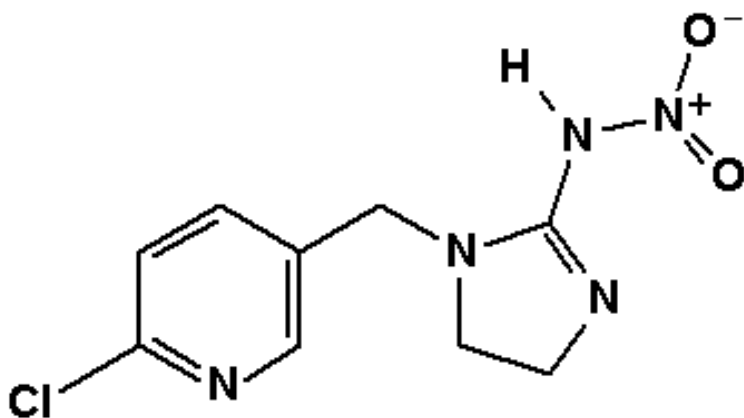


## Risk profile of neonicotinoid insecticides for arthropods



Presentation to the committee members of the  
Dutch Board for the Authorisation of Plant Protection Products and  
Biocides (Ctgb)  
at the Bee House in Wageningen on 25 May 2011

# The Discovery of Luc Belzunces

S. Suchail, D. Guez, and L. P. Belzunces (2001) Environ Toxicol Chem. 20:2482-6



- In 2001, Luc Belzunces, a bee researcher at INRA (the French agricultural institute) in Avignon, found an acute lethal dose of imidacloprid of only 40 ng per bee, which was much lower than most other insecticides.
- However, his greatest discovery was that the lethal dose from chronic exposure to imidacloprid was 4,000 times less.
- “Ingesting 1 pg per day was enough to kill a bee within 10 days” he told INRA magazine (June 2009). “Moreover, imidacloprid degrades into 6 metabolites, some of which are even more toxic.”
- He said that the capacity to measure very small traces of imidacloprid in pollen now shows that the concentration is in the range of microgrammes per kg of pollen and that this constitutes a risk for bees.

## Systemic application of insecticides causes contamination in pollen and nectar

- Systemic insecticides occur in small quantities (in the order of 1–10  $\mu\text{g}$  insecticide per kg) in pollen and nectar of plants consummated by honeybees (Rortais et al. 2005), such as  
  
sunflowers (Bonmatin et al. 2003; Schmuck et al. 2001),  
  
oil seed rape (Bonmatin et al. 2005)  
  
lacy phacelia (Wallner et al. 1999 cited in Decourtye et al. 2003)



# Systemic insecticides contaminate pollen and nectar which causes bee colonies to be chronically exposed to these materials

J. Kievits & Gh. De Roeck (2008) Maandblad van de Vlaamse Imkersbond:  
(May 2008 Monthly Bulletin from the Flemish Beekeepers' Federation)

- Contaminated pollen and nectar causes brood to be continuously and repeatedly exposed to systemic insecticides.

Contact with this compound is perpetuated during the time that bee larvae are fed or when bees consume contaminated honey from their stock.



# Harmful effects of bees' or a hive's exposure to systemic insecticides

- Lethal effect: exposed bees present increased mortality as opposed to non-exposed bees
- Sub-lethal effect: reduced performance of exposed bees (bee colonies) with regards to their development, fertility, life expectancy or behaviour. (Desneux et al. 2007).





## Risk Analysis of Clothianidin (02-11-2010)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Joseph DeCant, Ecologist & Michael Barrett, Chemist

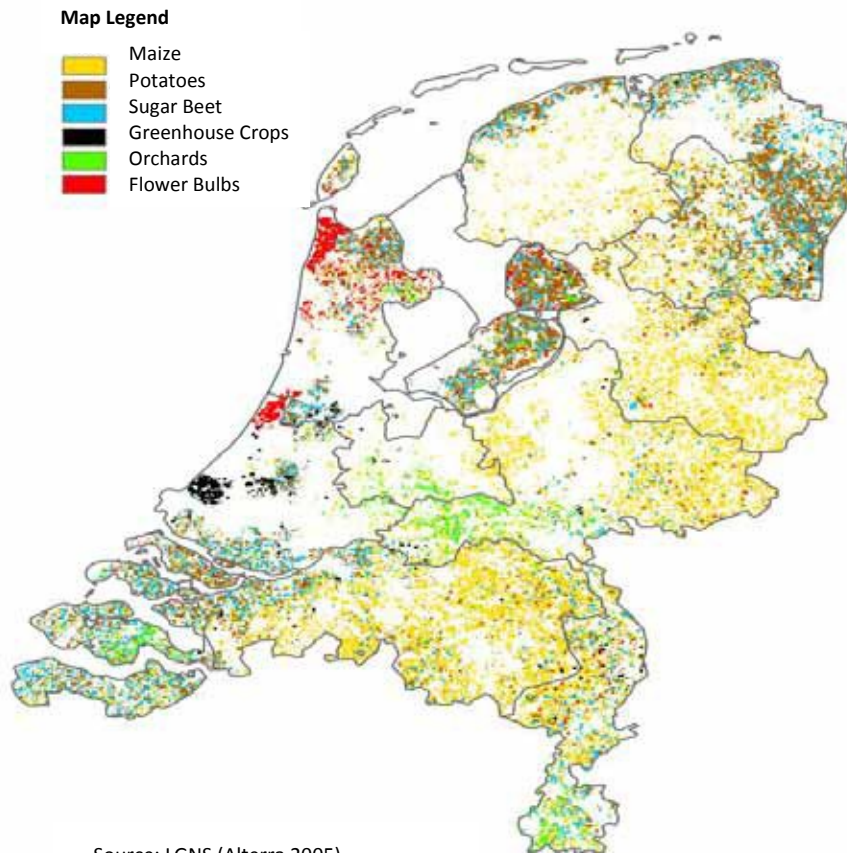
Environmental Risk Branch V, Environmental Fate and Effects Division (EFED)

- This compound is toxic to honey bees. The persistence of residues and potential residual toxicity of Clothianidin in nectar and pollen suggests the possibility of chronic toxic risk to honey bee larvae and the eventual instability of the hive.
- Clothianidin has the properties of a chemical which could lead to widespread groundwater contamination. The use of this chemical in areas where soils are permeable, particularly where the water table is shallow, may result in ground water contamination



# Application of Imidacloprid in The Netherlands causes Surface Water Pollution

Map courtesy of Planbureau voor de Leefomgeving (Planning Bureau for the Environment).



Source: LGNS (Alterra 2005)

- In 2005 and in 2006, the Rhineland measured pesticides in surface waters within their arable farming areas. The insecticide imidacloprid was considered one of the three problem substances in arable areas.
- In 2009, Rivierenland published its findings from the monitoring of plant resources in surface waters taken from agricultural sample points in 2008. Fruit was measured to contain the highest concentration of imidacloprid at 125 x above the norm.
- In 2008, 40% of all measurements of imidacloprid in surface waters of bulb cultivation areas were found to have an excess – by far the highest percentage since the beginning of these measurements in 2001.
- Delfland has a monitoring network that focuses on the glasshouse horticultural area. The results for 2005-2009 show that the concentrations of plant protection products are very high and considerably exceed the standards. The main problem substances in this framework are the insecticides imidacloprid, pirimicarb and pirimifos-methyl and the fungicide carbendazim.
- In 2005 and 2006, the Rhineland measured the pesticides in surface water on a monthly basis for the whole year on 5 orchard sites. The highest concentration of imidacloprid was in 2005 when it was 900x above the norm. In 2006, it was 400x above the norm.

