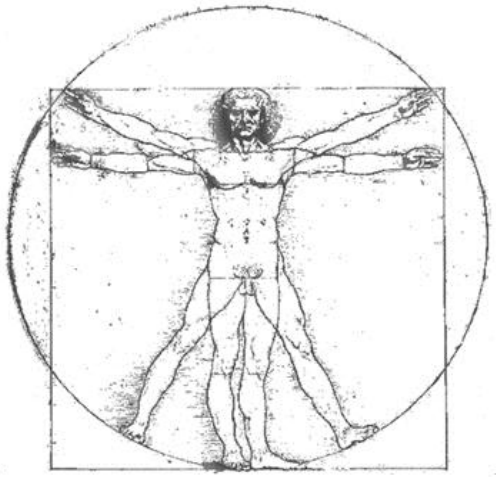
Henk Tennekes

Experimental Toxicology
Services Nederland



Time-Dependent Toxicity of Pesticides

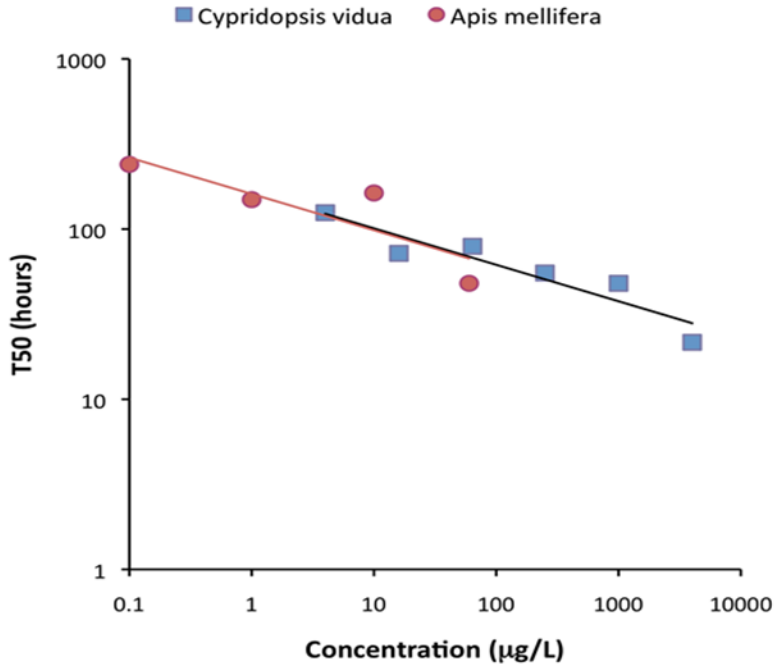
Dr. Henk Tennekes

12th International Fresenius Ecotox Conference
Mainz, 28 November 2012

The Dose:Response Characteristics of Genotoxic Carcinogens and Neonicotinoids are Strikingly Similar

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

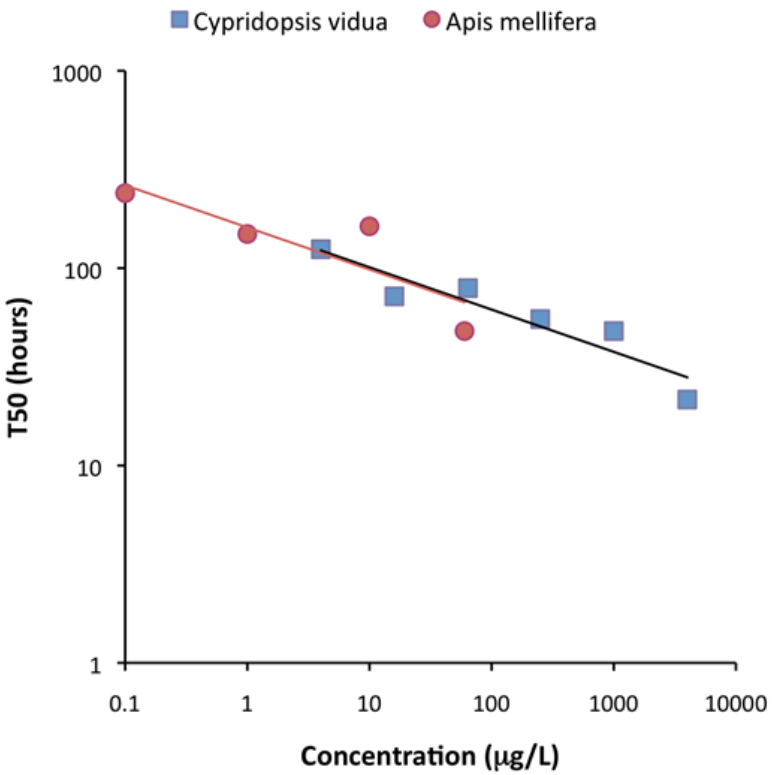
- In 2009, Henk Tennekes discovered that the dose response characteristics of the toxicity of widely used neonicotinoid insecticides to arthropods were strikingly similar to those of genotoxic carcinogens

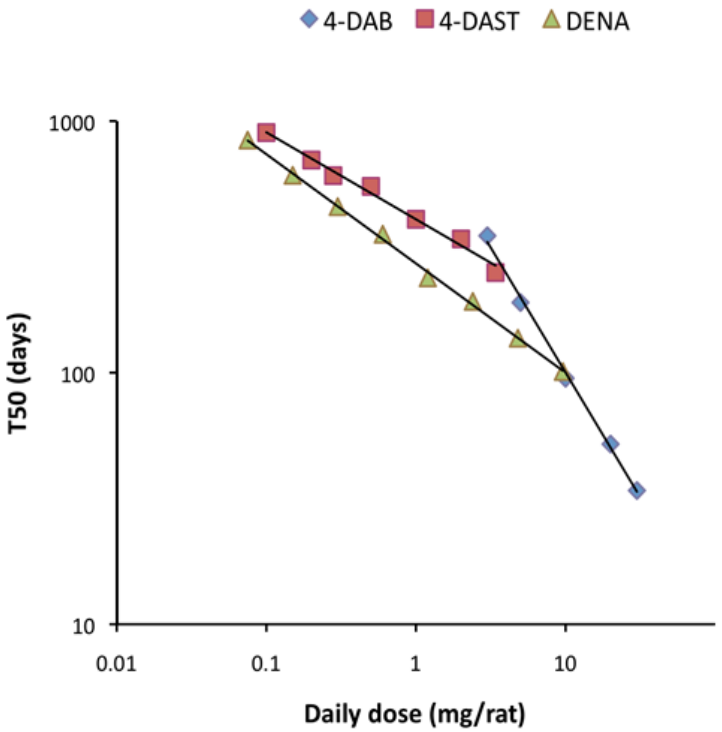


The Dose:Response Characteristics Of Genotoxic Carcinogens (Right) And Neonicotinoids (Left) Are Strikingly Similar

Druckrey-Küpfmüller Equations C x T50 ^{n} = constant, with n ≥ 1

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





Neonicotinoids Poison Non-Target Insects

- Neonicotinoid insecticides that are currently in use are water soluble (hydrophilic) and permeate the whole plant
- Advantage:
  Application rates are much lower than for traditionally used insecticides
- Disadvantage:
  Non-target insects such as honey bees or butterflies that collect pollen or nectar from the crop are poisoned

Lethal Effect of Imidacloprid on Honey Bees Toxicity Is Reinforced By Exposure Time

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 *lower* the exposure concentration**, the *longer* the time period up to a lethal effect,
  the *lower* the lethal dose

- **The Dose : Response Relationship is a Druckrey-Küpfmüller Equation**

  Ln T50 (hrs) = 5.11 – 0.22 Ln C (µg. L-1 or kg-1)

  or

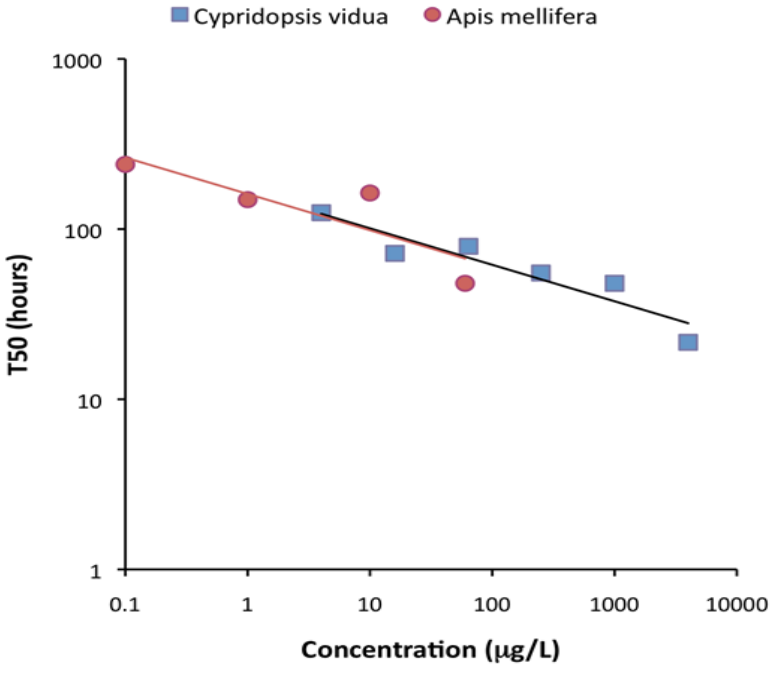
  $C \times T50^{4.5}$ = constant

Concentration C (µg/L)	Time to 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**

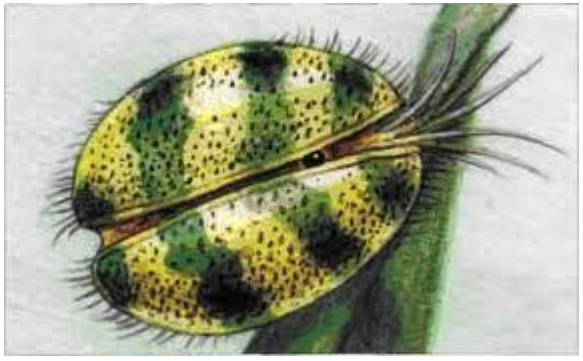
Lethal Effect of Imidacloprid on the Ostracod *Cypridopsis vidua* Toxicity Is Reinforced By Exposure Time

Sánchez-Bayo F. 2009. *Ecotoxicology* 18: 343-354
Tennekes HA. 2010. *Toxicology* 276, 1-4.
Tennekes HA, Sánchez-Bayo F. 2012. *J. Environment. Analytic Toxicol.* S4- 001

- **The *lower* the exposure concentration**,
  the *longer* the time period up to a lethal effect,
  the *lower* the lethal dose



