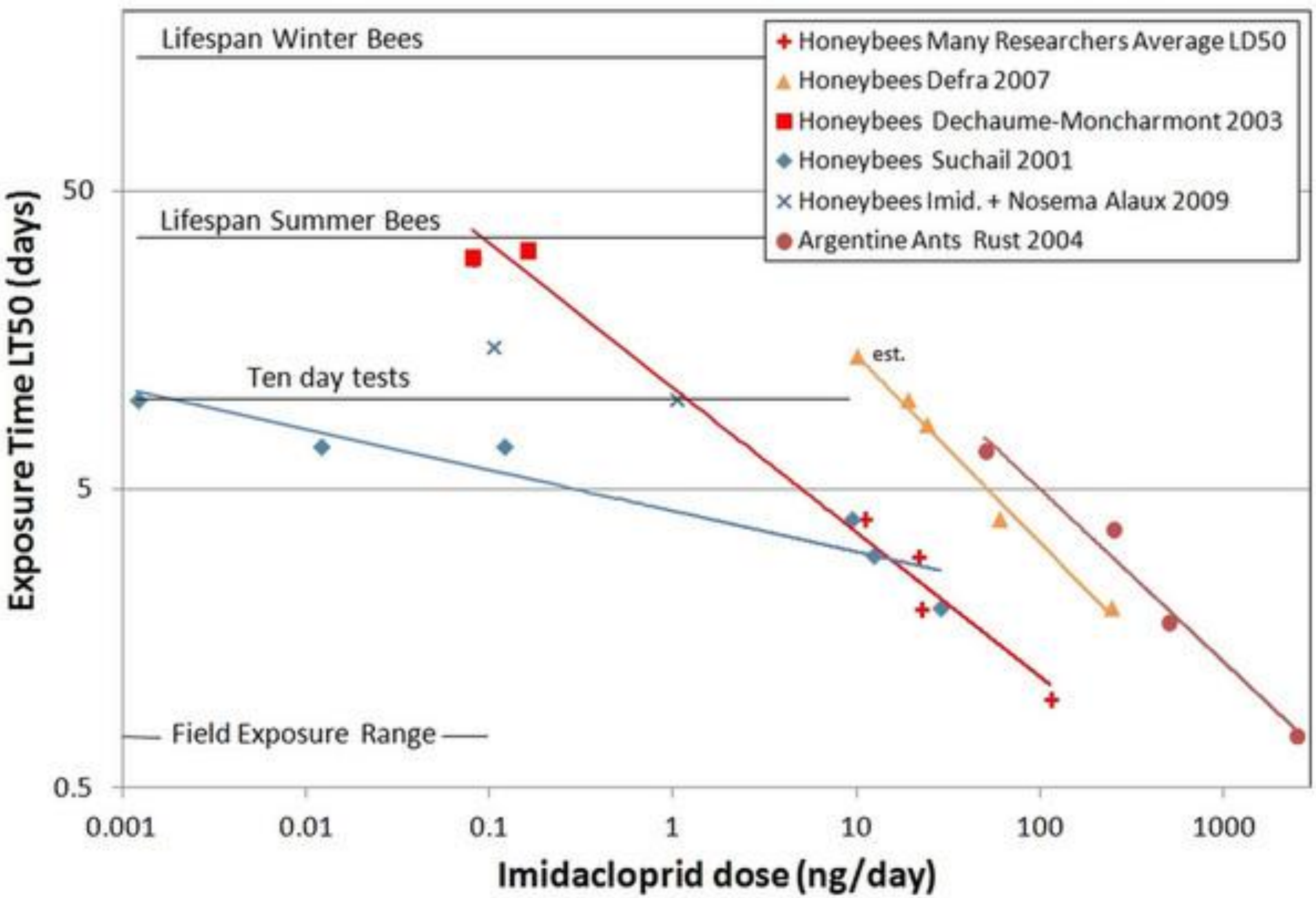
Dose-Response Relationships of Neonics in Bees and Argentine Ants

$C \times LT50^{n} = constant$, where $n \geq 1$

G. Rondeau, F. Sánchez-Bayo, H.A. Tennekes, A. Decourtye, R. Ramirez-Romero, N. Desneux

***Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites*.**

Nature Sci. Rep. 4, 5566; DOI:10.1038/srep05566



Dose-Response Relationships of Neonics in Termites

$C \times LT50^{n} = constant$, where $n \geq 1$

G. Rondeau, F. Sánchez-Bayo, H.A. Tennekes, A. Decourtye, R. Ramirez-Romero, N. Desneux

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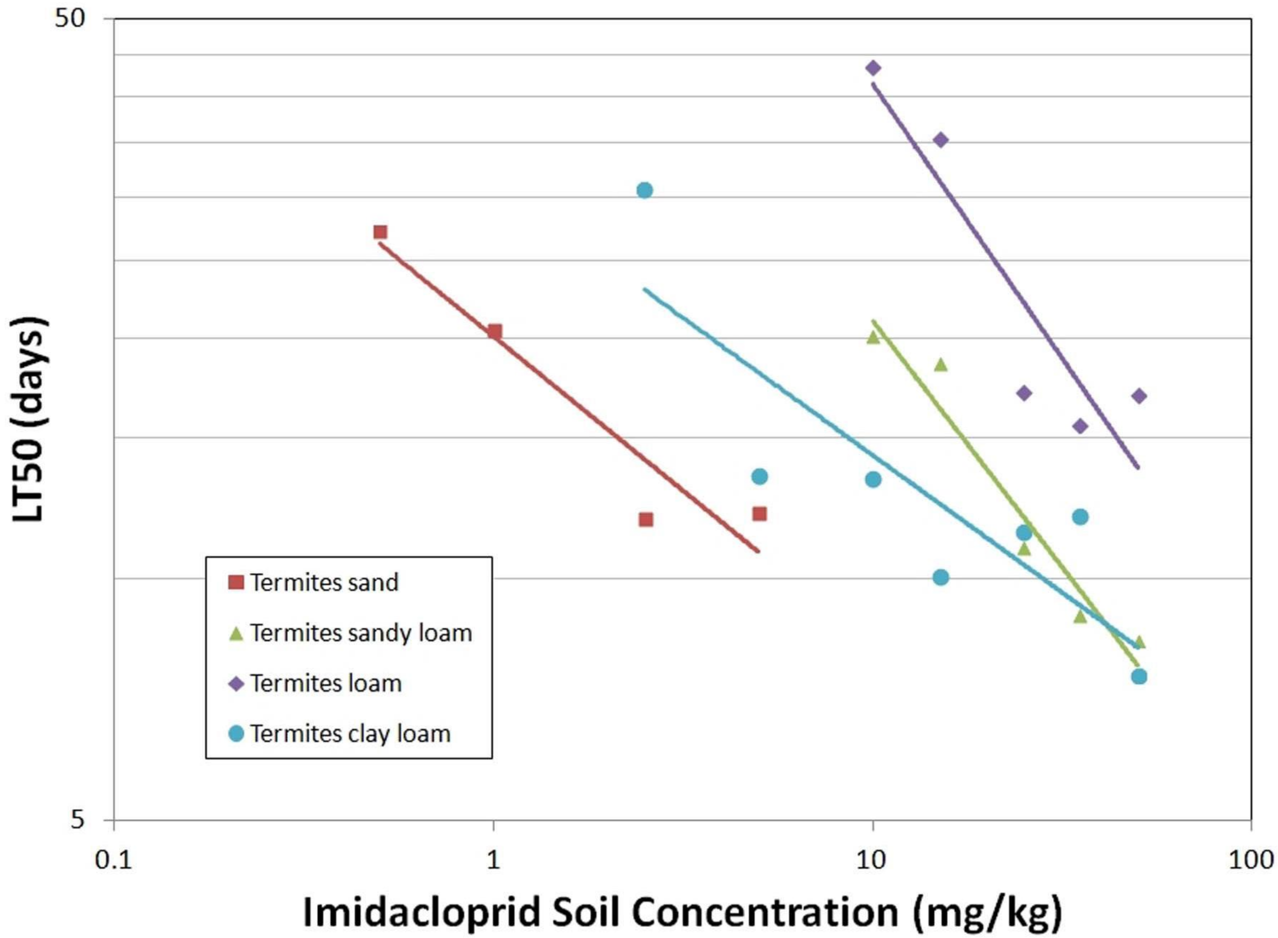
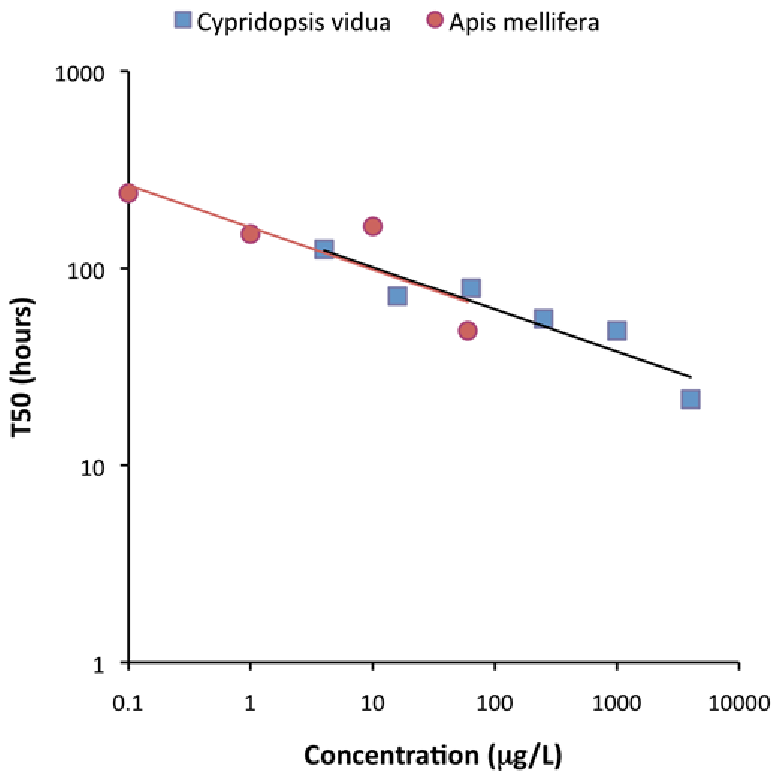
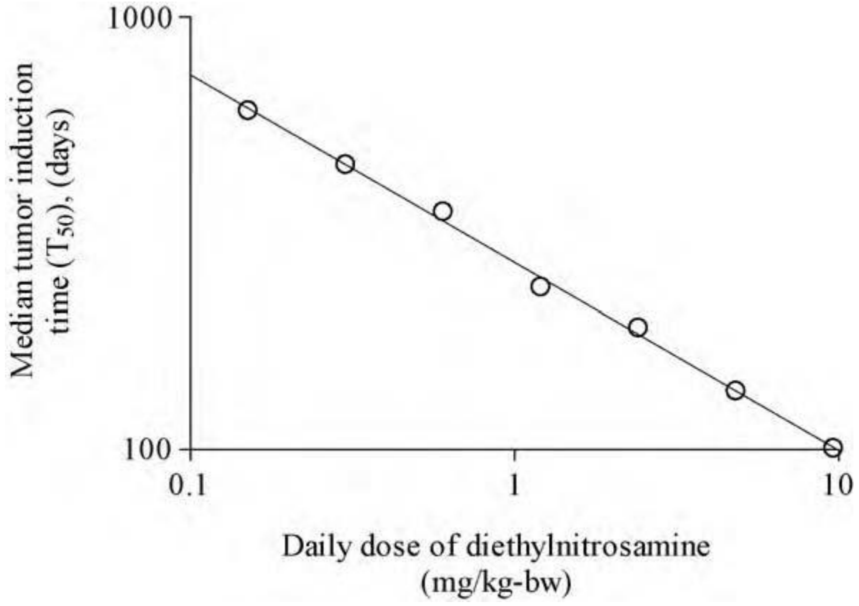