# Toxicity of neonicotinoid insecticides for arthropods

## Popular Species - Info, Specimens & More



Arachnids



Beetles



Butterflies



Cicada



Dragonflies



Grasshoppers



Walking Sticks

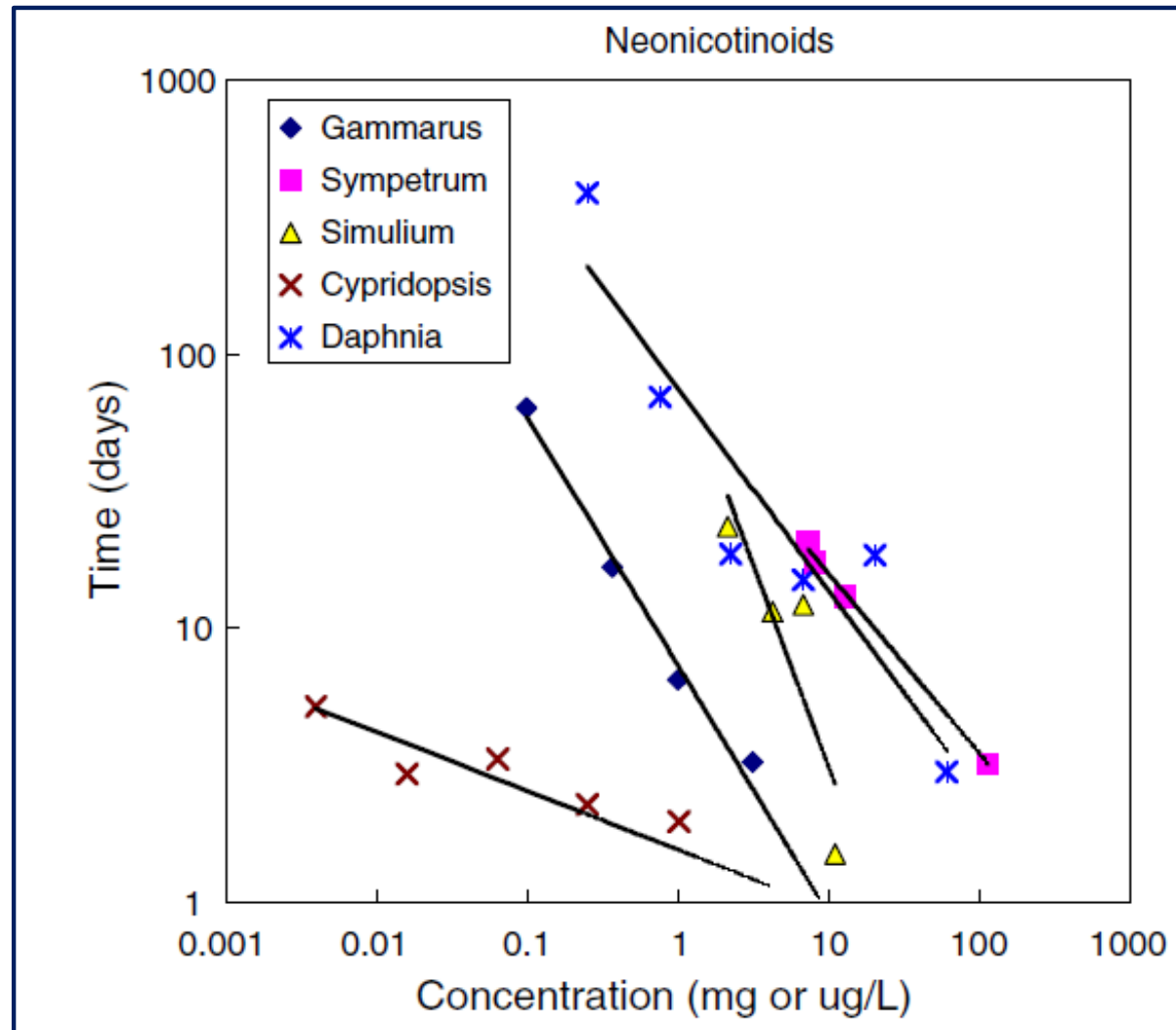


Moths



The lethal effects of imidacloprid and thiacloprid on arthropods.  
The relationship between concentration and length of exposure to lethal effect  
In a log-log plot the relationship appears as a straight line

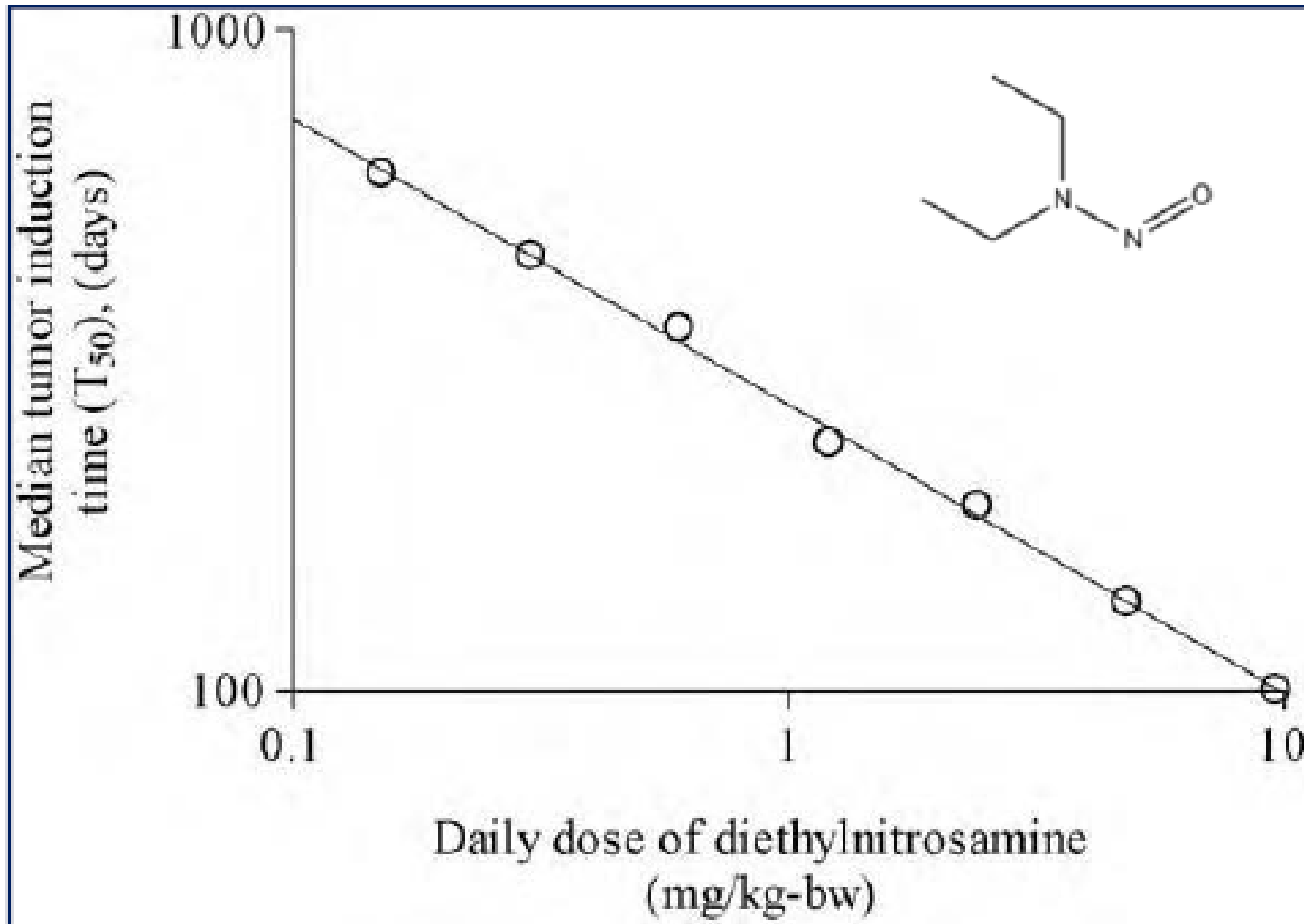
Sanchez-Bayo, F., 2009. Ecotoxicology 18, 343–354.



