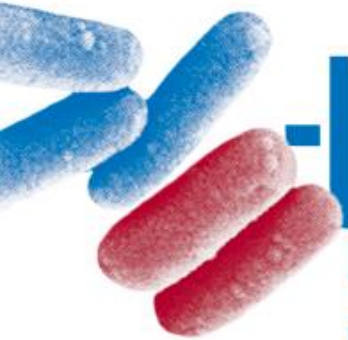

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# An Introduction to Chemical Risk Assessment *

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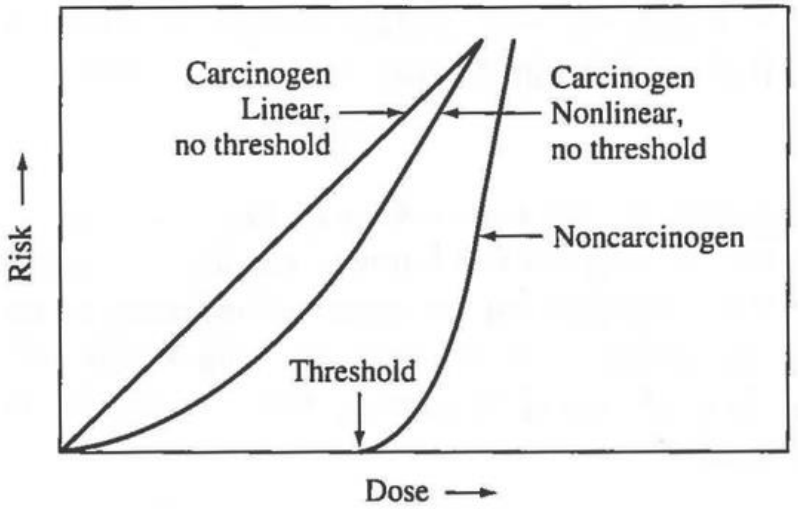
Gastcollege aan de Universiteit Utrecht
21 oktober 2016

* Based on: Druckrey, H. & Küpfmüller, K. (1949).
Dosis und Wirkung. Beiträge zur theoretischen Pharmakologie.
Editio Cantor GmbH, Freiburg im Breisgau, Germany

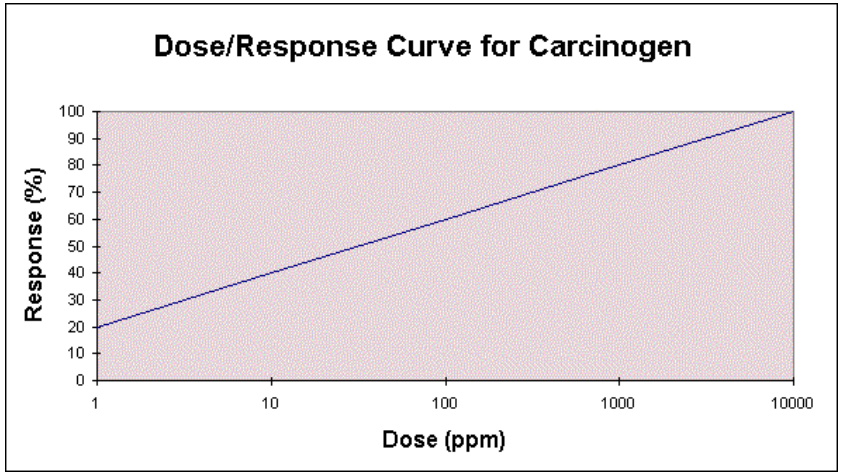
# Current Status of Chemical Risk Assessment
# No Universally Accepted Dose Response Model

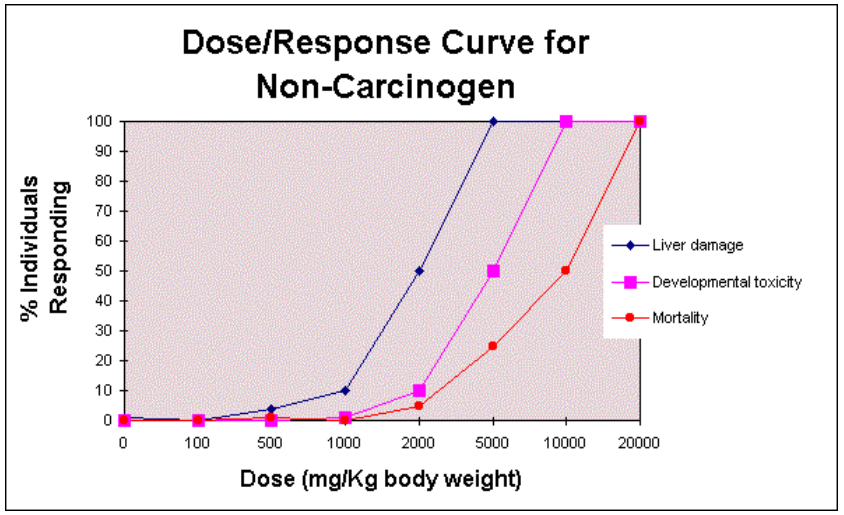
- A fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous substances for humans and the environment
- There is no universally accepted dose response model for risk assessment of low level exposures to potentially toxic substances
- Mainly because there is no consensus on presence or absence of a threshold below which no adverse effects can be assumed to occur
- The issue is readily apparent in carcinogenic risk assessment.



# Currrent Status of Chemical Risk assessment
# Carcinogenic Risk Assessment

- The point of departure for carcinogenic risk assessment is the assertion that the dose-response relationship for radiation-induced mutations is linear
- Likewise, it was assumed that “one hit” of a chemical carcinogen could cause a mutation and eventually result in cancer
- Any exposure level is assumed to be associated with a finite cancer probability
- By contrast, it was assumed that a threshold, or “safe” exposure level exists for non-carcinogens, and that an acceptable daily intake can be derived from animal experiments

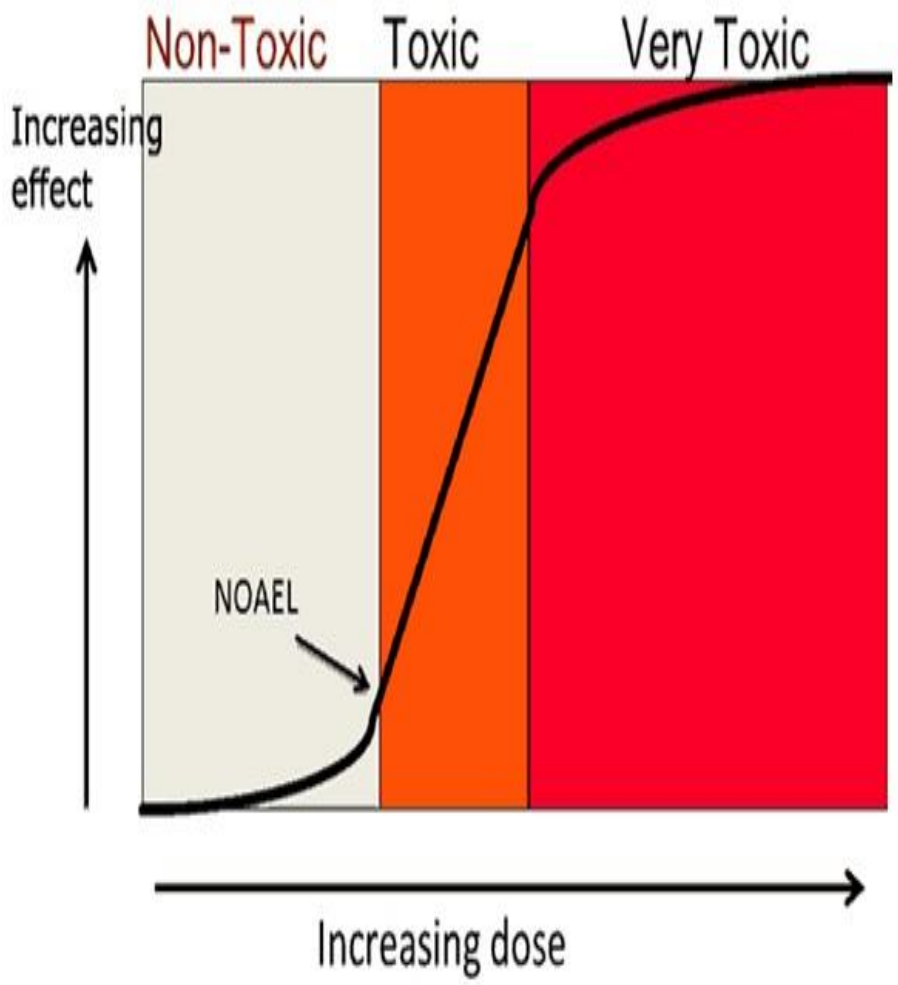


# Current Status of Chemical Risk Assessment

## Calculation of an Acceptable Daily Intake (ADI) for Non-Carcinogens

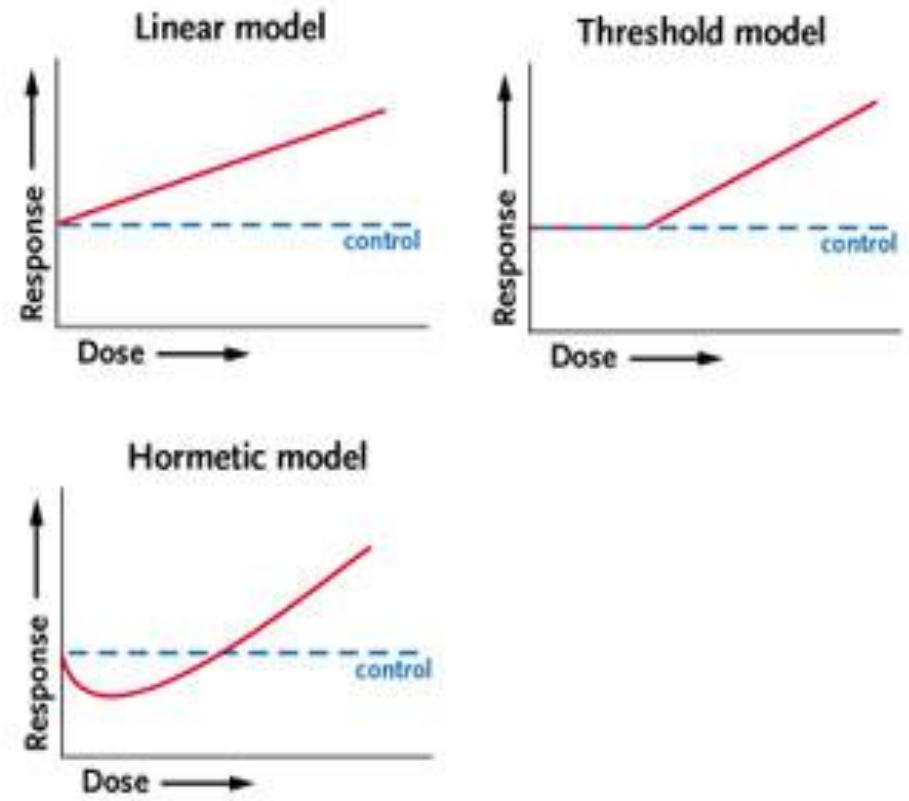
- Acceptable daily intake or ADI is a measure of the amount of a specific substance in food or drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk
- An ADI value is usually based on long-term animal studies. Usually the studies are performed with several doses including high doses
- First, a no-observed-adverse-effect level (NOAEL), the amount of a substance that shows no toxic effects, is determined
- Then, the NOAEL is divided by a safety factor, conventionally 100, to account for the differences between test animals and humans (factor of 10) and possible differences in sensitivity between humans (another factor of 10), to arrive at the ADI