Dose-Response Relationships of Neonics (Left) and Genotoxic Carcinogens (Right) are Identical

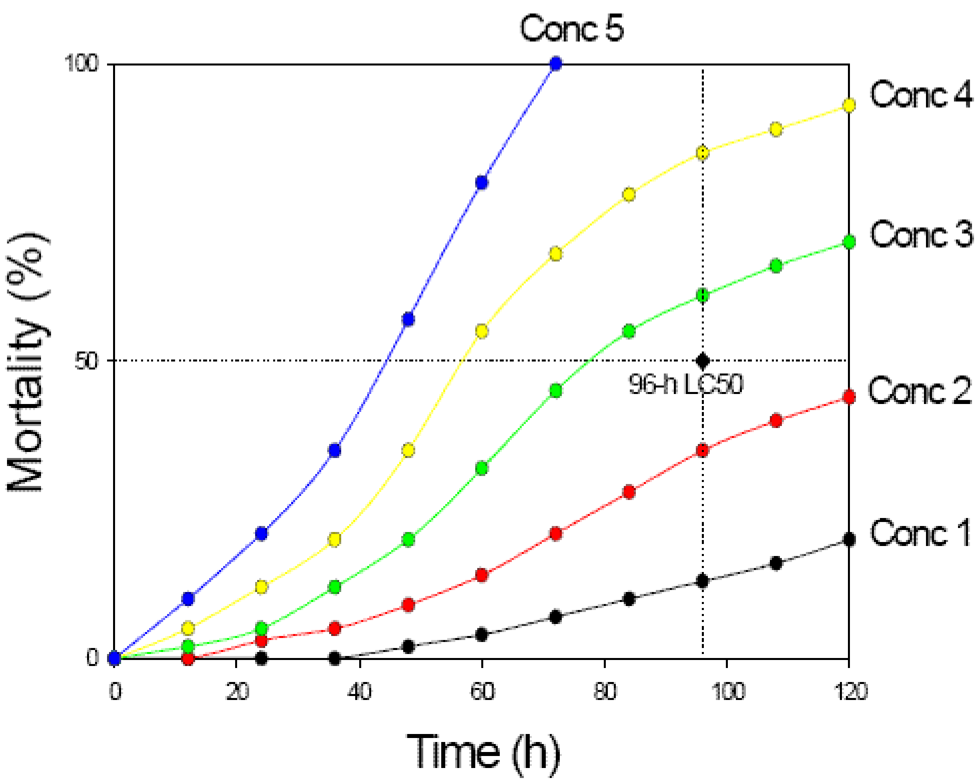
$C \times T50^{n} = constant$, where $n \geq 1$

Tennekes, H.A. (2010) Toxicology 276, 1–4.





Time To Event Methods Are Required For Pesticide Risk Analysis



An increasing number of researchers are using a variant of the traditional toxicity testing protocol which includes time to event (TTE) methods.

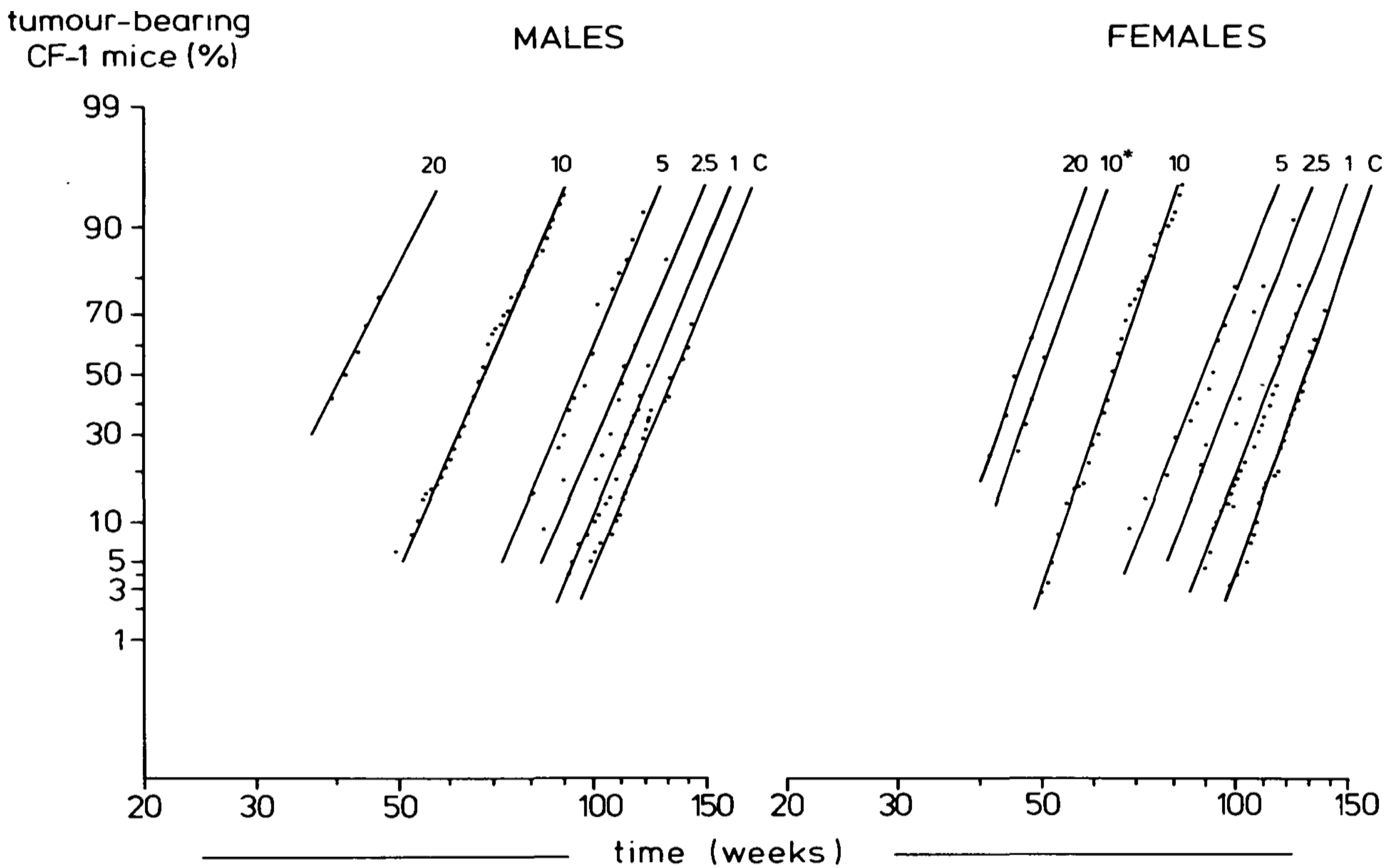
This TTE approach measures the times to respond for all individuals, and provides information on the acquired doses as well as the exposure times needed for a toxic compound to produce an effect on the organisms tested.