## Induction of liver cancer in rats by diethylnitrosamine

The relationship between daily dose and duration of exposure up to the initiation of cancer  
In a log-log plot the relationship appears as a straight line

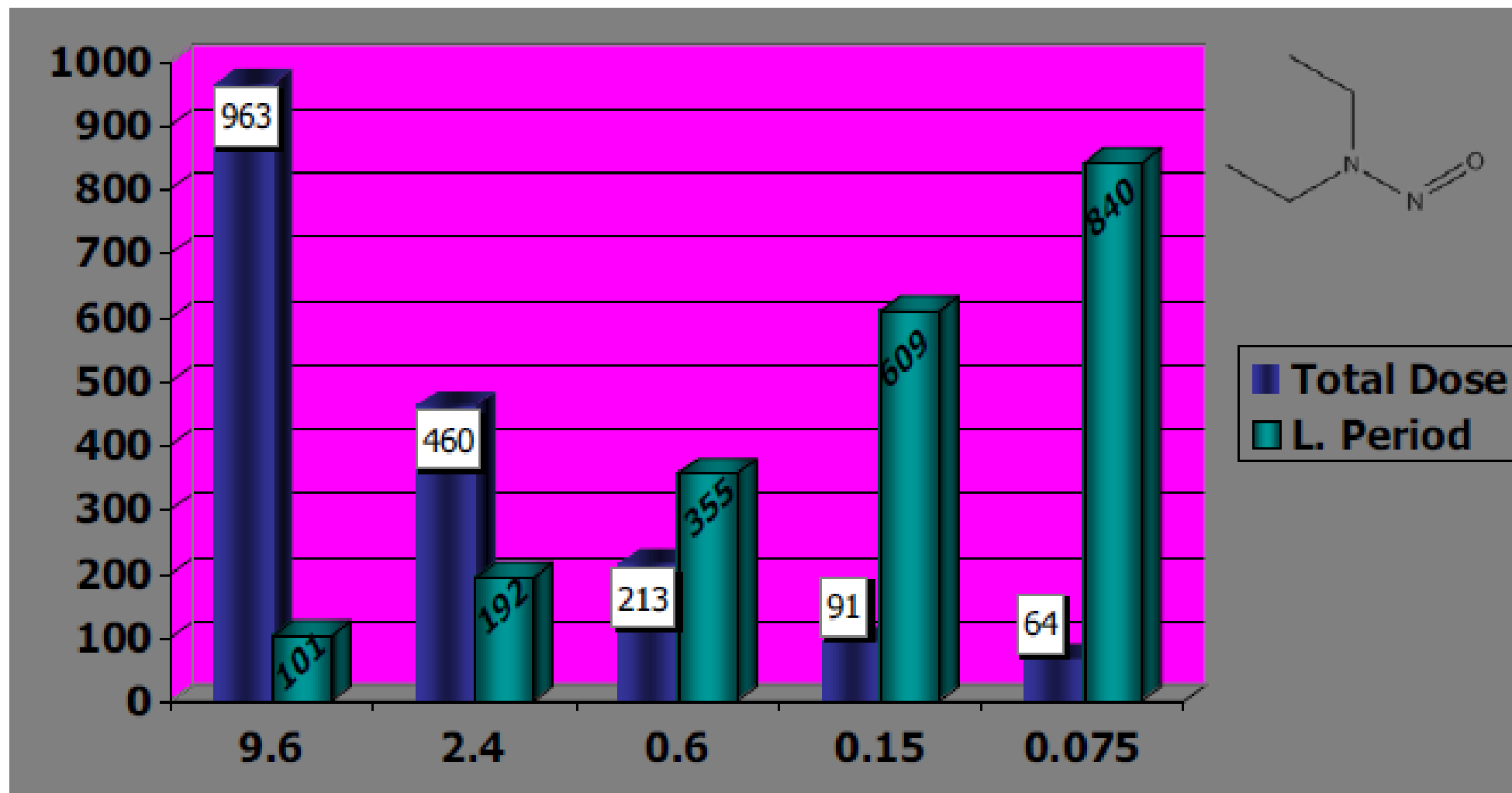
Druckrey, H., Schildbach, A., Schmaehl, D., Preussmann, R., Ivankovic, S., 1963. Quantitative analysis of the carcinogenic effect of diethylnitrosamine. *Arzneimittelforschung* 13, 841–851



# Induction of liver cancer in rats by diethylnitrosamine

## The carcinogenic effect is reinforced by exposure time

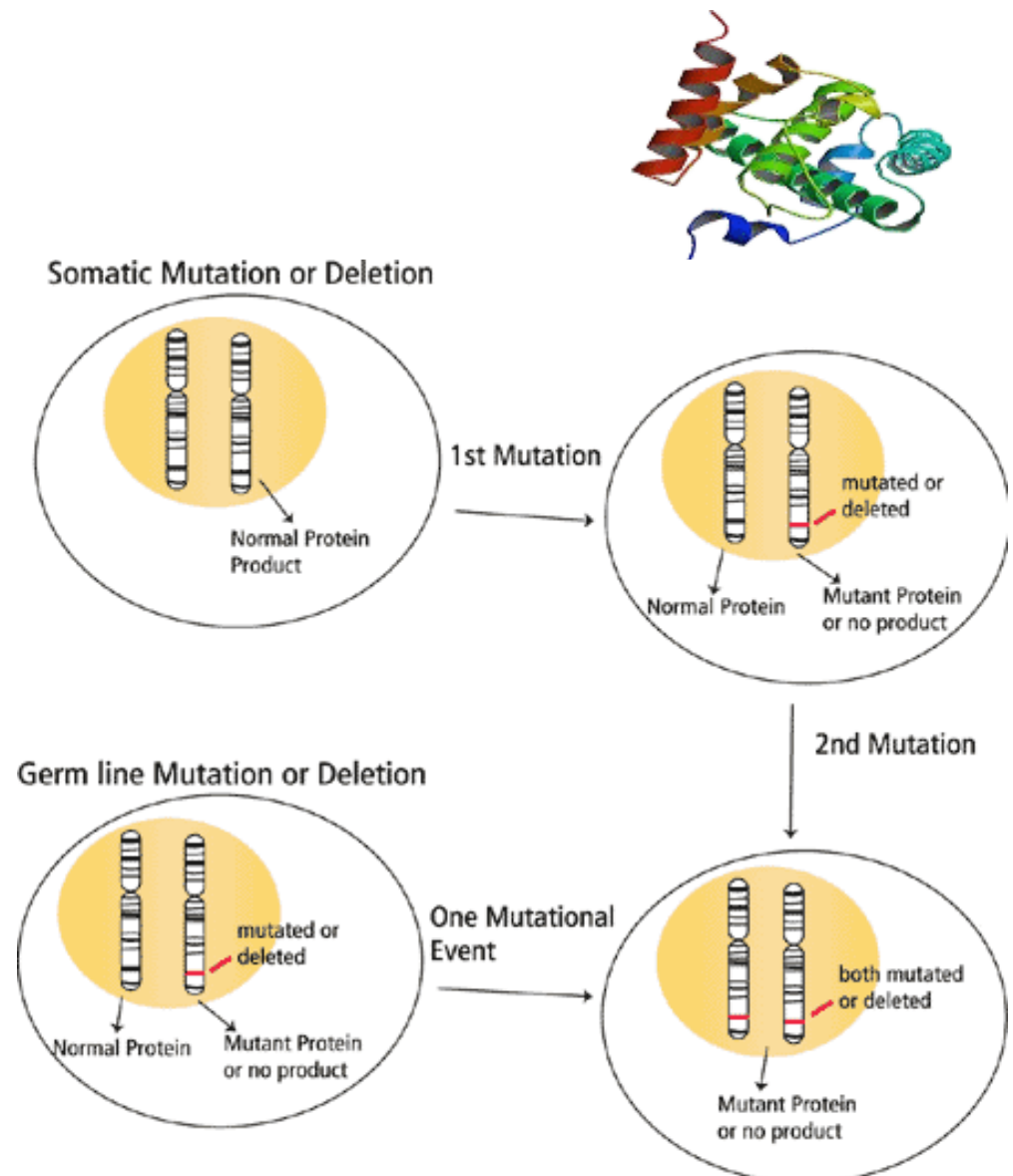
Druckrey, H., Schildbach, A., Schmaehl, D., Preussmann, R., Ivankovic, S., 1963. Quantitative analysis of the carcinogenic effect of diethylnitrosamine. *Arzneimittelforschung* 13, 841-851