# Derivation of the ADI using the NOAEL

## No Analysis of the Dose Response Relationship

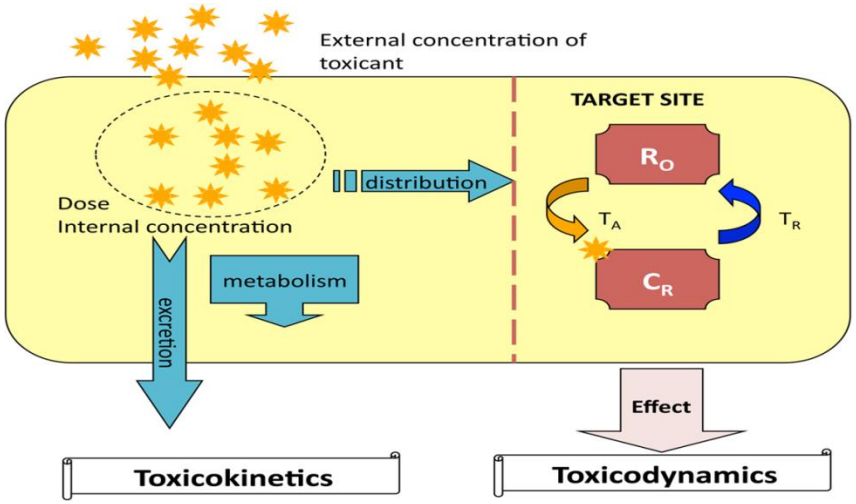
- In determining the NOAEL the data from the entire dose-response curve are not considered but rather only the data from a single dose group showing no response compared to the control group
- The NOAEL concept assumes the existence of a safe dose level or “threshold” without analysis of the dose response relationship
- However, the absence of a threshold would lead to underestimation of actual risk and there is increasing evidence that this is indeed the case for several environmental toxicants
- Examples of non-carcinogens without an apparent threshold (neonicotinoid insecticides, the rodenticide diphacinone, the insecticide dieldrin, endocrine disruptors, and sulfhydryl-reactive metals) will be discussed.
- Examples of underestimation of the risks of non-carcinogens without a threshold will be given

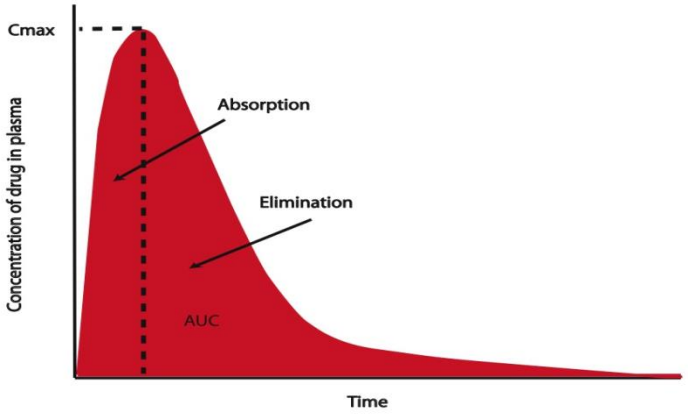


# Dose-Response Relationships Are Complex

## Toxicokinetics determine compound concentration at the site of (inter)action

## Toxicodynamics (Ergokinetics) determine compound interactions leading to an effect




- A pharmacological or toxic effect is a very complicated biological process
- A dose response relationship is the result of three sequential processes which are also complex in nature
- 1. Toxicokinetics: Absorption which in conjunction with metabolism and elimination determines the concentration of the compound (or a metabolite) at the site of (inter)action
- 2. Toxicodynamics (Ergokinetics): Interaction of the compound (or a metabolite) with a functional organic macromolecule in the organism and
- 3. The biological effect resulting from this interaction
- Can we make sense of the vast array of empirical dose response relationships?
- More importantly, can we obtain certainty on presence or absence of a threshold from an analysis of the dose-response relationship?

# I am going to approach Chemical Risk Assessment with Mathematics


**Why do children dread mathematics? Because of the wrong approach.**
**Because it is looked at as a subject - Shakuntala Devi**

**The aim of this presentation is to achieve understanding of the mechanisms of action that determine dose – response relationships**

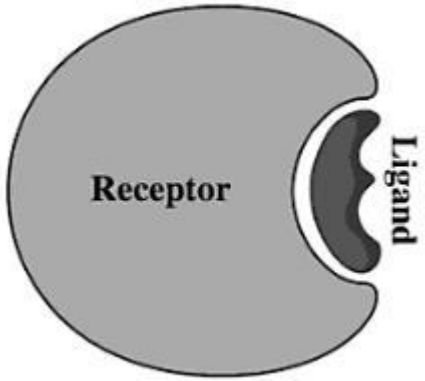
**MATHEMATICS**
is not about
numbers, equations,
computations, or
algorithms:
it is about
**UNDERSTANDING.**

*William Paul Thurston*

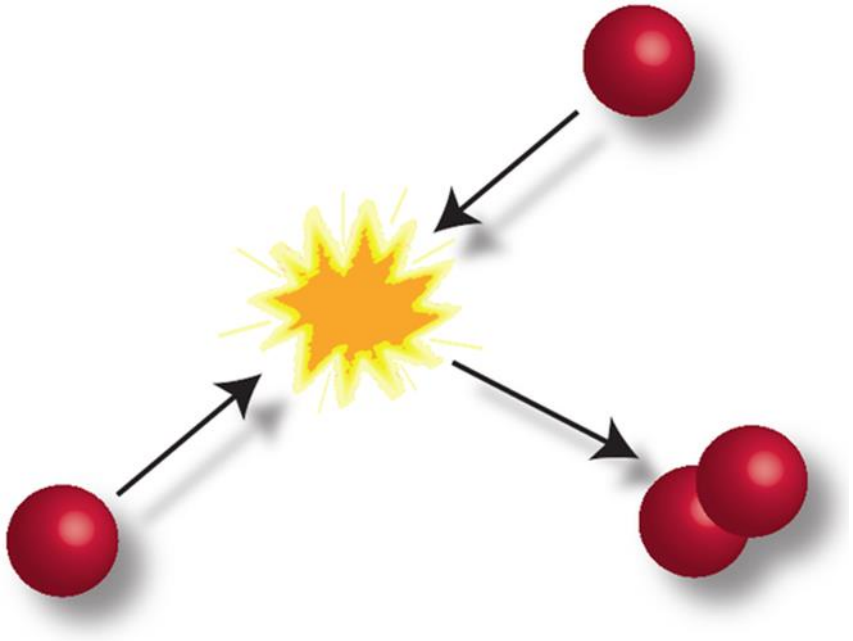
# Analysis of Ergokinetics

## Analysis of the Kinetics of Receptor Binding

**The biological effect of a drug is assumed to result from binding to a functional organic macromolecule referred to as a specific receptor, in a bimolecular reaction**




**The rate of receptor binding is directly proportional to the product of the concentrations of the reactants (law of mass action)**



# The Rate of Receptor Binding

## According to the Law of Mass Action

*The rate of receptor binding is determined by the product of*

*1. The concentration of the drug at the site of interaction with the receptor* $C$

*2. The concentration of free (unbound) receptors,*

*i.e. by the difference between the initial concentration of free receptors* $R_0$ *and the concentration of bound receptors* $C_R$*:* $R_0 - C_R$

**Applying the law of mass action the rate of receptor binding is**

$$K (R_0 - C_R) C$$

**where K is a reaction constant**

# The Reaction Kinetics of Receptor Binding

## The Difference between the Rate of Receptor Binding (Association) and the Rate of Dissociation of Bound Receptors

*The reaction kinetics of receptor binding are determined by the difference between*
*the rate of receptor binding (association)*

$$K (R_0 - C_R) C$$

*and the rate of dissociation which is determined by the reversibility of receptor binding. Instead of a reaction constant we write the reciprocal value of a time constant because it has the dimension of a time*

$$C_R / T_R$$

*So we obtain the following equation for the reaction kinetics of receptor binding*

$$dC_R / dt = K (R_0 - C_R) C - C_R / T_R$$

# Reaction Kinetics of Receptor Binding
# Effect Occurs when $C_R \ll R_0$ (First Order Kinetics)

*The reaction kinetics of receptor binding are*

$$dC_R / dt = K (R_0 - C_R) C - C_R / T_R$$

*Now assume the effect occurs under circumstances where $C_R \ll R_0$, then $R_0$ remains practically constant, in which case*

$$dC_R / dt = K R_0 C - C_R / T_R$$

*Because $K$ and $R_0$ are constant we can now define the time constant for association*

$$K R_0 = 1 / T_A$$

*and the reaction kinetics then simplify to*

$$dC_R / dt = C / T_A - C_R / T_R$$

# First Order Reaction Kinetics of Receptor Binding in Equilibrium

$$dC_R / dt = C / T_A - C_R / T_R$$

In equilibrium where $dC_R / dt = 0$

$$C_R / T_R = C / T_A$$

$$C_R = C [T_R / T_A]$$

Assuming the effect is determined by the relative concentration of bound receptors $C_R / R_0$ we replace $C_R$ by $C_R / R_0$ and obtain

$$\text{Effect} \sim C_R / R_0 = [1 / R_0] [T_R / T_A] C$$

This equation is dimensionless and generally applicable

# Determinants of Dose Response

**Effect is determined by the relative concentration of bound receptors $C_R / R_0$**

$$C_R / R_0 = [1 / R_0] [T_R / T_A] C$$

**Effect is**

- **inversely proportional to the concentration of specific receptors $R_0$**

  ***So if the concentration of specific receptors $R_0$ is low, as may be the case with endocrine effects, pronounced effects may be induced by very low drug concentrations C. Many pesticides are alleged to cause endocrine disruption and there is much discussion about safe exposure levels, which some argue may not exist***

- **proportional to the quotient of time constants $T_R / T_A$**

  ***So if the quotient of time constants $T_R/T_A \ll 1$, i.e., if receptor binding is quickly reversible, we are not really dealing with a poison in the strict sense of the word, but toxic effects are still possible at very high drug concentrations C. This is the theoretical explanation of the Paracelsus paradigm Dosis facit venenum***