Consequently, extrapolations and predictions of toxic effects for any combination of concentration and time are now made possible.

Time-Dependent Toxicity of Non- Genotoxic Pesticides

Liver Tumor Enhancement in Mice Exposed to Dieldrin

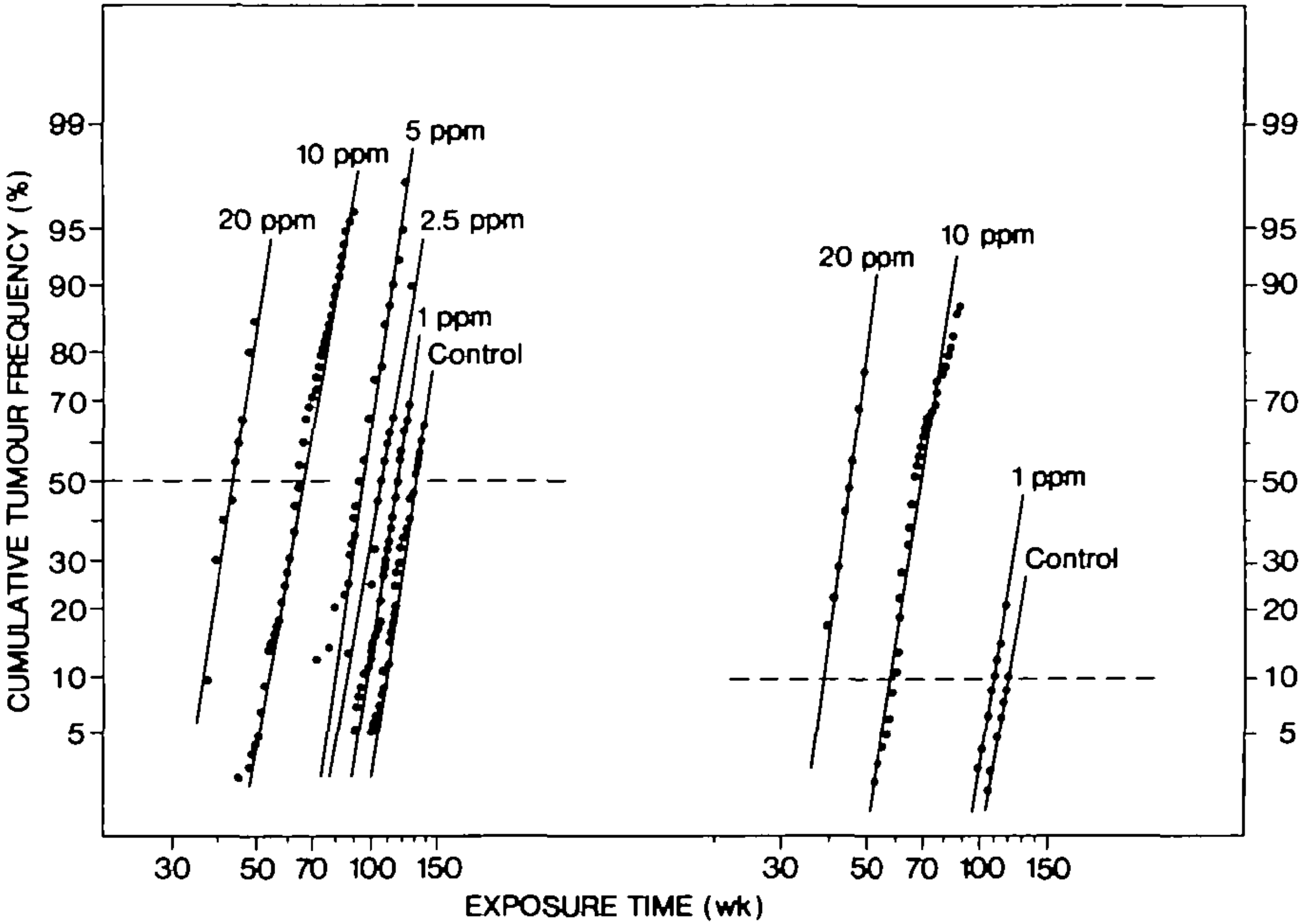
H. A. Tennekes et al. (1982) Carcinogenesis 3, 941-945



New Approach to Pesticide Risk Analysis:

Relate the Velocity of a Toxic Process to the Exposure Level of the Toxicant

H. Tennekes et al. (1985) Carcinogenesis 6, 1457-1462



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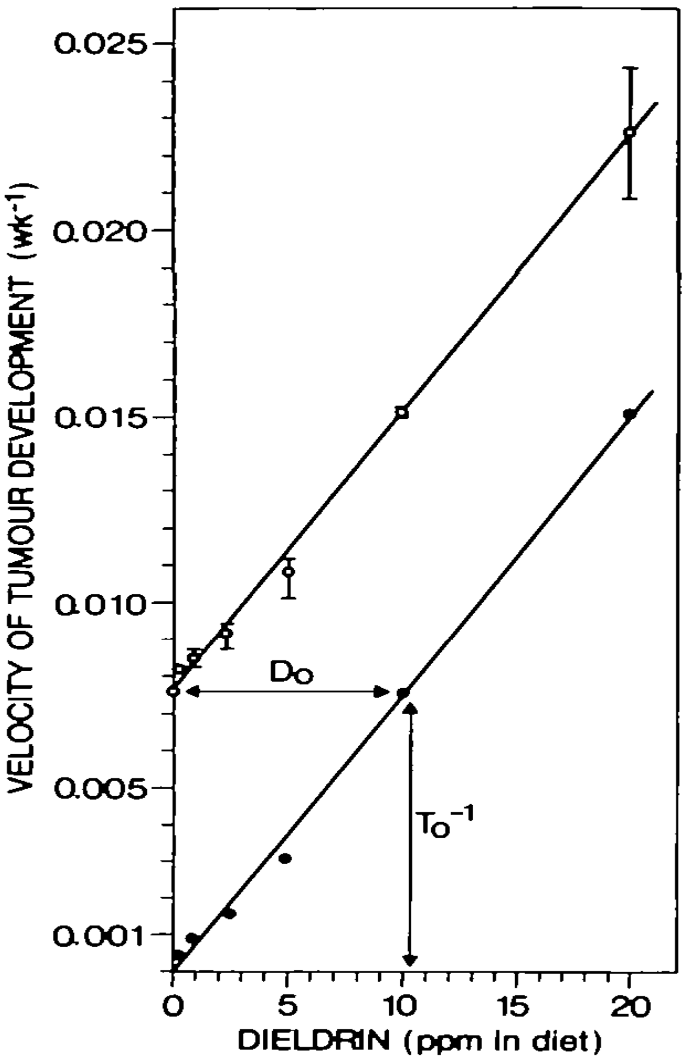
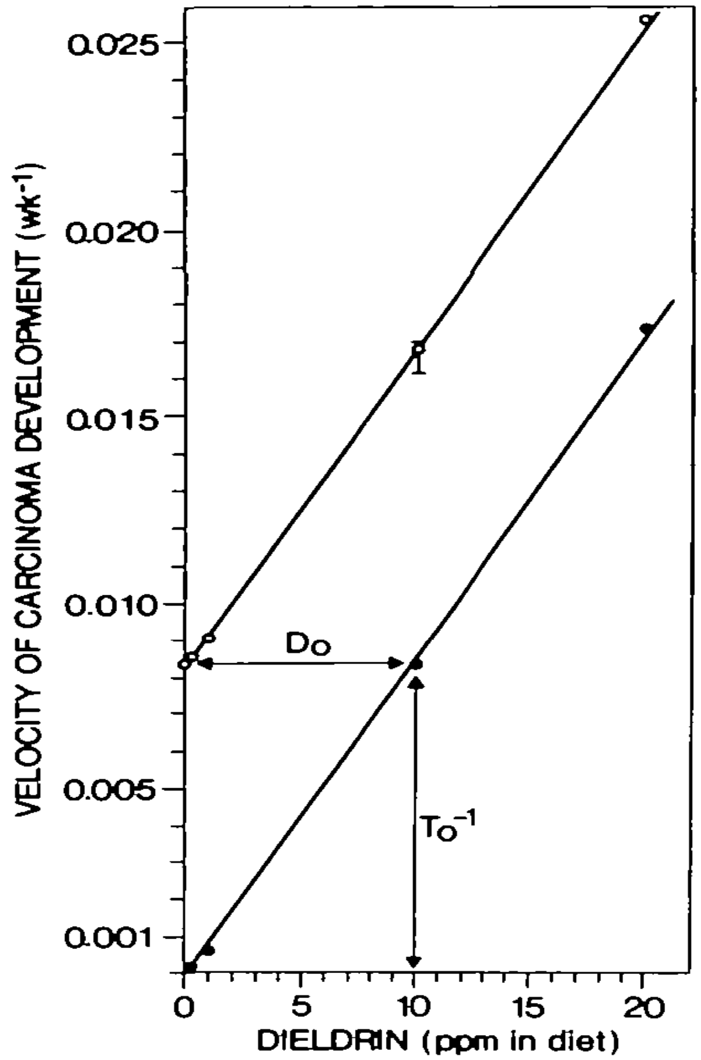