# A safe level of exposure for carcinogens cannot be defined

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

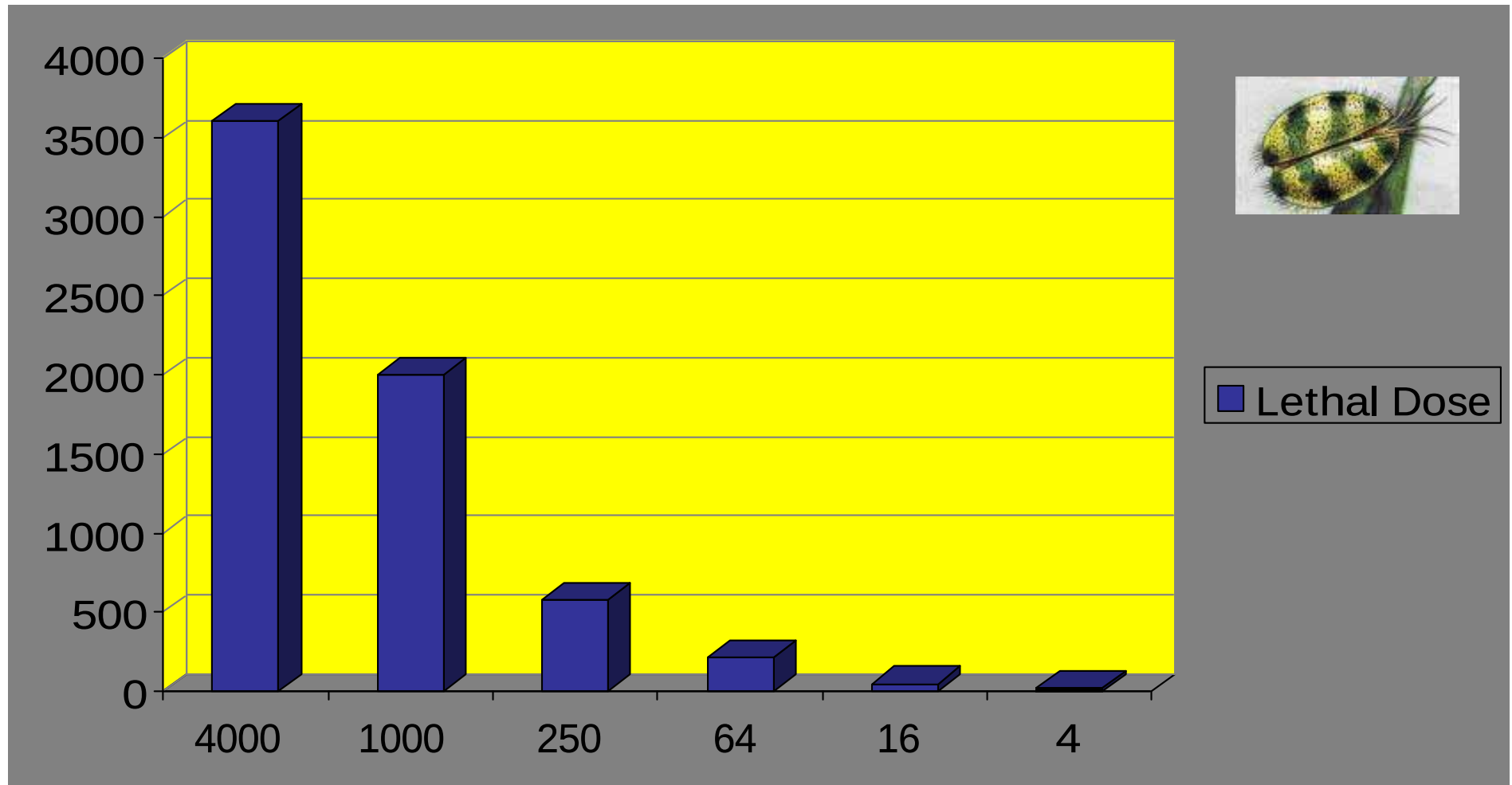
- **A single hit may suffice to induce cancer.**
- The retinoblastoma protein (Rb) is a tumor suppressor protein that is dysfunctional in many types of cancer
- Mutated Rb may be inherited and the mutated gene is recessive
- Should a cell sustain only one mutation in the other Rb gene is also mutated by a substance, all pRb in this cell will be ineffective.



# Lethal effect of Imidacloprid on *Cypridopsis vidua*

## The lethal effect is reinforced by exposure time

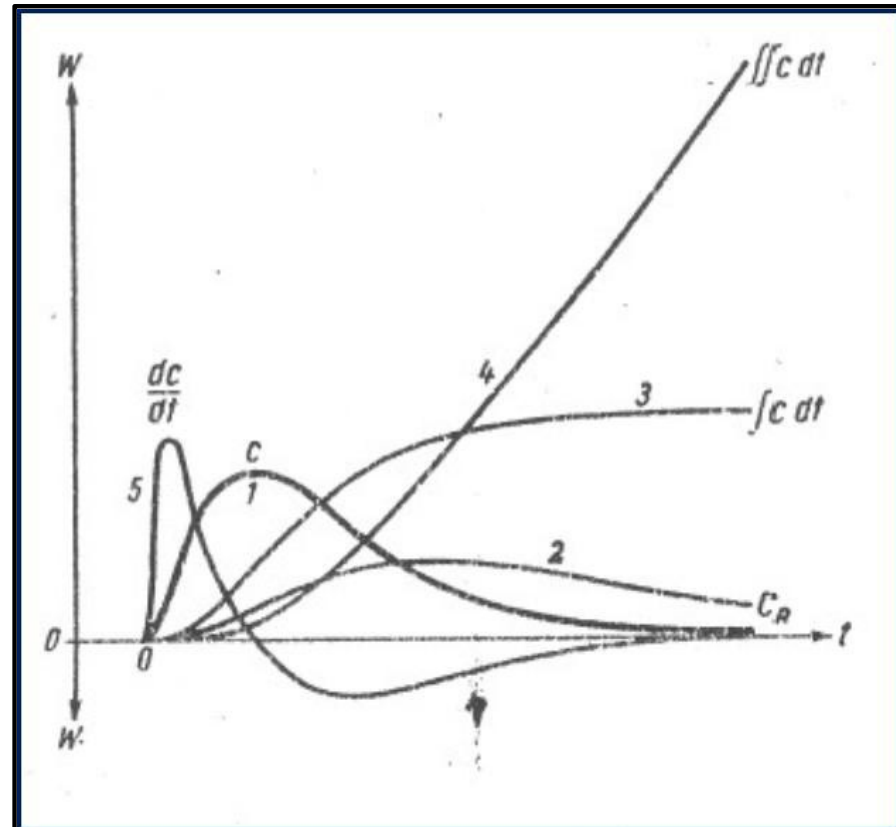
Tennekes, H.A. (2010) Toxicology 276, 1-4



# Reinforcement of the effect through exposure time is explained when both receptor binding and the effect are irreversible

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

- If both receptor binding and the effect are irreversible, the effect will be determined by the double integral of compound concentration over time.
- This is exemplified by curve 4, whereas curve 1 exemplifies the concentration of poison at the site of action over time.
- In this case, the effect is reinforced by exposure time.