# Dependence of the Effect on Compound Concentration at the Target Site

*The reaction kinetics of receptor binding are*

$$dC_R / dt = K (R_0 - C_R) C - C_R / T_R$$

*Replacing the concentration of bound receptors $C_R$ by the relative concentration of bound receptors $C_R/R_0$ and the reaction constant $K R_0$ by $1 / T_A$ (where $T_A$ is regarded as the time constant for association), we obtain*

$$\frac{[dC_R / R_0]}{dt} = \frac{[C (1 - C_R/R_0)]}{[R_0 T_A]} - \frac{[C_R / R_0]}{T_R}$$

*This Equation can be simplified to indicate the relative concentration of bound receptors $C_R/R_0$ (leading to the effect) in steady-state, i.e. when $[dC_R / R_0] / dt = 0$ then*

$$\text{Effect} \sim C_R / R_0 = \frac{[C/R_0)].[T_R/T_A]}{1 + [C / R_0].[T_R/T_A]}$$

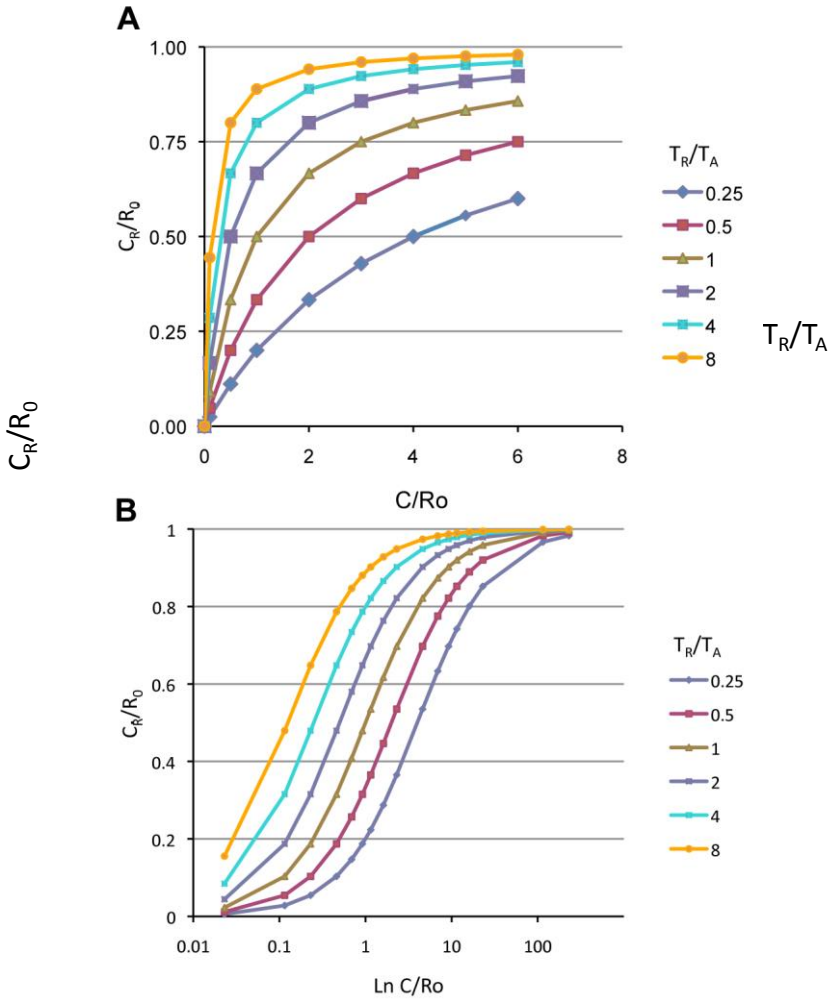
# Dependence of the Toxic Effect on Relative Compound Concentration at the Target Site

In steady state, the relationship between relative concentration of bound receptors $C_R / R_0$ (leading to the toxic effect) and relative toxicant concentration $C/R_0$ is invariably a hyperbole

The higher the $T_R / T_A$ ratio, the higher the toxicity

A linear concentration : effect relationship may occur whenever receptor binding leading to an effect is less than 25%

A logarithmic concentration : effect relationship may occur whenever the effect requires a high degree of receptor binding (20-80%)



# Concentration-Dependent Toxicity

## Dissociation is a Fast Process

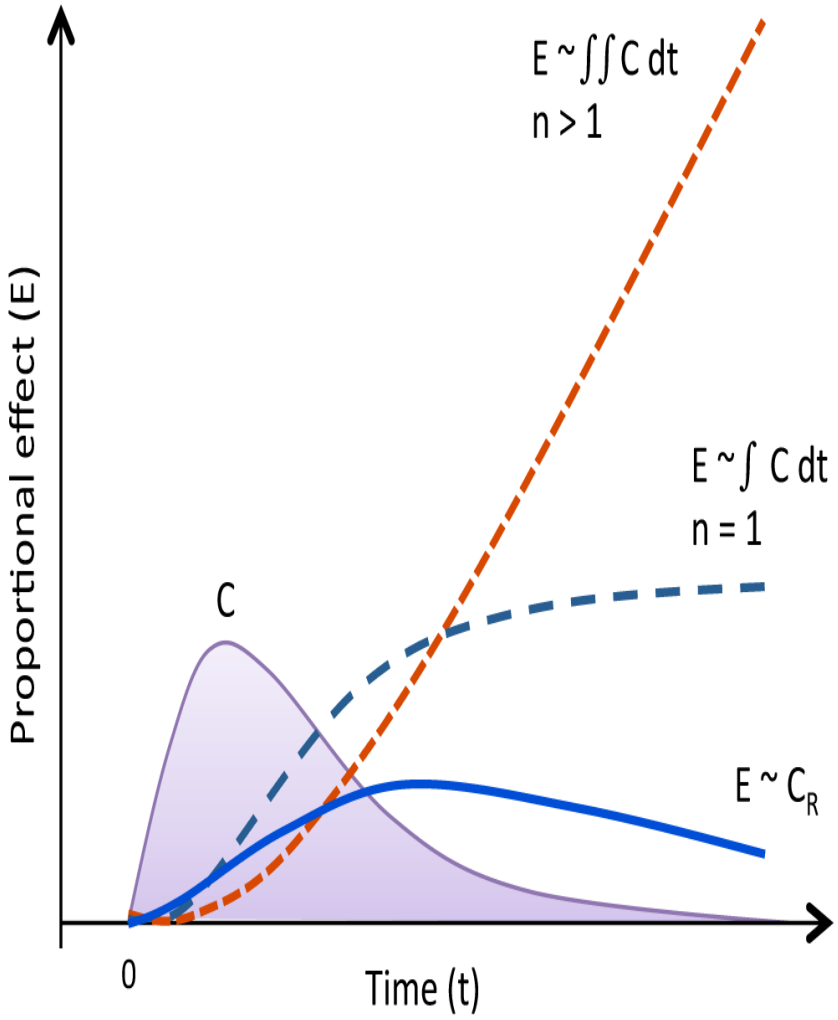
**Effect is determined by $C_R$ / $R_0$**

$$C_R / R_0 = [1 / R_0] [T_R / T_A] C$$

**If $T_R$ is low, i.e. when dissociation is a fast process, the equilibrium between C and receptor binding (and effect) will be established quickly but the toxic effect will also regress quickly.**

**The time course of the effect will be the same as the time course of the concentration at the site of action C, and the maximum effect will occur when the concentration at the site of action C is at its maximum.**

**The effects will thus be strictly concentration-dependent**



# Time-Dependent Toxicity

## Slowly Reversible Receptor Binding

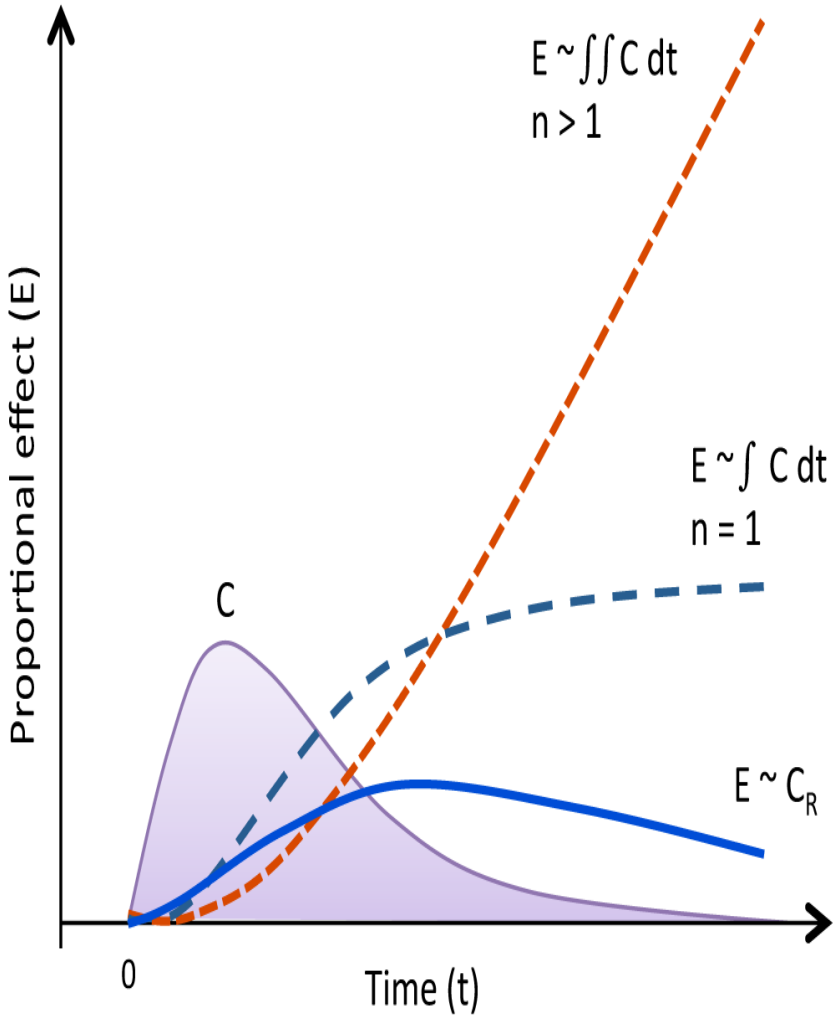
**Effect is determined by the relative concentration of bound receptors $C_R / R_0$**

$$C_R / R_0 = [1 / R_0] [T_R / T_A] C$$

**If the time constant for dissociation $T_R$ is high, i.e. when receptor binding is only slowly reversible, the time to maximum effect will be delayed, and the toxic effect will also be slowly reversible.**

**Because equilibrium between C and receptor binding will be established very slowly, toxicity becomes a process that takes place in time. Upon repeated exposure in quick succession, there may be cumulative effects.**

