

# Neonicotinoids and pollinators

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## European Research project ALARM:

*The trend of world wide pollinator loss is a major threat to biodiversity*

*Causes include: new pesticides, land use change and climate change*

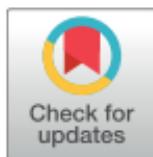
## RESEARCH ARTICLE

# More than 75 percent decline over 27 years in total flying insect biomass in protected areas

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## OPEN ACCESS

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## Abstract

Global declines in insects have sparked wide interest among scientists, politicians, and the general public. Loss of insect diversity and abundance is expected to provoke cascading effects on food webs and to jeopardize ecosystem services. Our understanding of the extent and underlying causes of this decline is based on the abundance of single species or taxonomic groups only, rather than changes in insect biomass which is more relevant for ecological functioning. Here, we used a standardized protocol to measure total insect biomass using Malaise traps, deployed over 27 years in 63 nature protection areas in Germany (96 unique location-year combinations) to infer on the status and trend of local entomofauna. Our analysis estimates a seasonal decline of 76%, and mid-summer decline of 82% in flying insect biomass over the 27 years of study. We show that this decline is apparent regardless of habitat type, while changes in weather, land use, and habitat characteristics cannot explain this overall decline. This yet unrecognized loss of insect biomass must be taken into account in evaluating declines in abundance of species depending on insects as a food source, and ecosystem functioning in the European landscape.

## Insectageddon: farming is more catastrophic than climate breakdown

George Monbiot

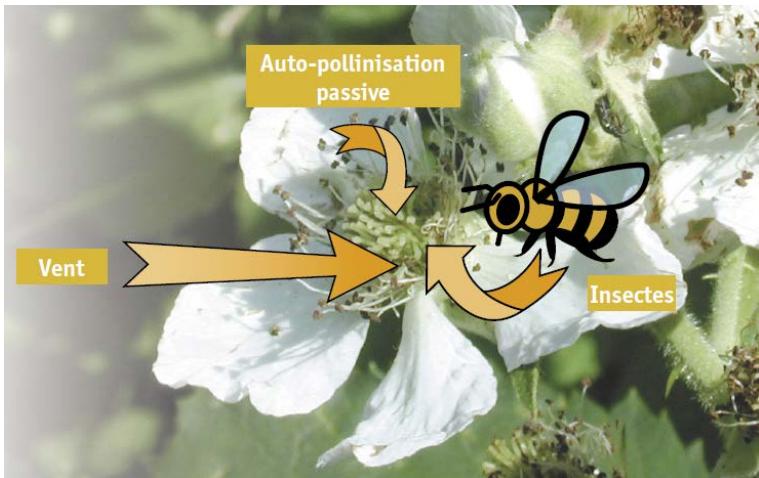


The shocking collapse of insect populations hints at a global ecological meltdown

**“The impact on wildlife of changes in farming practice (and the expansion of the farmed area) is so rapid and severe that it is hard to get your head round the scale of what is happening. A study published this week in the journal Plos One reveals that flying insects surveyed on nature reserves in Germany have declined by 76% in 27 years. The most likely cause of this Insectageddon is that the land surrounding those reserves has become hostile to them: the volume of pesticides and the destruction of habitat have turned farmland into a wildlife desert.”**

# The importance of pollinators

- 90 major crops (35% world food production volume) depend on pollinators
- Key nutrients: 90-100% from pollinator mediated crops (vit C, antioxidants, lycopene,  $\beta$ -tocopherol, vit A and folic acid)
- Value in Europe: 14.2 billion Euro / yr
- 87% of all flowering plants on earth depends on 25000 bee species for reproduction and evolution



Alfalfa  
Apple  
Almond  
Artichoke  
Asparagus  
Blackberry  
Blueberry  
Broccoli  
Brussels sprouts

## Some crops pollinated by bees<sup>3</sup>

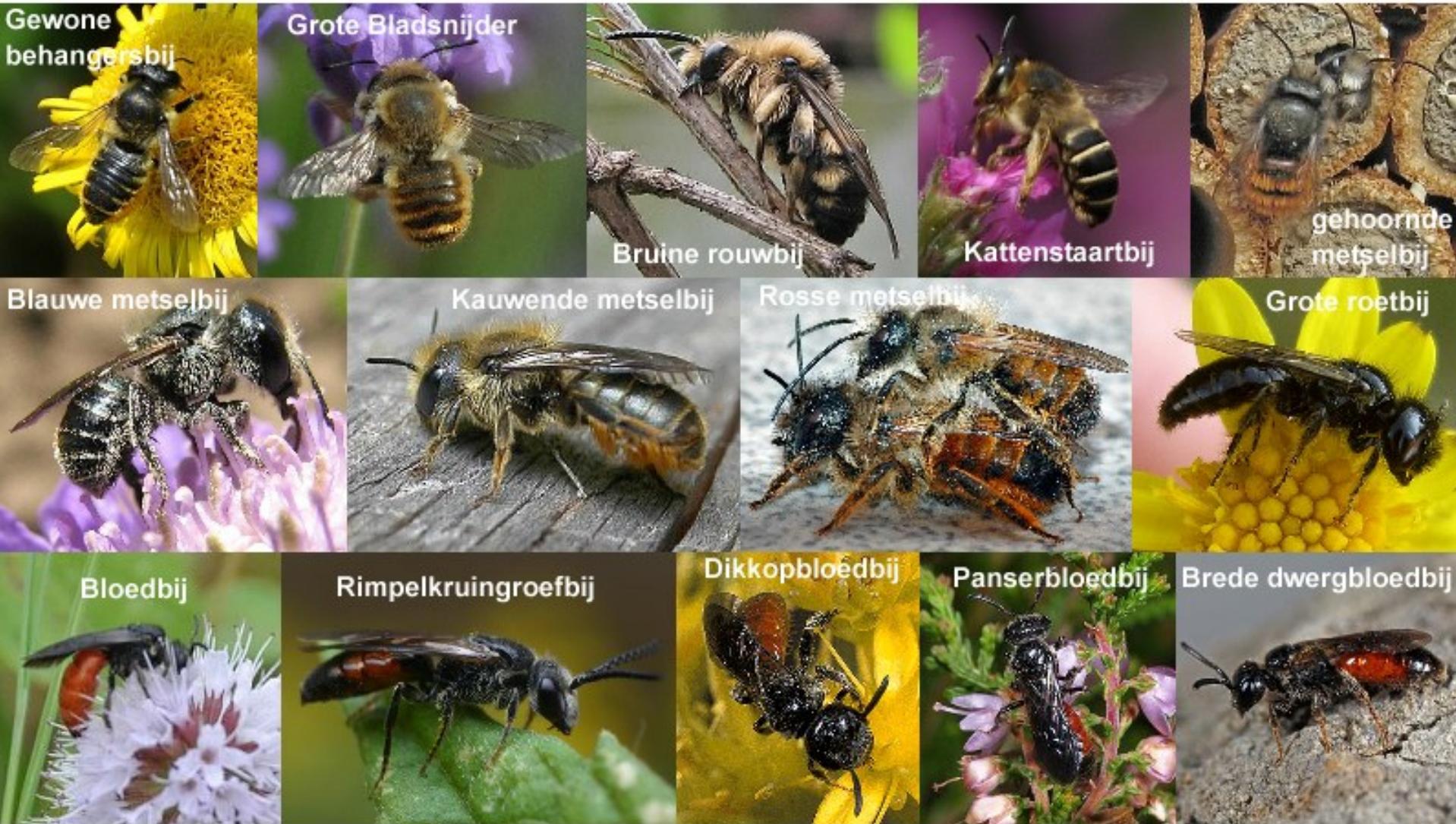
Cabbage  
Cacao  
Cantaloupe  
Carrot  
Cashew  
Cauliflower  
Celery  
Cherry  
Citrus  
Dill  
Eggplant/  
Aubergine  
Fennel  
Garlic

Kale  
Kola nut  
Leek  
Lychee  
Macadamia  
Mango  
Mustard  
Nutmeg  
Onion  
Passion fruit  
Peach  
Pear  
Plum  
Pumpkin

Raspberry  
Sapote  
Squash  
Sunflower  
Tangerine  
Tea  
Watermelon



**World wide: 25000 bee species  
In NL about 350 bee species, 181 of them  
are on the Red List / at risk of extinction**



2013

Late lessons from early warnings:  
science, precaution, innovation

ISSN 1725-9177



European Environment Agency 

## Patterns of widespread decline in North American bumble bees

Sydney A. Cameron<sup>a,1</sup>, Jeffrey D. Lozier<sup>a</sup>, James P. Strange<sup>b</sup>, Jonathan B. Koch<sup>b,c</sup>, Nils Cordes<sup>a,2</sup>, Leellen F. Solter<sup>d</sup>, and Terry L. Griswold<sup>b</sup>

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Edited<sup>a</sup> by Gene E. Robinson, University of Illinois, Urbana, IL, and approved November 24, 2010 (received for review October 3, 2010)

Bumble bees (*Bombus*) are vitally important pollinators of wild study in the United States identified lower genetic diversity and

2011

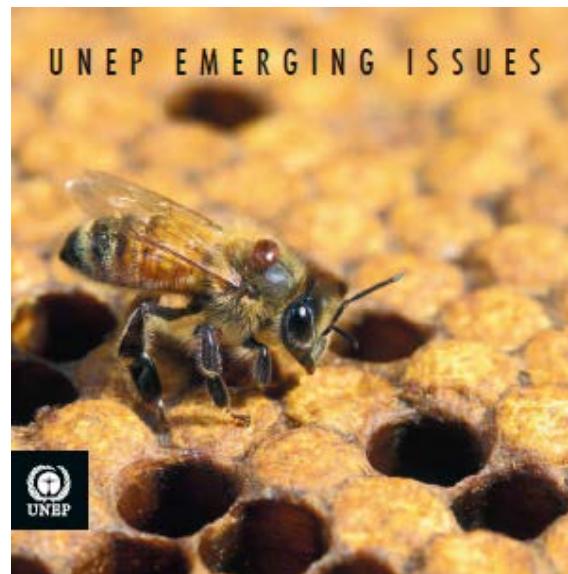
intensive nationwide surveys of >16,000 specimens. We show that the relative abundances of four species have declined by up to 96% and that their surveyed geographic ranges have contracted by 23–87%, some within the last 20 y. We also show that declining populations have significantly higher infection levels of the microsporidian pathogen *Nosema bombi* and lower genetic diversity compared

# Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands

2006

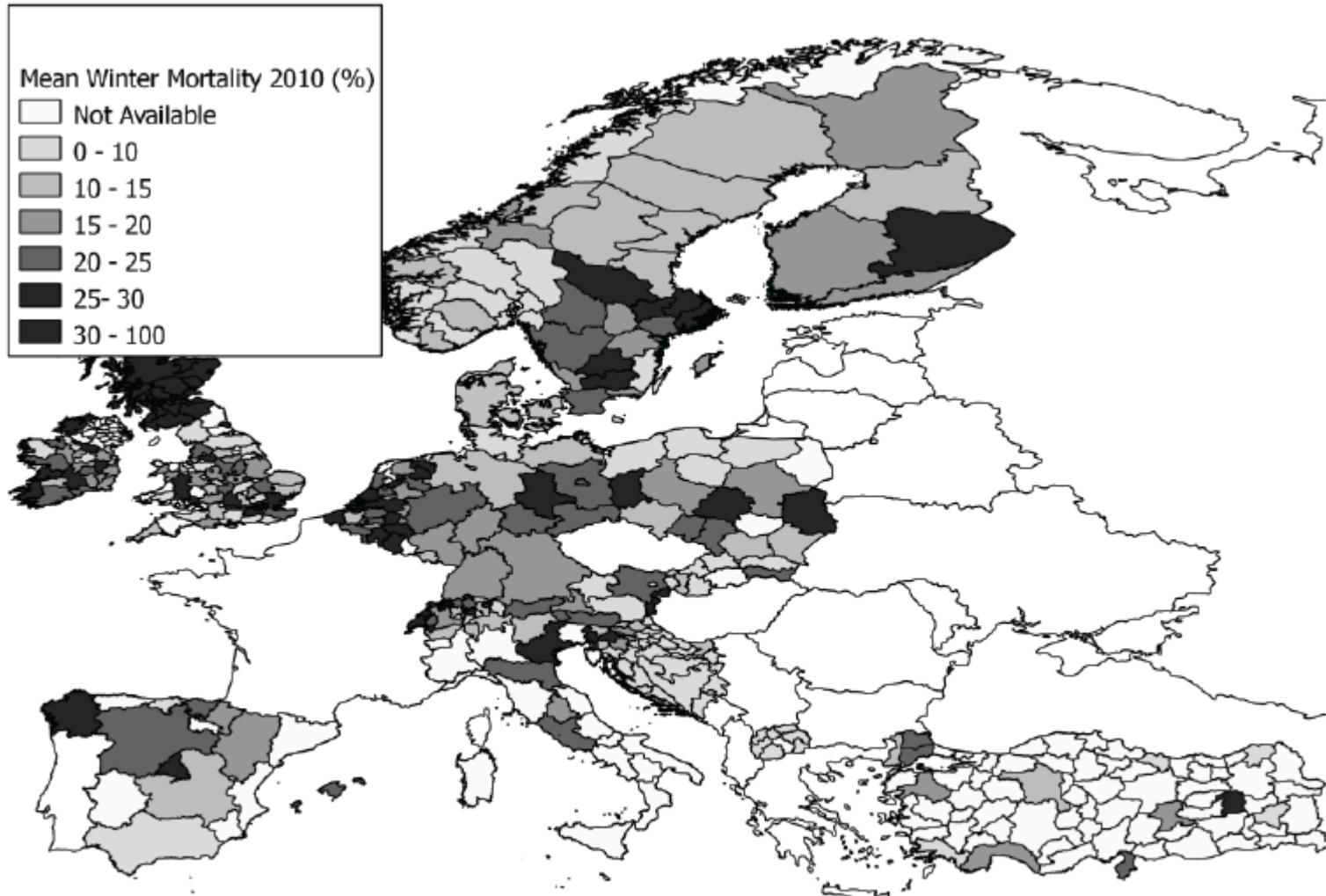
J. C. Biesmeijer,<sup>1\*</sup> S. P. M. Roberts,<sup>2</sup> M. Reemer,<sup>3</sup> R. Ohlemüller,<sup>4</sup> M. Edwards,<sup>5</sup> T. Peeters,<sup>3,6</sup> A. P. Schaffers,<sup>7</sup> S. G. Potts,<sup>2</sup> R. Kleukers,<sup>3</sup> C. D. Thomas,<sup>4</sup> J. Settele,<sup>8</sup> W. E. Kunin<sup>1</sup>

Despite widespread concern about declines in pollination services, little is known about the patterns of change in most pollinator assemblages. By studying bee and hoverfly assemblages in Britain and the Netherlands, we found evidence of declines (pre- versus post-1980) in local bee diversity in both countries; however, divergent trends were observed in hoverflies. Depending on the assemblage and location, pollinator declines were most frequent in habitat and flower specialists, in univoltine species, and/or in nonmigrants. In conjunction with this evidence, outcrossing plant species that are reliant on the declining pollinators have themselves declined relative to other plant species. Taken together, these findings strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species.