# Imidacloprid is a **strong agonist** with a **high affinity** for nicotinic acetylcholine receptors in the central nervous system of insects

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

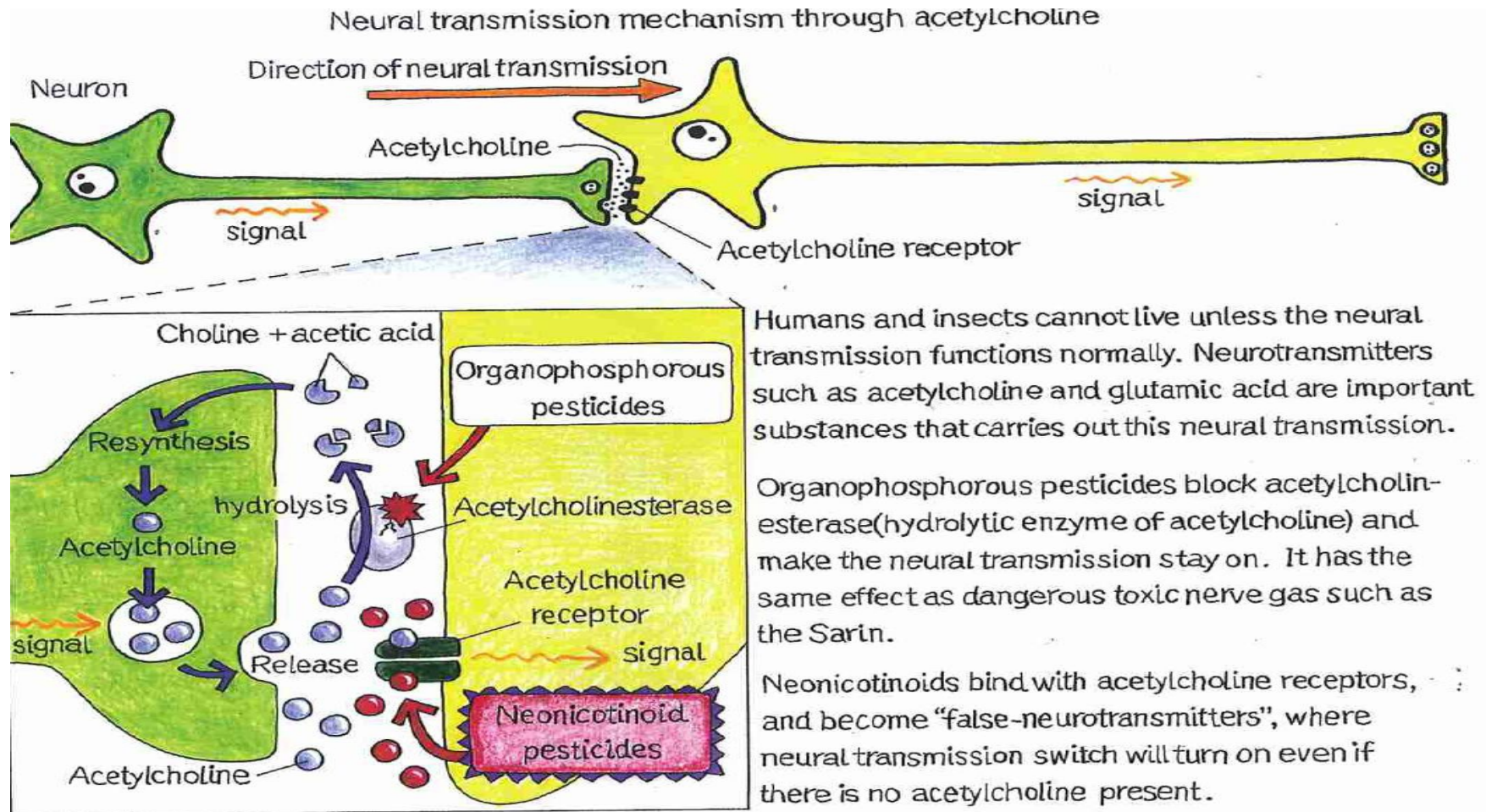
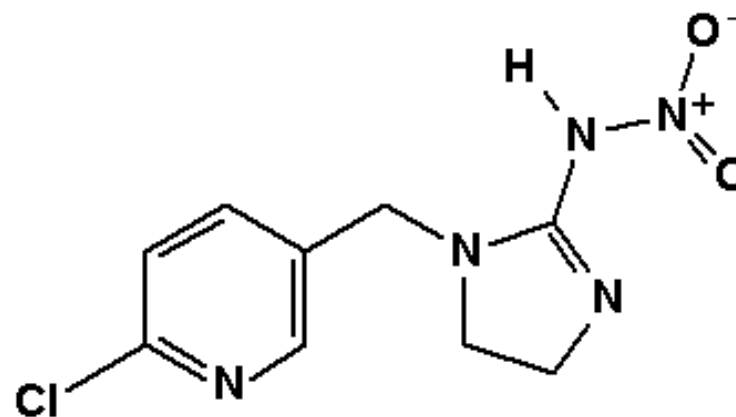


Illustration: Saori Yasutomi

# Imidacloprid is a **weak agonist** with a **high affinity** for nicotinic acetylcholine receptors in the human central nervous system

P. Li, J. Ann, G. Akk, Journal of Neuroscience Research, article first published online: 28 April 2011

- The  $\alpha 4\beta 2$  receptor forms most of the high-affinity nicotine binding sites in the brain.
- Our data suggest that imidacloprid is a low-efficacy but high-affinity agonist of the human  $\alpha 4\beta 2$  receptor. Coapplication of imidacloprid strongly inhibited currents elicited by ACh.
- The inhibitory effect was especially noticeable at lower transmitter concentrations, i.e., conditions under which the  $\alpha 4\beta 2$  receptors operate in vivo.
- The data thus suggest that imidacloprid-containing insecticides may have stronger side effects on humans.



# Application of Imidacloprid over several years has a deadly effect on hexapods, collembola, thrips, and beetles living in the soil

D.C. Peck (2009) *Pedobiologia* 52, 287-299

- A study was conducted over 3 years on an experimental home lawn to detect, measure and contrast the effects of white grub control products on the abundance of soil-active arthropods.
- A consistent short-term effect due to individual applications was not detected. But the cumulative results of three consecutive yearly applications to the same field plots showed that imidacloprid suppressed numbers of total hexapods, Collembola, Thysanoptera and Coleoptera adults by 54-62%.
- Trichlorfon, halofenozide, sulfur and nematodes had no discernible impact on the abundance of non-target arthropods as measured in this study.