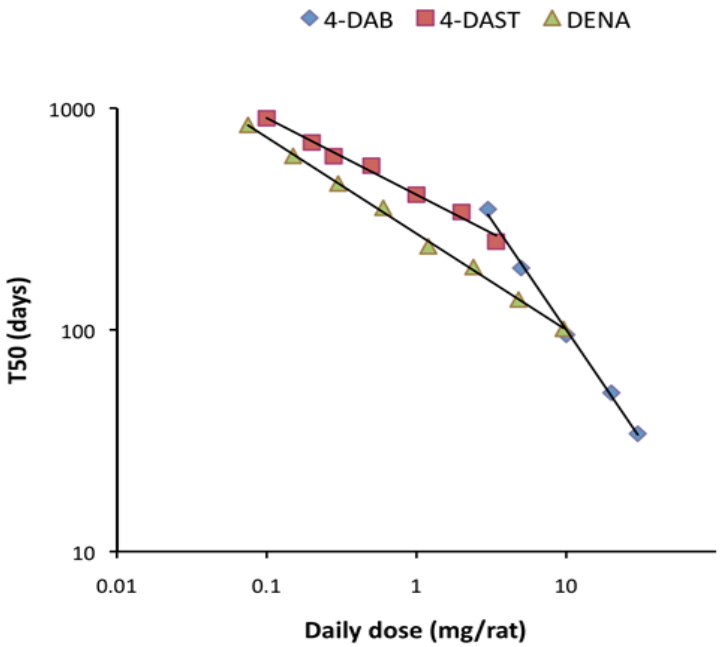
Concentration (µg/L)	Time to Effect (days)	Lethal Dose (µg/L x days)
4,000	0.9	**3,600**
250	2.3	**575**
64	3.3	**211.2**
4	5.2	**20.8**



Induction of Liver Cancer In Rats By Diethylnitrosamine Toxicity Is Reinforced By Exposure Time

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

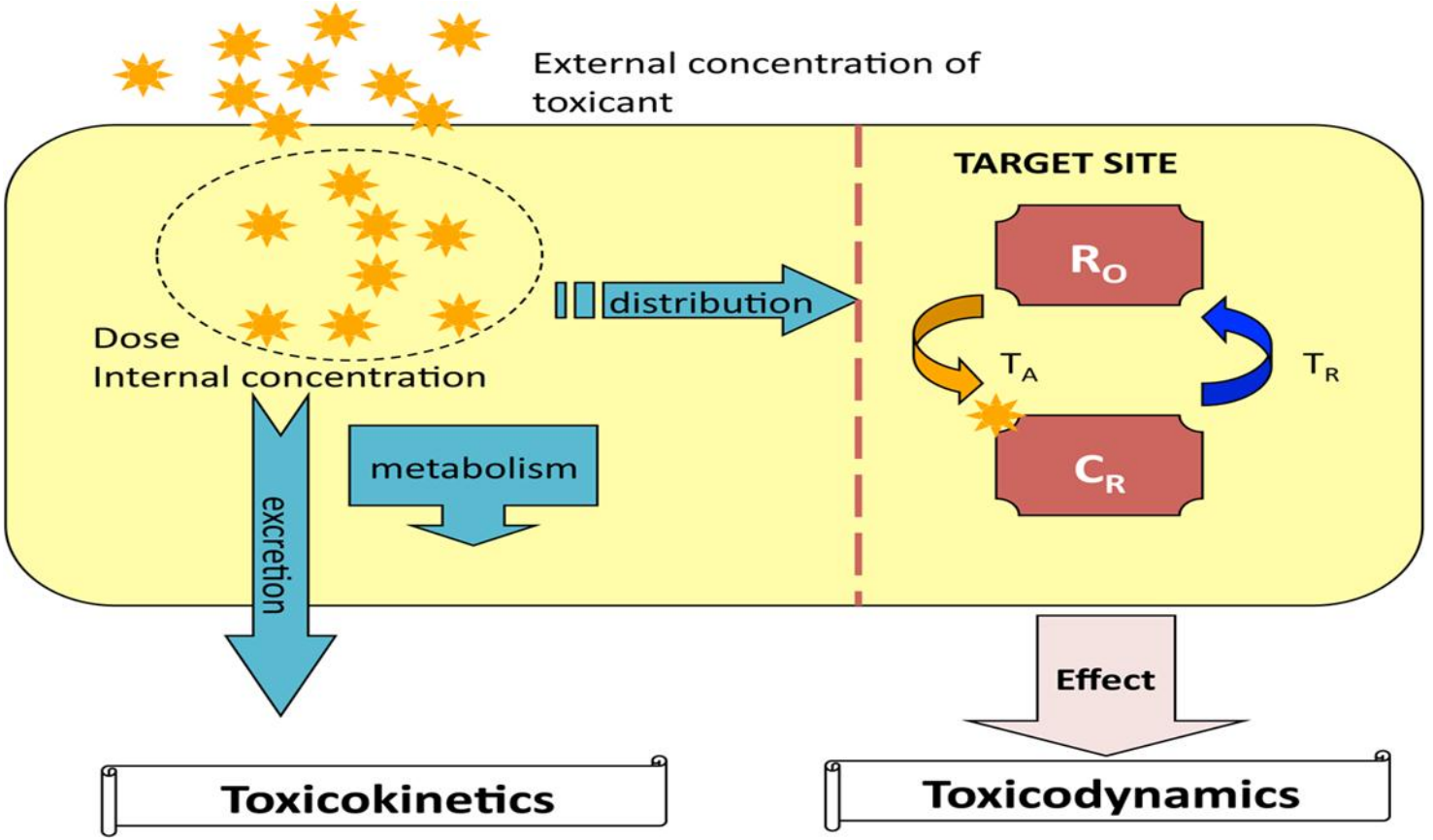
- **The *lower* the exposure concentration,**
  the *longer* the time period up to a carcinogenic effect,
  the *lower* the carcinogenic dose



Daily Dose (mg/kg)	Time to Effect (Days)	Carcinogenic Dose (mg/kg)
9,6	101	**963**
1,2	238	**285**
0,3	457	**137**
0,075	840	**64**

The Dose:Effect Characteristics of Imidacloprid in Arthropods are Determined by the Reversibility of Receptor Binding

At the target site (CNS), imidacloprid 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 : effect relationship

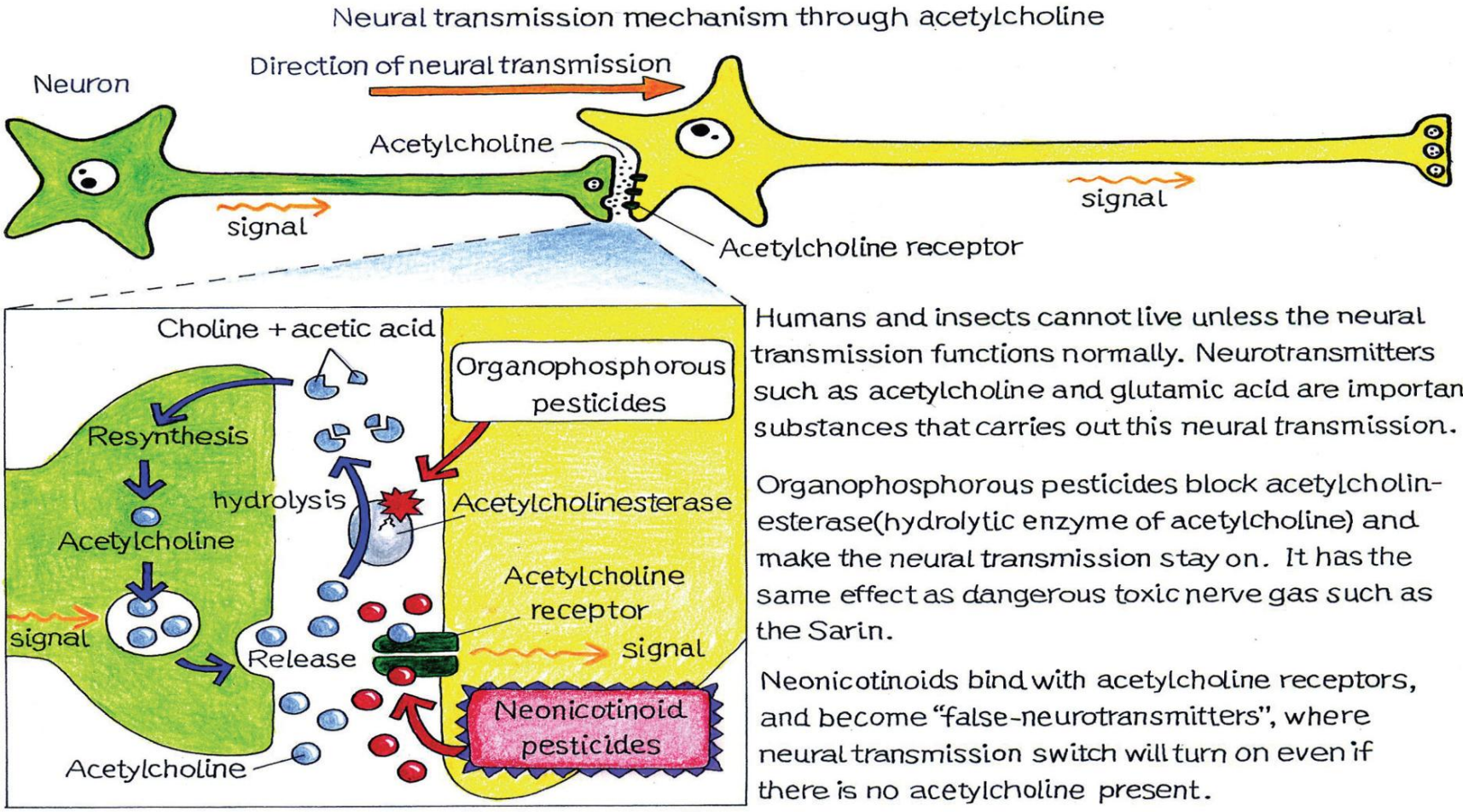


Bayer's Description of The Mode of Action of Neonicotinoids:

„Their Mode Of Action Derives From Virtually Irreversible Blockage Of Postsynaptic Nicotinic Acetylcholine Receptors"

Abbink, J. (1991) Pflanzenschutz-Nachrichten Bayer, Serial ID-ISSN 0340-1723C.

Neonicotinoid / Organophosphorous pesticides disrupt the neural transmission



Humans and insects cannot live unless the neural transmission functions normally. Neurotransmitters such as acetylcholine and glutamic acid are important substances that carries out this neural transmission.

Organophosphorous pesticides block acetylcholinesterase(hydrolytic enzyme of acetylcholine) and make the neural transmission stay on. It has the same effect as dangerous toxic nerve gas such as the Sarin.

Neonicotinoids bind with acetylcholine receptors, and become "false-neurotransmitters", where neural transmission switch will turn on even if there is no acetylcholine present.