GLOBAL HONEY  
BEE COLONY  
DISORDERS  
AND OTHER  
THREATS  
TO INSECT  
POLLINATORS

2011



Mean winter losses of bee hives 2009-10 Europe,  
Turkey & Israel (Van der Zee e.a., 2012; Coloss)  
<http://dx.doi.org/10.3896/IBRA.1.51.1.12>



# The work of the honeybee

- Colony 10,000 to 80,000 bees
- Empty honeybee 80 to 100 mg
- Maximum “freight”: 70 mg/honeybee.
- Water “freight” up to 40 microliter per bee
- One colony visits 21 million flowers / day
- 700 flowers visited per honeybee per day
- 1 liter nectar requires 20,000 to 100,000 flights
- Worker honeybee lives ca. 20 - 42 days, winterbees 170 days and more.

Modified from: [http://www.unaf-apiculture.info/presse/DOSSIER\\_DE\\_PRESSE\\_2009\\_L\\_ABEILLE\\_SENTINELLE\\_DE\\_L\\_ENVIRONNEMENT.pdf](http://www.unaf-apiculture.info/presse/DOSSIER_DE_PRESSE_2009_L_ABEILLE_SENTINELLE_DE_L_ENVIRONNEMENT.pdf)

# New pollinator emerging in China



# Pollinator decline: interaction of mutually reinforcing causes

PPPP

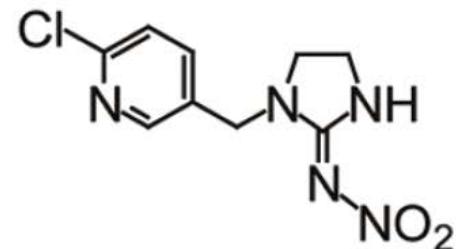
- Pollen
- Pathogens
- Pesticides
- Places (for nesting)



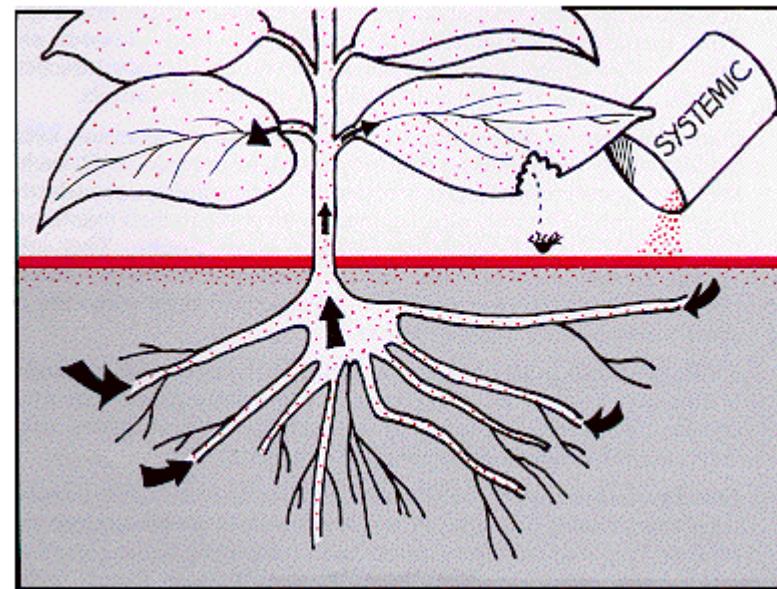
monoculture



# Systemic insecticides: revolution in plant protection



imidacloprid (1991)

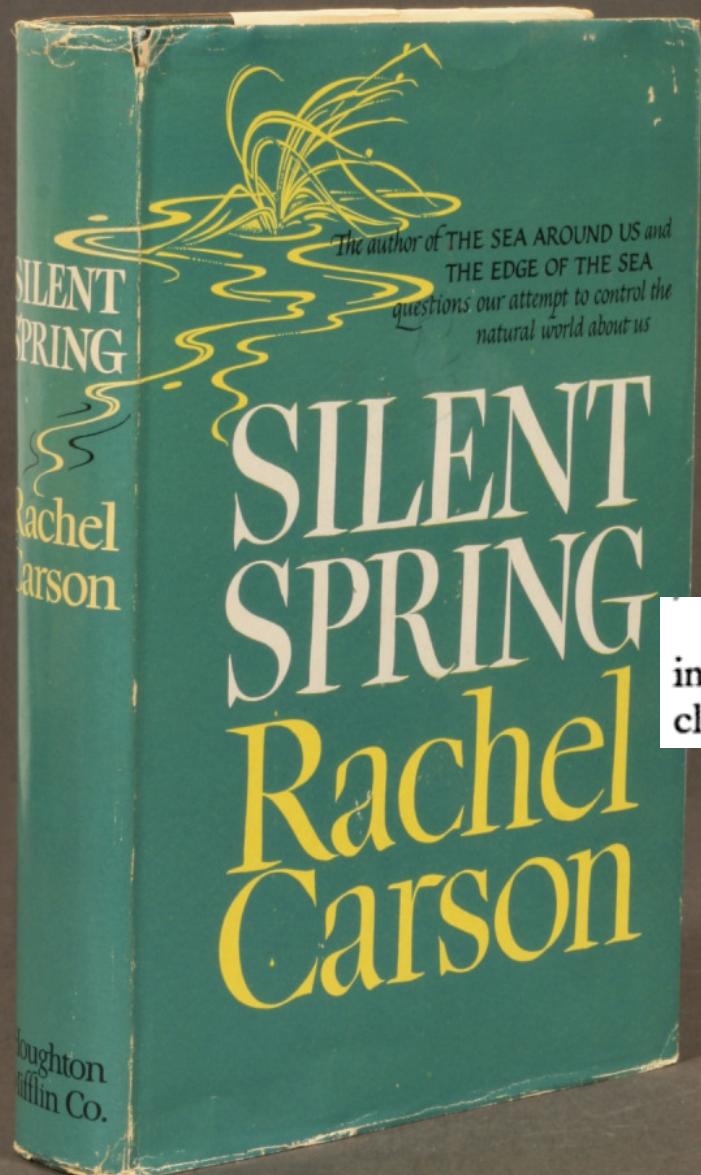


Shinzo Kagabu

*Systemic = crop takes it up into its plantsap: chemical makes plant toxic from inside*

Professor Shinzo Kagabu received the **2010 American Chemical Society International Award for Research in Agrochemicals** in recognition of his discovery of imidacloprid (IMI) and thiacloprid, which opened the **neonicotinoid era of systemic pest management**.

(Tomizawa & Casida, 2010, [DOI:10.1021/jf103856c](https://doi.org/10.1021/jf103856c))



1962

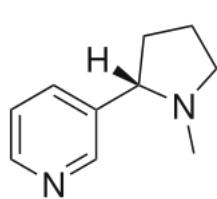


The world of systemic insecticides is a weird world, surpassing the imaginings of the brothers Grimm — perhaps most closely akin to the cartoon world of Charles Addams. It is a

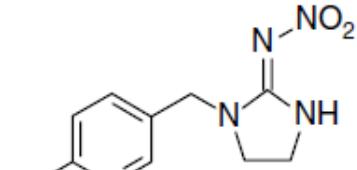
ELIXIRS OF DEATH

33

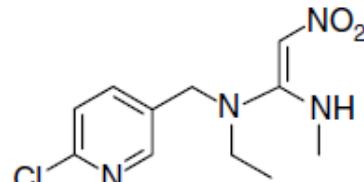
world where the enchanted forest of the fairy tales has become the poisonous forest in which an insect that chews a leaf or sucks the sap of a plant is doomed. It is a world where a flea bites a dog, and dies because the dog's blood has been made poisonous, where an insect may die from vapors emanating from a plant it has never touched, where a bee may carry poisonous nectar back to its hive and presently produce poisonous honey.



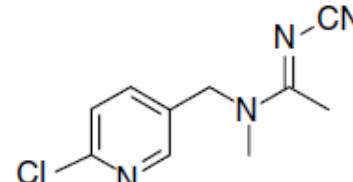
Nicotine



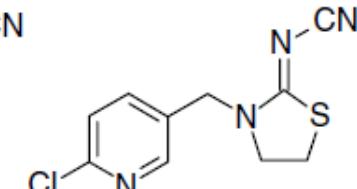
Imidacloprid (1)



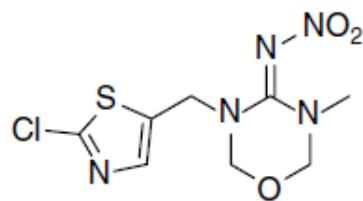
Nitenpyram (2)



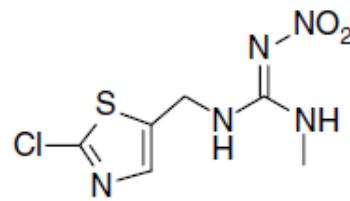
Acetamiprid (3)



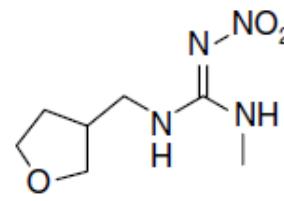
Thiacloprid (4)



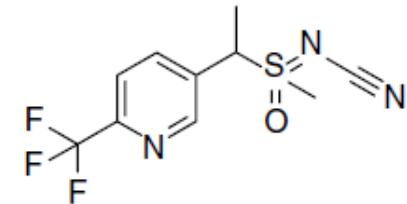
Thiamethoxam (5)



Clothianidin (6)



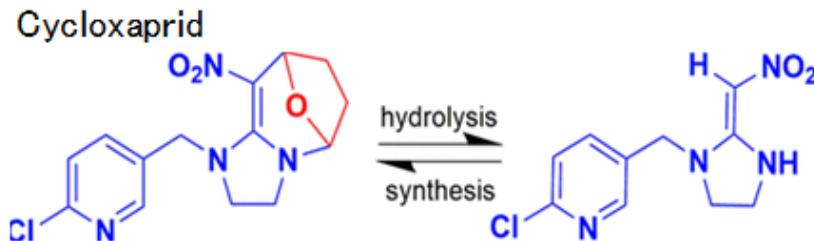
Dinotefuran (7)



Sulfoxaflor (8)

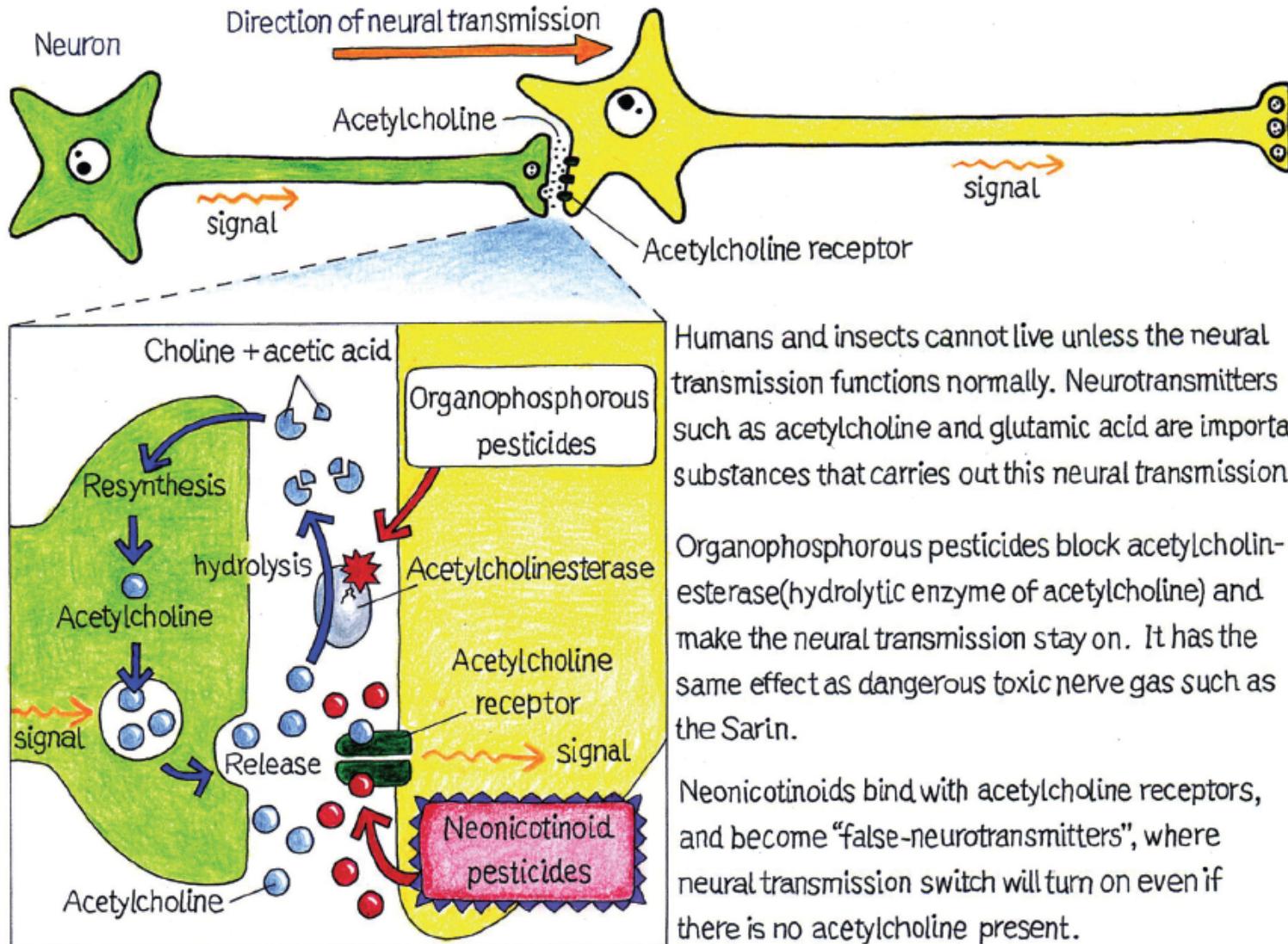
## Imidacloprid + Thiamethoxam + Clothianidin in EU:

>200 products  
>1000 allowed  
applications



# Neonicotinoid/Organophosphorous pesticides disrupt the neural transmission

## Neural transmission mechanism through acetylcholine



# Toxicity of neonicotinoids

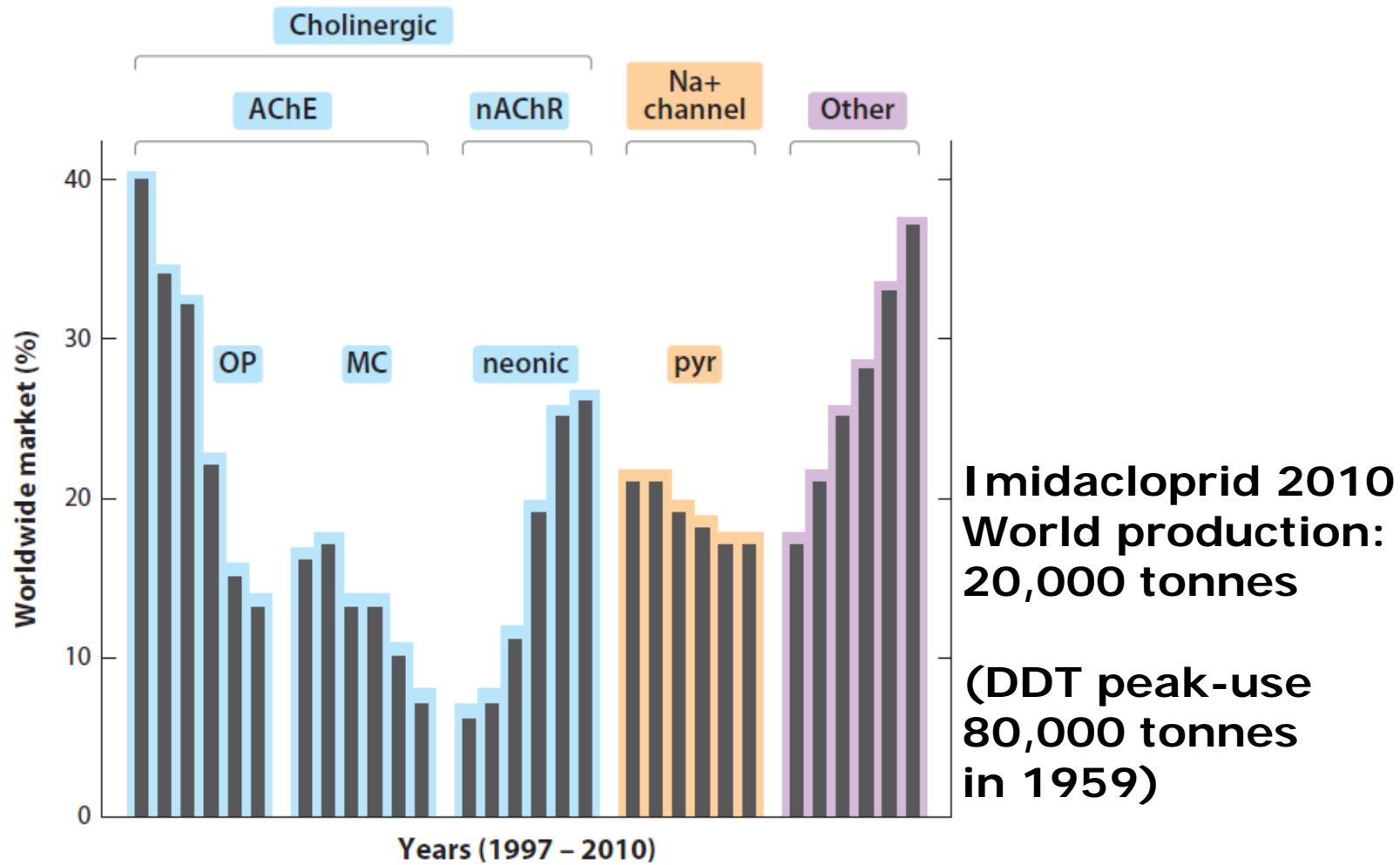
Pesticide	®	Use	LD50 (ng/honeybee)	Toxicity index relative to DDT
DDT	Dinocide	insecticide	27000	1
Amitraz	Apivar	insecticide / acaricide	12000	2
Coumaphos	Perizin	insecticide / acaricide	3000	9
Tau-fluvalinate	Apistan	insecticide / acaricide	2000	13.5
Methiocarb	Mesurol	insecticide	230	117
Carbofuran	Curater	insecticide	160	169
λ-cyhalothrin	Karate	insecticide	38	711
Deltamethrine	Decis	insecticide	10	2700
Thiamethoxam	Cruise	insecticide	5	5400
Fipronil	Regent	Insecticide	4.2	6475
Clothianidine	Poncho	Insecticide	4.0	6750
Imidacloprid	Gaucho	Insecticide	3.7	7297

Toxicity of insecticides to honeybees compared to DDT. The final column expresses the toxicity relative to DDT. (Source: Bonmatin, 2009)

<http://www.bijensterfte.nl/images/Bonmatin-conclusions-sentinelle-gb-2009.pdf>

# Systemic insecticides

- Systemic: Contamination of nectar and pollen
- Very high toxicity for honeybees
- A long persistence in soils ( $t_{1/2} = 9$  months) and water (160 days)
- Main metabolites as toxic as imidacloprid for bees
- Acute effects (overdosing, sowing...)
- Sublethal effects and chronic exposure
- Risks in fields :  $PEC/PNEC >> 1$
- Synergies with other pesticides
- Synergies with other pathogens (Nosema, Wing Deform Virus)
- **Major weakening factor of bee colonies**



**Figure 4**

Source: Casida and Durkin, 2013 doi: 10.1146/annurev-ento-120811-153645

Changes in use of insecticide classes between 1997 and 2010 showing decreases for organophosphates (OPs), methylcarbamates (MCs), and pyrethroids (pyr) and increases for neonicotinoids (neonic) and other compounds. Abbreviations: AChE, acetylcholinesterase; nAChR, nicotinic acetylcholine receptor. Data shown for the years 1997, 2000, 2002, 2005, 2008, and 2010 from T.C. Sparks (personal communication) are similar to those from his coauthored paper (95).

# Uses: **spray, seed treatment, other**

## Spray uses in Europe (70%)



<b>thiamethoxam</b>	potato, nut trees, pome fruit, stone fruit, cucurbits, brassicas, citrus fruits, cotton, vines, salad, herbs, ornamentals, peppers, tobacco, tomato, floriculture crops, tree nursery, flower bulbs, cereals, carrot, sunflower, onions, oilseed rape, cotton,
<b>clothianidin</b>	potato, stone fruit, pome fruit
<b>imidacloprid</b>	ornamentals, potato, pome fruit, hops, vines, stone fruit, tobacco, pepper, flower bulbs, floriculture crops, tree nursery, stone fruit, tomato, almonds, cucurbits, artichoke, beans, brassicas, celery, citrus fruits, hazel, olives, salad, palm trees, peppers, forestry, alfalfa, cereals, strawberry.
<b>acetamiprid</b>	pome fruit, ornamentals, oilseed rape, turnip rape, salad, herbs, stone fruit, maize, potato, tobacco, brassicas, forestry, cucurbits, soft fruits, tomato, peppers, floriculture crops, tree nursery, flower bulbs, citrus fruits, fig, artichoke, clover, lucerne, cotton, strawberry, citrus fruits
<b>thiacloprid</b>	stone fruit, pome fruit, strawberry, oilseed rape, potatoes, cereals, mustard, ornamentals, soft fruits, salad, herbs, nut trees, fennel, asparagus, carrot, brassicas, celeriac, celery, onions, cucurbits, leeks, garlic, shallot, flower bulbs, beans, ornamentals, floriculture crops, tree nursery, sugar beet, fodder beet, hemp, strawberries, sunflower, maize, cotton, alfalfa, olive trees, fig trees,



## Seed treatment uses in Europe (20%)

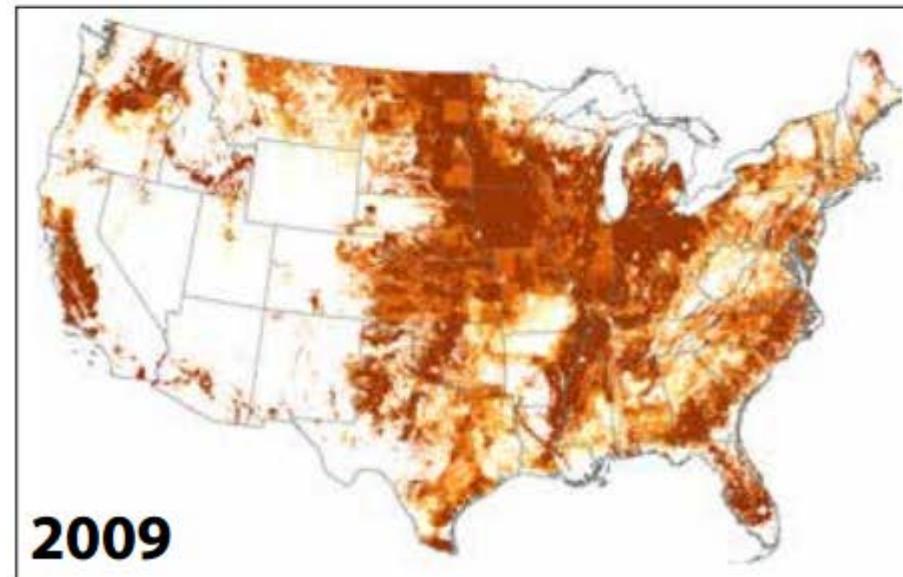
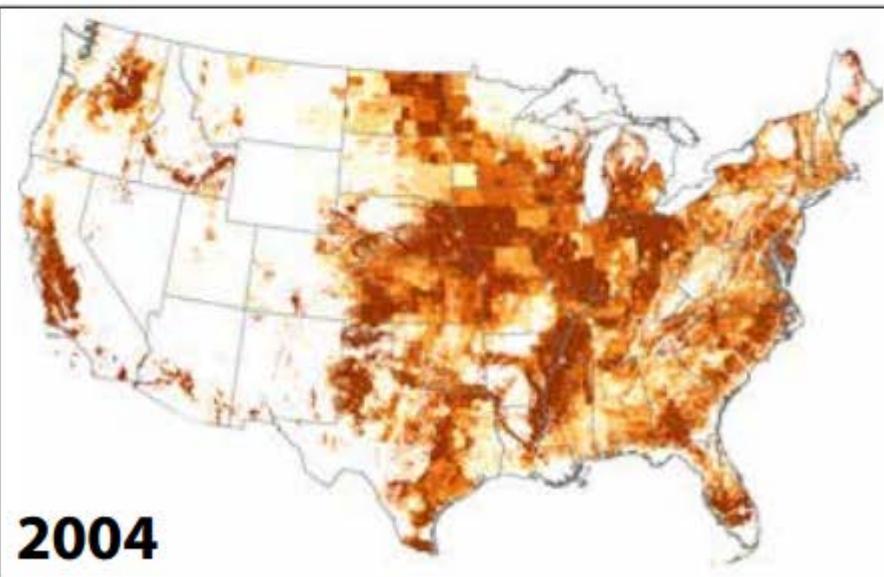
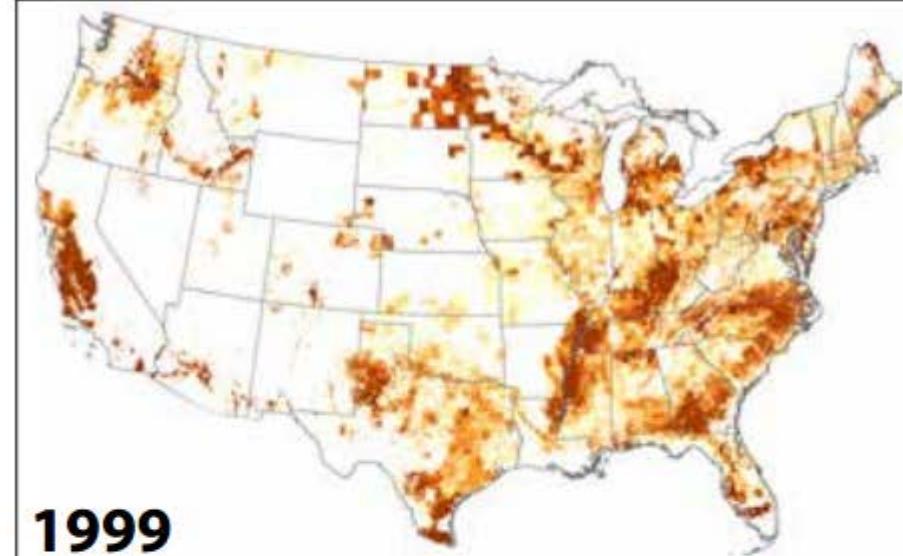
<b>thiamethoxam</b>	beet seeds, cabbage, cauliflower, cotton, kale, lettuce, maize, mustard, peas, potato, oilseed rape, sorghum, sunflower, wheat, barley, rye, oat, triticale
<b>clothianidin</b>	beet seeds, cereals (wheat, barley, oat, rye, triticale), maize, oilseed rape, potato, sunflower
<b>imidacloprid</b>	beet seeds, oat, asparagus, broccoli, cauliflower, barley, bulb crops, corn, lettuce, cabbage, brassicas, hop, leek, linseed, maize, onion, peas, potato, pumpkin seeds, oilseed rape, sunflower, wheat
<b>thiacloprid</b>	maize