# Imidacloprid contaminated soil is toxic to earthworms after one week of exposure

N. Dittbrenner et al (2010) Ecotoxicology

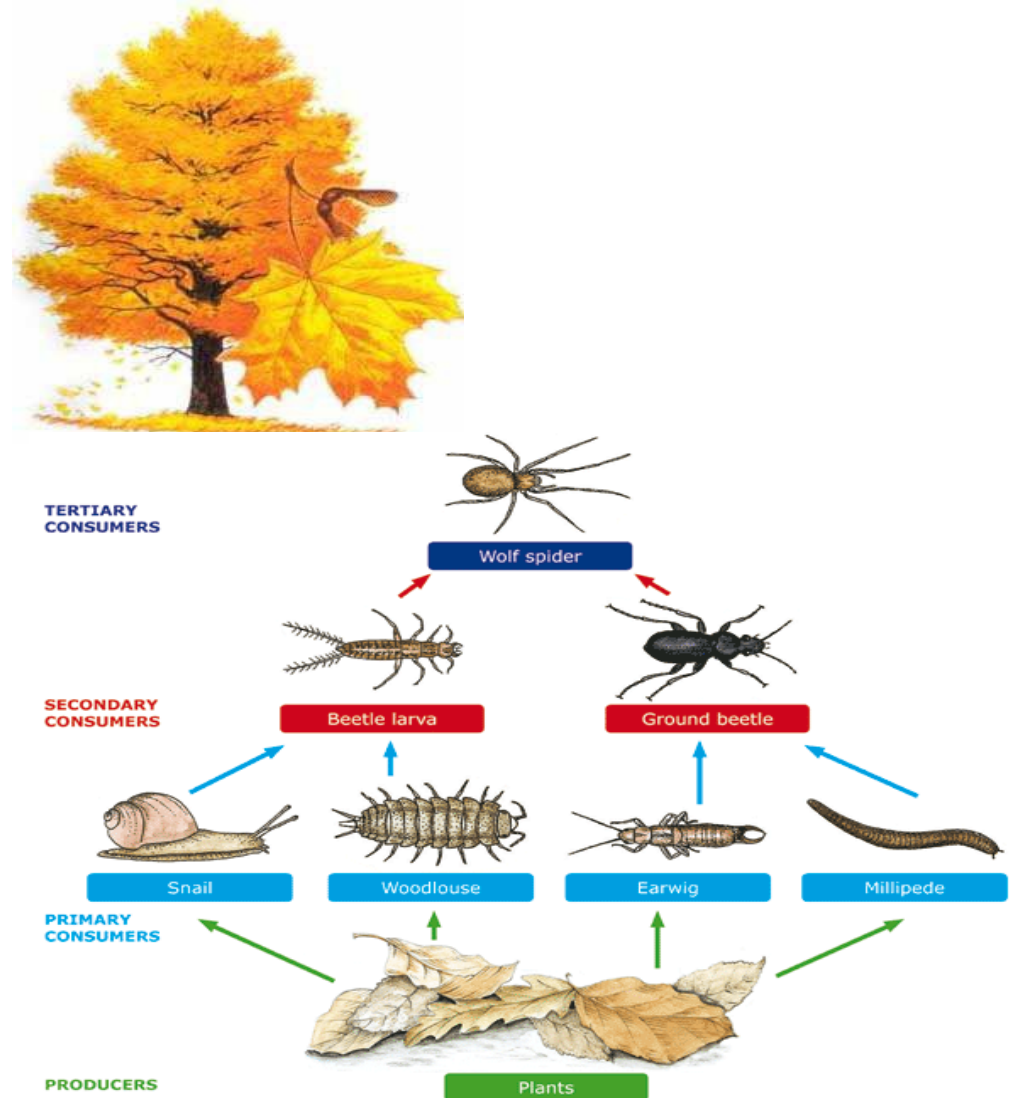
- In laboratory experiments we have assessed sub-lethal effects of imidacloprid on two earthworm species commonly found in different agricultural soils (*Lumbricus terrestris* and *Aporrectodea caliginosa*).
- After 7 days of exposure in contaminated soil, a significant loss of body mass was found in both species exposed to imidacloprid concentrations as low as 0.66 mg kg<sup>-1</sup> dry soil. These losses ranged from 18.3 to 39% for *A. caliginosa* and from 7.4 to 32.4% for *L. terrestris*, respectively.
- The detected sub-lethal effects were found close to the predicted environmental concentration (PEC) of imidacloprid, which is in the range of 0.33–0.66 mg kg<sup>-1</sup> dry soil.



# Systemic imidacloprid inhibits decomposition of autumn leaves through sub-lethal effects on arthropods

Kreutzweiser DP et al (2009) Ecotoxicology and Environmental Safety 72: 1053-1057

- Applications to deciduous trees result in foliar concentrations of imidacloprid that could pose a risk of harm to non-target decomposer invertebrates when autumn-shed leaves fall to forest floors or adjacent water bodies
- Our results indicate that imidacloprid at realistic concentrations in leaves can inhibit leaf litter breakdown through adverse sub-lethal effects on decomposer invertebrates



## **Sowing neonicotinoid-laced corn causes bee mortality**

- The corn sowing posed a significant threat to honeybees, with thiamethoxam being the most probable toxic agent.

P. Tremolada et al (2010) Field Trial for Evaluating the Effects on Honeybees of Corn Sown Using Cruiser® and Celest xl® Treated Seeds. Bull Env Contam Toxicol 85, 229-234

- Collected data indicate that the higher number of bee losses events occurred in intensively cultivated flat areas, located in the North of Italy, mainly during or after corn sowing. The chemical analyses of dead bees revealed the presence of three neonicotinoid residues.

L. Bortolotti et al. Spring honey bee losses in Italy.

In: Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group, Julius-Kühn-Archiv 423, 2009

- In late April and early May 2008 a bee mortality occurred in parts of South-West Germany, which affected approximately 12,000 colonies of bees, Very soon, maize seeds which had been treated with the insecticidal substance clothianidin were suspected as a possible cause. Only two weeks later a clothianidin poisoning was confirmed.

R. Forster. Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008. In: Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group, Julius-Kühn-Archiv 423, 2009



## Use of sowing machines with deflectors does not appear to prevent bee mortality in Austria

- Every year we have the same tragedy: No sooner have the insecticide-laced corn and pumpkin seeds been sown than the surrounding styran beekeepers report bee mortality – and so it is again this year. Once again, the eastern districts are mostly affected. However, damage claims are also being reported from Upper and Lower Austria.



GLOBAL 2000: Bienenschäden durch chemisch behandeltes

Mais-Saatgut nun schon das dritte Jahr! (mai 2010)

- GLOBAL 2000 – Bee mortality through chemically treated corn, now for the third year running! (May 2010)



# The harmful effect of systemic neonicotinoid insecticides on non-target insects

R. A. Cloyd and J. A. Bethke (2011) Pest Management Science 67, 3-9

- Systemic neonicotinoid insecticides are harmful to non-target insects (including natural predators associated with biological control such as predatory insects and parasitoids) when they:



(1) feed on pollen, nectar or plant tissues which are contaminated with the active ingredient;

(2) consume the active ingredient while ingesting plant sap;



(3) feed on preys that are contaminated with the active ingredient in plant sap.

# Neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds are lethal to bees

A Tapparo et al (2011) J. Environ. Monit

- **The researchers show that concentrations of neonicotinoids present in drops collected from the leaves of corn plants are high enough to kill bees within a few minutes.**
- Although Tapparo doesn't think the mechanism they identify is the primary cause - noting that bee decline is probably due to many different factors - he says it can't be ignored. 'It must not be neglected that bees can be exposed to high doses of insecticides,' he says.



# Systemic imidacloprid is lethal for the parasitoid *Anagyrus pseudococchi*

Krischik VA et al (2007) Environ. Entomol. 36(5): 1238-1245

Behavior was altered and survivorship was reduced when parasitoids, *Anagyrus pseudococchi* (Girault) (Hymenoptera: Encyrtidae), were fed flowers from buckwheat, *Fagopyrum esculentum* L. (Polygonaceae), treated with soil applications of imidacloprid (Marathon 1% G).



**Parasitoids at 1 d had significantly reduced survivorship of  $38 \pm 6.7\%$  on label rate and  $17 \pm 4.2\%$  on twice label rate compared with  $98 \pm 1.2\%$  on untreated flowers.**

Parasitoids trembled 88% on label rate and 94% on twice label rate compared with 0% on untreated flowers.