Time to Effect Analysis (Adopted by EFSA) For Imidacloprid in Bees Using Druckrey-Küpfmüller Equation

Ln t50 (h) = 5.19–0.17 Ln c (µg L−1 or kg−1)

c x t50 ^ 5.9 = konstant

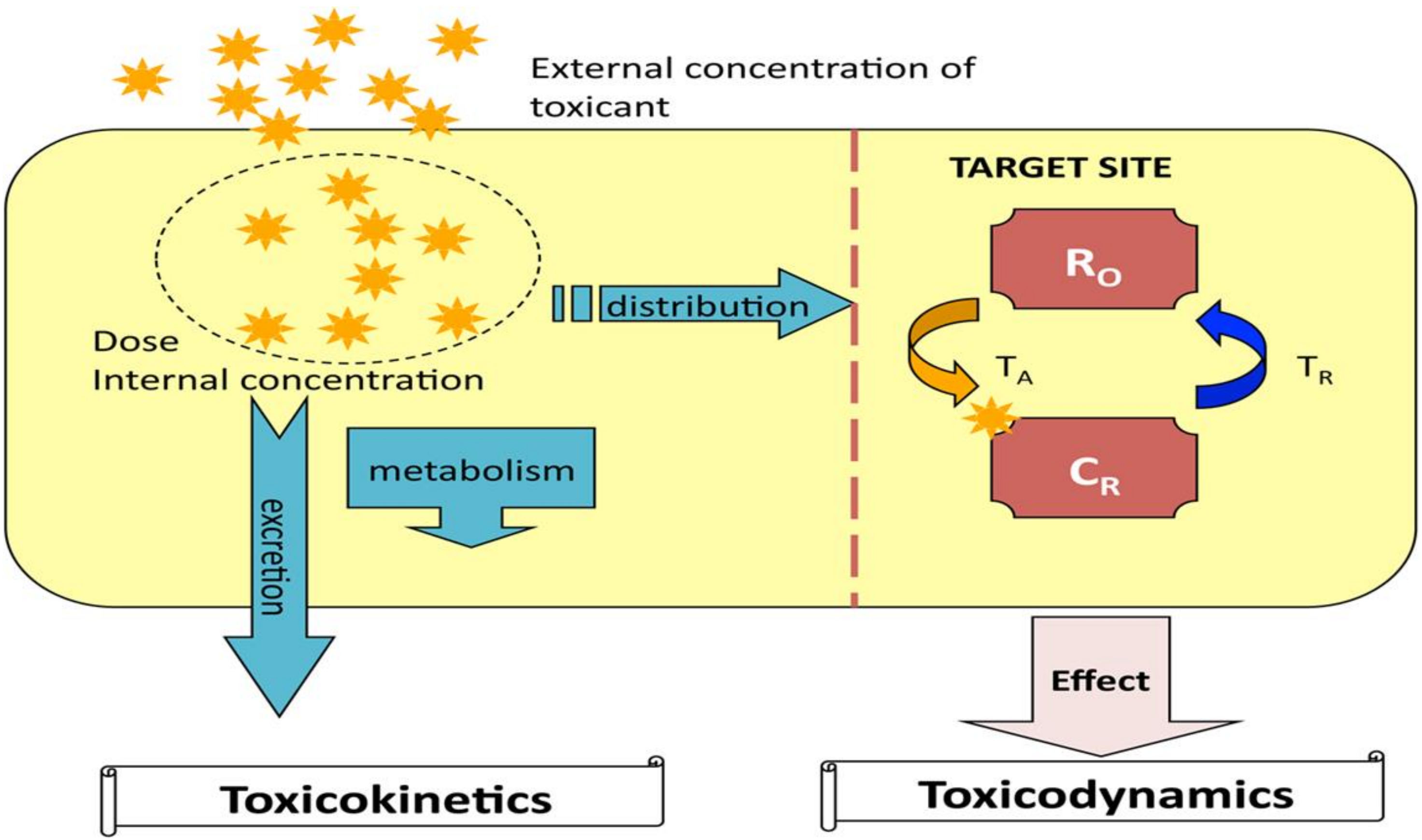
Residues	Imidacloprid (PEC) (µg L−1 or kg−1)	Aver. Exposure Concentration c PEC × frequency (11%) (µg L−1 or kg−1)	Predicted time to lethal effect t50 (hrs)	Percentage of average life expectancy
Nectar	1	0.11	263	26
	3	0.33	218	22
Pollen	0.7	0.08	280	28
	10	1.1	177	18

Tennekes H.A., Sánchez-Bayo, F., 2013. Toxicology 309, 39– 51

Introduction To The Druckrey-Küpfmüller Theorem

At the target site, toxicant molecules bind to critical receptors and produce a toxic effect. *The value of the time constant for dissociation* ***(T_R), which determines the reversibility of receptor binding,*** *is the critical variable that determines the nature of the dose : response relationship*

Druckrey, H. & Küpfmüller, K. (1949). Dosis und Wirkung. Beiträge zur theoretischen Pharmakologie, Editio Cantor GmbH, Freiburg im Breisgau
Tennekes, H.A. (2010) Toxicology 276, 1–4



Irreversible Receptor Binding Can Lead To Reinforcement Of The Effect

Druckrey, H. & Küpfmüller, K. (1949).
Dosis und Wirkung. Beiträge zur theoretischen Pharmakologie, Editio Cantor GmbH, Freiburg im Breisgau
Tennekes, H.A. (2010) Toxicology 276, 1–4.

- **If receptor binding happens to be virtually irreversible, the concentration of bound receptors C_R would be proportional to the integral of the toxicant concentration at the target site C over time:**

  $C_R \sim \int C\,dt$ (1)

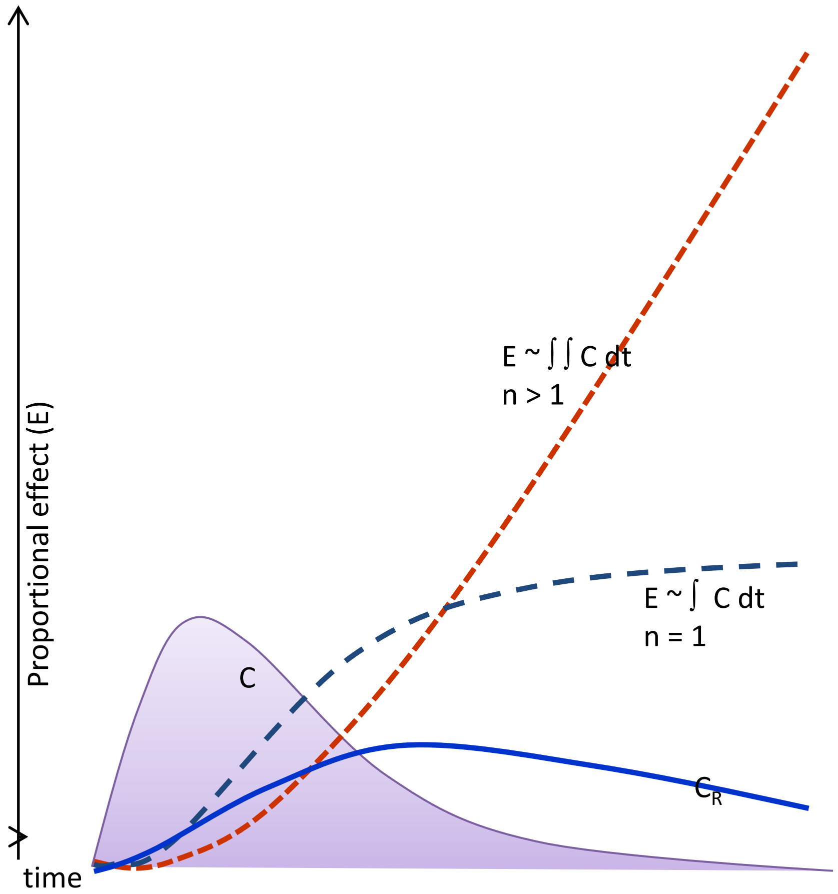
- **If the effect produced by receptor binding would happen to be irreversible as well (e.g. gene mutations, perturbations of cognitive functions or immune response), the effect E would be proportional to the integral of the concentration of bound receptors C_R over time:**

  $E \sim \int C_R\,dt$ (2)

- **So, in cases of irreversible receptor binding and an irreversible effect, *the effect E would be proportional to the double integral of the toxicant concentration at the target site C over time*, as the combination of eq. (1) and (2) shows:**

  $E \sim \int\int C\,dt$ (3)

- **This explains the dose : response relationship of neonics and genotoxic carcinogens where exposure time reinforces the effect**



Mechanism of Action of Genotoxic Carcinogens

SJ Lee et al. (2013) Nature Scientific Reports 3, Article number: 2783 doi:10.1038/srep02783

- Most genotoxic carcinogens are electrophiles that interact directly with DNA through the formation of covalent bonds, resulting in DNA-carcinogen complexes (DNA adducts).
- These complexes lead to various types of DNA damage, including the formation of cross-links between the two helices, chemical bonds between adjacent bases, removal of DNA bases (hydration) and cleavage of the DNA strands, all of which result in modifications to the information stored within the DNA (mutations).
- So, *receptor binding and the effect are essentially irreversible*