## Other uses in Europe

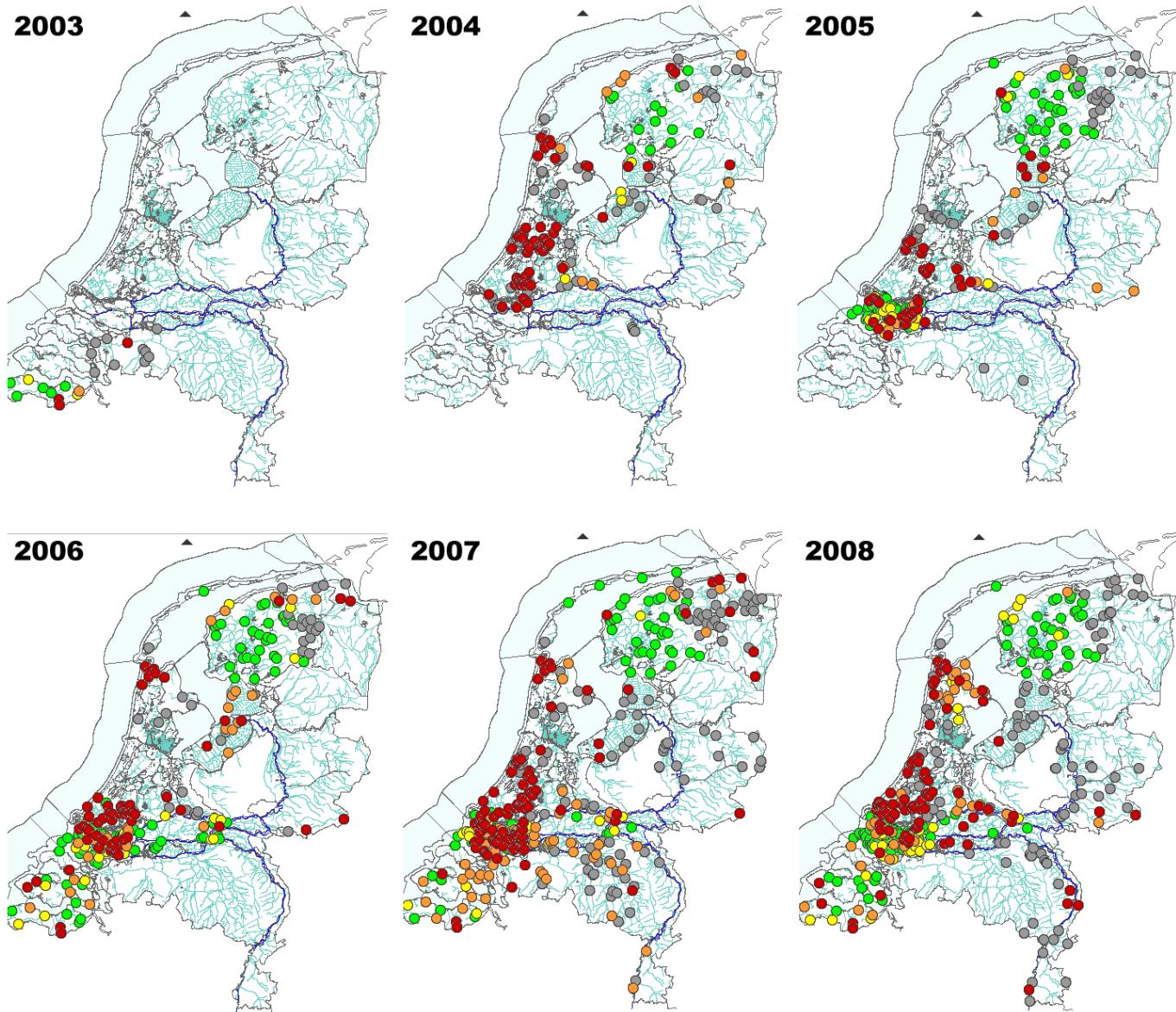
<b>thiamethoxam</b>	cucurbits, beans, brassicas, citrus fruit, salad, herbs, ornamentals, palm trees, peppers, tomato, flower bulbs, potato, house plants, pome fruit, forestry, citrus fruits, peppers,
<b>clothianidin</b>	maize, potato, sorghum, sorghum, poppy
<b>imidacloprid</b>	grassland, hops, salad, herbs, potato, brassicas, chicory–roots, beans, citrus fruits, cucurbits (eggplant, melons, cucumber), palm tree, peppers, tomato, rice, forestry, pome fruit, stone fruit, artichokes, vines, alfalfa, tobacco, olive trees, ornamentals, strawberries, hops,
<b>acetamiprid</b>	non fruiting trees and bushes
<b>thiacloprid</b>	ornamentals, tree nursery

Imidacloprid use on farms. Darker color indicates greater quantity used per square mile.



Source: USGS National Water-Quality Assessment Program Pesticide National Synthesis Project, [http://water.usgs.gov/nawqa/pnsp/usage/maps/compound\\_listing.php](http://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php) (accessed 9/16/13).

Only 1.6 to 20%  
of applied  
neonicotinoid is  
absorbed by the  
growing crop  
(Sur & Stork  
2003)  
**80 to 98.4%**  
**leaches** to soil &  
water!



# Effects on honeybees

- Acute intoxication
- Chronic intoxication
- Sublethal effects
- Synergy effects

# Corn coated with Gaucho® (imidacloprid)



75 gram imidacloprid / ha corn  
100000 seeds / ha

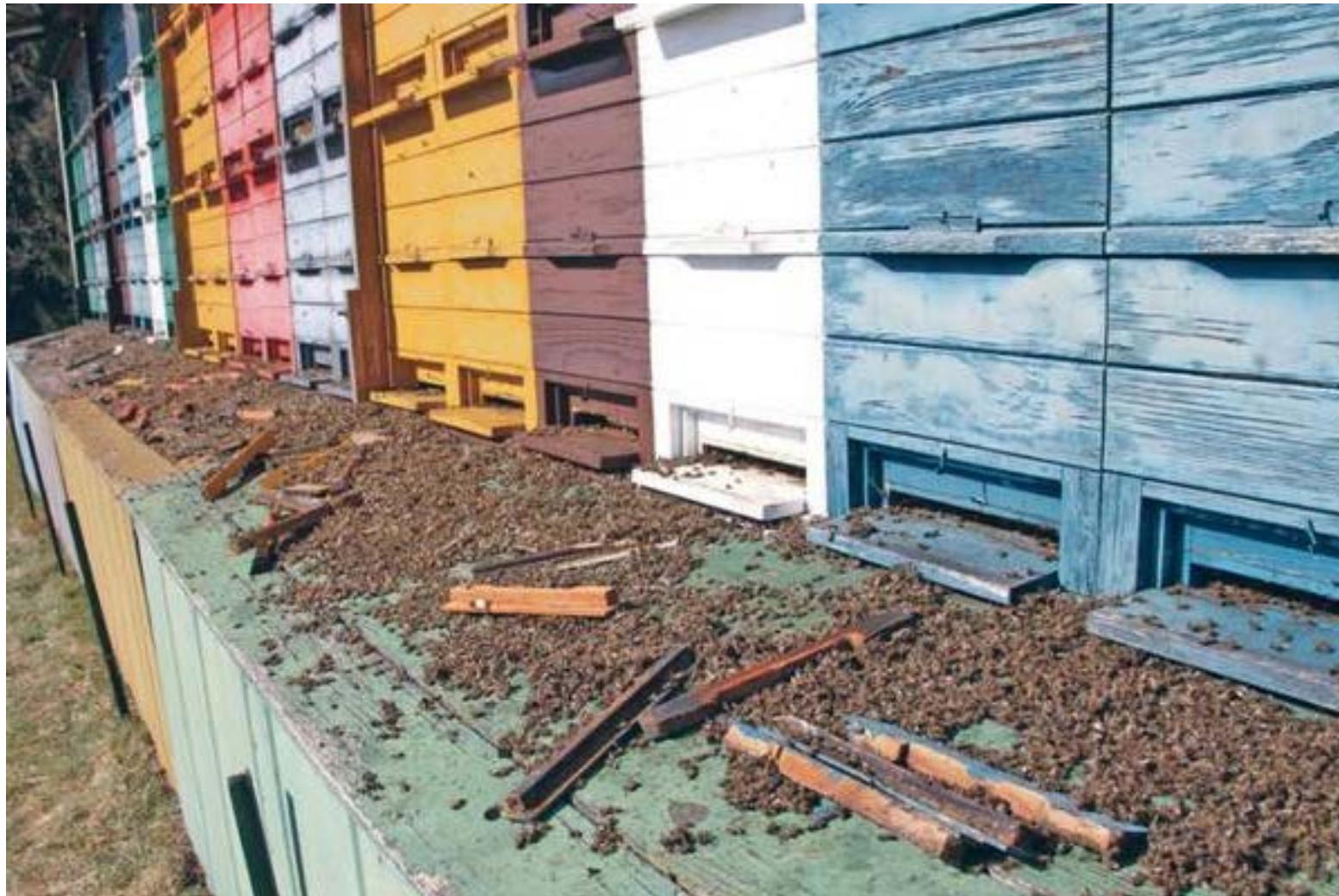
0.00075 gram imidacloprid / seed



3.7 nanogram = LD50 Honeybees

202702 LD50 / corn seed

# Pomurje, Slovenia April 2011, sowing period clothianidin corn



Damage  
2500  
colonies  
lost

> 100  
million  
bees

# Field test in Padua

Deadly dust cloud

< 30 seconds 10m away:  
300 to 4000 ng imidacloprid  
per bee



# What are exposure pathways?

- Treated crops
  - Contact
  - Pollen (delayed consumption!, Bee bread etc.)
  - Nectar (delayed consumption!, honey)
  - Extrafloral nectar
  - Honey-dew (from aphids)
  - Guttation (plant sap excreted by the plant)
  - Dew/rain (waterdrops from the atmosphere)
  - Sweet remains of e.g. sugarbeets, etc.
- Systemic uptake by untreated wild plants and trees on same soil
- Systemic uptake of contaminated water by wild plants and trees
- Spray drift / dust drift to flowering fields
- Direct contact with dust (flying through the dust cloud)
- Foraging on polluted surface water (for drinking and COOLING!)
- Residues in sugar used for sugar syrup supplementary feeding
- Residues in water used by beekeepers to make sugar syrup (violation of drinkingwater norm in NL > 100 ng/liter)
- Can it travel trough the air? On PM2.5? On diessel soot/black carbon? On airosol-water?
- Brabant, NL scandal 2011: Waste-sand from treated Lilly bulbs used for trails in protected nature area
- Etc.....



アブラムシ

# Krupke e.a. 2012 study



**Table 6.** Pesticide concentrations found in unplanted fields near apiary during planting period in 2011, all concentrations shown are expressed as parts per billion.<sup>1</sup>

SAMPLE TYPE	Sample wt. (g)	THIAMETHOXAM LOD = 1.0	CLOTHIANIDIN LOD = 1.0	METOLACHLOR LOD = 0.5	ATRAZINE LOD = 0.2	AZOXYSTROBIN LOD = 0.2	COUMAPHOS LOD = 1.0
Soil, unplanted field 1, Soybeans 2010 (2 samples)	5.15, 5.01	ND	6.0±0.3	1014±14	771±170	0.2±0.1	ND
Soil, unplanted field 2, Soybeans 2010 (2 samples)	5.28, 5.43	ND	8.9±0.1	8.3±0.7	160±15	26±17	ND
Dandelions near maize field	2.96	ND	1.4	49	677	ND	ND
Dandelions near maize field	3.81	1.6	5.9	64	1133	ND	ND
Dandelions near maize field	4.51	1.3	3.1	28	522	ND	ND
Dandelions near maize field	4.05	2.9	1.1	60	269	ND	ND
Dandelions near maize field	3.10	1.1	1.6	5.7	125	ND	ND
Dandelions near maize field	3.44	ND	9.4	295	1004	ND	ND
Dandelion, CAES (non-agricultural area)	3.93	ND	ND	ND	0.3	ND	ND

When two aliquots of the same sample were analyzed the results are expressed as ± the standard deviation of the two analyses.

<sup>1</sup>ND = Not detected.

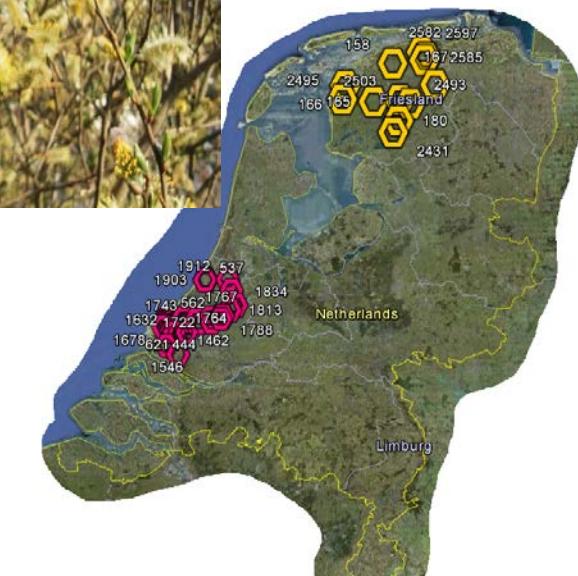
doi:10.1371/journal.pone.0029268.t006

Krupke e.a. 2012. Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. <http://dx.doi.org/10.1371/journal.pone.0029268>



# Do trees translocate imidacloprid from surface water into pollen & nectar?

*In NL we took samples from willow trees (*Salix*) in polluted areas*





**Exposure  
via guttation?**

**Via water  
for drinking  
and cooling?**



**Figure 9.1** The spreading of water droplets by nurse bees when a colony's broodnest is threatened by overheating. Spreading water, combined with fanning the wings to expel hot air from the hive, causes evaporative cooling of the brood combs. After Park 1925.