The Druckrey-Küpfmüller Theorem: Irreversible Effects

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 imidacloprid concentration at the target site C over time:

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

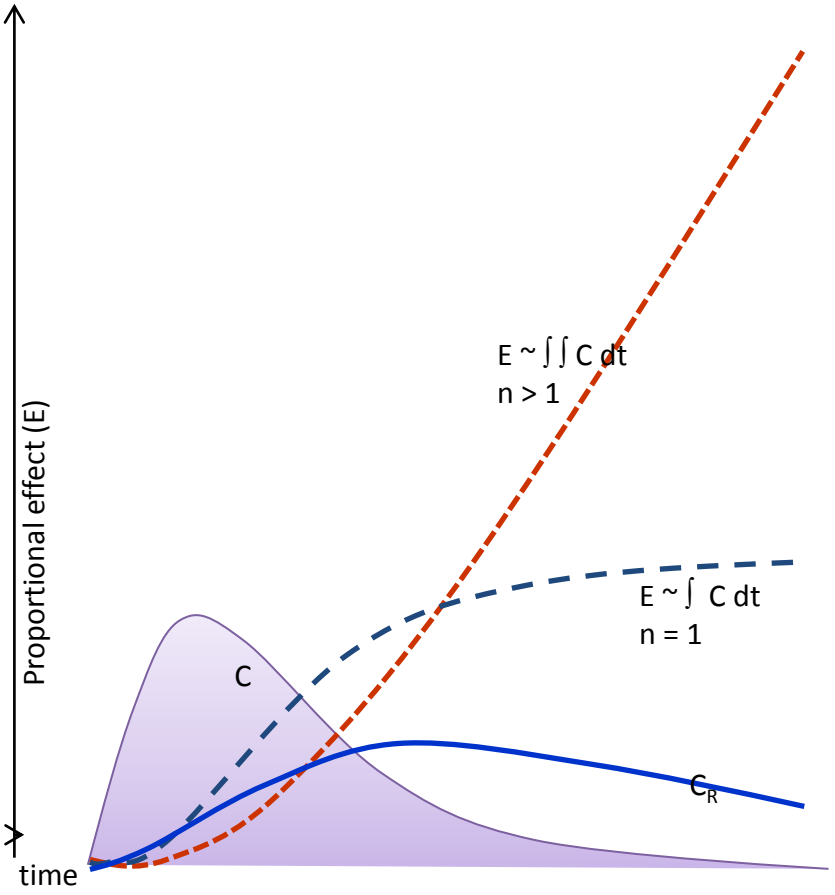
- If the subsequent effect would happen to be irreversible as well (e.g. perturbations of cognitive functions), the effect E would be proportional to the integral of the concentration of bound receptors C_R over time:

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

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

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

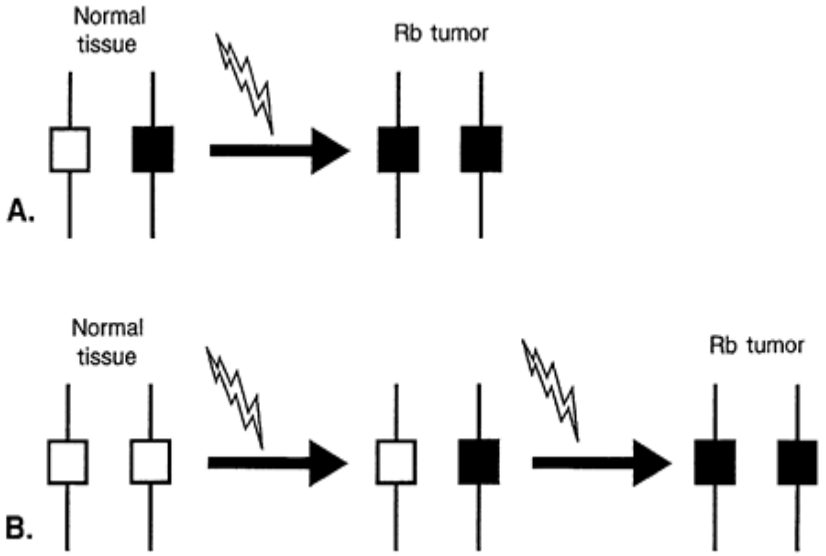
- This explains the imidacloprid dose : response relationship where exposure time reinforces the effect, as seen for genotoxic carcinogens



The Druckrey-Küpfmüller Equation C x T50 ^{n} = constant, with n ≥ 1
A Safe Dose Can Not Be Defined For Genotoxic Carcinogens

Knudson AG (1971) Mutation and cancer: statistical study of retinoblastoma Proc Natl Acad Sci U S A. 68(4):820-3

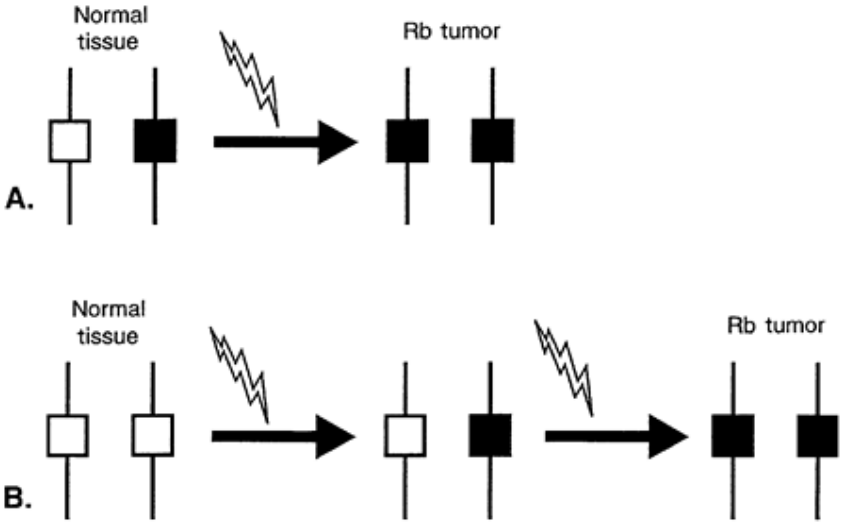
- "one hit" could cause a mutation and eventually result in cancer The retinoblastoma (Rb) protein is a tumor suppressor protein that is dysfunctional in many types of cancer
- Mutated Rb can be inherited. The mutated gene is recessive
- Should a cell sustain only one mutation in the other Rb gene all pRb proteins in that cell would be ineffective



Risk Assessment of Genotoxic Carcinogens

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

- 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



The Risk Of Imidacloprid For Honey Bees Is Underestimated

Suchail S, Guez D, Belzunces LP, 2001. Environ. Toxicol. Chem. 20: 2482-2486
Bonmatin JM et al., 2007. Environmental fate and ecological effects of pesticides. Pp. 827-834
Mullin CA et al, 2010. PloS One 5, e9754

- **Druckrey-Küpfmüller Equation**

  Ln T50 (hrs) = 5.11 – 0.22 Ln C (µg. L-1 or kg-1)

  or

  $$C \times T50^{4.5} = \text{constant}$$

- ***The concentrations of imidacloprid detected in nectar or pollen cause lethal effects in honey bees within a week***

Food Source	Imidacloprid Content C (µg/kg or ppb)	Expected Time to Effect (T50) (Days)
Nectar	1	**6.9**
	3	**5.4**
Pollen	0.7	**7.5**
	10	**4.2**

Current Toxicological Risk Assessment Can Lead To Serious Underestimates Of Actual Risk

Neonicotinoids Are A Case In Point

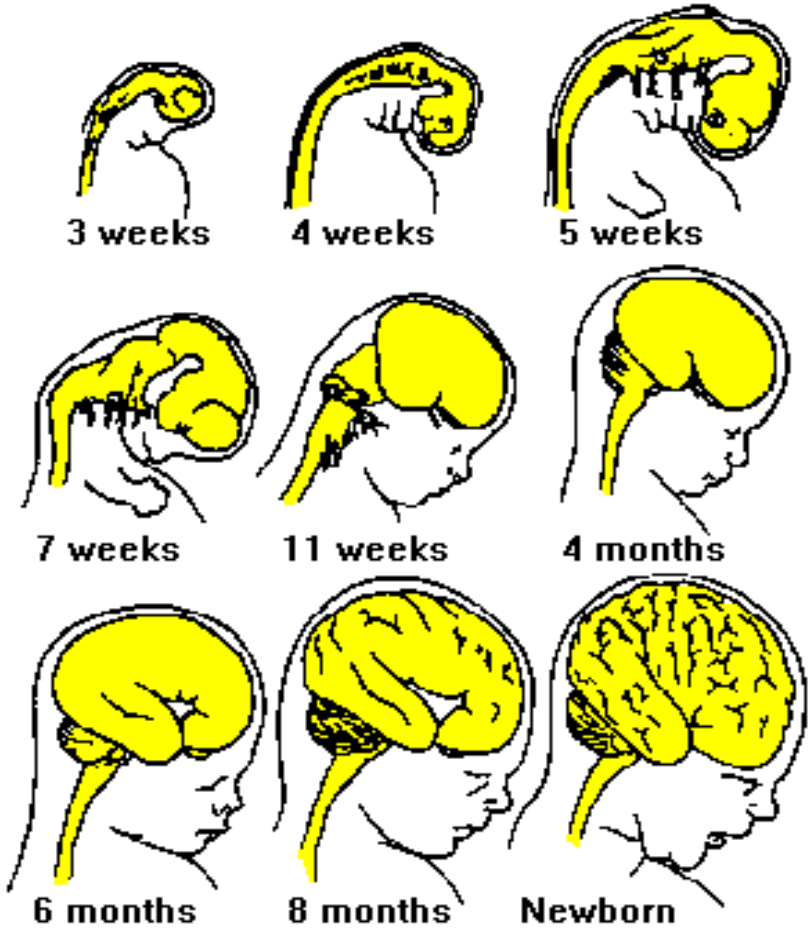
Tennekes HA, Sánchez-Bayo F (2011) J Environment Analytic Toxicol S4:001. doi:10.4172/2161-0525.S4-001

- The traditional approach to toxicity testing is to consider dose (concentration)-effect relationships at arbitrarily fixed exposure durations which are supposed to reflect 'acute' or 'chronic' time scales.
- This approach measures the proportion of all exposed individuals responding by the end of different exposure times.
- Toxicological databases established in this way are collections of endpoint values obtained at fixed times of exposure. As such these values cannot be linked to make predictions for the wide range of exposures encountered by humans or in the environment.
- 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 any level of effect on the organisms tested.
- Consequently, extrapolations and predictions of toxic effects for any combination of concentration and time are now made possible.

Nicotine Is A Neuroteratogen
Nicotine Alters The Developmental Trajectory Of The Brain

Eppolito AK et al. (2010) Neurotoxicology and Teratology 32 : 336–345
Dwyer JB et al. (2009) Pharmacol Ther. 122 : 125–139
Kimura-Kuroda J et al. (2012) PLoS ONE 7(2): e32432. doi:10.1371/journal.pone.0032432

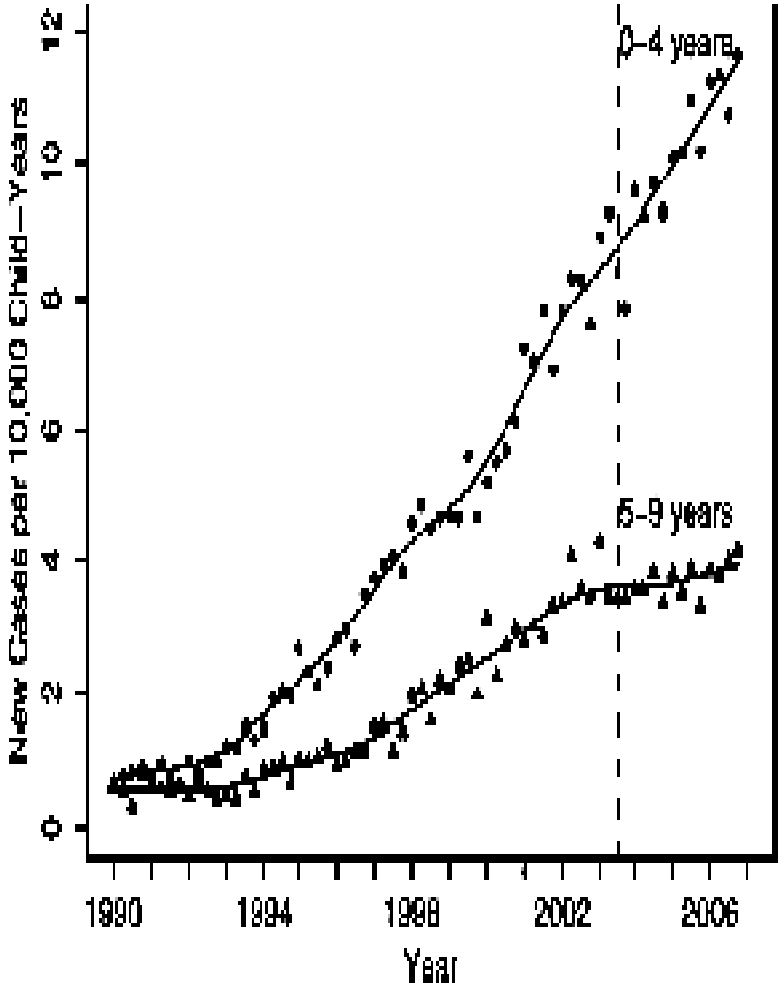
- Nicotinic acetylcholine receptors (nAChRs) regulate critical aspects of brain maturation during the prenatal, early postnatal, and adolescent periods
- Nicotine disrupts the normal developmental influences of acetylcholine
- Neonicotinoids as well as nicotine directly act on mammalian nAChRs and, therefore, may have various adverse effects on the human health, especially on the developing brain.