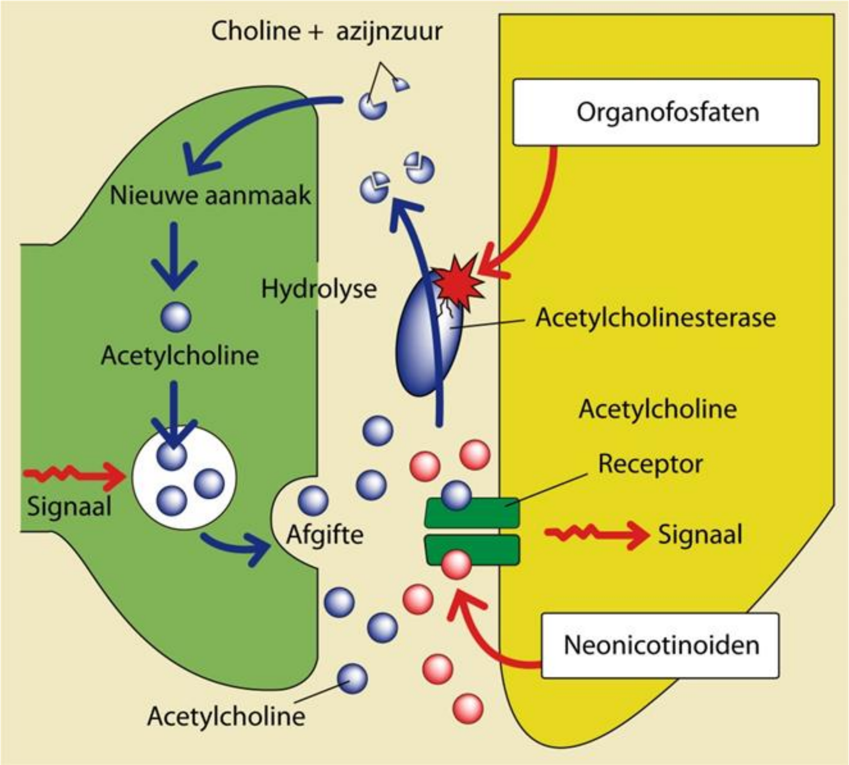
Mechanism of Action of Neonics

Abbink, J. (1991) Pflanzenschutz-Nachrichten Bayer, Serial ID-ISSN 0340-1723C.
Di Prisco, G. et al. PNAS 110, 18466–18471, doi:10.1073/pnas.1314923110 (2013)

Mechanism of action

- „Their Mode Of Action Derives From Virtually Irreversible Blockage Of Postsynaptic Nicotinic Acetylcholine Receptors“
- Neonicotinoids impair cognition and downgrade the innate immunity pathway governed by NF-kB
- Neonicotinoids account for worker bees neglecting to provide food for eggs and larvae, for a breakdown of the bees' navigational abilities, and for increased susceptibility to infectious diseases

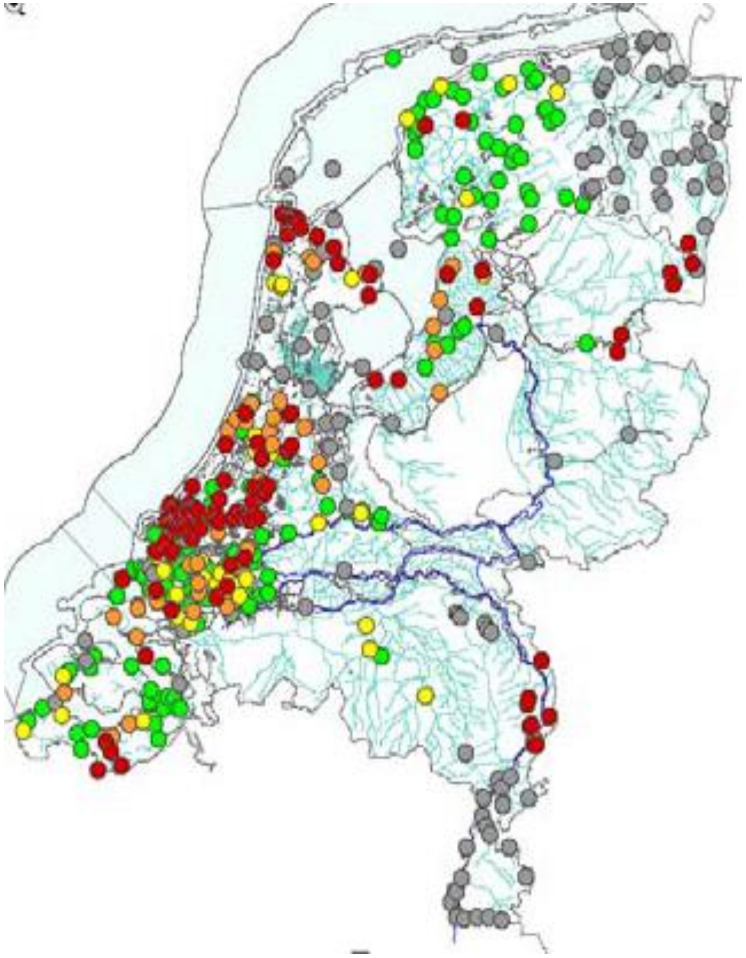
Irreversible Blockage of nAChRs



Issue of Concern: Imidacloprid Frequently Exceeds The Maximum Permissible Risk Level (MTR) in Dutch Surface Water

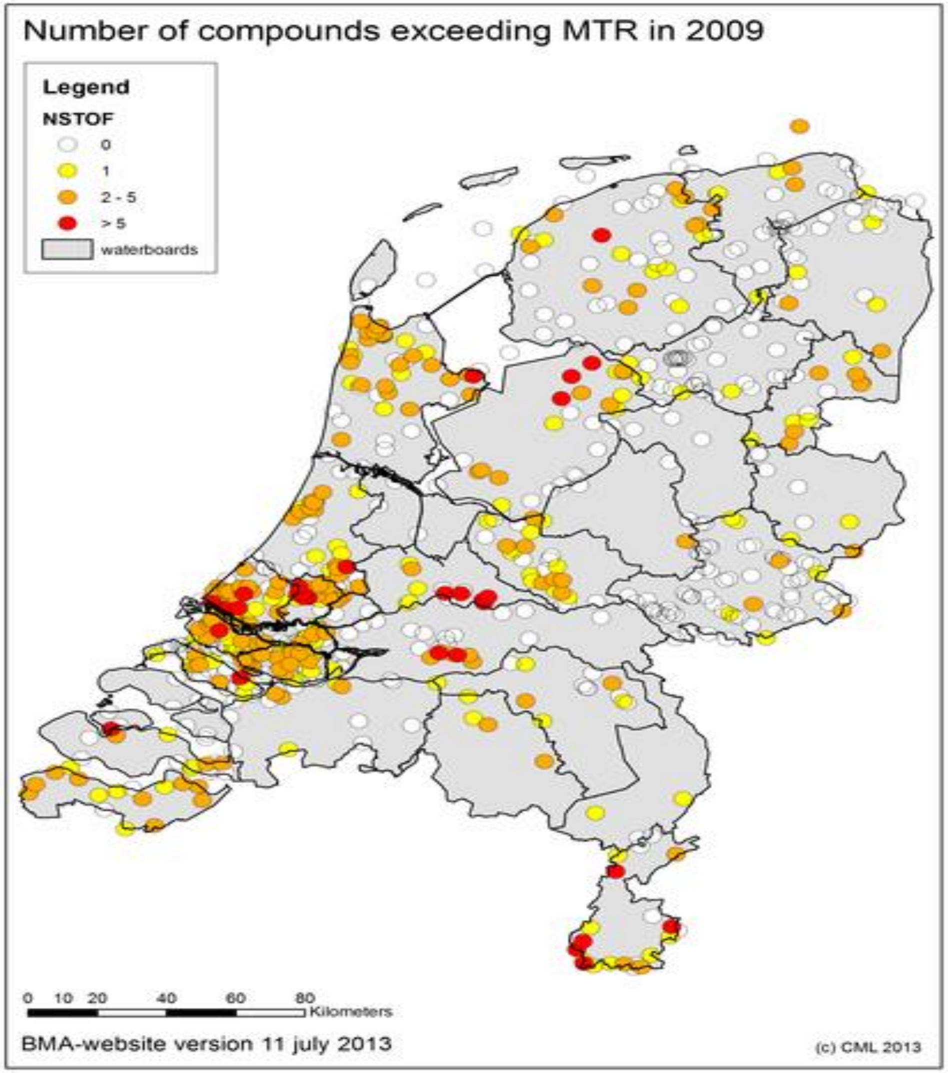
MTR = 13 nanogram per liter

- > 5 MTR
- > 2MTR < 5MTR
- > MTR < 2MTR
- < MTR
- undetermined



Sources:
- Bestrijdingsmiddelenatlas (CML, 2013)
- C.E. Smit | D. Kalf. Bestrijdingsmiddelen in oppervlaktewater. Vergelijking tussen Nederland en andere Europese landen. RIVM briefrapport 601714026/2014

Imidacloprid Is One of Many Pesticides Polluting Dutch Surface Water



Vijver MG, van den Brink PJ (2014) PLoS ONE 9(2): e89837. doi:10.1371/journal.pone.0089837