**There will be a latency period up to a defined effect**



# Reaction Kinetics of Irreversible Receptor Binding
# Haber's Rule

*The reaction kinetics of receptor binding are*

$$dC_R / dt = K (R_0 - C_R) C - C_R / T_R$$

*Now assume the effect occurs under circumstances where $C_R \ll R_0$, then $R_0$ remains practically constant, in which case*

$$dC_R / dt = K R_0 C - C_R / T_R$$

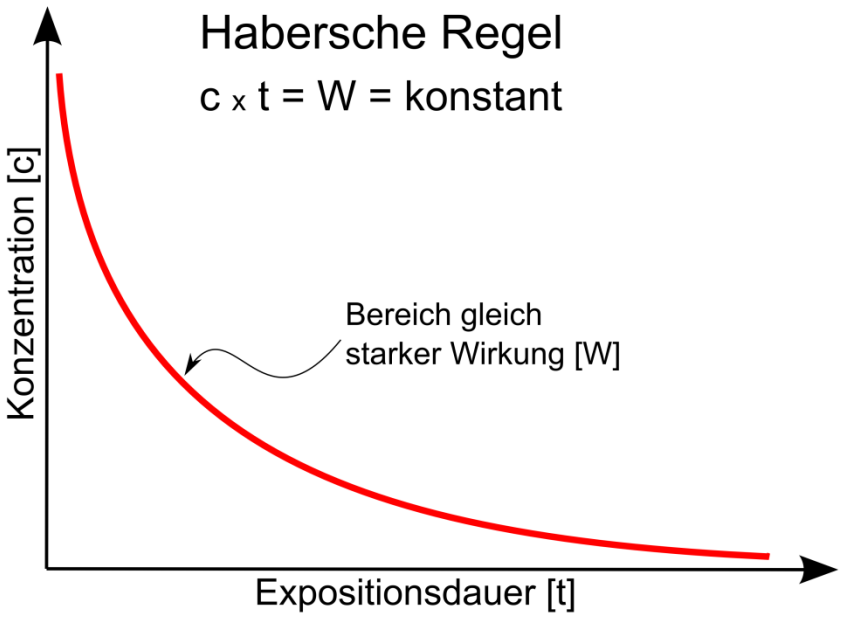
*If receptor binding is irreversible, then $T_R$ approaches infinity and we obtain*

$$dC_R / dt = K R_0 C$$

*If the drug concentration C remains constant during the study, integration yields*

$$C_R / R_0 = K C t$$

**A defined effect is determined by the product of exposure concentration C and duration t**



# Haber's Rule or Haber's Law

**C x t = constant**

may characterise the dose response relationship of substances with irreversible effects, such as carcinogenic or lethal effects.

A defined effect is determined by the product of exposure concentration and exposure duration, that is by the total dose administered.

The toxicity is cumulative, i.e. the toxic effects of even the smallest doses persist, strongly suggesting that a threshold may not exist

**Liver cancer induced in rats by 4-dimethylaminoazobenzene (4-DAB)**

Druckrey, H. Klin. Wochenschr. 1943, 22: 532

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

# Haber's Rule or Haber's Law

## Threshold concentration may not exist

**The famous British pharmacologist AJ Clarke arrived at similar conclusions when he expanded Haber's rule to characterise the action of a number of drugs**

**$(C - C_m)(t - t_m) = \text{constant}$**

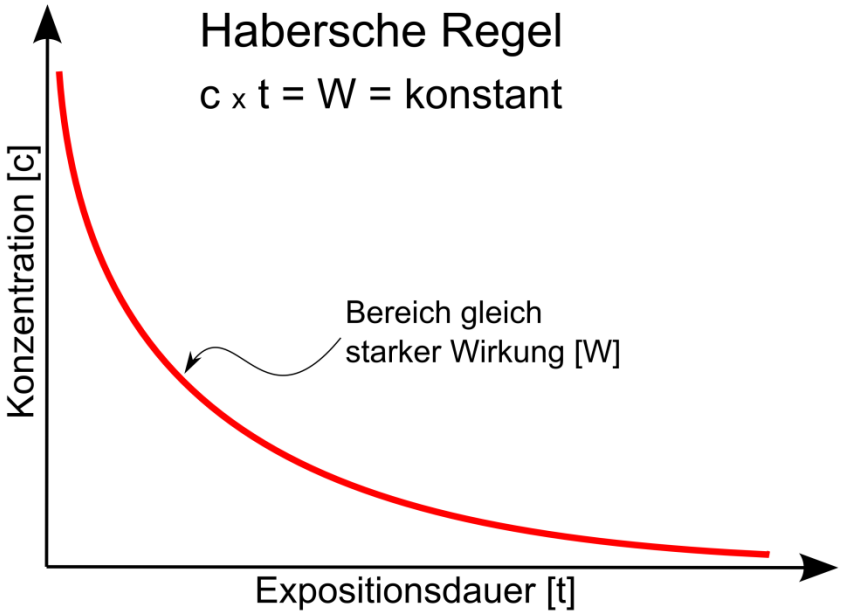
**where $C_m$ is a threshold concentration, and**

**$t_m$ 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"***



# Haber's Rule Will Only Apply Under Certain Conditions

Haber's rule or Haber's law

$C \times t = \text{constant}$

Proportionality between the exposure concentration $c$ and the concentration at the site of action $C$, which must also increase over time in a strictly linear fashion

$\frac{dC}{dt} = K c$

Effect $E$ has to be proportional to the concentration at the site of action $C$ (and thus to exposure concentration $c$) as well, so that

$\frac{dE}{dt} = K c$ and $E = K \int c \, dt$

If, under such circumstances, the exposure concentration $c$ is kept constant then

$E = K c t$

and the toxicant will follow Haber's rule, that is the velocity of the effect $E/t$ will be linearly related to the exposure concentration $c$

$E/t = K c$

# Reinforcement Of An Effect By Exposure Time

## Druckrey-Küpfmüller Equation C x T50 $^{n}$ = constant, with n > 1

**If receptor binding is irreversible, the concentration of bound receptors $C_R$ is proportional to the integral of the drug concentration at the target site C over time:**

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

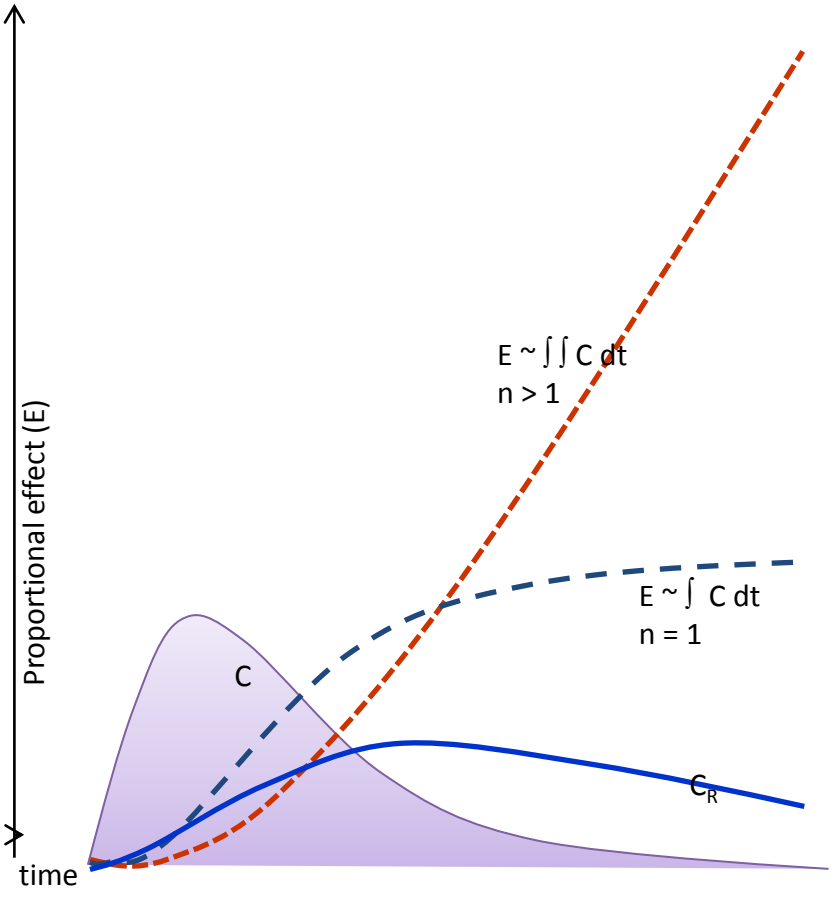
**If the subsequent effect is irreversible as well the effect E is proportional to the integral of the concentration of bound receptors $C_R$ over time:**

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

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

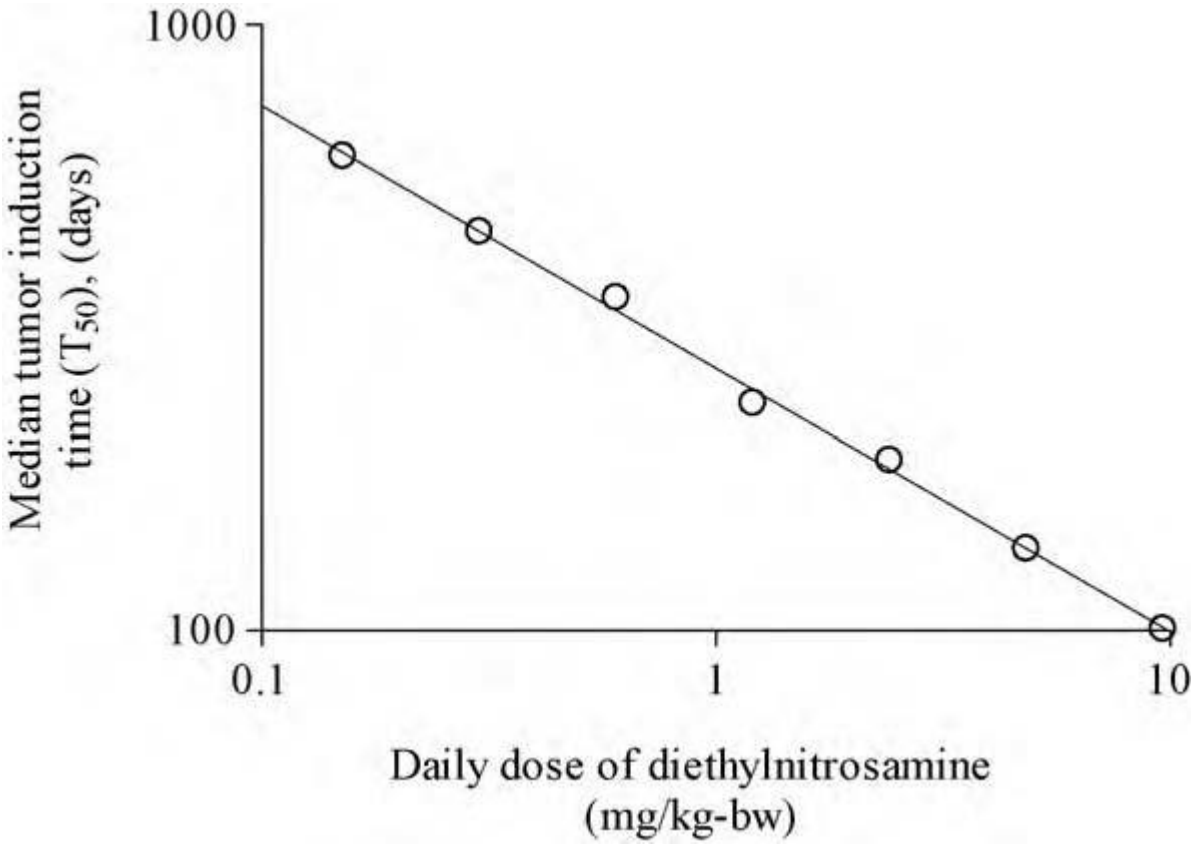
$E \sim \int \int C\,dt$ (3)