**T. Seeley, The wisdom of the hive  
Chapter 9 regulation of water collection**

# Chronic toxicity imidacloprid for bumblebees



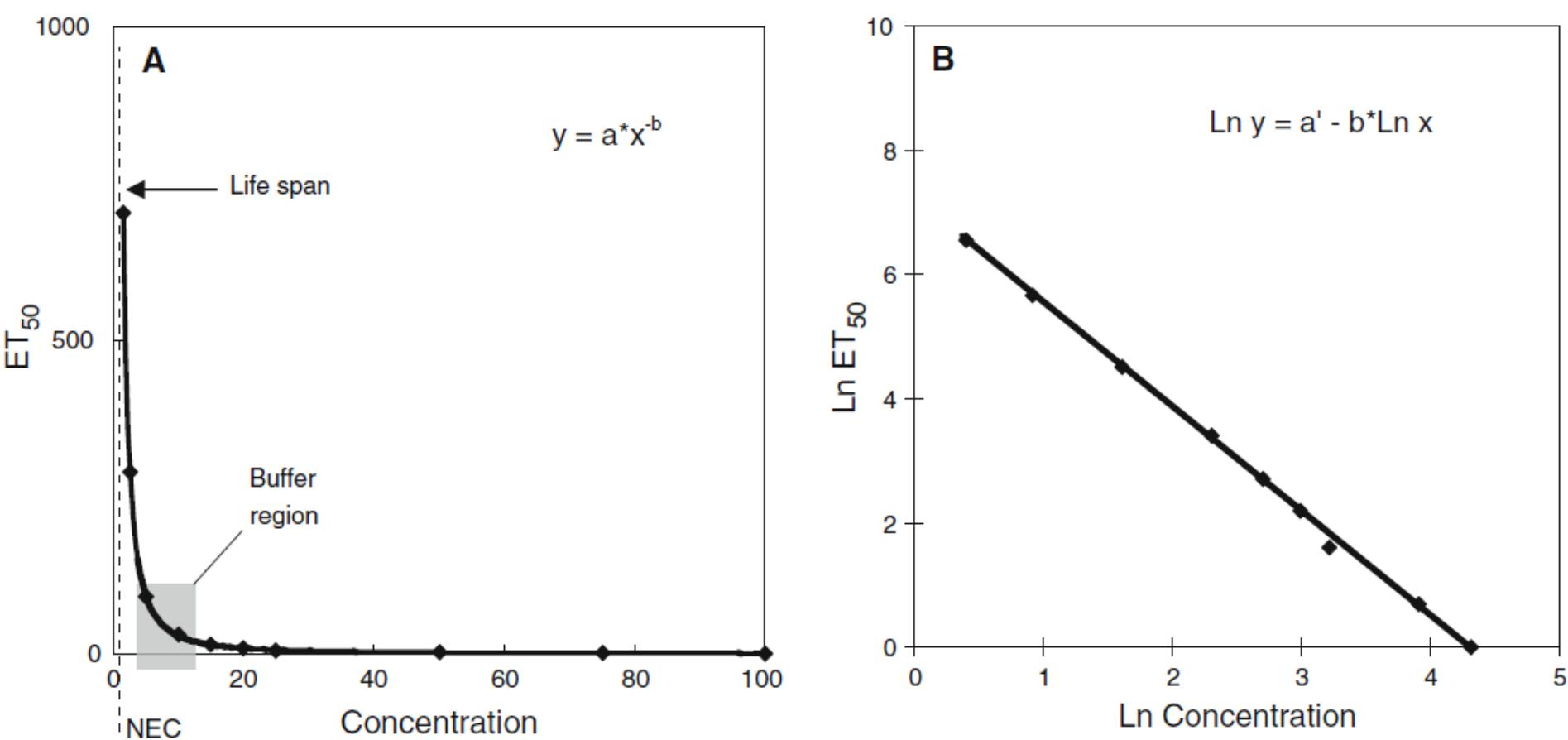
Micro colonies fed with imidacloprid at

- 200 ppm 100% mortality few hours
- 20 ppm 100% mortality 14 days
- 2 ppm 100% mortality 28 days
- 0.2 ppm 100% mortality 49 days,
- 20 ppb 15% mortality (77 days)
- 10 ppb 0% mortality (77 days)

NOEC reproduction <2.5 ppb

<http://dx.doi.org/10.1007/s10646-009-0406-2> Mommaerts e.a. 2010

**Mismatch with EU regulatory 10 day test!!**



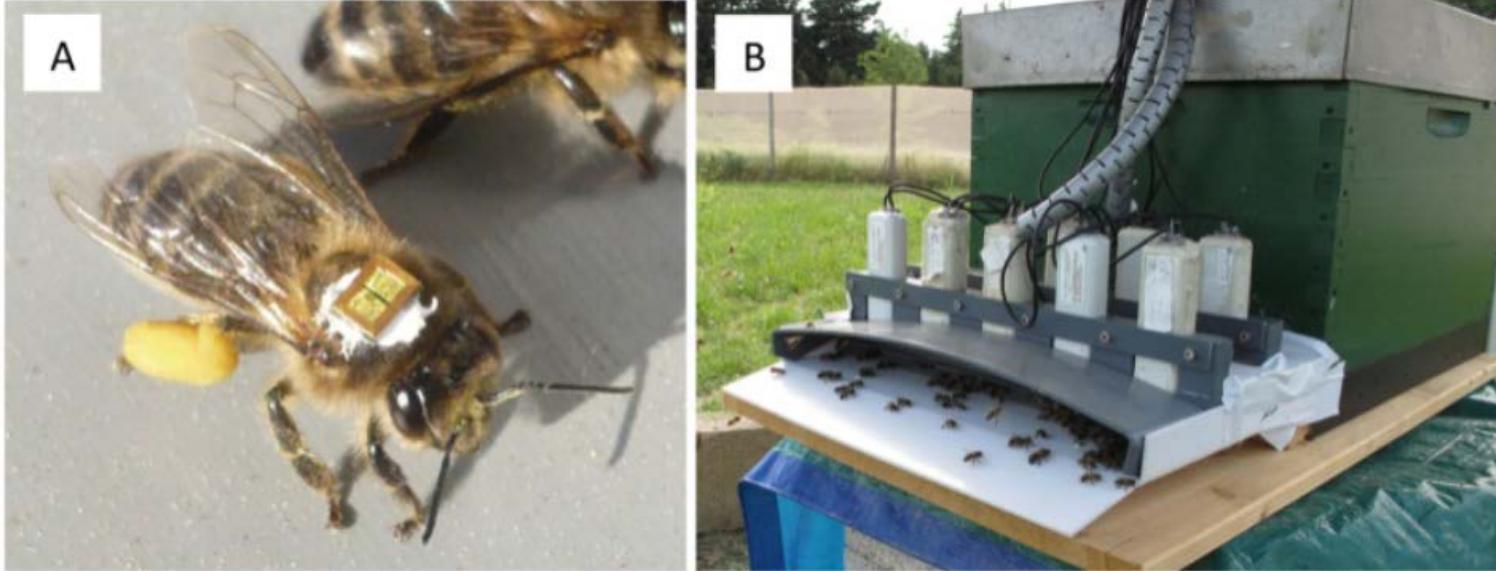
Relationship between neonicotinoid concentration and time to 50% effect ( $ET_{50}$ ) in the organisms exposed follows a hyperbolic curve asymptotic on the y axis; in reality, this asymptote is determined by the no-effect concentration (NEC), while the upper limit of the curve is determined by the life span of the organism. (Sanchez-Bayo, 2009)

# Sublethal effects

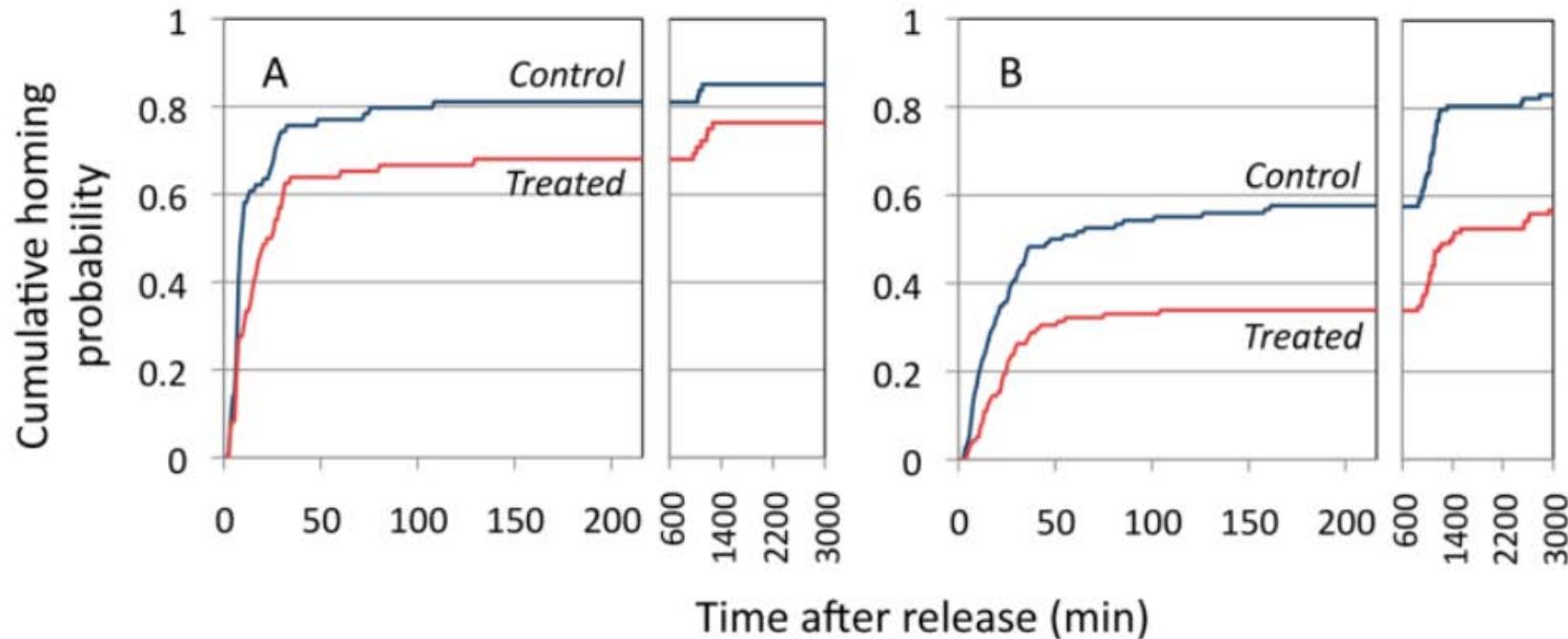
- Foraging behaviour / navigation
- Task differentiation in the hive
- Grooming
- Immune system
- Brood
- Larval development
- etc/.

Henry ea.  
2012  
*Science*  
studie

## Radio ID chips



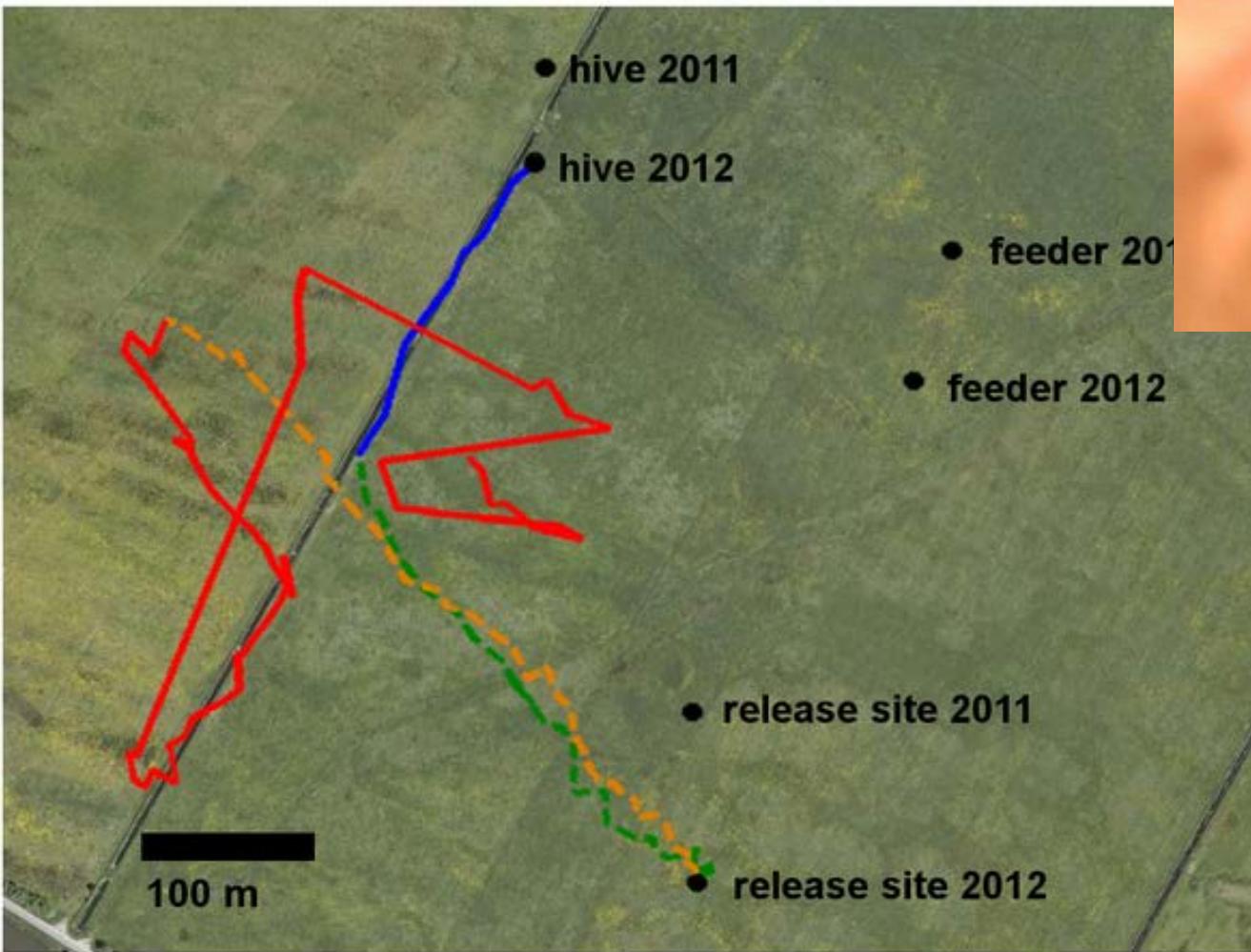
Dose per bee: 1.34 ng thiamethoxam in 20- $\mu$ l sucrose solution