PLOS | ONE

Common Pesticides Polluting Dutch Surface Water

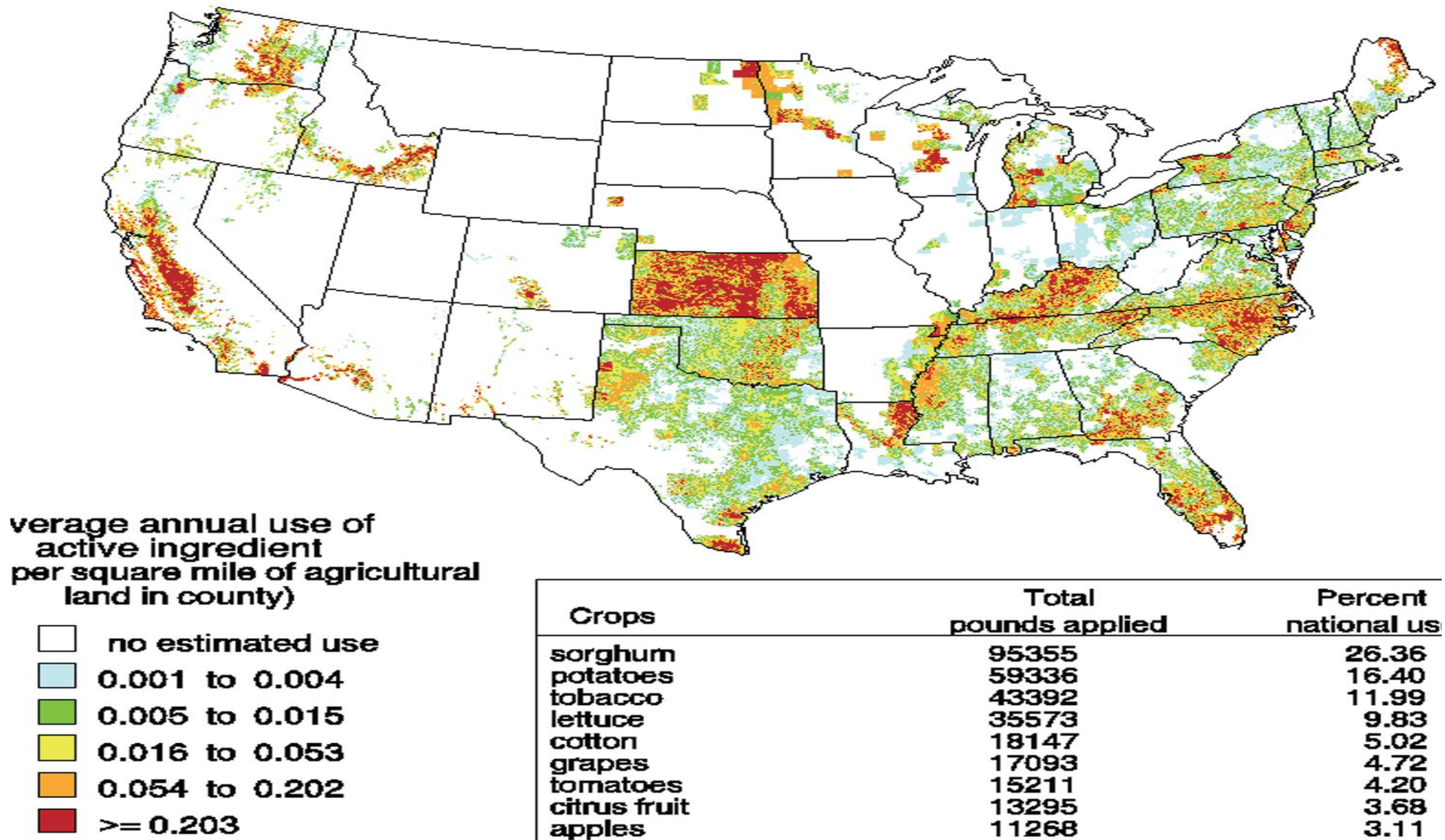
University of Hertfordshire (2013) The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006-2013

Dutch pesticides atlas website. Available: http://www.bestrijdingsmiddelenatlas.nl, version 2.0. Institute of Environmental Sciences (CML) at Leiden university and Waterdienst of the Dutch Ministry of Infrastructure and Environment

No.	Pesticide	Pesticide type	Water solubility at 20 °C (mg/L)	Soil degradation, aerobic (typical DT50 in days)[1]	Soil adsorption and mobility		GUS leaching potential index	Environmental Quality Standard (EQS) in the Netherlands		
					K_{oc} (ml/g)	K_{foc} (ml/g)		MTR[4] (ng/L)	AA-EQS[5] (ng/L)	Exceeded EQS limits > 5-fold in
1	**azoxystrobin**	Fungicide	6.7	**78**	589	423	2.60	56		2012
2	**carbendazim**	Fungicide, Metabolite	8.0	40	-	225	2.64		600	2012
3	**desethyl-terbuthylazine**	Metabolite	327.1	**70.5**	-	**78**	**3.90**	2.4		2012
4	**dicamba**	Herbicide	**250000**	4.0	-	**12.36**	1.75	130		2010
5	**dichlorvos**	Insecticide, Acaricide, Metabolite	**18000**	2	**50**	-	0.69		**0.6**	2012
6	**dimethoate**	Insecticide, Acaricide, Metabolite	**39800**	2.6	-	**28.3**	1.06		70	2011
7	**esfenvalerate**	Insecticide	0.001	44	5300	-	0.45		**0.1**	2012
8	**imidacloprid**	Insecticide	610	**191**	-	**225**	**3.76**	13	67	2012
9	**fipronil**	Insecticide	3.78	**142**	-	727	2.45	**0.07**		2012
10	**metolachlor**	Herbicide	530	**90**	120	163	**3.49**	200		2011
11	**permethrin**	Insecticide	0.2	13	100000	-	-1.11	**0.2**		2010
12	**pirimiphos-methyl**	Insecticide, Acaricide	11	39	1100	170	**2.82**		**0.5**	2010
13	**propoxur**	Insecticide, Acaricide	1800	**79**	**30**	-	**4.79**	10		2010

Use of Imidacloprid in US Agriculture in 2002

US Geological Survey National Water-Quality (NAWQA) Program



Crops	Total pounds applied	Percent national us
sorghum	95355	26.36
potatoes	59336	16.40
tobacco	43392	11.99
lettuce	35573	9.83
cotton	18147	5.02
grapes	17093	4.72
tomatoes	15211	4.20
citrus fruit	13295	3.68
apples	11268	3.11
pecans	10001	2.76

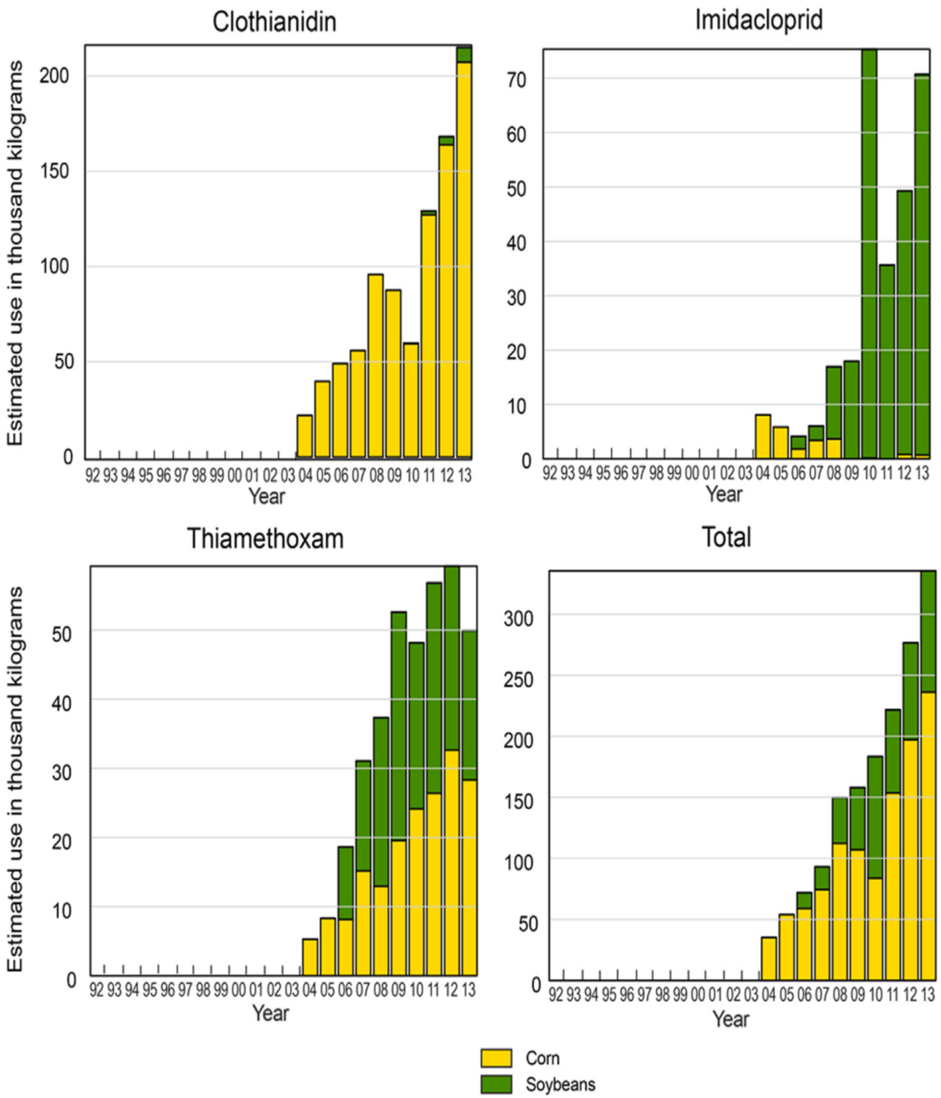
Contamination of Surface Water with Imidaclopride in California's Central Valley