Nicotine Causes Many Adverse Effects On The Normal Development Of A Child

Kimura-Kuroda J et al. (2012) PLoS ONE 7(2): e32432. doi:10.1371/journal.pone.0032432

- Neonicotinoids as well as nicotine directly act on mammalian nAChRs which regulate critical aspects of brain maturation during the prenatal, early postnatal, and adolescent periods
- Perinatal exposure to nicotine is a known risk factor for sudden infant death syndrome, low-birth-weight infants, attention deficit/hyperactivity disorder (ADHD), autism
- The Graph on the Right →: The rise of autism in California since the introduction of the neonicotinoid insecticides in the early 1990s



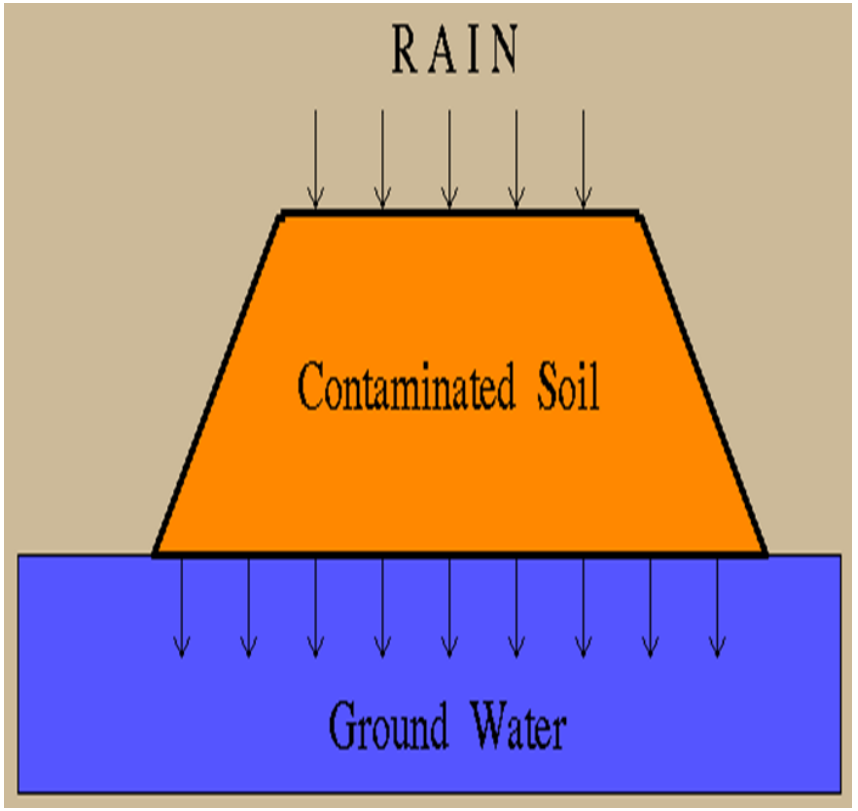
A Generation in Jeopardy

How pesticides are undermining our children's health & intelligence

PESTICIDE ACTION NETWORK NORTH AMERICA , OCTOBER 2012

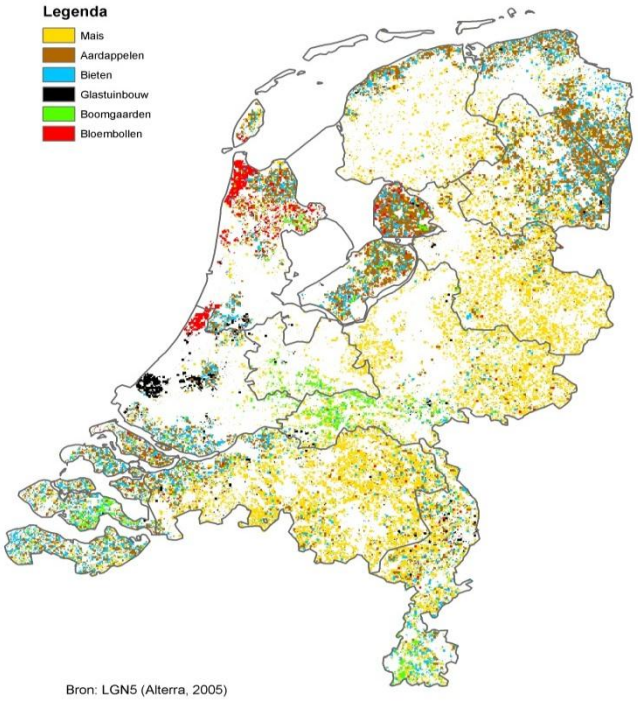
Neonicotinoids May Be Washed Out Of The Soil Into Waterways and Groundwater

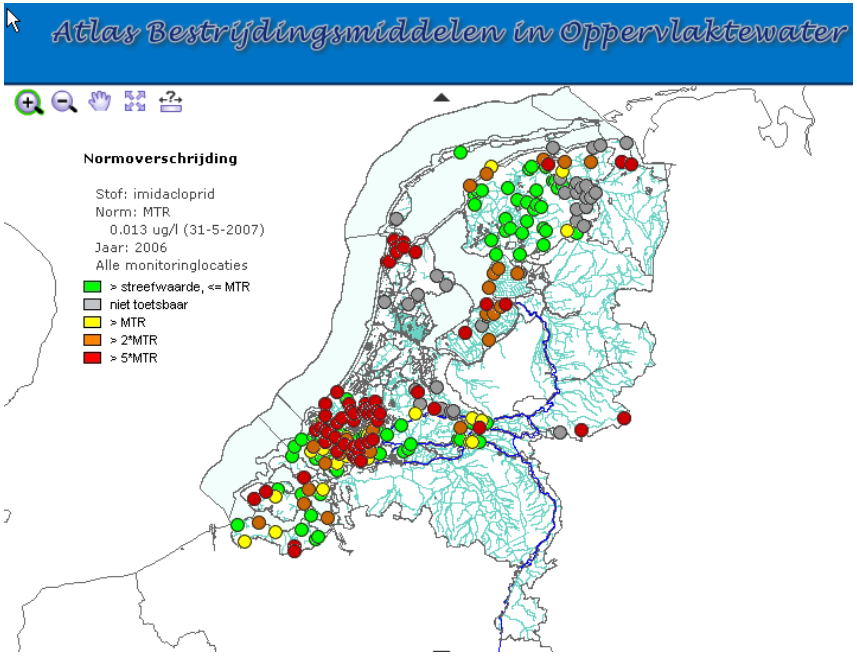
- Not only are neonicotinoids water soluble and mobile in soil, they are also quite persistent in soil and water.



The Widely Used Neonicotinoid Insecticide Imidacloprid Has Caused Major Contamination Of Dutch Surface Water Since 2004

Red dots (right hand side) : Maximum Permissible Risk Level (MRL) Exceeded At Least Five Times
In 2005, MRL Exceeded 25,000 Times at Noordwijkerhout (Flower Bulb Cultivation Area)

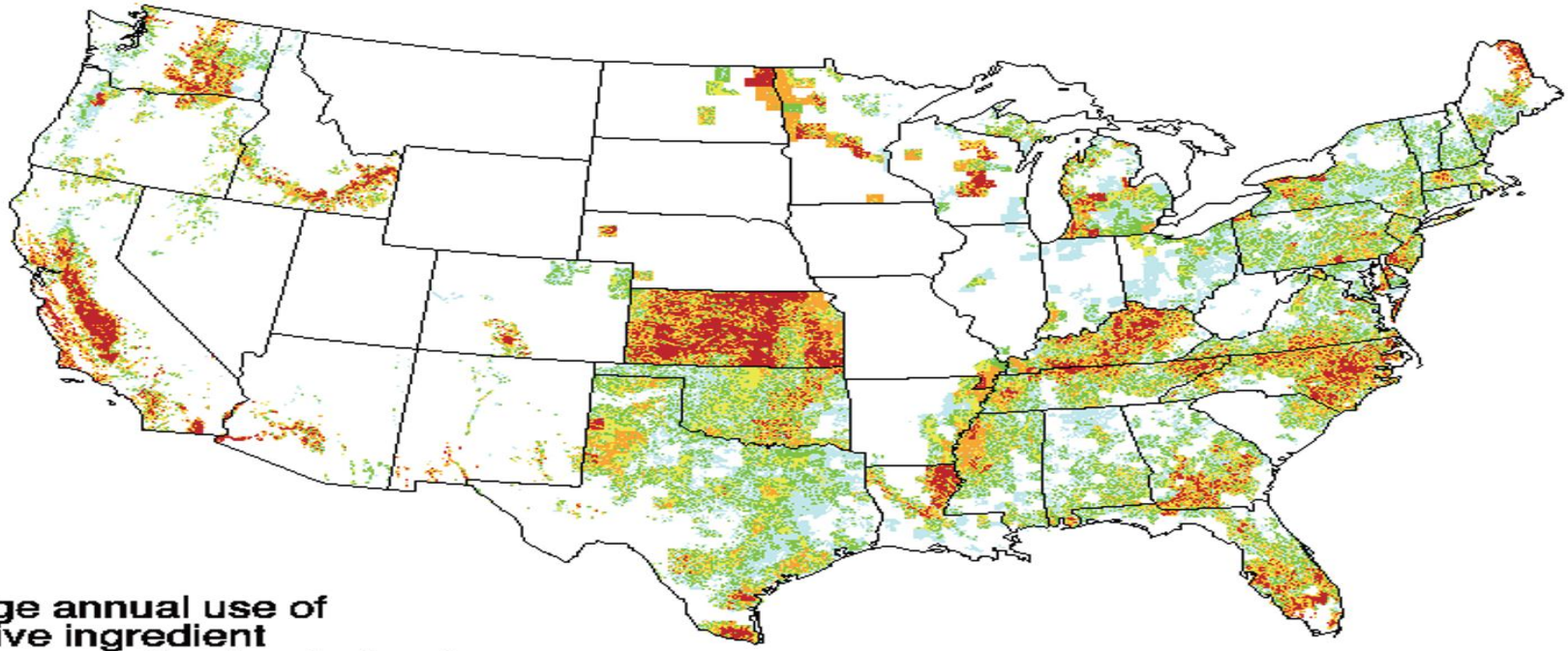




Imidacloprid

Estimated Annual Agricultural Use In The US In 2002

US Geological Survey National Water-Quality (NAWQA) Program



verage annual use of
active ingredient
per square mile of agricultural
land in county)

- no estimated use
- 0.001 to 0.004
- 0.005 to 0.015
- 0.016 to 0.053
- 0.054 to 0.202
- >= 0.203

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

Imidacloprid Contaminates Surface Waters in Agricultural Regions of California

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%).