A: familiar location; B random location

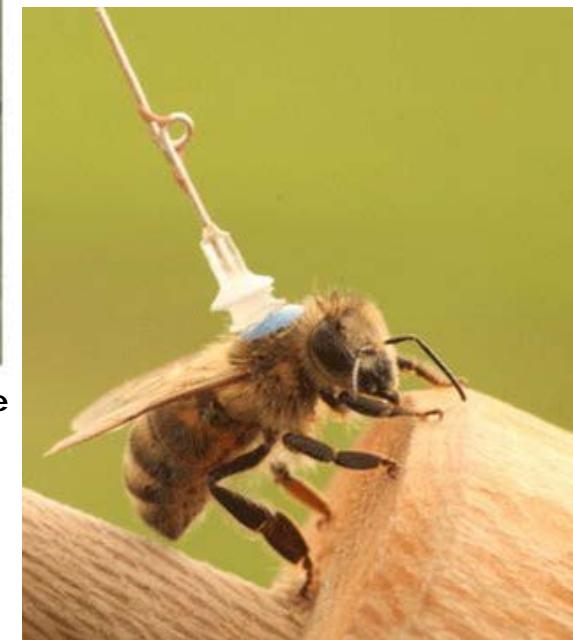
<http://dx.doi.org/10.1126/science.1215039>

# Radar-tracking experiment Randolph Menzel: Bees exposed to neonicotinoids loose orientation



**Yellow-Red  
Thiacloprid-bees**

**Green-Blue  
Control bees**



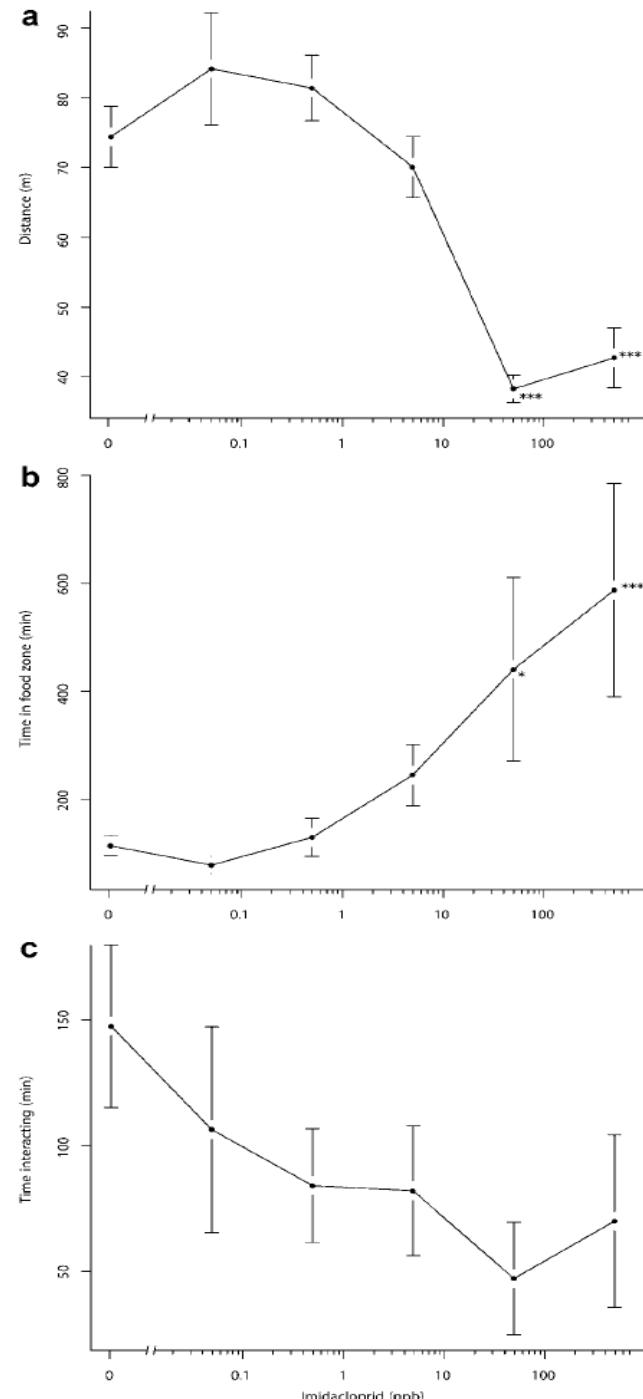
Fischer J, Müller T, Spatz A-K, Greggers U, et al. (2014) Neonicotinoids Interfere with Specific Components of Navigation in Honeybees. PLoS ONE 9(3): e91364. doi:10.1371/journal.pone.0091364

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0091364>

# Using video-tracking to assess sublethal effects of pesticides on honey bees

- Bees exposed to 0.05, 0.5, 5.0, 50, and 500 ppb imidacloprid in a sugar agar cube
- significant reduction in distance moved at 50 and 500 ppb imidacloprid ( $p < 0.001$ ).
- Obvious biological gradient

Figure: a=distance, b=time in foodzone, c=time interacting  
<http://dx.doi.org/10.1002/etc.1830>



# Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production

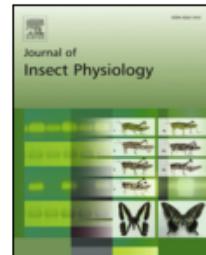


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Growing evidence for declines in bee populations has caused great concern due to the valuable ecosystem services they provide. Neonicotinoid insecticides have been implicated in these declines as they occur at trace levels in the nectar and pollen of crop plants. We exposed colonies of the bumble bee *Bombus terrestris* in the lab to field-realistic levels of the neonicotinoid imidacloprid, then allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered an 85% reduction in production of new queens compared to control colonies. Given the scale of use of neonicotinoids, we suggest that they may be having a considerable negative impact on wild bumble bee populations across the developed world.



## The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.)



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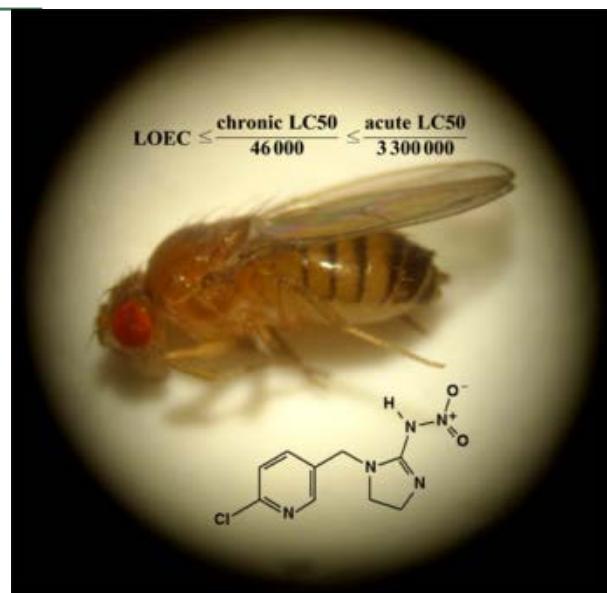
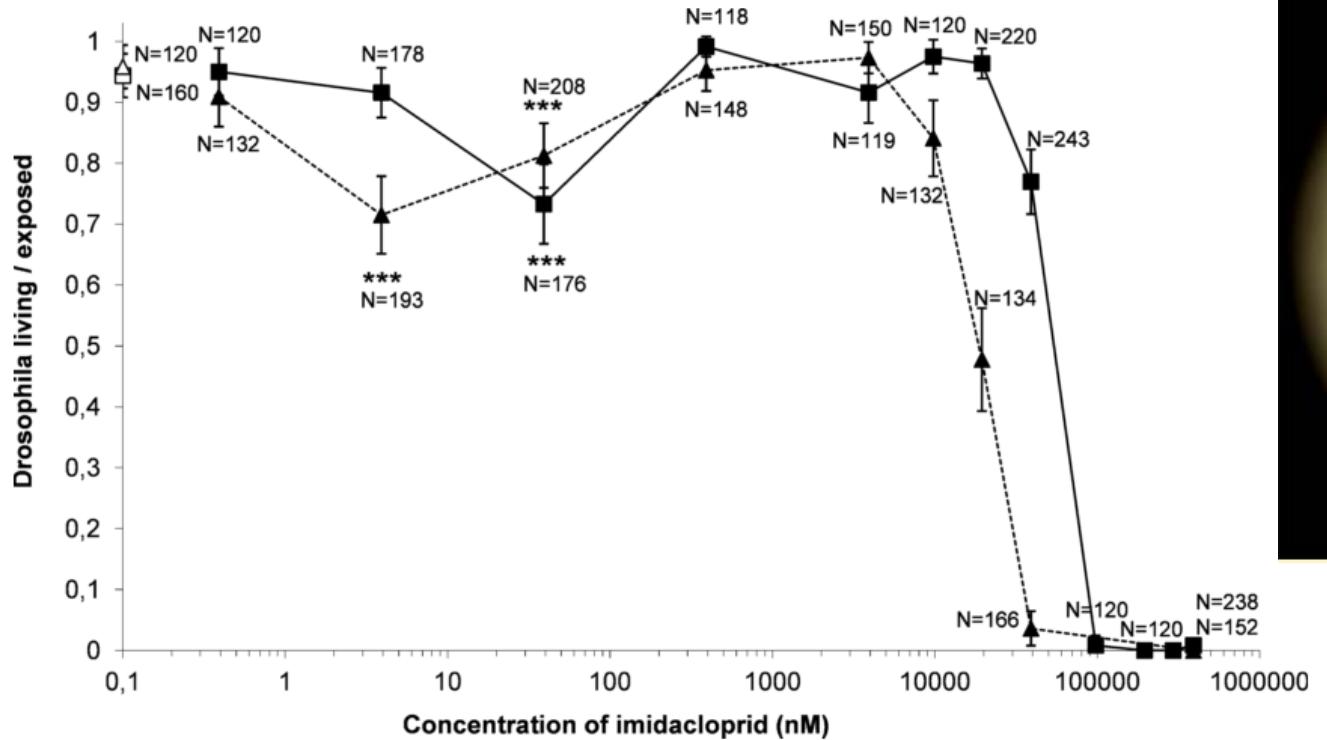
Antimicrobial activity

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### ABSTRACT

A strong immune defense is vital for honey bee health and colony survival. This defense can be weakened by environmental factors that may render honey bees more vulnerable to parasites and pathogens. Honey bees are frequently exposed to neonicotinoid pesticides, which are being discussed as one of the stress factors that may lead to colony failure. We investigated the sublethal effects of the neonicotinoids thiacloprid, imidacloprid, and clothianidin on individual immunity, by studying three major aspects of immunocompetence in worker bees: total hemocyte number, encapsulation response, and antimicrobial activity of the hemolymph. In laboratory experiments, we found a strong impact of all three neonicotinoids. Thiacloprid (24 h oral exposure, 200 µg/l or 2000 µg/l) and imidacloprid (1 µg/l or 10 µg/l) reduced hemocyte density, encapsulation response, and antimicrobial activity even at field realistic concentrations. Clothianidin had an effect on these immune parameters only at higher than field realistic concentrations (50–200 µg/l). These results suggest that neonicotinoids affect the individual immunocompetence of honey bees, possibly leading to an impaired disease resistance capacity.

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**Figure 1.** Average ratios of surviving drosophila after chronic exposure. Data are reported for adult flies: males (■) and females (▲). Concentrations of imidacloprid ranged from 0.391 nM to 0.391 mM. Ratios are given from the number of flies still alive, after chronic exposure to imidacloprid (8 days), over the number of flies exposed (see the Experimental Section). N: number of flies. Bars corresponding to 95% confidence intervals (CI95) are reported for each data point. Ratios for controls are indicated on the vertical axis: males (□) and females (△). Significant differences are indicated in the low concentration range only (\*\*\* when  $p < 0.001$ ).