**This is the theoretical explanation of the Druckrey-Küpfmüller Equation C x T50 $^{n}$ = constant, with n > 1**



# Druckrey-Küpfmüller Equation C x T50 $^{n}$ = constant, with n = 2.3
# Liver Cancer Induction in Rats by Diethylnitrosamine
# Suggesting Irreversible Receptor Binding with Irreversible Effects

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



# Mechanism of Action of Genotoxic Carcinogens

## Formation of DNA adducts leading to mutations

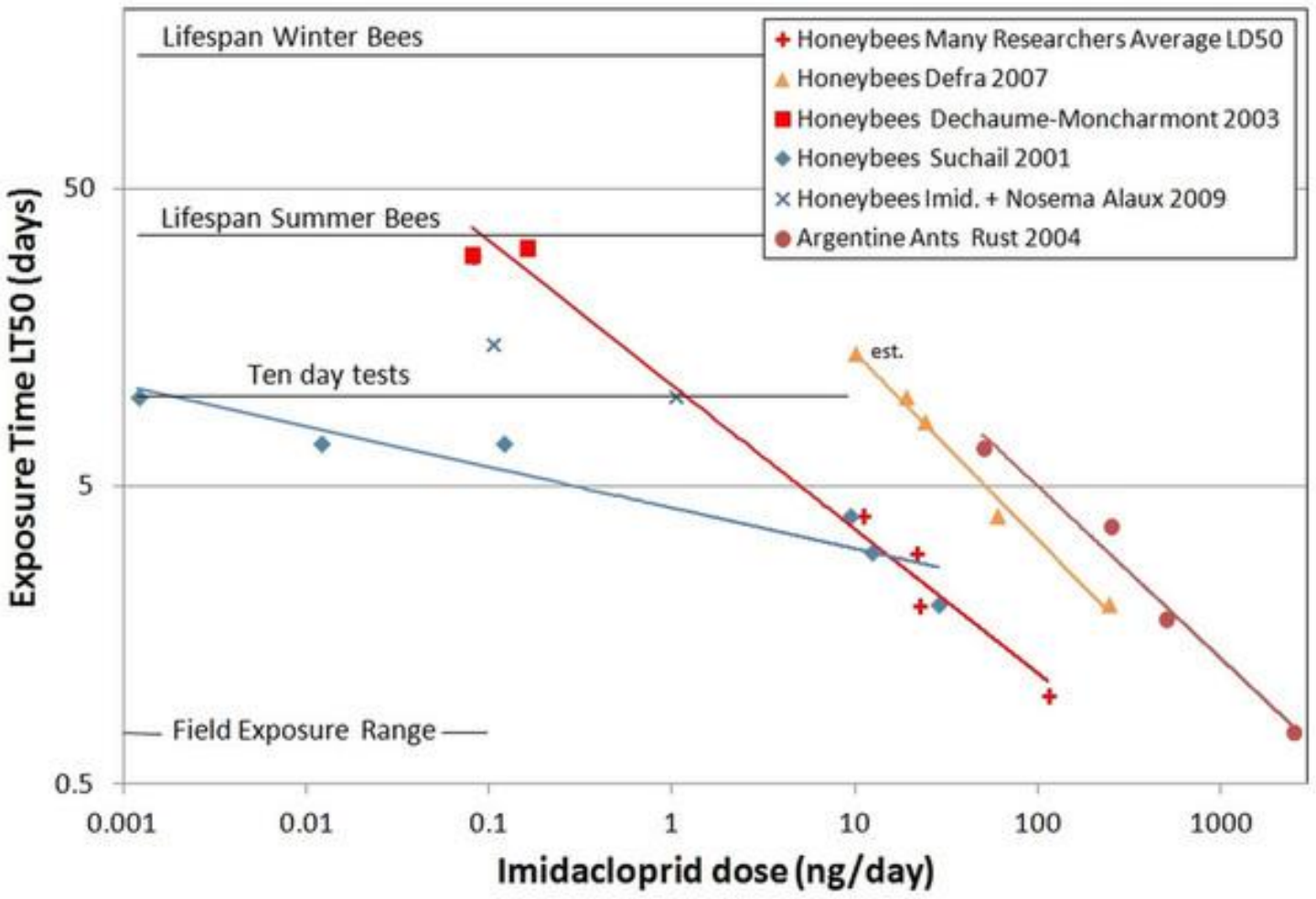
- **Most genotoxic carcinogens are electrophiles that interact directly with DNA through the formation of covalent bonds, resulting in DNA-carcinogen complexes (DNA adducts = irreversible receptor binding).**
- **These complexes lead to various types of DNA damage, including the formation of cross-links 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 = irreversible effect).**

# Druckrey-Küpfmüller Equation C x T50 n = constant, with n > 1

## Time-Cumulative Effects of a Neonicotinoid Insecticide in Bees and Ants

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

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



# IMIDACLOPRID

## Time-Cumulative Toxicity in Various Species

**Druckrey-Küpfmüller Equation $C \times T50^{n} = \text{constant}$, with $n > 1$**

**The dose response of Imidacloprid in the water flea *Daphnia magna* follows**
**$C \times T50^{2.4} = \text{constant}$**

**The dose response of Imidacloprid in the hymenopteran parasitoid *Chelonus blackburni* follows**
**$C \times T50^{1.5} = \text{constant}$**

**The dose response of Imidacloprid in honey bees *Apis mellifera* follows**
**$C \times T50^{5.8} = \text{constant}$**

**The dose response of Imidacloprid in the ostracod *Cypridopsis vidua* follows**
**$C \times T50^{4.7} = \text{constant}$**

**A description of the mechanism of action of imidacloprid by Bayer CropScience experts confirms irreversible interactions and effects:**

***Irreversible Receptor Binding:***
***The compound led to a continuous blockage of insectspecific nicotinic-acetylcholine receptors (nAChR), causing tetanic muscle contractions within minutes of exposure. This manifested as intense trembling of the legs and pumping movements of the body.***

***Irreversible Effects:***
***The affected flea stages remained motionless while the nerves and muscles were constantly and irreversibly destroyed due to hyperactivity. The ganglia of the head and thorax and the striated muscles of the flea body and legs were damaged first, whereas the intestinal movements (e.g., visible in larvae) took longer to exhibit damage.***

**Mehlhorn H et al. Parasitol Res (1999) 85: 625-637**

# 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)

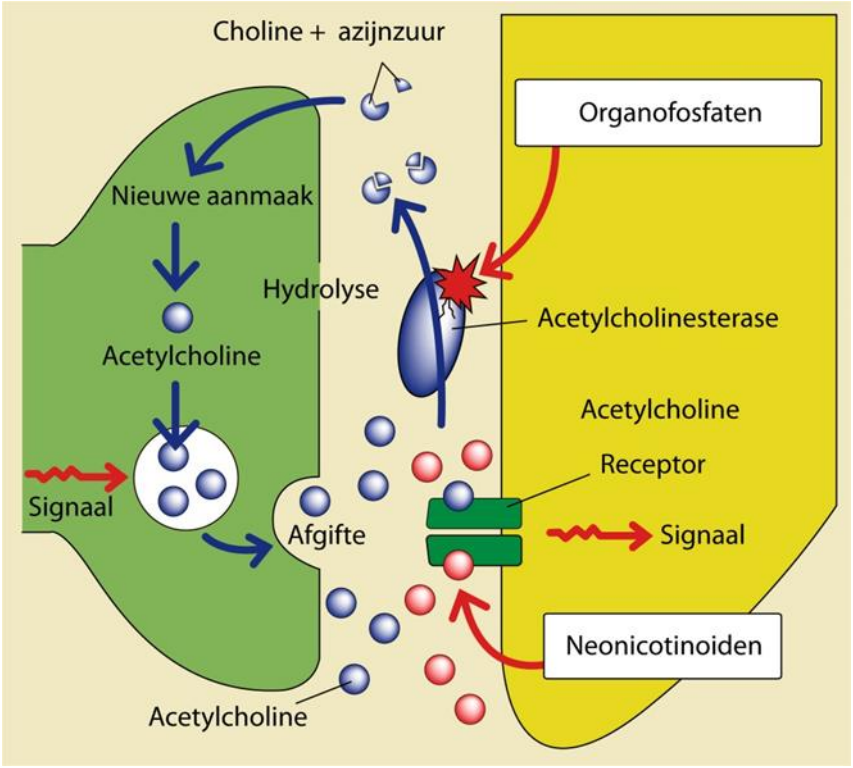
## Mechanism of action

**„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



# Rodenticide Diphacinone
# Time-Cumulative Toxicity in *Falco sparverius*

**The anticoagulant rodenticide diphacinone fed to American kestrels (*Falco sparverius*) shows reinforcement of mortality over time**

**The Druckrey-Küpfmüller Equation is**

**$C \times T50^{1.6} = \text{constant}$**

**This dose response relationship points to irreversible receptor binding associated with an irreversible effect.**

**Diphacinone binds irreversibly to vitamin K epoxide reductase, impairing the carboxylation of the serine protease coagulation factors that result in hemorrhages and ultimately death**