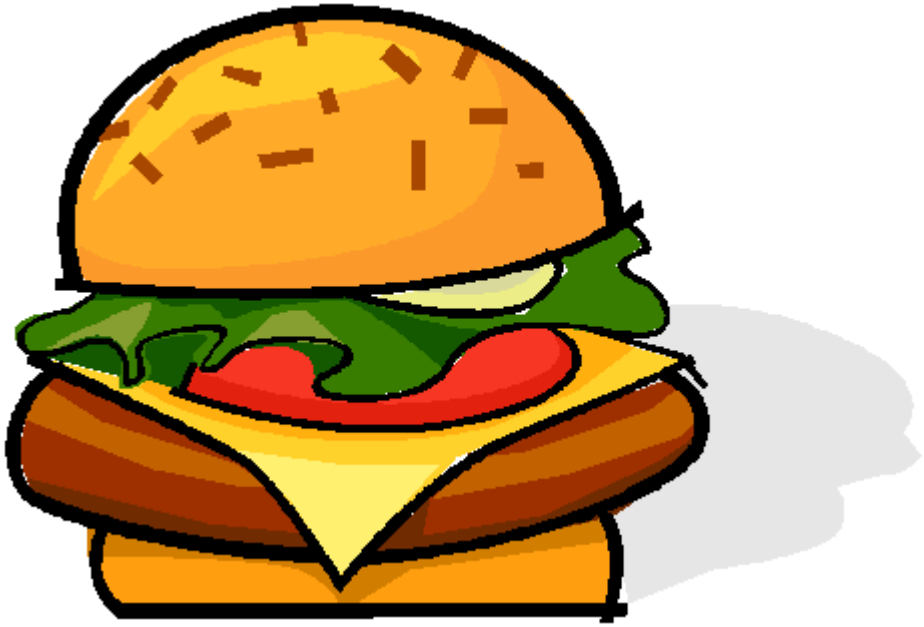
Neonicotinoids Are Diffusing Through The Environment
Are They Breaking The Food Chain?

- Imidacloprid is diffusing through the environment in California and the Netherlands, killing or debilitating non-target insects and possibly other arthropods, and by doing so progressively reducing invertebrate prey for higher organisms

From Big Mac to McBun

May Berenbaum (Entomologist, University of Illinois) NZZ Folio 07/01 - Theme: Käfer und Co

- A McDonald's Big Mac burger in an insect-free world would have no meat, no lettuce, no cheese, no pickle, no onion, and no ketchup; basically, it would be a McBun.



Our Fiber Needs Are Met In Large Part As A Result Of Insect Activity

May Berenbaum (Entomologist, University of Illinois) NZZ Folio 07/01 - Theme: Käfer und Co

- The **cotton** plant is insect – pollinated

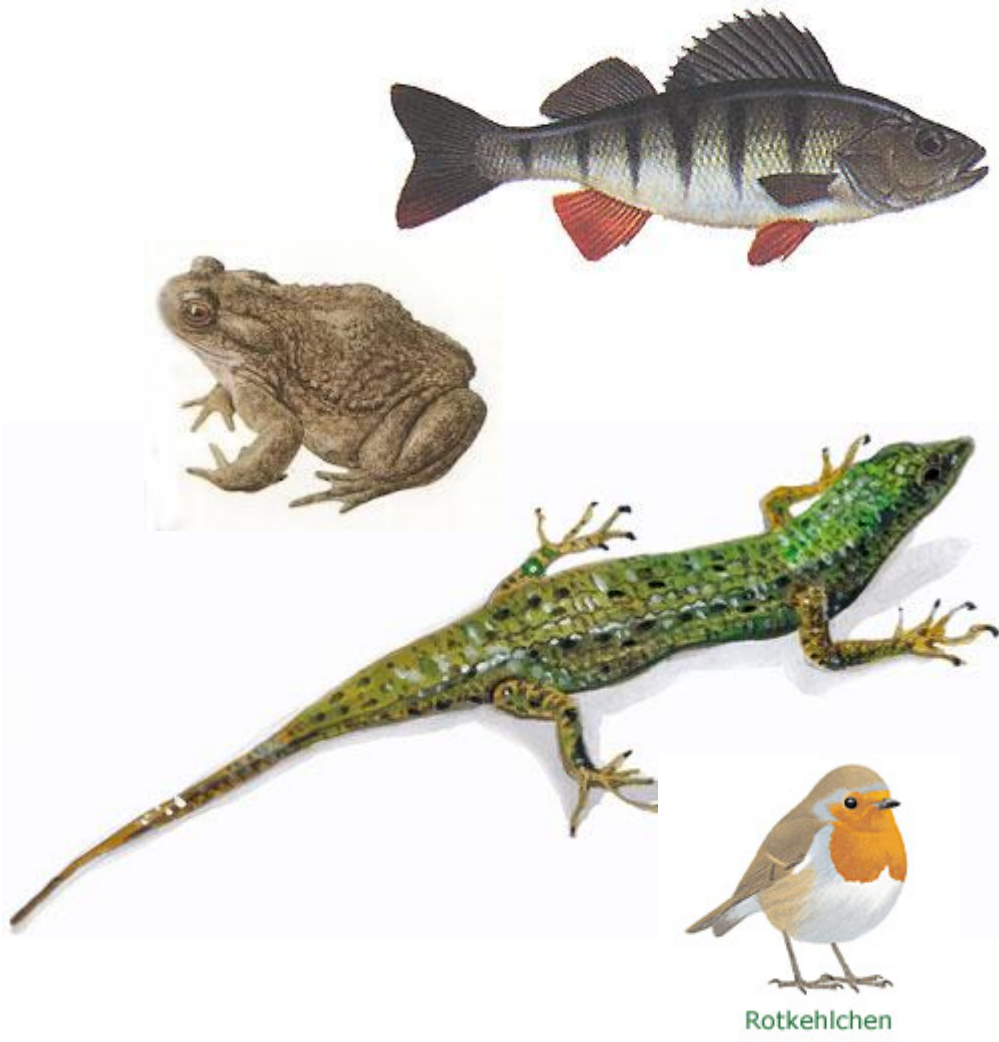
- **Wool** and **leather** come for the most part from sheep and cattle that have eaten insect-pollinated legumes in their diet.

- **Silk**, of course, is a natural fiber produced directly by an *insect—Bombyx mori*, the Japanese silkworm

Most Vertebrates Rely Heavily On Insects In Their Diet

May Berenbaum (Entomologist, University of Illinois) NZZ Folio 07/01 - Theme: Käfer und Co

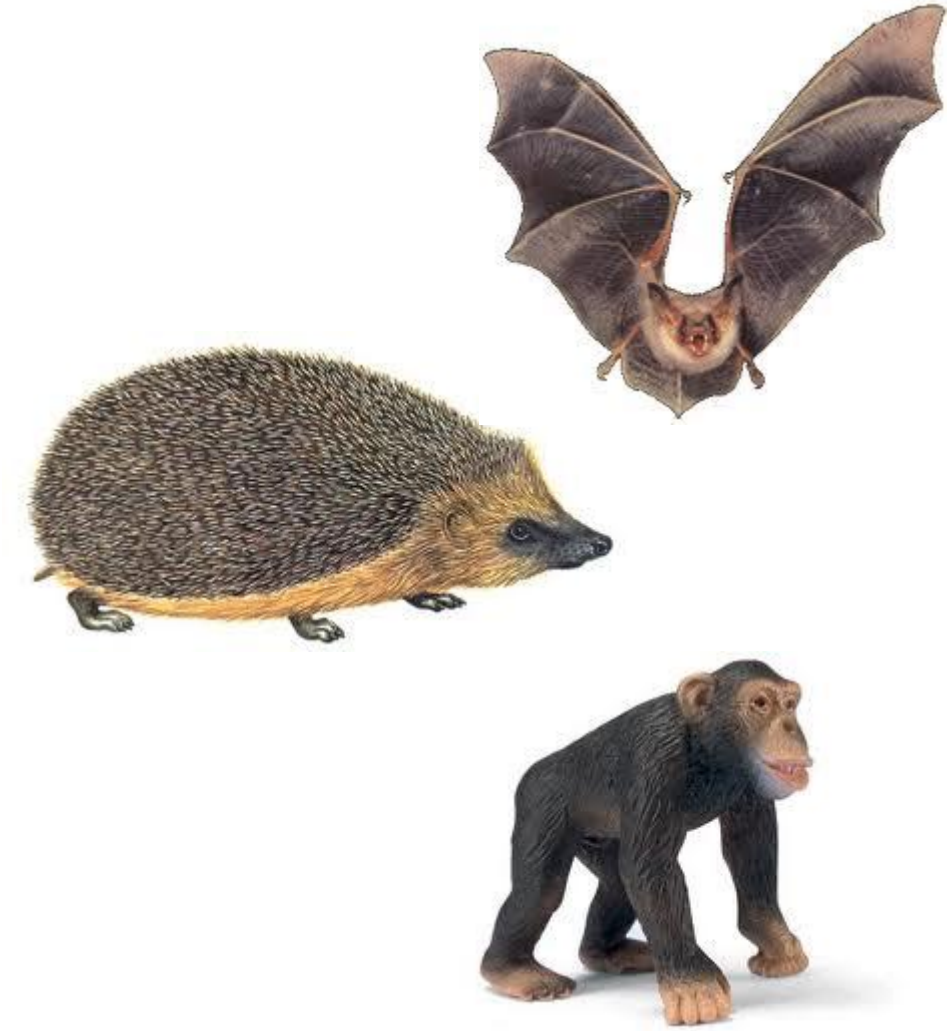
- Approximately 40 to 90% of the diet of **freshwater fish** consists of insects
- Among the **amphibians**, frogs, toads, and salamanders depend on insects; about 75% of the diet of the common toad is made up of insects
- Among the **reptiles**, insects are the food of choice for lizards, chameleons, green glass snakes, and horned toads.
- About one-third of the diet of **game birds and songbirds** are insects and their relatives