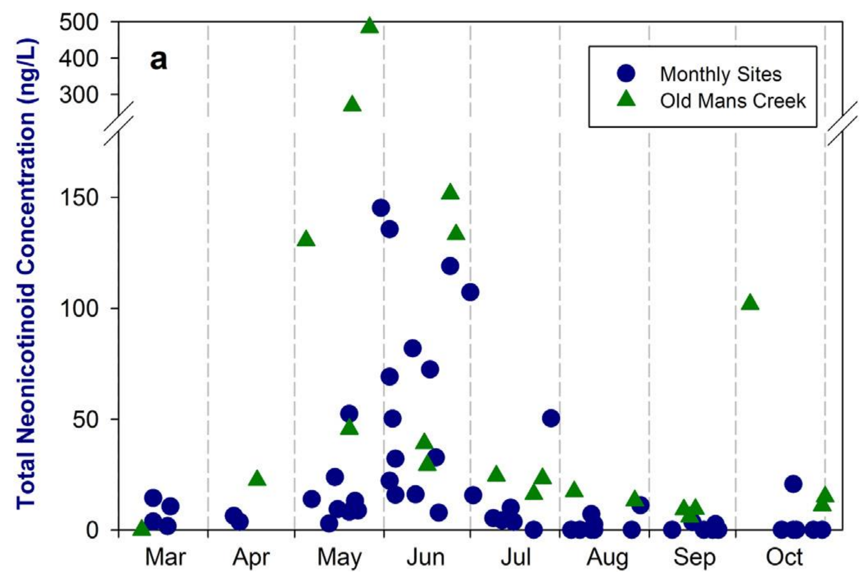
K Starner and KS Goh (2012) Bulletin of Environmental Contamination and Toxicology DOI: 10.1007/s00128-011-0515-5

- 75 surface water samples from three agricultural regions of California were collected and analyzed for contamination with imidacloprid
- Imidacloprid was detected in 67 samples (89%);
- *Concentrations exceeded the U.S. Environmental Protection Agency's (EPA) chronic invertebrate Aquatic Life Benchmark of 1.05 µg/L (micrograms per liter) in 14 samples (19%).*

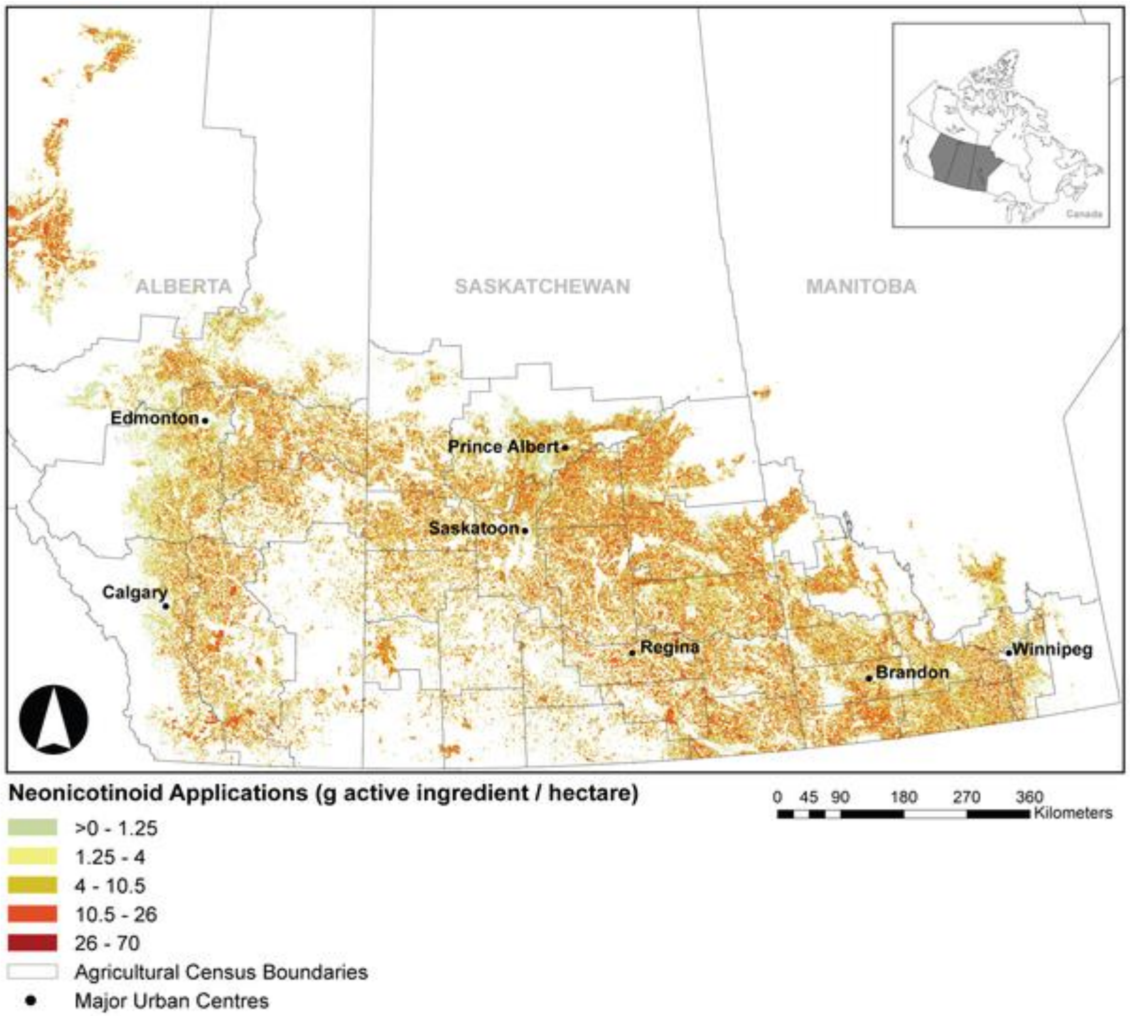
Surface Water Pollution with Neonics in Iowa in an Area of Intense Corn and Soybean Production

M.L. Hladik et al. / Environmental Pollution 193 (2014) 189-196





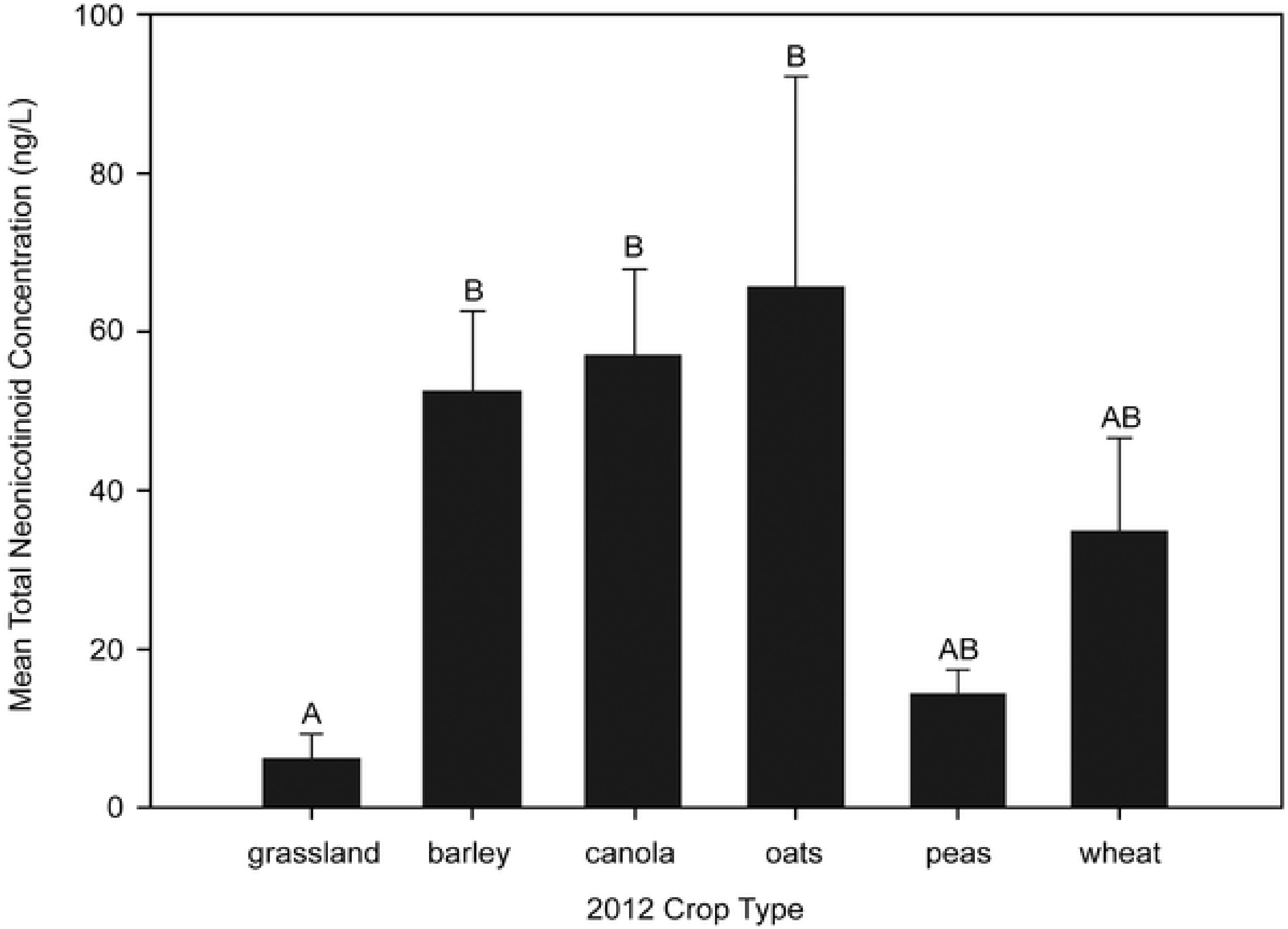
Map of Modelled Distribution of Neonicotinoid Use Across Prairie Canada (2012)



Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ, et al. (2014) Widespread Use and Frequent Detection of Neonicotinoid Insecticides in Wetlands of Canada's Prairie Pothole Region. PLoS ONE 9(3): e92821. doi:10.1371/journal.pone.0092821
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0092821

PLOS | ONE

Mean Total Neonicotinoid Water Concentrations in Wetlands in Central Saskatchewan



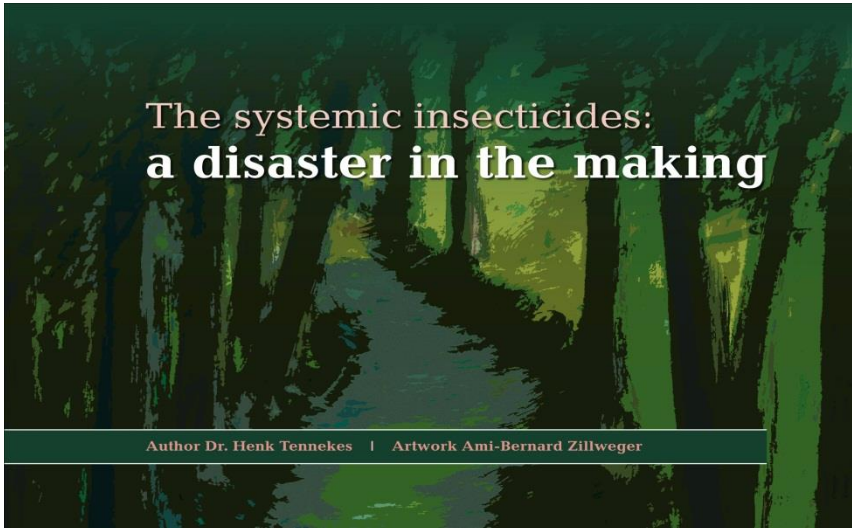
Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ, et al. (2014) Widespread Use and Frequent Detection of Neonicotinoid Insecticides in Wetlands of Canada's Prairie Pothole Region. PLoS ONE 9(3): e92821. doi:10.1371/journal.pone.0092821
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0092821

PLOS | ONE

„Knowing what I do,
there would be no future peace for me if I kept silent…“

Rachel Carson

- In 2010, realising the dire consequences of environmental pollution with neonicotinoid insecticides, Henk Tennekes published a book to warn the general public about an impending environmental catastrophe



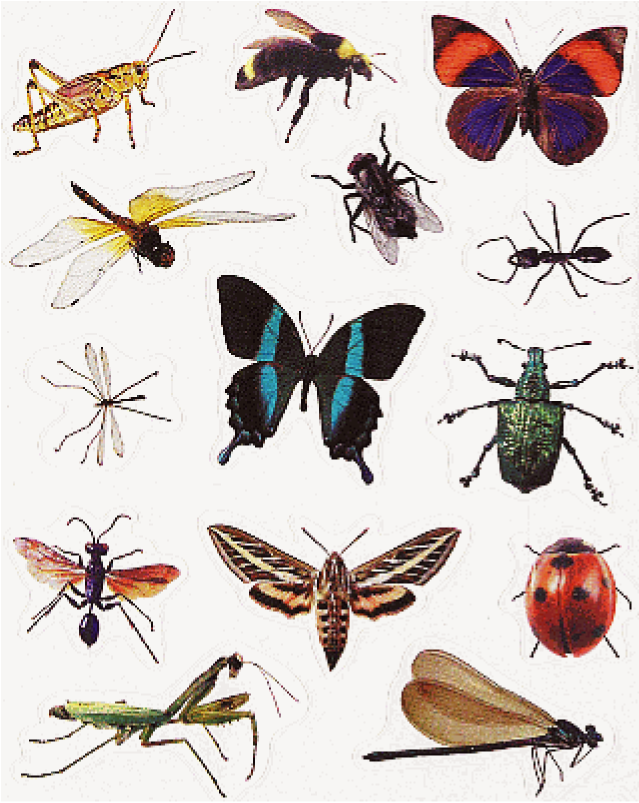
German Edition of ‘A Disaster in the Making’

Preface by Professor Hubert Weiger, Chairman, *Friends of the Earth Germany*

German Translation: Sven Buchholz Tomas Brückmann Patricia Cameron

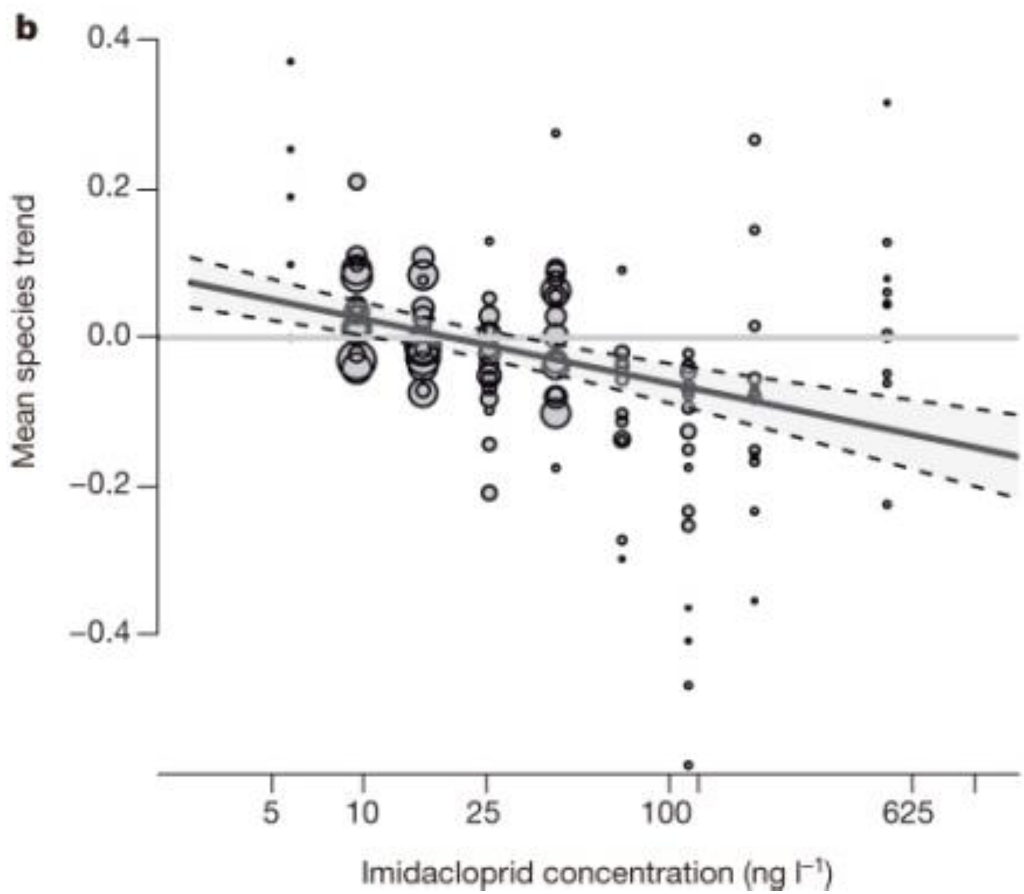
The Systemic Insecticides: A Disaster in the Making

Tennekes, H.A. (2010) ETS Nederland BV, Zutphen, The Netherlands.



- Dr Tennekes' book catalogues a tragedy of monumental proportions regarding the loss of insects and subsequent losses of the insect-feeding bird populations in all environments in the Netherlands.
- The disappearance can be related to the neonicotinoid insecticide imidacloprid, which is a major contaminant of Dutch surface water since 2004
- What, in effect, is happening is that the use of ***imidacloprid is creating a toxic landscape, in which insects are killed off***

Surface Water Pollution with Imidacloprid Correlates With Decline of Insectivorous Birds



nature

CA Hallmann et al. Nature 000, 1-3 (2014) doi:10.1038/nature13531
H A Tennekes (2010) The Systemic Insecticides: A Disaster in the Making. ETS Nederland BV, Zutphen, The Netherlands

May Common Sense Prevail !

Thank You For Your Attention