# Viability of Rat Cerebrocortical Neurons Exposed to Methylmercury

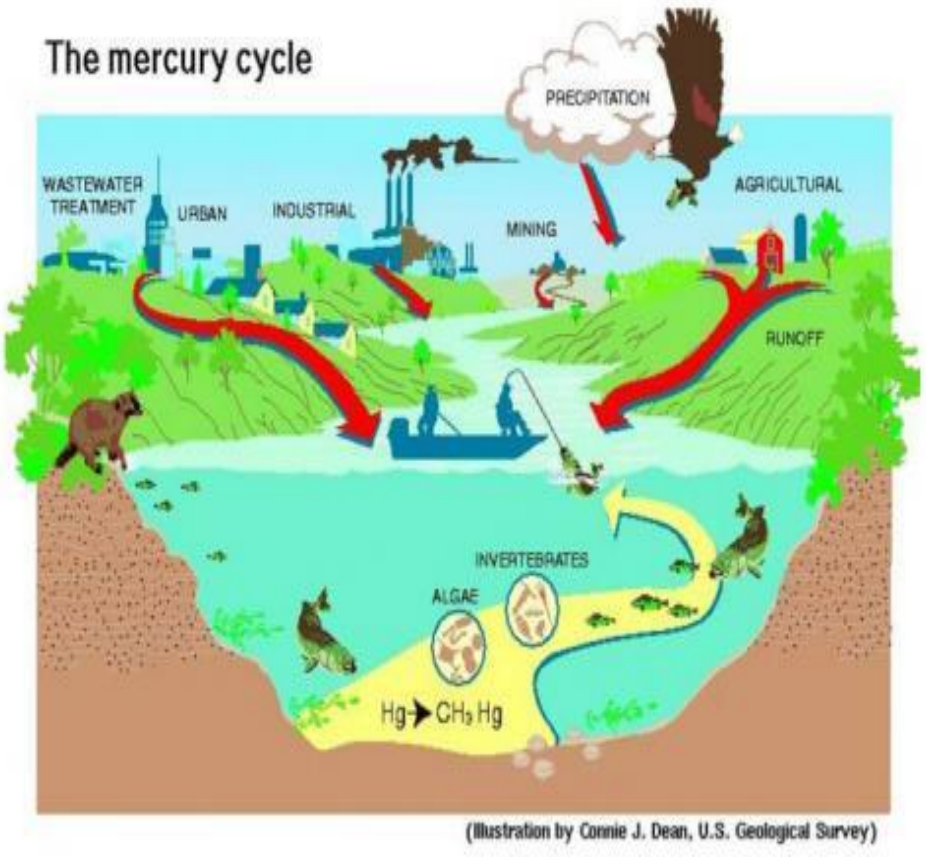
**MeHg follows a pattern of toxicity that is reinforced with time of exposure**

**C x T50 $^{1.7}$ = constant**

**Formed by anaerobic microorganisms in aquatic sediments, MeHg is biomagnified through the food chain.**

**As with inorganic mercury, MeHg also forms covalent bonds with sulfide groups in proteins, but it is more toxic than mercury because it penetrates the tissues and reaches the central nervous system**

**Its neurotoxicity symptoms include motor difficulties, sensory problems and mental retardation, an irreversible condition known as Minamata disease**



# The Druckrey-Küpfmüller Equation As Indicator of Time-dependent and Time-Cumulative Toxicity

**The Druckrey-Küpfmüller Equation**

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

**can serve as a screening tool to identify toxicants that show time-dependent or time-cumulative toxicity**

**Haber's rule is a special case of the Druckrey-Küpfmüller Equation**

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

**where $n = 1$**

**If a compound is shown to follow the Druckrey-Küpfmüller equation, it may be impossible to define a threshold, i.e. a safe level of exposure, and its use should be severely restricted and preferably be prohibited**

**Time To Event Methods Are Required For Risk Analysis**

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

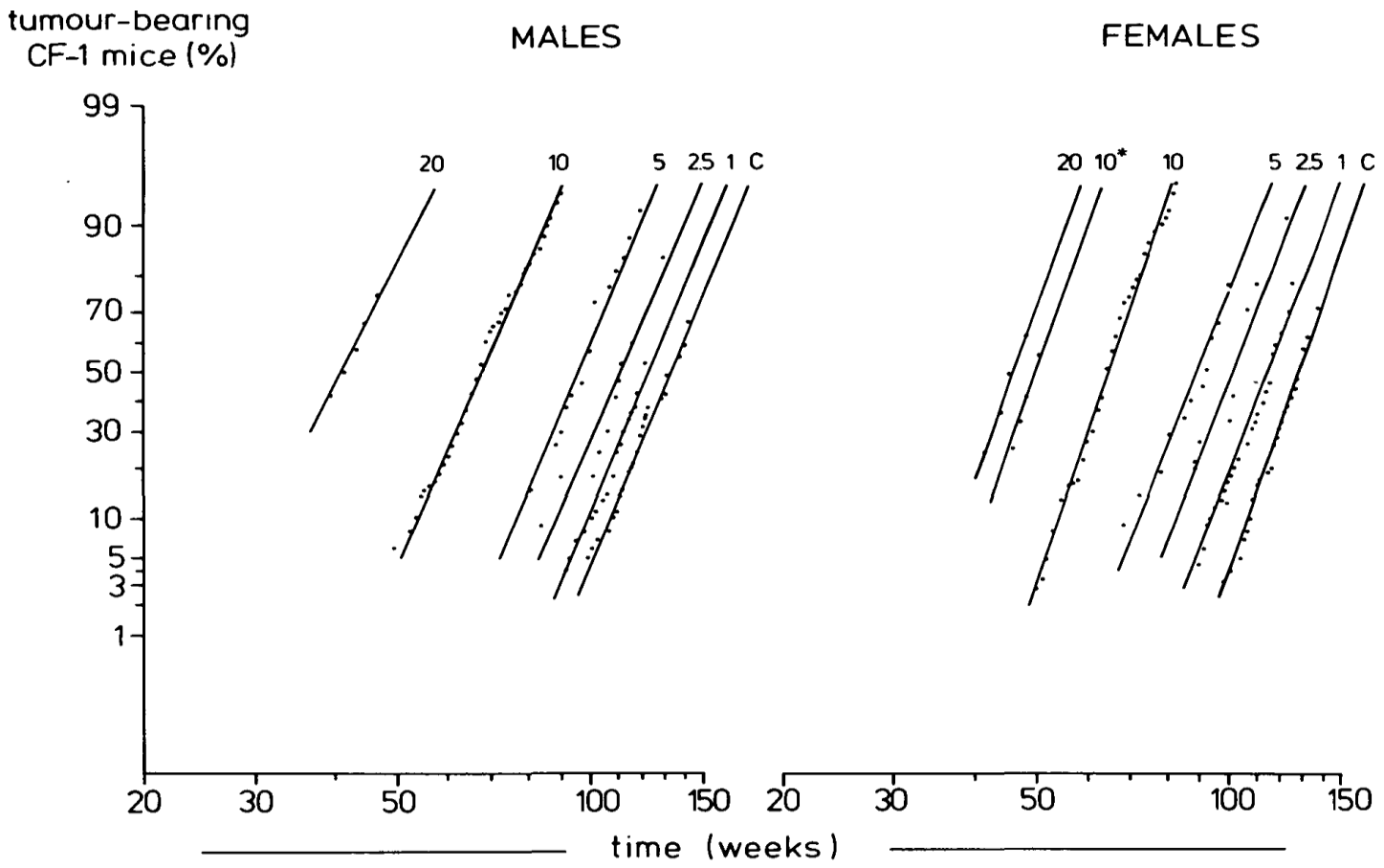
**This TTE approach measures the times to respond for all individuals, and provides information on the acquired doses as well as the exposure times needed for a toxic compound to produce an effect on the organisms tested.**

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

# Revised Approach to Risk Assessment

## Liver Tumor Promotion in Mice Exposed to the Insecticide Dieldrin

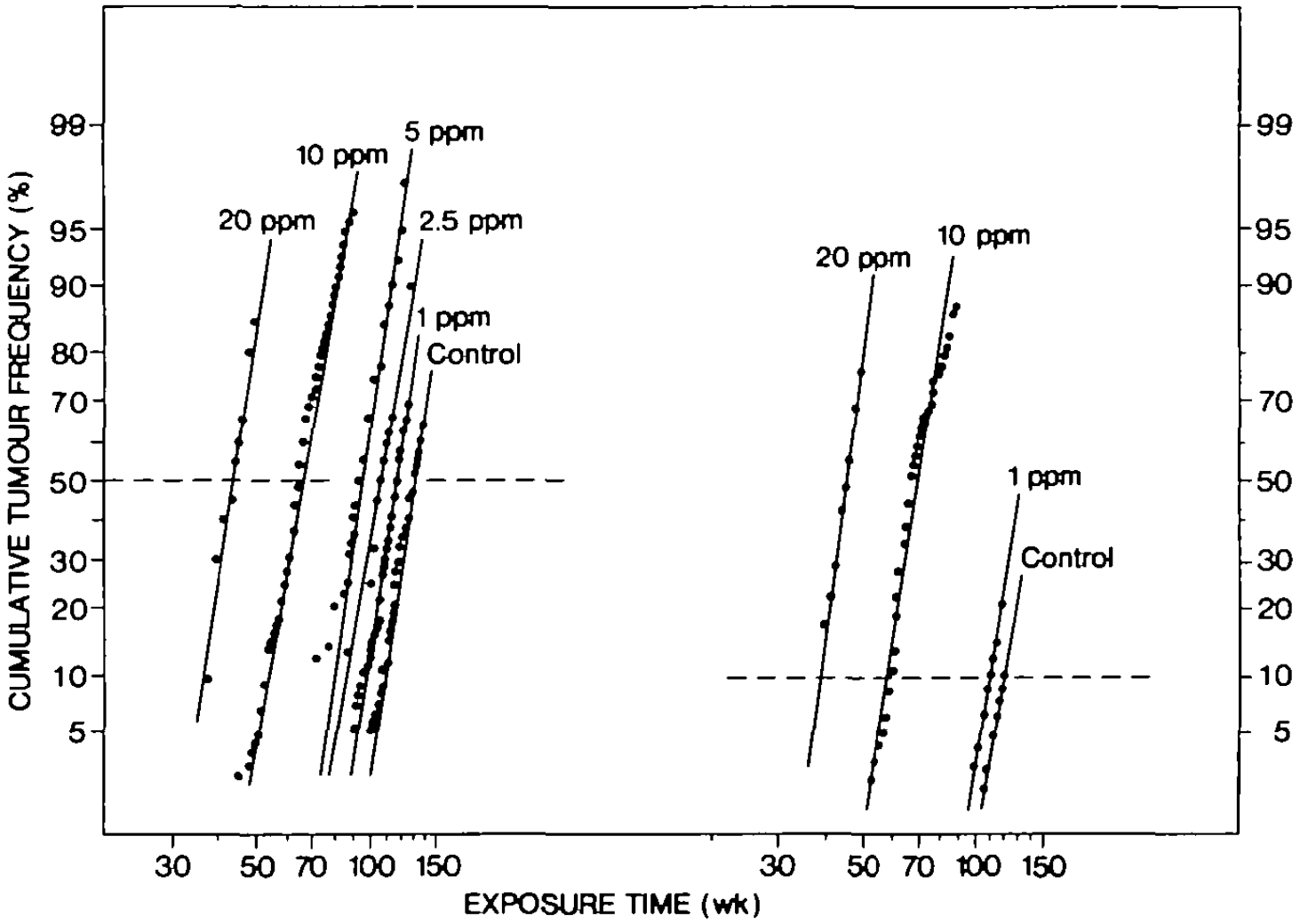
H. A. Tennekes et al. (1982) Carcinogenesis 3, 941-945