Lu e.a. 2012 Harvard University- 5 Apr 2012

# In situ replication of honey bee colony collapse disorder

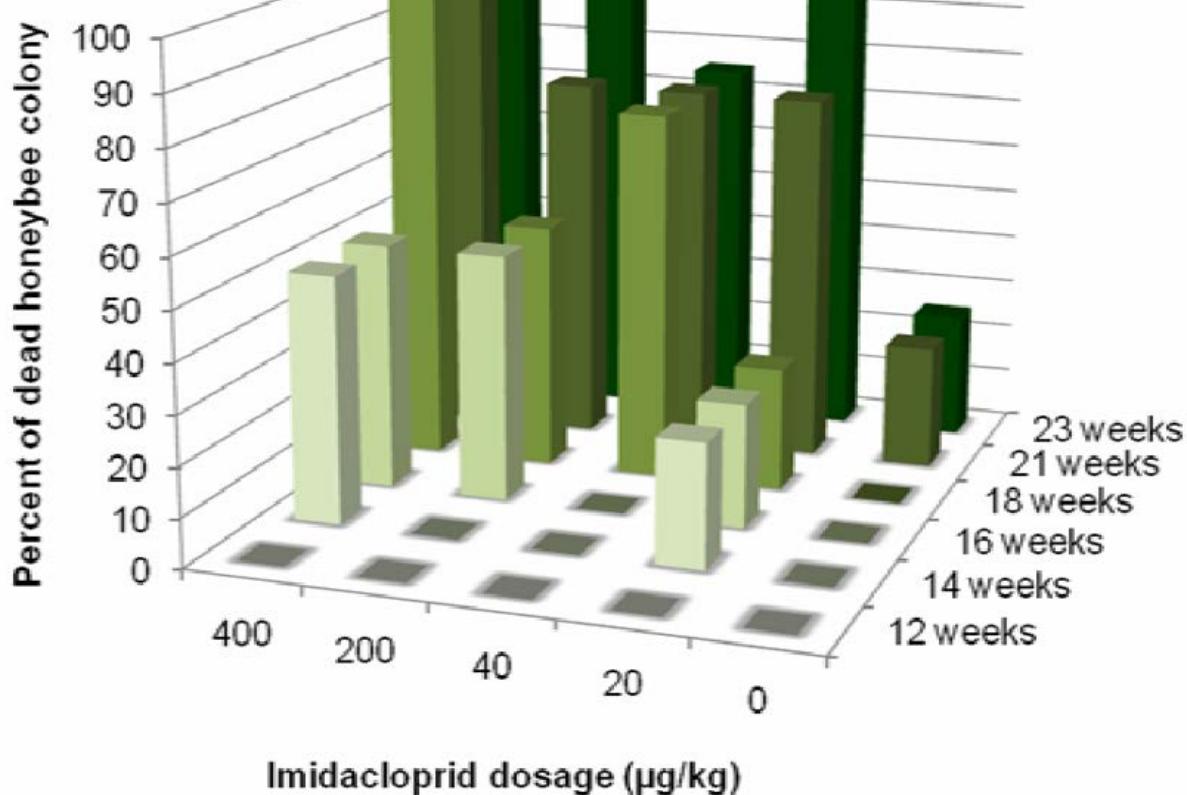


Figure 3. Dead hive (ID<sup>#</sup> 4-4) treated with 20  $\mu\text{g}/\text{kg}$  of imidacloprid which shows the abundance of stored honey and some pollen, but no sealed brood or honey bees. Photo was taken on February 24<sup>th</sup>, 2011.

<http://goo.gl/1a0Aa>

# Yamada 2012

D= clothianidin 10, 50, 100x diluted  
S= dinotefuran 10, 50, 100x diluted  
Compered to recommended field rate

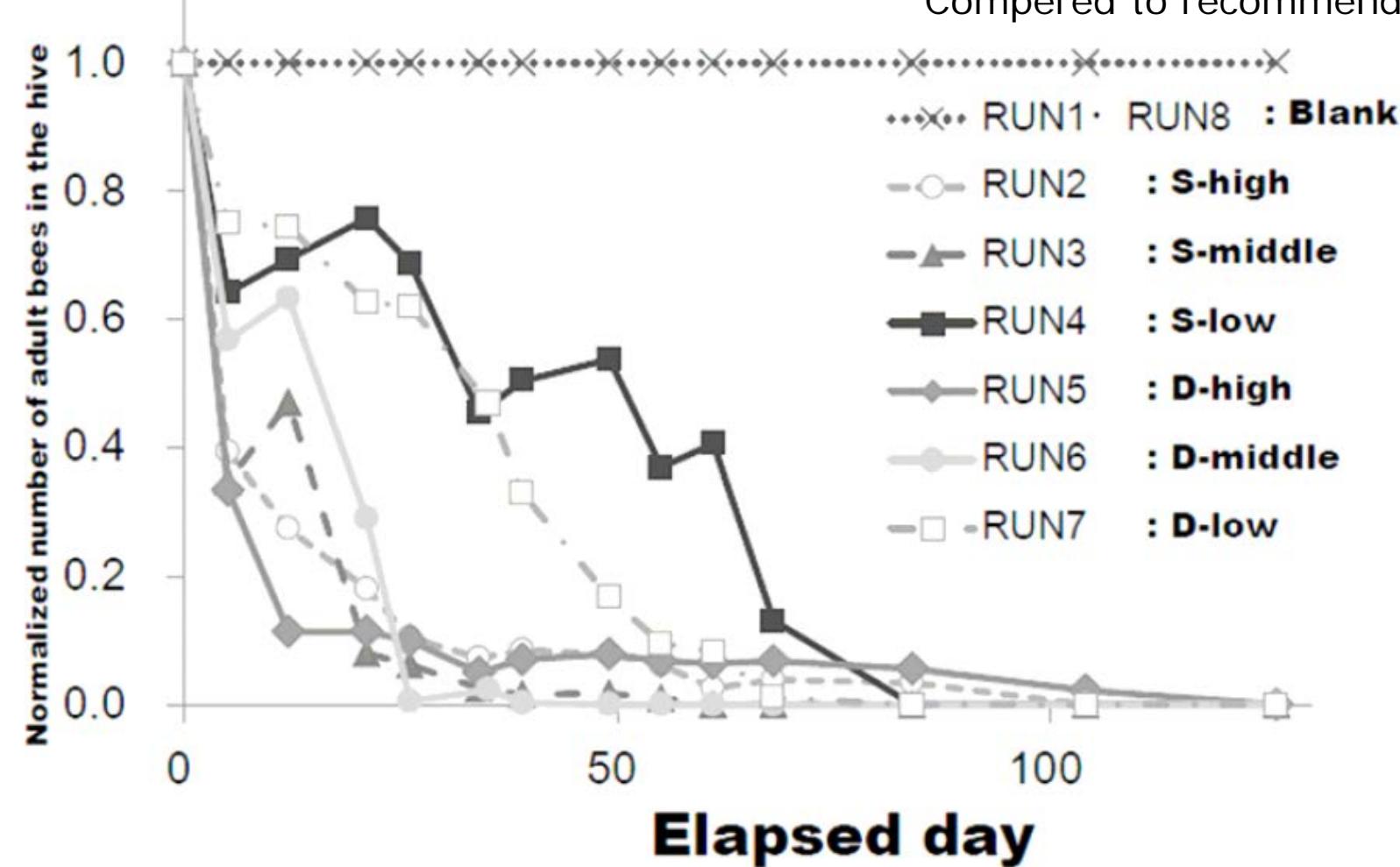
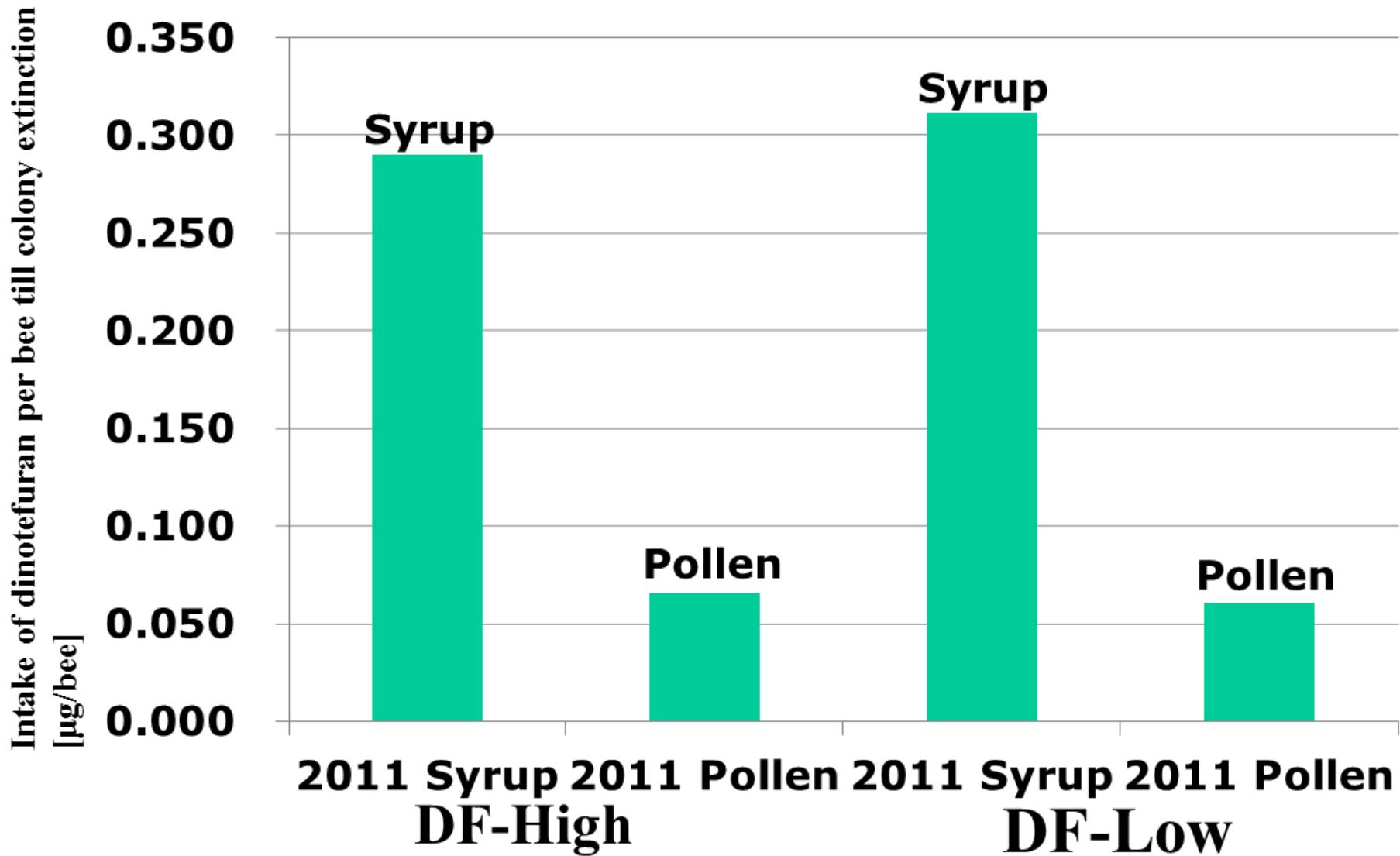


Figure 1 Normalized number of adult bees in the hive with the elapsed days

# Required dose of neonicotinoid per bee till colony extinction



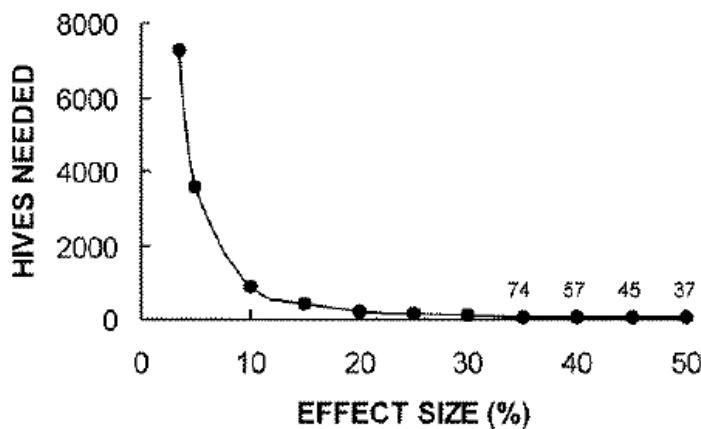
(Yamada, 2013)

# Problems with field studies

- Some field studies have  $n=1$  (Schmuck 2001)
- Statistical power:
  - 75 hives needed to detect 35% drop in colony performance
  - >2000 hives to detect 5% drop in colony performance
- Many flaws in experimental set-up of field studies used for authorization. Transport of nectar & pollen ≠ exposure!
- Many field studies turned out to have a hidden sponsor: Bayer Cropscience – Flawed-by-design!
- Example: Cutler and Dupree 2007 study
- In authorization protocols field studies (even flawed ones and  $n=1$  ones) get more weight than lab studies, but from a scientific point of view lab studies are more reliable!

# Toxicity tests available for risk assessment

## Field test problems



The number of honey bee hives needed to detect specified increases in mortality rate (percentage effect size) using dead bee traps.  
A field-scale experiment using conventional dead bee traps would require 74 hives to detect an effect of 35% with a significance level of 0.05.

(bron: Robert Luttik, 2013)

# **Plurality and uncertainty in risk assessment: lessons learned**

- **Diversity of the knowledge base:**
  - It must be based on the full spectrum of available scientific knowledge;
- **Robustness of the knowledge claims**
  - Include uncertainty, dissent and criticism in the analysis, synthesis and assessments;
- Make thorough **Knowledge Quality Assessment the key task in the science policy interface** and develop a joint language to communicate limitations to our knowledge and understanding clearly and transparently
- Make use of **information of non-scientific sources** (local knowledge)
  - But scrutinize this information and be clear on its status;
- **Clarify values, stakes and vested interests** that play a role in research and in the political and socioeconomic context within which the research is embedded.

(Maxim and van der Sluijs, 2007, 2012)

If time allows...

A few more concerns ...