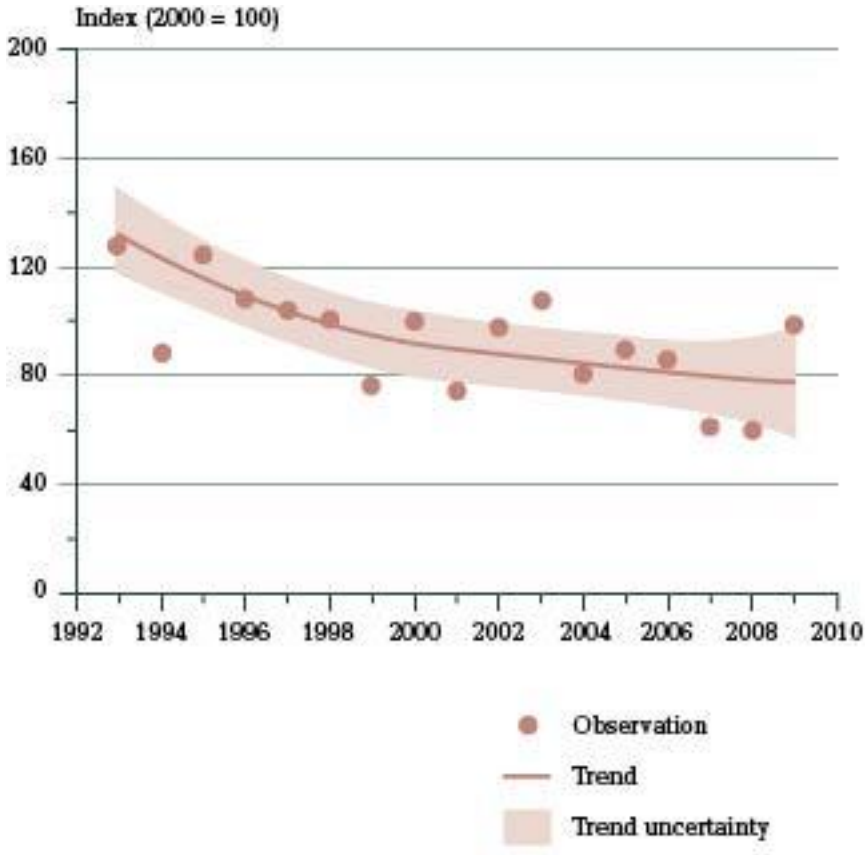
Most Orders Of Mammals Contain Insect-Eating Species

May Berenbaum (Entomologist, University of Illinois) NZZ Folio 07/01 - Theme: Käfer und Co

- spiny anteaters, duck-billed platypuses, opossums, cuscuses, caenolestid rat opossums, bandicoots, marsupial moles, hedgehogs, moles, tenrecs, solenodons, shrews, most bats, anteaters, armadillos, pangolins, some mice, and raccoons all consume insects on a regular basis.
- Even among the primates, our closest relatives, insect-eating is the norm; lemurs, aye-ayes, lorises, tarsiers, marmosets, and several of the great apes are to various degrees entomophagous.
- Both gorillas and chimpanzees fashion sticks into tools to help them extract termites and ants from their nests.



The Number Of Butterflies In The Netherlands Is Presently At The Lowest Point Ever Recorded

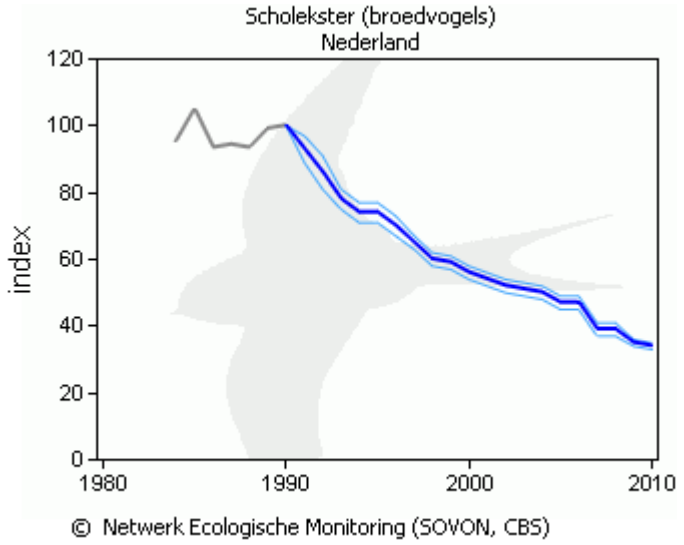


Source: NEM (Vlinderstichting, CBS)

CBS/jul09/1386
www.compendiumvoordeleefomgeving.nl

The Decline of Grassland Birds In The Netherlands

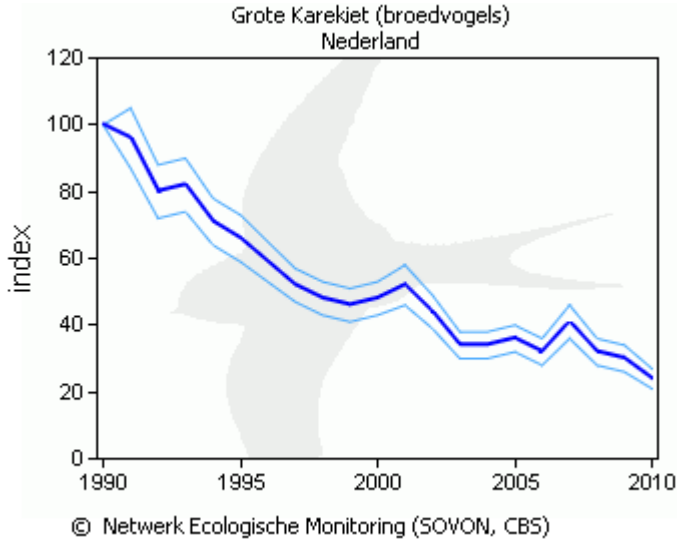
Oystercatcher



- Sharp decline observed in Germany as well

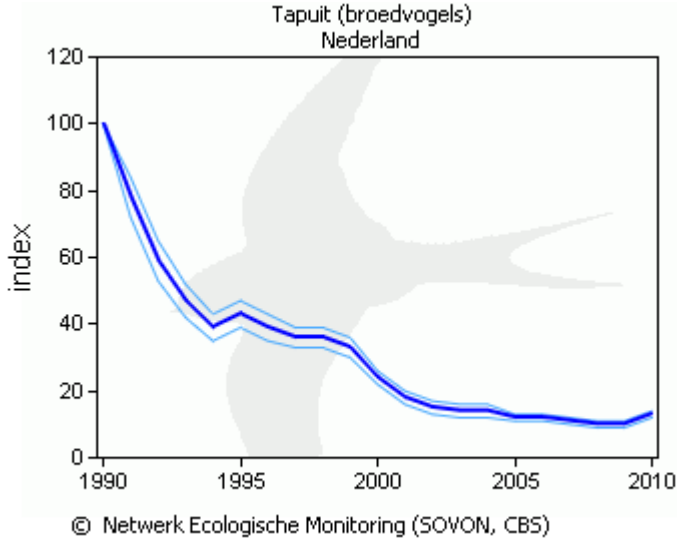
The Decline of Marsh Birds In The Netherlands

Great Reed Warbler



The Decline of Heath Land Birds In The Netherlands

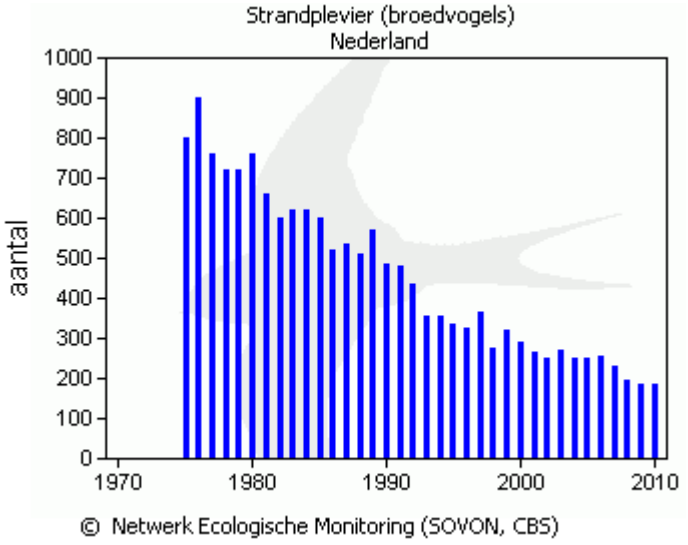
Northern Wheatear



- **On the brink of extinction in Germany**

The Decline of Coast Birds In The Netherlands

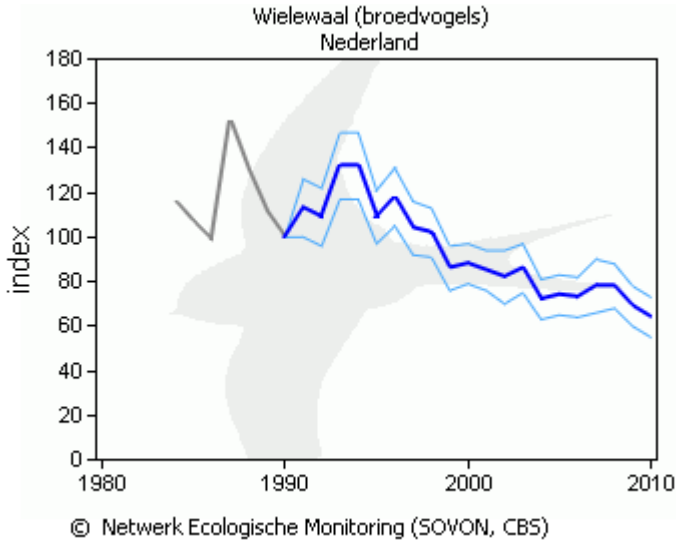
Kentish Plover



- **On the brink of extinction in Germany**

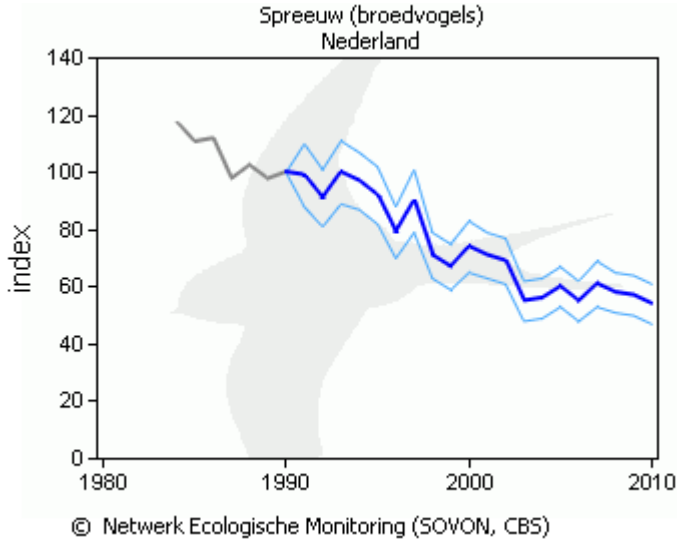
The Decline of Woodland Birds In The Netherlands

Golden-Oriole



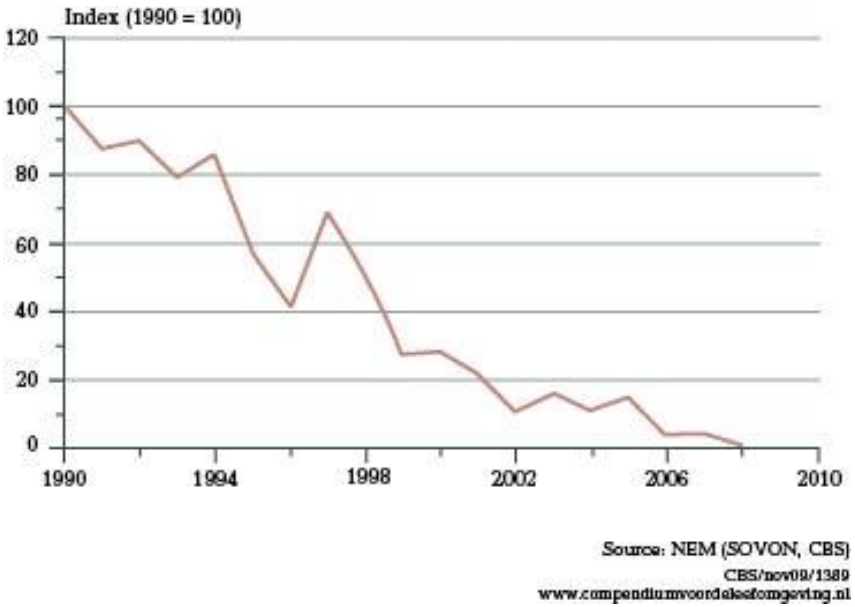
The Decline of Settlement Birds In The Netherlands