# Revised Approach to Risk Analysis

## Relate the Velocity of Liver Tumor Induction to the Dieldrin Exposure Level

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

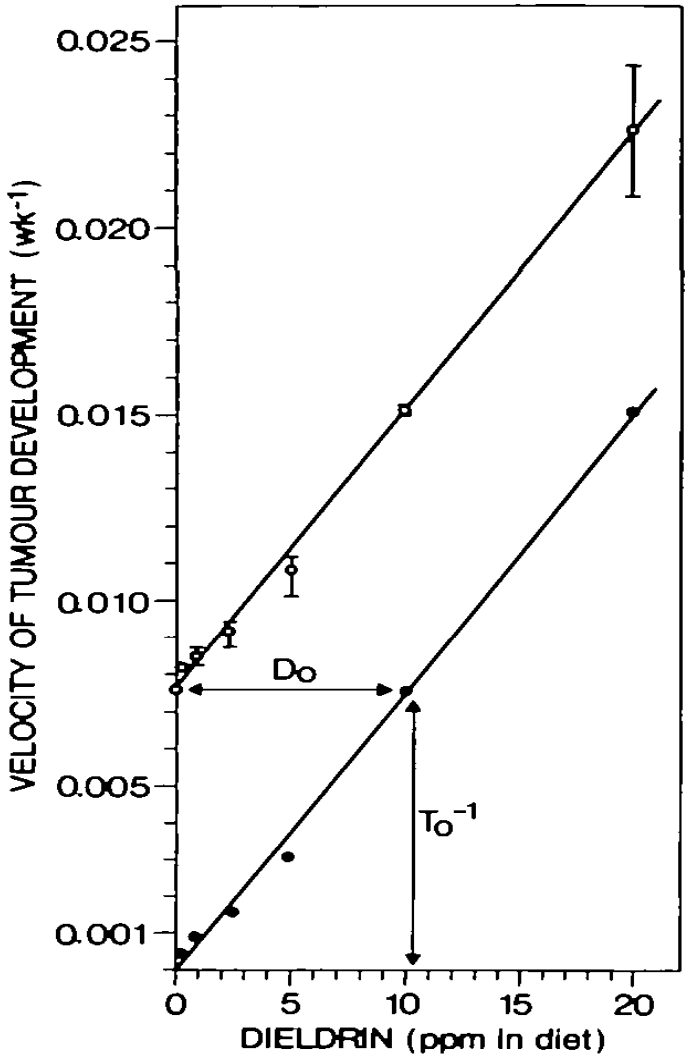


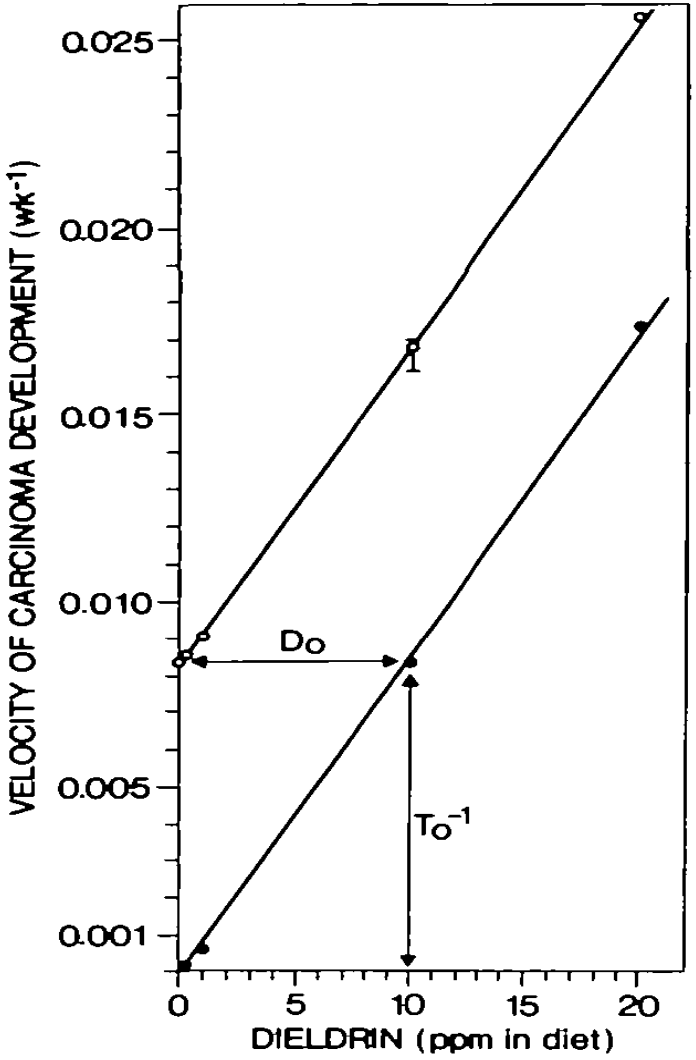
# Revised Approach to Risk Analysis

## The Velocity of Mouse Liver Tumor Promotion by Dieldrin is Linearly Related to Exposure Concentration

## There is No Threshold Concentration (No Safe Exposure Level)

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



# 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 = constant**

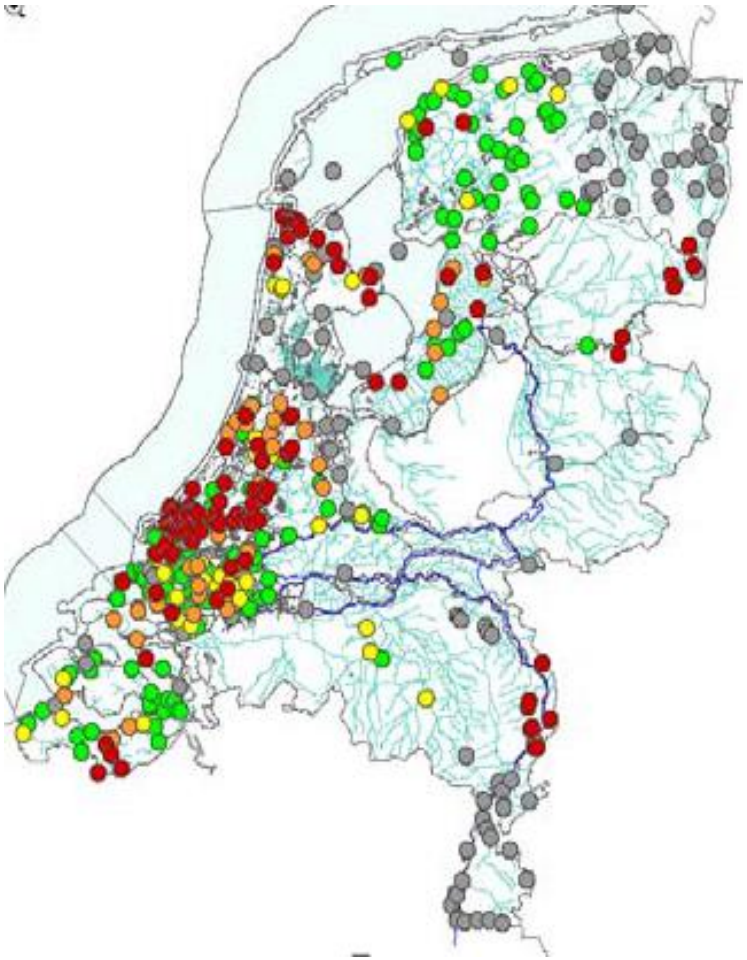
| Residues | Imidacloprid (PEC) (µg L–1 or kg–1) | Aver. Exposure Concentration c PEC × frequency (11%) (µg L–1 or kg–1) | Predicted time to lethal effect t50 (hrs) | Percentage of average life expectancy |
|---|---|---|---|---|
| Nectar | 1 | 0.11 | 263 | 26 |
| | 3 | 0.33 | 218 | 22 |
| Pollen | 0.7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

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

# 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



**Imidacloprid is prone to leach from soils into groundwater and runoff to surface water. A safe dose for insects cannot be defined**

**This insecticide should never have been allowed in areas with high groundwater levels, such as the western parts of Holland**


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

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

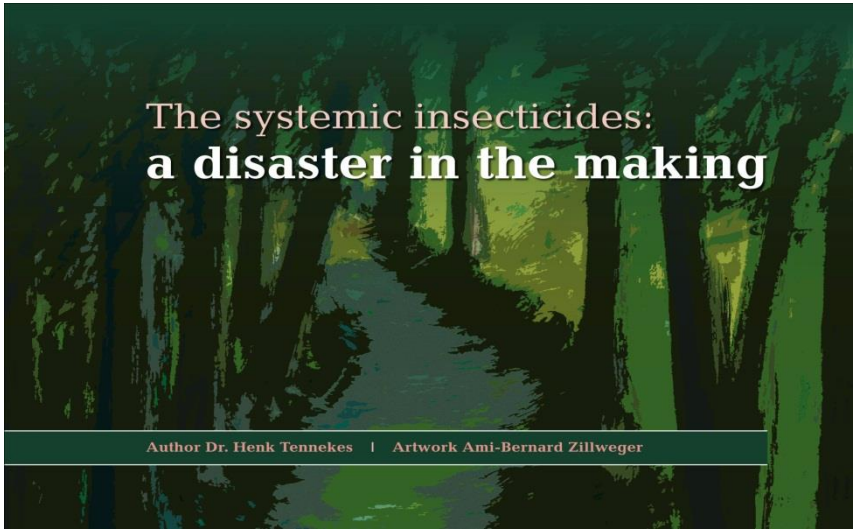
**Rachel Carson**

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

**The 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**

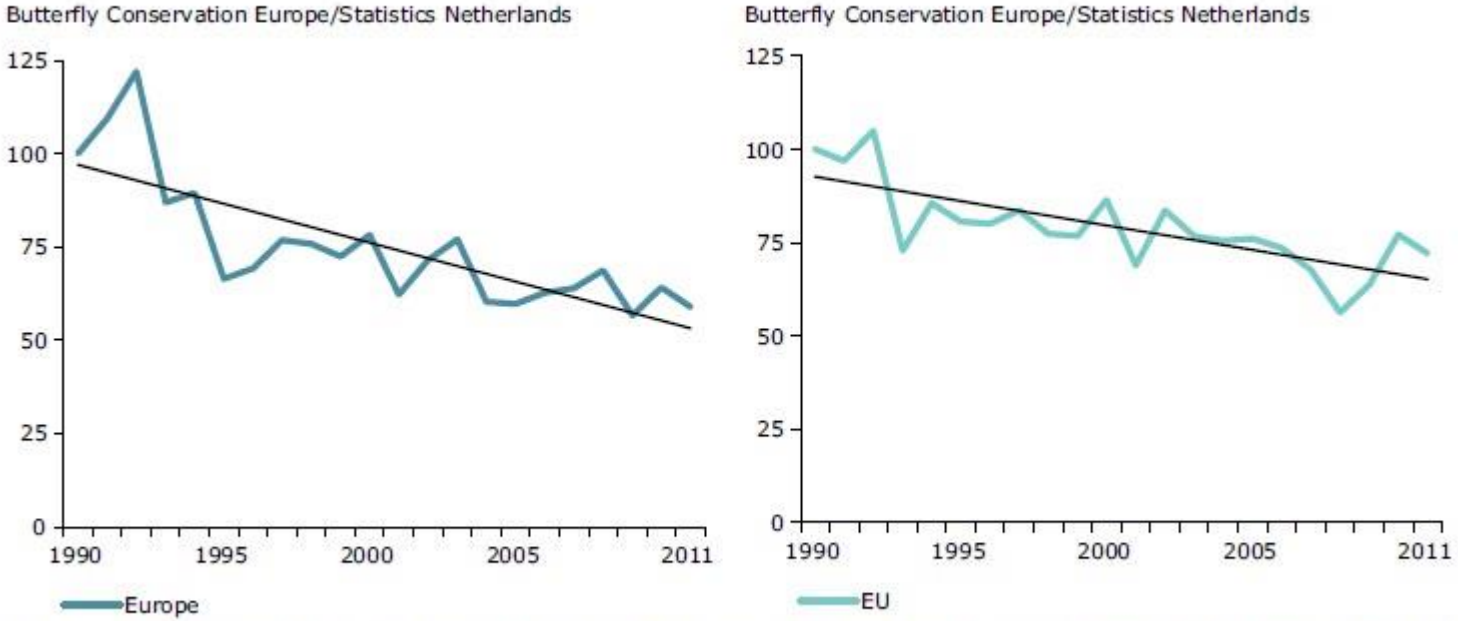
**The disappearance can be related to the neonicotinoid insecticide imidacloprid, which is a major contaminant of Dutch surface water since 2004**