# Feeding predaceous ladybirds on aphids reared on (imidacloprid) systemic treated beans had an adverse effect on their development, longevity and fecundity

D. P. Papachristos et al (2008) Biological Control 47(1): 77-81

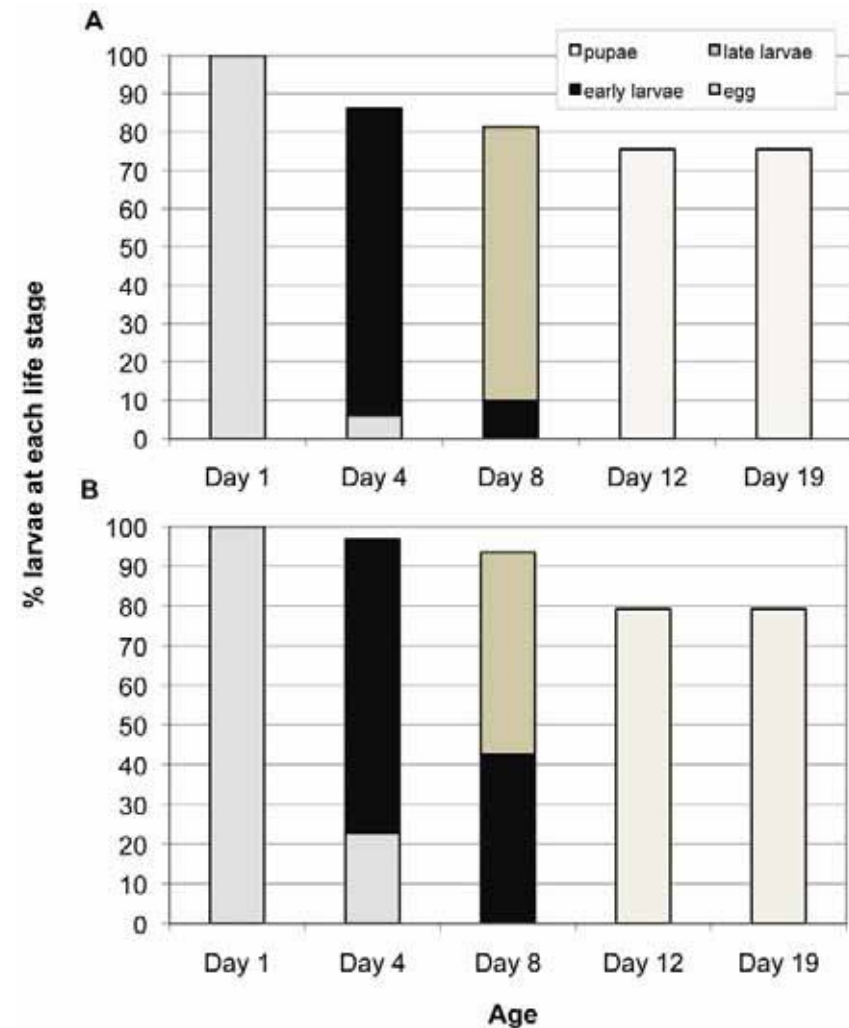
- We studied, under laboratory conditions, the possible effects of sublethal doses of soil-applied imidacloprid on development, survival and fecundity of the predator *Hippodamia undecimnotata*.
- For studies, predator fed upon *Aphis fabae* that was reared on *Vicia faba* plants treated with systemic imidacloprid.
- Survival of immature stages in insecticide treatment (52.2%) was lower than control (77.4%).
- Adult average longevity was significantly higher for the control than the insecticide treatment.
- Moreover, females oviposited fewer eggs in insecticide treatment than the control (33% reduction in average fecundity for imidacloprid).



# Delayed development in worker honey bees with exposure to neonicotinoid pesticides (increases the fertility of the Varroa mite)

Wu JY et al. (2011) Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker HoneyBee (*Apis mellifera*) Development and Longevity. PLoS ONE 6(2): e14720

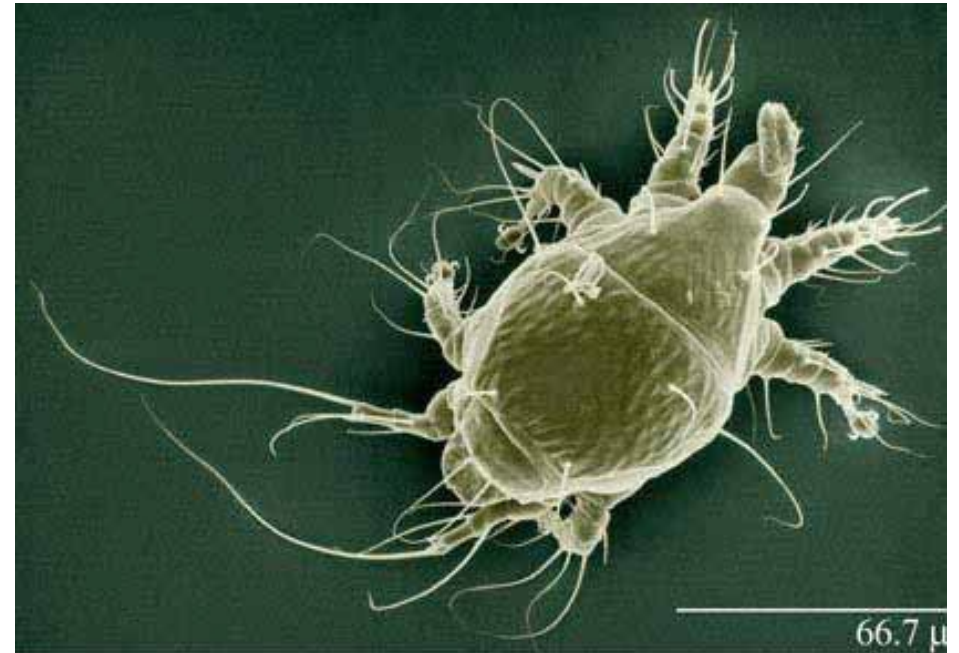
- Delayed development occurred in bees reared in treatment comb containing 17 different pesticides, including 9 systemic compounds and 5 neonicotinoid insecticides (clothianidin, dinotefuran, imidacloprid, thiacloprid, thiamethoxam), particularly in the early stages (day 4 and 8) of worker bee development
- Delayed adult emergence may provide a reproductive advantage for a gravid foundress Varroa mite invading a cell occupied by a developing bee larva and laying four eggs in 30 hour intervals. The first egg results in a male, with subsequent eggs developing into multiple daughter mites.
- With delayed adult bee emergence the likelihood that the third daughter mite will successfully reach maturity and mate increases.



# Imidacloprid weakens the resistance of bee colonies to pathogenic organisms

Cédric Alaux et al. Environ Microbiol. 2010 March; 12(3): 774–782

- We demonstrated that the interaction between the microsporidia *Nosema* and a neonicotinoid (imidacloprid) significantly weakened honeybees.
- This provides the first evidences that interaction between an infectious organism and a chemical can also threaten pollinators