Starling



The Decline of Farmland Birds In The Netherlands

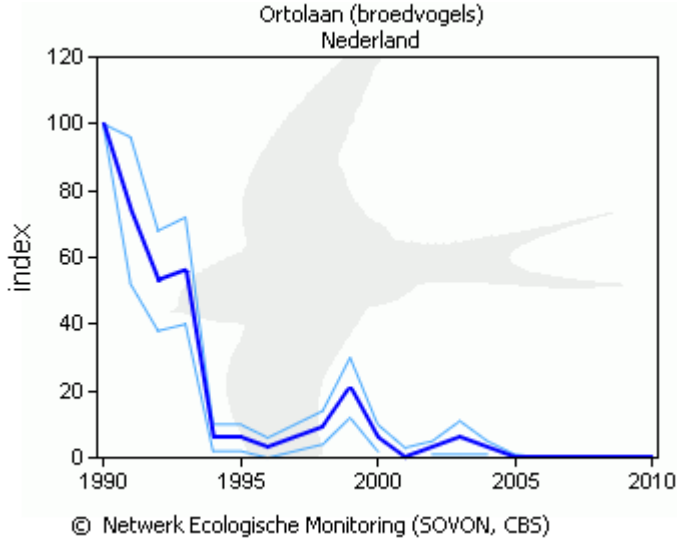
Corn Bunting



- **Threathened in Germany**

The Decline of Farmland Birds In The Netherlands

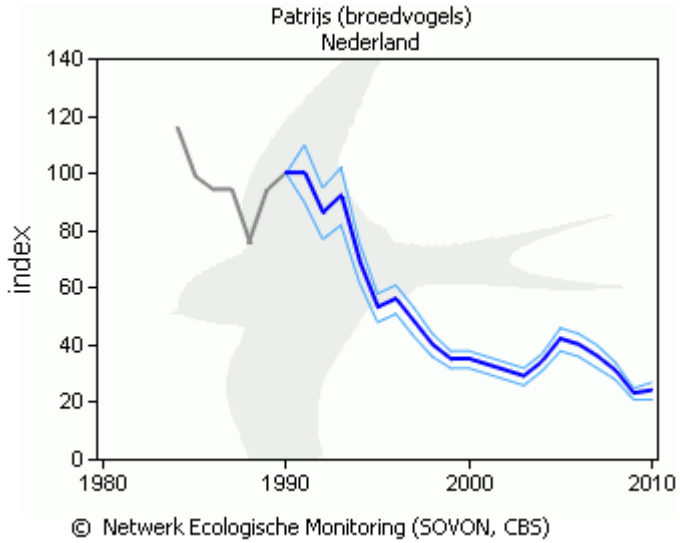
Ortolan Bunting



- **Threathened In Germany**

The Decline of Farmland Birds In The Netherlands

Grey Partridge



- **Threathened in Germany**

Immune Suppression by Neonicotinoid Insecticides At The Root Of Global Wildlife Declines

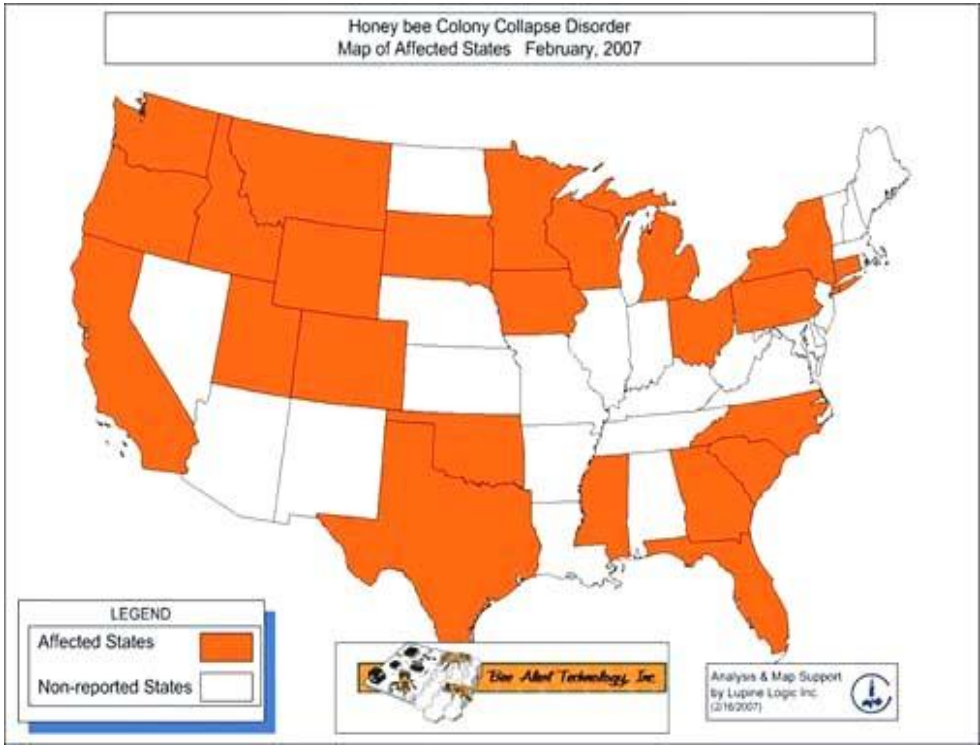
R Mason, H Tennekes, F Sánchez-Bayo, P Uhd Jepsen. Journal of Environmental Immunology and Toxicology 2012; X:XX-XX (in press)

- There is experimental evidence of immune suppression in bees and fish by neonicotinoids
- There have been outbreaks of infectious diseases in honey bees, bumble bees, fish, amphibians, bats and birds in the past two decades
- The disease outbreaks started in countries and regions where neonicotinoid insecticides were used for the first time, and later they spread to other countries

Honey Bee Declines in the US and Europe Are Linked To Immune Suppression and Infections

Cédric Alaux et al. Environ Microbiol. (2010) 12(3): 774–782
Pettis, JS et al. (2012) Naturwissenschaften DOI 10.1007/s00114-011-0881-1

- Neonicotinoids are weakening the insects' immune systems, and thus allowing infections to spread through a hive
- One thing common to bee colonies that go on to collapse is a greater variety and higher load of parasites and pathogens than other colonies



The Massive Bumble Bee Declines in the US and Europe Are Linked To Infections

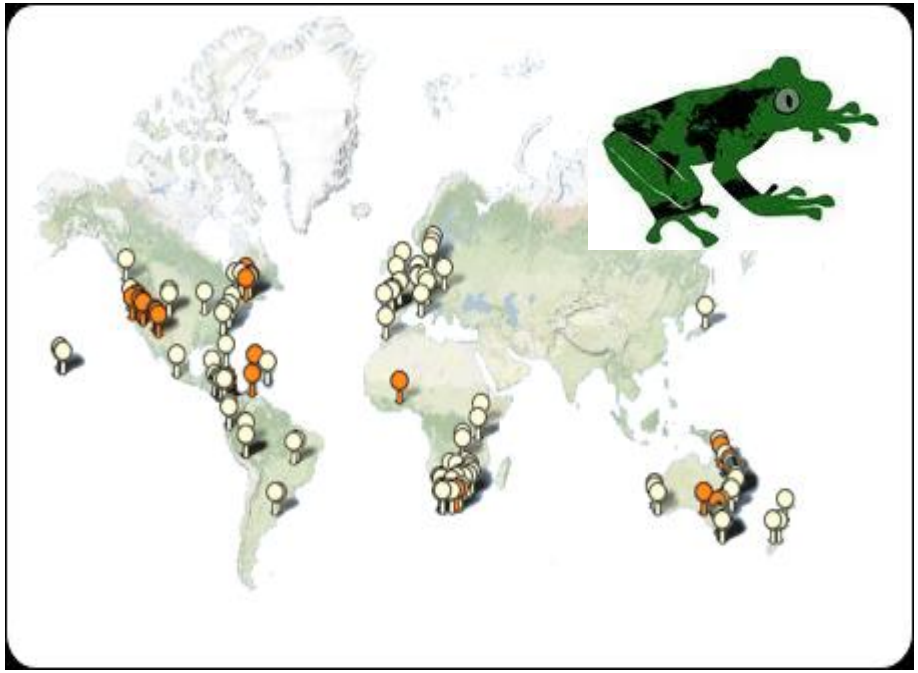
Cameron, S.A. et al. (2011) Proc. Natl Acad. Sci. USA 108, 662-667

- Comparing results with museum records of bumble bees showed that the relative abundances of four species had declined historically by up to 96%. Geographical ranges had contracted by 23-87%, some within the past two decades.
- Those species that had declined had significantly higher infection levels of the pathogen ***Nosema bombi***
- Exposure to neonicotinoid insecticides is likely to have occurred and may have weakened immune systems, such that they became more susceptible to pathogens

The Massive Declines in Amphibian Populations Are Linked To Infections
The Chytrid Fungus Is Devastating Frog Populations

Symposium held at the Zoological Society of London: 20/21 November 2008. Halting the global declines in amphibians. Research & Practice

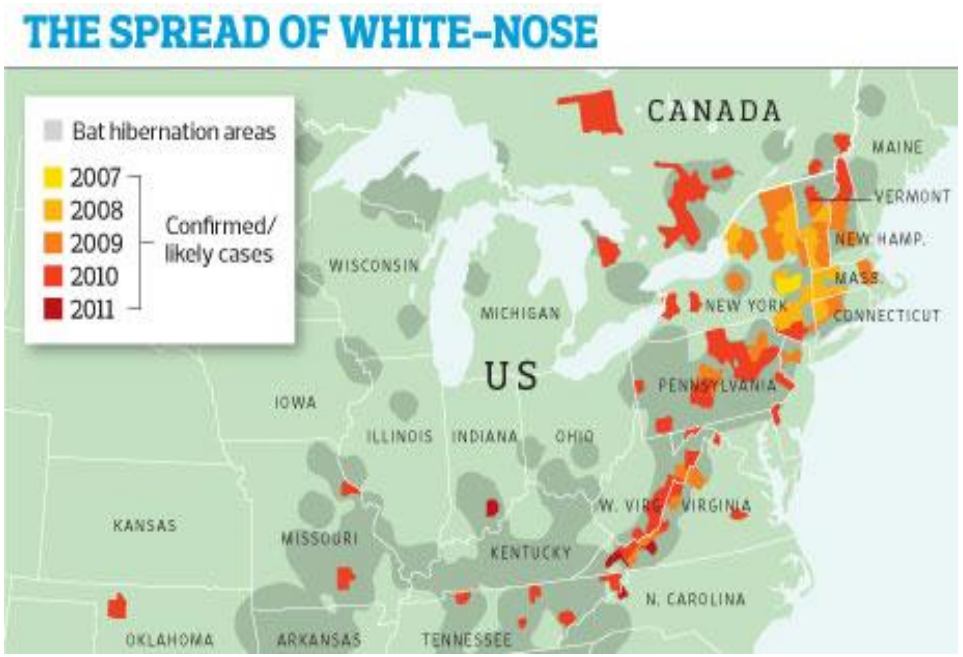
- Two species of once common frogs that had inhabited the thousands of lakes and ponds in California's Sierra Nevada are being wiped out by ***chytridiomycosis***
- Exposure to small doses of neonicotinoid insecticides is likely to have occurred and may have weakened the amphibian immune systems, such that they became more susceptible to pathogens