**What, in effect, is happening is that the use of imidacloprid is creating a toxic landscape, in which insects are killed off**



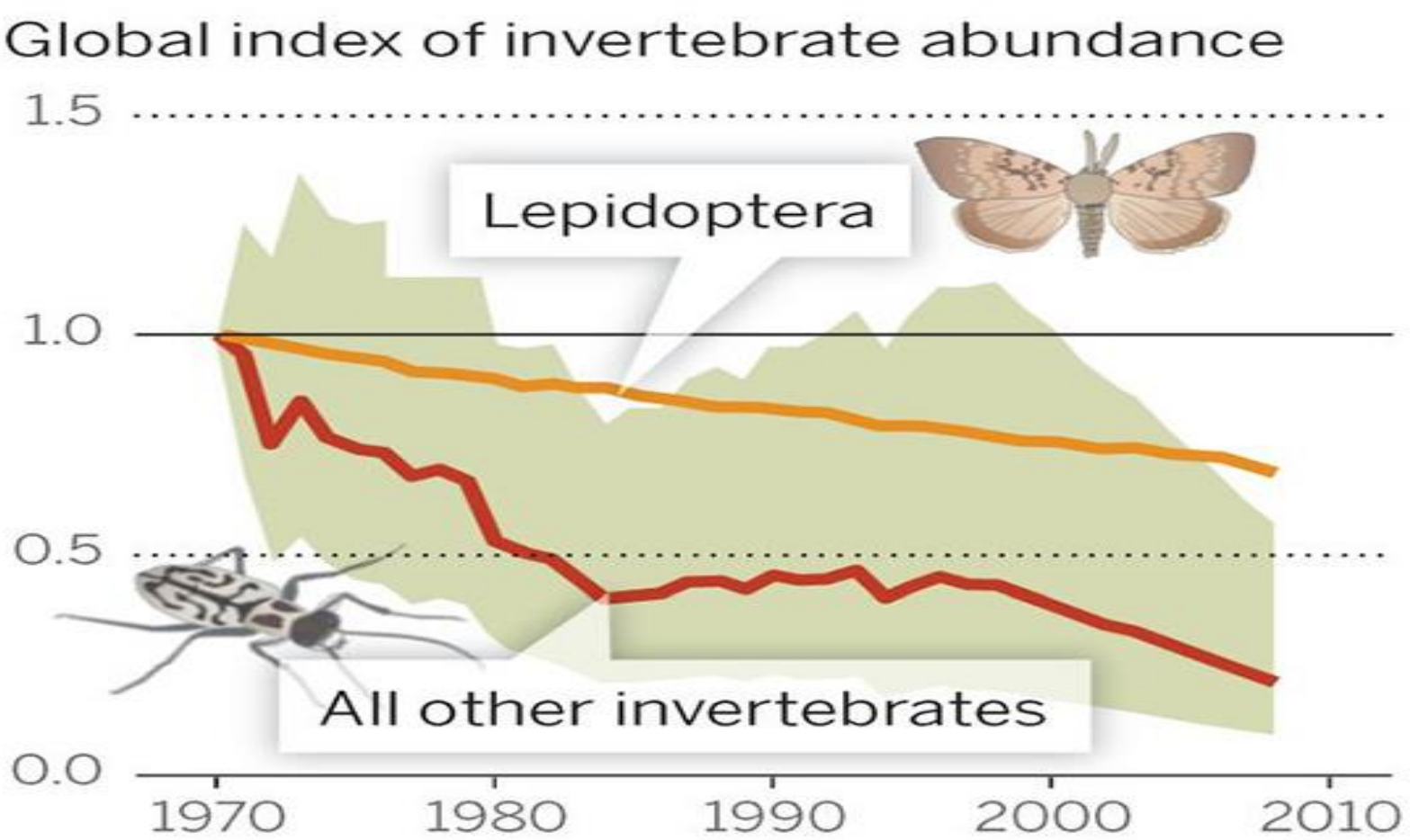
# Decline of Butterflies in Europe

**Figure 4.1 The Grassland Butterfly Indicators for Europe (left) and the EU (right)**



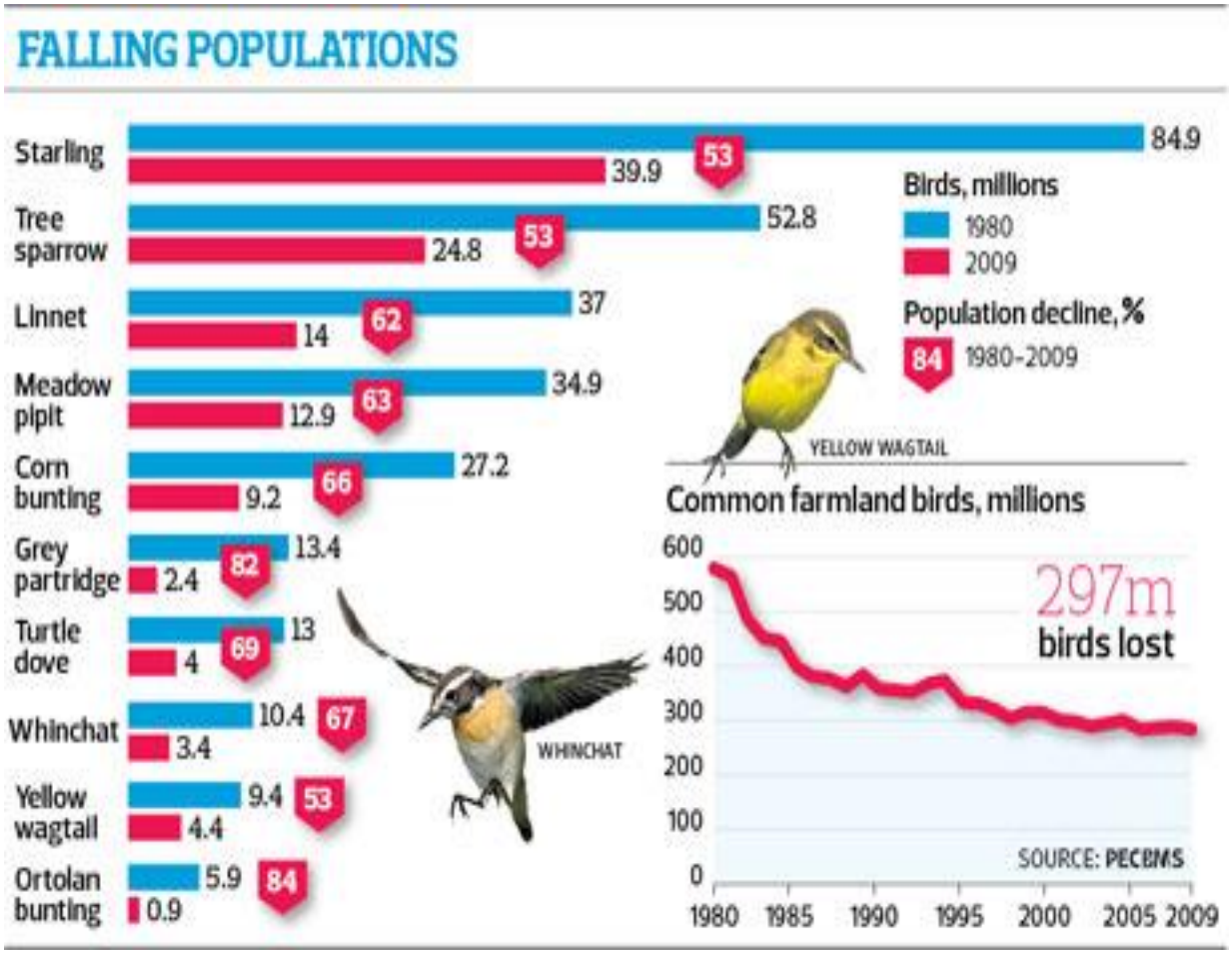

**Note:** The indicators (blue lines) are based on the countries in Map 1.1 and characteristic grassland butterfly species in Figure 2.1 (the black line represents the significant trend). Both indicators show a marked decline.

**According to global monitoring data for 452 species, there has been a 45 percent decline in invertebrate populations over the past 40 years**



# Decline of Farmland Birds in Europe

**The Guardian, 26 May 2012**



# Sustainable Agriculture

Geiger F et al. (2010) Basic Appl Ecol 11(2):97–105
van Lenteren JC BioControl DOI 10.1007/s10526-011-9395-1

- Agriculture is a major source of pollution and chemical pesticides have serious negative effects on biodiversity
- If biodiversity is to be restored in Europe there must be a Europe-wide shift towards farming with minimal use of pesticides over large areas
- Biological pest control is to be strongly encouraged

# Chemical Risk Assessment

## The Take Home Messages

- **Dose – Response Relationships Are Complex**
  **Toxicokinetics determine compound concentration at the site of (inter)action**
  **Ergokinetics determine compound interactions with receptors leading to an effect**
- **The Time Constant for Dissociation of Bound Receptors is a Major Determinant of Dose – Response Relationships**
  **Low values may lead to concentration-dependent toxicity**
  **High values may lead to time-dependent toxicity**
  **Values approaching infinity may lead to Haber's rule C x t = constant**
- **Irreversible Receptor Binding with an Associated Irreversible Effect Leads to Time-Cumulative Toxicity**
  **The Druckrey-Küpfmüller Equation $C \times T50^{n} = constant$ not only describes the dose response of carcinogens but can serve as a general screening tool to identify toxicants that show time-dependent or time-cumulative toxicity. A threshold level may not exist**
- **A new risk assessment is needed to evaluate adverse effects that chemicals may have on humans and the environment.**
- **We must adopt time-to-effect approaches in chemical risk analyses to identify chemicals with time-cumulative effects and severely restrict or preferably prohibit their application**