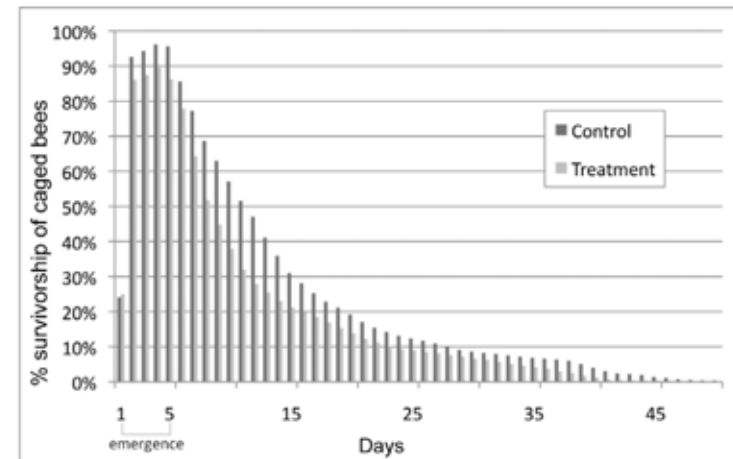


# Neonicotinoids can reduce longevity in honey bees

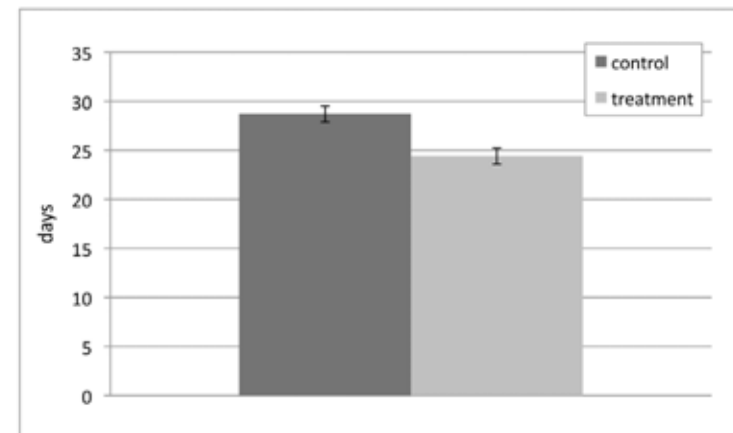
Wu JY et al. (2011) Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker HoneyBee (*Apis mellifera*) Development and Longevity. PLoS ONE 6(2): e14720

- Adult longevity was reduced by 4 days in bees exposed to residues of neonicotinoids in contaminated brood comb during development.
- Combined effects from honey bee exposure to neonicotinoid residue in brood comb, such as reduced adult longevity, increased brood mortality, higher fecundity of Varroa mites (due to delayed development and emergence of adult bees) and increased susceptibility to pathogens, may contribute to reduced honey bee colony health.

A



B





# Sublethal dosages of imidacloprid can have a negative effect on the foraging and navigation behaviour in foraging honey bee workers

E. C. Yang et al (2008) Journal of Economic Entomology 101(6):1743-1748.

- The foraging behavior of the honey bee workers was investigated to show the effects of imidacloprid.
- When bees were treated with an imidacloprid concentration higher than 1,200 µg/liter, they showed abnormalities in revisiting the feeding site.
- Some of them went missing, and some were present again at the feeding site the next day. Returning bees also showed delay in their return trips.
- Our results demonstrated that sublethal dosages of imidacloprid were able to affect foraging behavior of honey bees.



# A recent meta-analysis of 14 studies under laboratory and semi-field conditions showed that traces of imidacloprid in nectar reduced the performance in honey bees

James E. Cresswell, *Ecotoxicology* (2010)

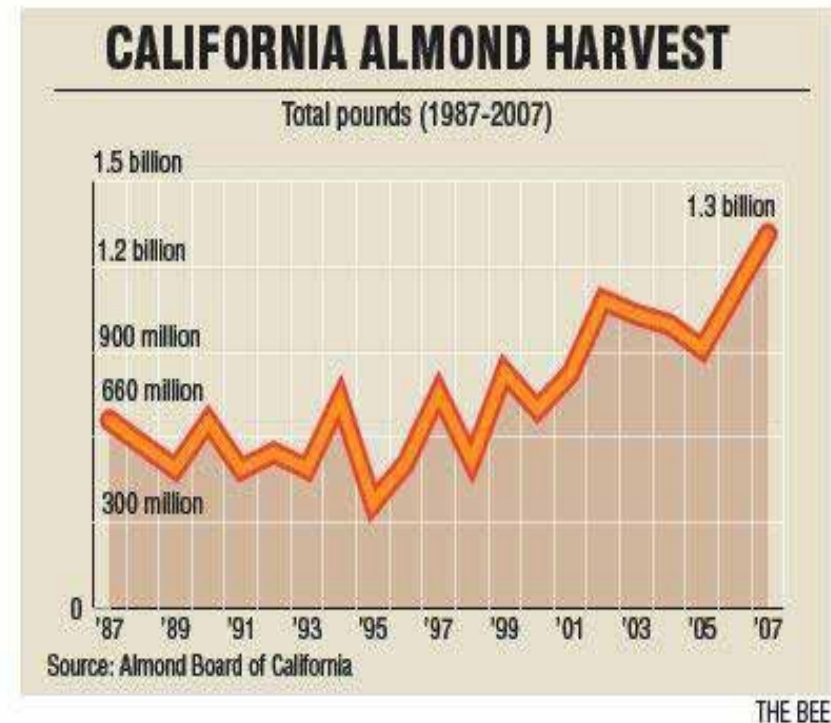
- In a meta-analysis of fourteen published studies of the effects of imidacloprid on honey bees under laboratory and semi-field conditions that comprised measurements on 7073 adult individuals and 36 colonies, fitted dose–response relationships estimate that trace dietary imidacloprid at field-realistic levels in nectar will have no lethal effects, but will reduce expected performance in honey bees by between 6 and 20%.



# An increase in honey bee colony losses jeopardises global food production

Rabobank Industry Note 252-2011: Why the Loss of Honey Bee Colonies May Sting Global Agriculture

- Agriculture in the United States has been identified as a risk region. While production of pollinated crops has quadrupled since 1961, the number of bee colonies has halved. The average number of bee colonies per pollination-requiring hectare has declined by nearly 90%.
- This development illustrates the growing inherent risk of colony losses as the average colony is responsible for a greater pollination task.



# Conclusions I

## Mechanism of action of neonicotinoid insecticides

- The mode of action of neonicotinoid insecticides derives from almost complete and virtually irreversible blockage of postsynaptic nicotinic AcetylCholine Receptors (nAChRs) in the central nervous system.
- Imidacloprid is a weak agonist in humans and a strong agonist in insects.
- The toxicity of imidacloprid and thiacloprid to arthropods is reinforced by exposure time. This also applies to honey bees.
- Their dose-response characteristics are similar to those of carcinogens for which a safe level of exposure is not definable. It is therefore questionable if indeed there is a safe neonicotinoid level of exposure for non-target arthropods.
- This question is also relevant because any application of imidacloprid will contaminate groundwater and surface water in the Netherlands, whereby wild flora (and thus non-target insects) will consequently be exposed to imidacloprid.

# Conclusions II

## Toxicity of neonicotinoids to arthropods

- After several years of application, Imidacloprid is found to have a lethal effect on arthropods in the soil. Imidacloprid in the soil is harmful to non-target insects that are used for biological control. Imidacloprid is toxic to earthworms after only one week in the soil.
- Sowing neonicotinoid-laced corn seeds causes bee mortality, which cannot be prevented in Austria despite the implementation of deflectors on their corn sowing machines.
- Imidacloprid weakens and reduces the performance of a honey bee colony and makes it susceptible to parasites. There are indications that neonicotinoids can delay the development of worker honey bees (and thus increase the fertility of the Varroa mite) and shorten their longevity. Sub-lethal dosages of imidacloprid can have a negative effect on the foraging and navigation behaviour in foraging honey bee workers.
- To summarise, there are clear indications that there is a correlation between chronic exposure of bee colonies to neonicotinoids and increased bee mortality over the last few years, which poses a threat to global food production.

# The admission policy of neonicotinoids fails to protect honey bee colonies

- The mode of action, the dose-response characteristics and fundamental research provide clear indications that neonicotinoids, even in minute quantities, have, long-term, undesirable and deleterious effects on organisms crucial to the ecosystem.
- In view of the worrying bee mortality in recent years, it should now be obvious that measures are needed to prevent chronic exposure of bees to any amount of neonicotinoids under any circumstances and thus to minimise any risks
- To date however, these measures depend on compelling evidence for a causal link between the use of neonicotinoids and bee mortality (which is virtually impossible to deliver).