The Massive Decline Of Bat Populations Is Linked To Infections

www.fws.gov/whitenosesyndrome

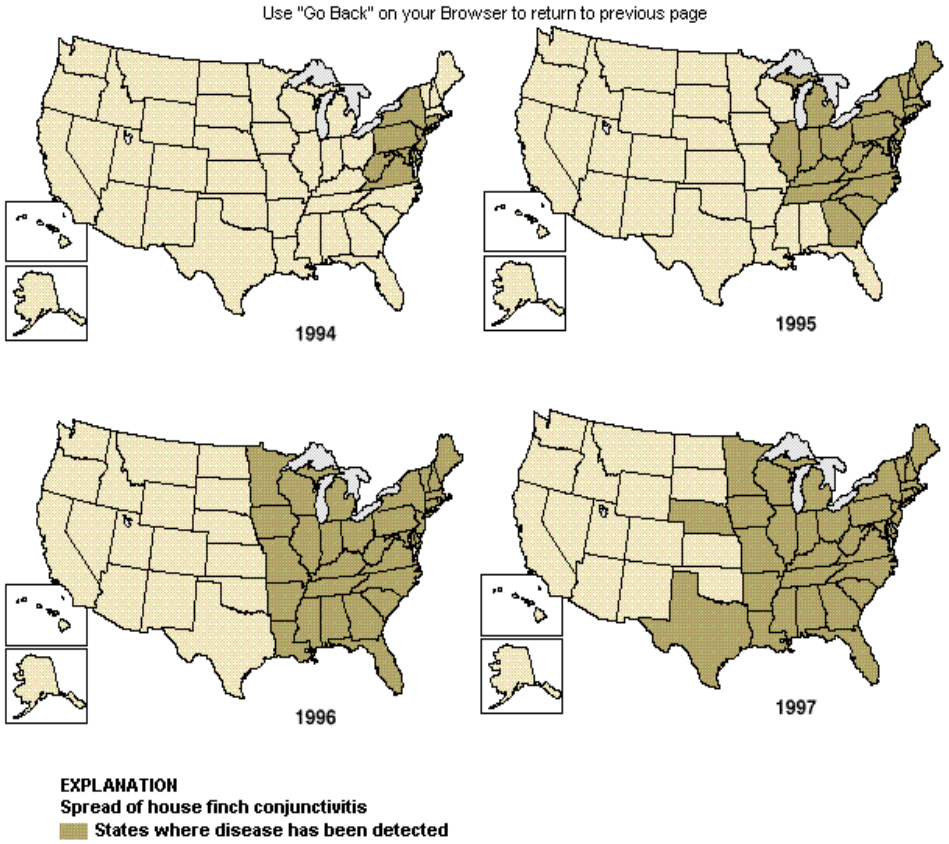
- A powdery white nose tip was pathognomonic of the disease and when the powder was cultured a fungus, ***Geomyces destructans*** was grown. This infected the skin and wing membranes of bats and was associated with unprecedented numbers of deaths
- It was first found in a cave in New York State in the 2005/6 winter and rapidly spread through the north-eastern states
- the thousands of invertebrates consumed in their diet will inevitably have exposed bats to small cumulative doses of neonicotinoids



Declines Due To Pathogens In Birds In the US

Fischer JR, Stallknecht DE, Luttrell P, et al. Emerg Infect Dis 1997; 3(1):69-72.

- A mycoplasmal conjunctivitis was first reported in wild house finches (*Carpodacus mexicanus*) in February 1994 in suburban Washington, DC.
- It was identified as ***Mycoplasma gallisepticum***, a pathogen of poultry that had not previously been associated with wild songbirds.
- In the first three years it killed an estimated 225 million finches. There was a dramatic spread of disease to house finches in the mid-West and South East

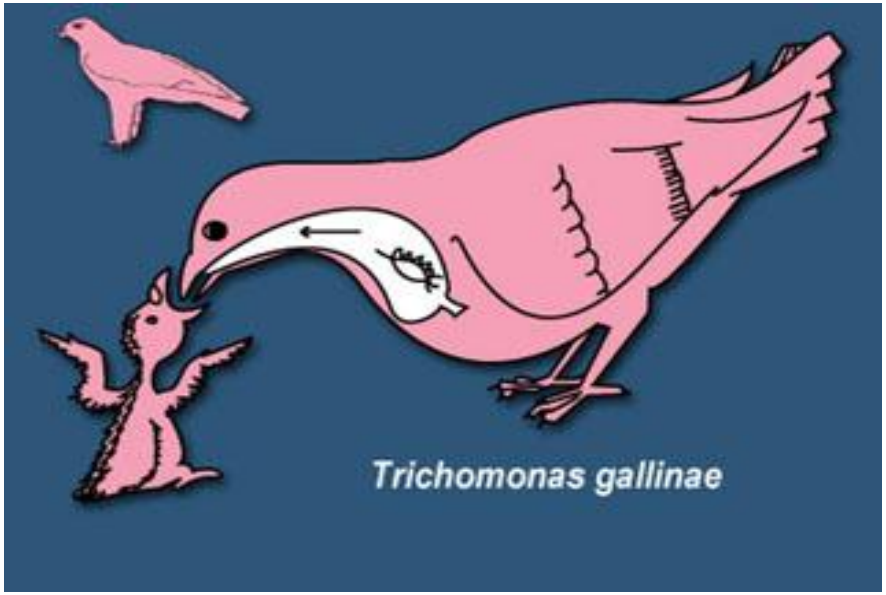


Reported geographic spread of house finch inner eyelid inflammation (conjunctivitis) since the initial 1994 observation. (Data adapted from reports in the scientific literature and personal communications between the National Wildlife Health Center and other scientists.)

Declines Due To Pathogens In Birds In Europe

Robinson RA, Lawson B, Toms MP, et al. PLoS One 2010; 5(8):e12215.
Lawson B, Malnick H, Pennycott TW, et al. Vet J 2011; 188(1):96-100

- In Europe epidemics caused by a variety of novel pathogens in wild birds began in early 2000
- Greenfinch (*Carduelis chloris*) numbers in Europe have been devastated by infections with ***Trichomonas gallinae***, a protozoal organism which invades the bird's crop and mucosal lining of the beak. Deaths started in the UK around 2005
- At the same time, chaffinches (*Fringilla coelebs*) appeared in gardens with white, crusty growths on their legs and feet caused by a ***papilloma virus***. The mortality is said to be about 20%, so the disease kills more slowly than with the Greenfinch *Trichomonas* infections



Declines Due To Pathogens In Birds In Europe

Friedrich-Loeffler-Institute. Federal Research Institute for Animal Health. News 16/09/2011

- In September 2011, mass deaths of Blackbirds (Turdus merula) were reported in the Rhine-Neckar area of Germany
- The Bernhard-Noct Institute for Tropical Diseases and the Friedrich-Loeffler Institute examined four birds and confirmed that it was the tropical **Usutu Virus** from Africa
- It was first seen in Austria in 2001, followed by reports from Italy, Hungary and Switzerland. In birds it first causes apathy, then signs of a central nervous system disorder, with unnatural movements of the head
- An estimated 300,000 blackbirds were killed by the disease

Summary

- Neonicotinoid insecticides act by causing *virtually irreversible* blockage of postsynaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects.
- The damage is cumulative, and with every exposure more receptors are blocked. In fact, there may not be a safe level of exposure.
- The nAChRs play roles in many cognitive processes and neonicotinoids account for worker bees neglecting to provide food for eggs and larvae, and for a breakdown of the bees' navigational abilities. Very small quantities of neonicotinoid insecticides are sufficient to cause collapse of bee colonies
- Food residues of neonicotinoids may adversely affect human health, especially the developing brain
- Neonicotinoid insecticides are persistent and mobile in soil, soluble in water and stable to breakdown by water at neutral pH, and - as a result of these properties - the compounds may leach from soils.
- Major contamination of surface water with imidacloprid has been detected in the Netherlands and in California
- Consequently, high concentrations of imidacloprid have been diffusing through the environment, killing or debilitating non-target insects and other arthropods, decimating invertebrate prey for higher organisms. Moreover, imidacloprid causes immune suppression in bees and freshwater fish and may be at the root of global wildlife declines
- Breaking one link on the food chain means that all of the higher organisms above that link are in threat of extinction

Conclusion

A Disaster In The Making - Neonicotinoids Break Food Chains

- **Invertebrate-dependent bird species in the Netherlands have been declining on a massive scale in recent times, in all kinds of habitats** (grasslands, marshes, heathlands, at the coast, woodlands, settlements, farmlands):
- *Skylark, Yellow Wagtail, Oystercatcher, Black-tailed Godwit, Northern Lapwing, Common Redshank, Meadow Pipit, Willow Tit, Spotted Flycatcher, Wood Warbler, Pied Flycatcher, Wood Nuthatch, Willow Warbler, Marsh Tit, Grey-faced Woodpecker, Wryneck, Common Crossbill, Golden-Oriole, Northern House Martin, Barn Swallow, Common Swift, Starling, House Sparrow, Common Redstart, Great Reed Warbler, Bearded Tit and Spotted Crake*

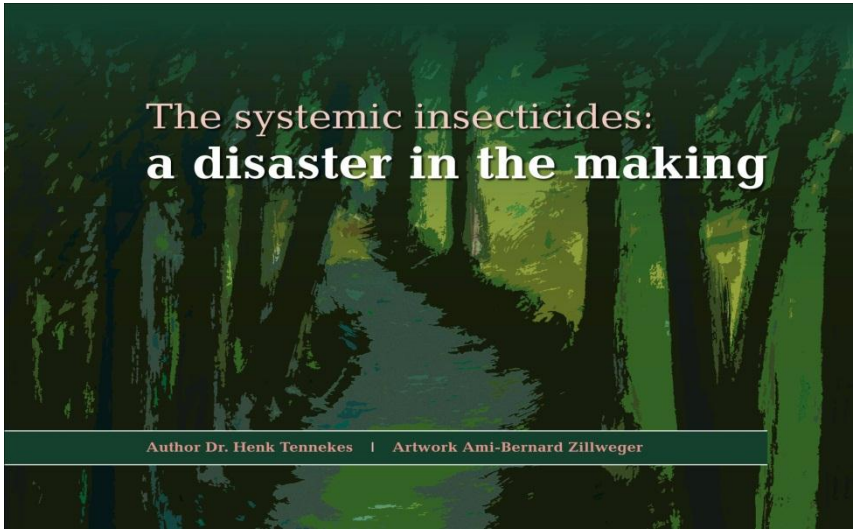
- Ground and surface water contamination with persistent insecticides that cause irreversible and cumulative damage to aquatic and terrestrial (non-target) insects must lead to an environmental catastrophe.
- The data presented here show that **an environmental catastrophe is actually taking place before our eyes**, and that

IT MUST BE STOPPED

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

Rachel Carson

- Realising the dire consequences of environmental pollution with neonicotinoid insecticides, Henk Tennekes decided to write 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

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