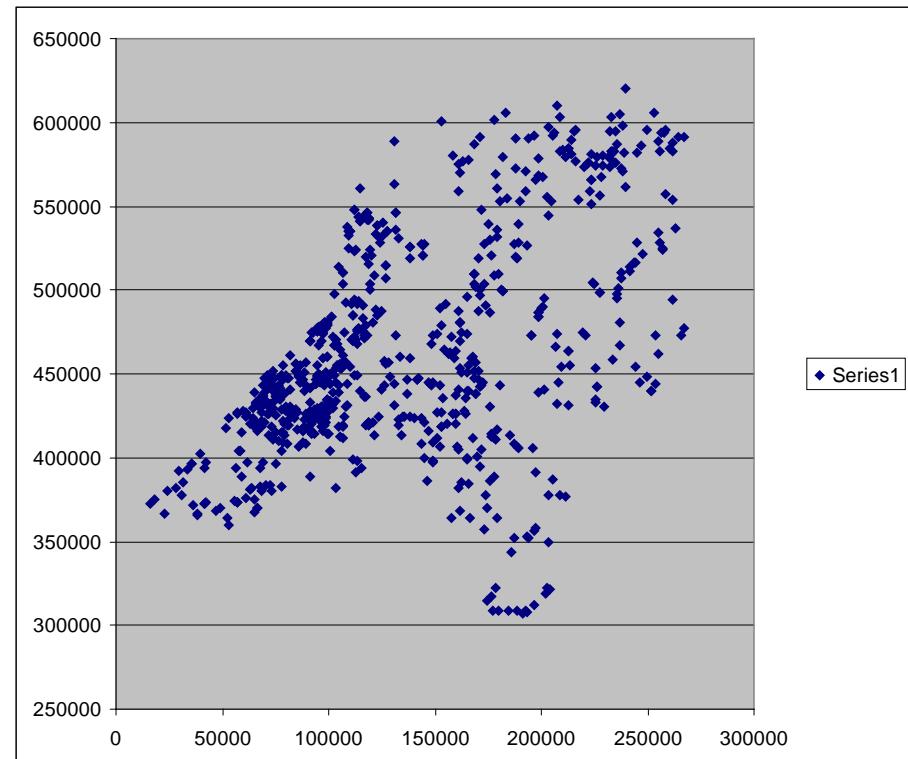
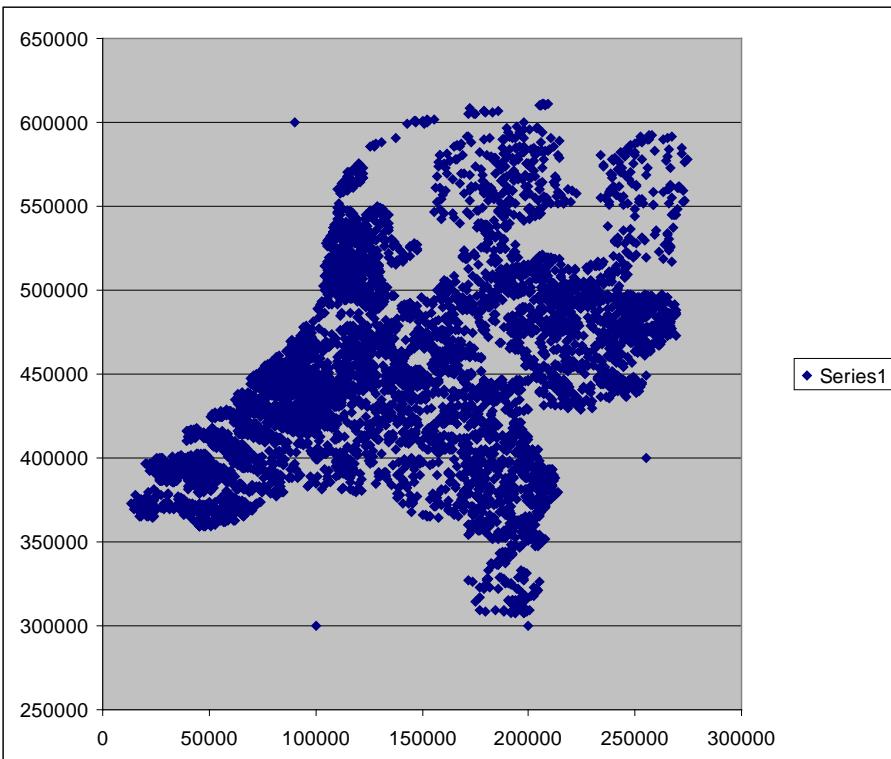
# ECOSYSTEM FUNCTIONING

*Impacts cascade through the ecosystem weakening its stability*

- Persistence & solubility led to large scale contamination of:
  - soils and sediments
  - ground and surface water
  - treated and non-treated vegetation
- Multiple routes chronic & acute exposure non-target species
- Toxicity increased by the duration of exposure
- Effects lethal & sublethal: behaviour, immune suppression, reproduction
- Levels resulting from authorized uses frequently exceed 'lowest observed adverse effect concentrations' for wide range of non-target species.

# Macro-invertebrate decline in Dutch surface waters polluted with imidacloprid

- Species abundance data: 7381 locations (left)
- Imidacloprid database: 801 locations (right)
- Years 1998, 2003-2009 pooled
- 18898 points with IMI data within 1 km radius & < 160 days
- 4009 species from 92 orders included

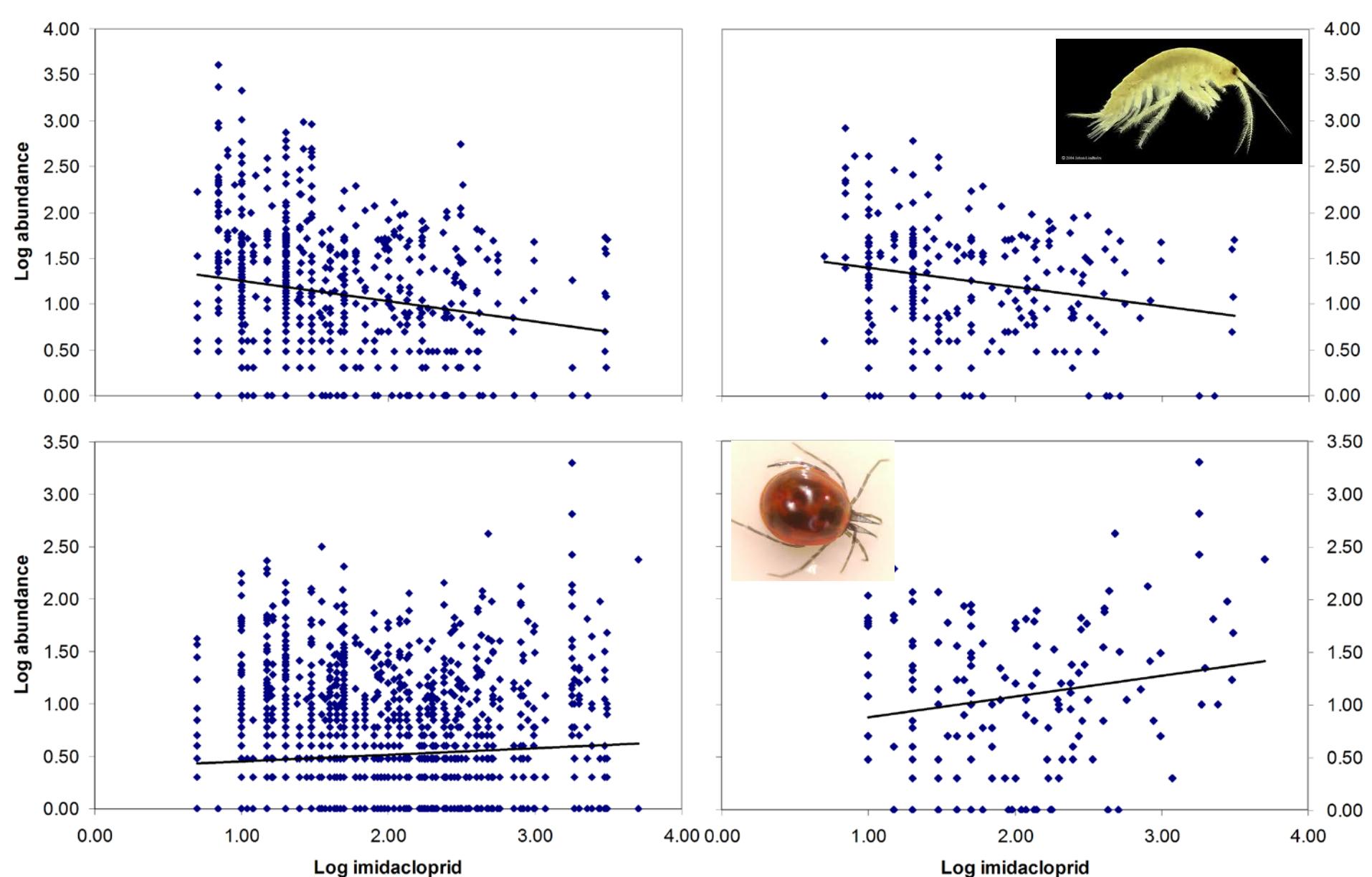


# **Significant negative relationship between species abundance and imidacloprid concentration found for:**

- All orders pooled
- Amphipoda (crustaceans)
- Diptera (true flies)
- Ephemeroptera (mayflies)
- Isopoda (crustaceans)
- Odonata (dragonflies & damselflies)
- Basommatophora (snails)
- All macro invertebrates pooled



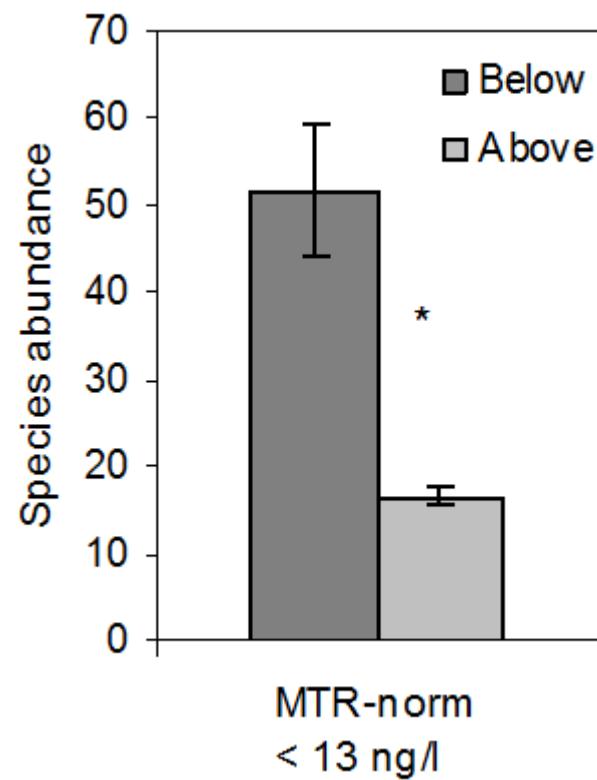
For one order we found significant positive relation: Actinedida

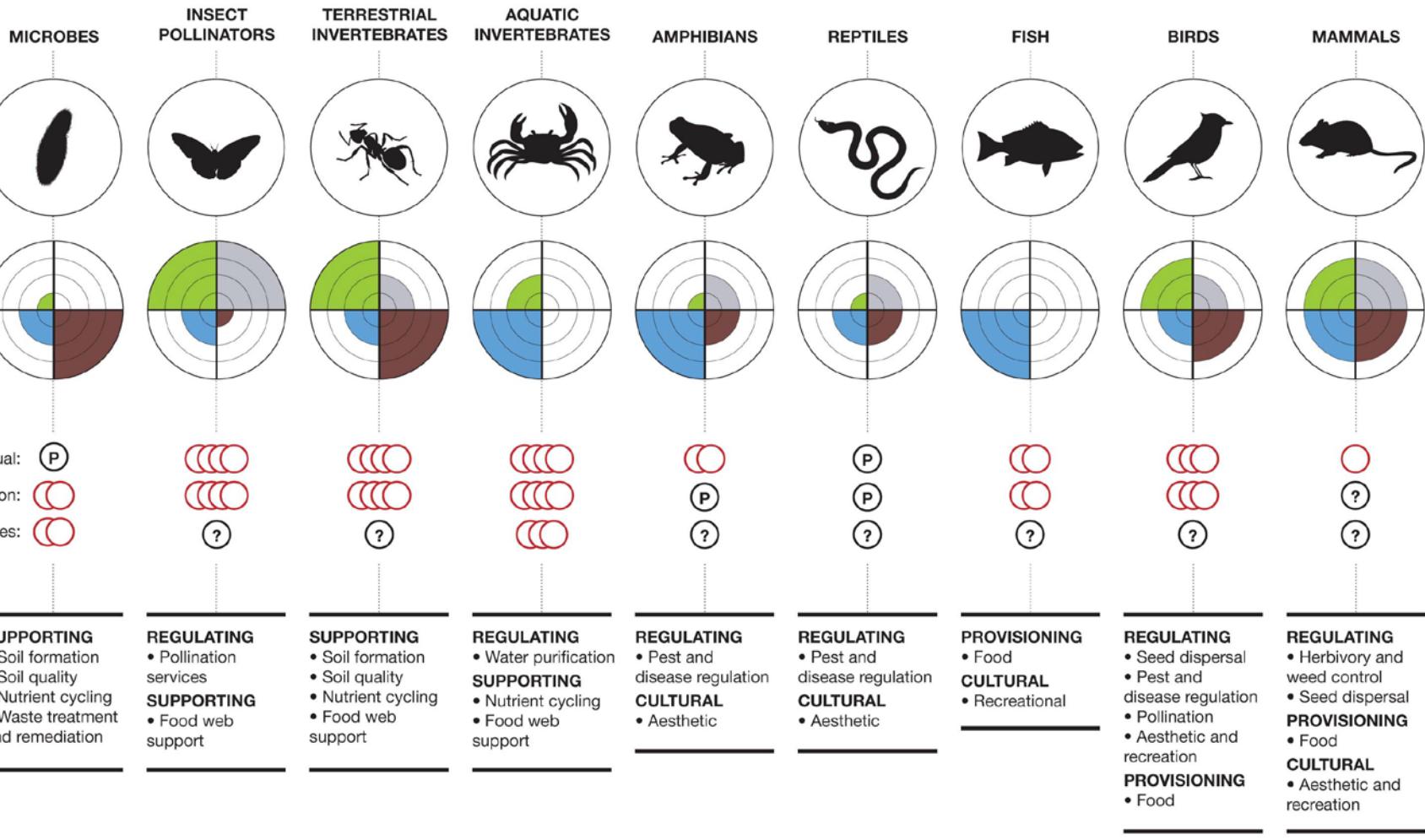


log10 imidacloprid concentration (ng/l) versus log10 macro-invertebrate species abundance in surface water for a) *Amphipoda*, b) its most abundant species *Gammarus tigrinus*, c) *Actinedida* and d) its most abundant species *Limnesia undulata*.

# Findings on aquatic ecosystems

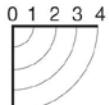
- 45% of all samples ( $n=9037$ ) on 801 locations: imidacloprid exceeds MTR ( $>13 \text{ ng/l}$ )
- 70% reduction in macrofauna abundance in polluted water
- Permanent leaching of Imidacloprid year round from fields to surface water
- Meeting MTR requires reduction of use by at least 90%





#### \*EXPOSURE

- 0: No route of exposure
- 1: Potential route of exposure assumed negligible
- 2: Relevant route of exposure low
- 3: Relevant route of exposure moderate
- 4: Relevant route of exposure high



- Plants
- Air
- Soil
- Water

#### †ECOTOXICOLOGICAL EFFECT

- 1: Potential effects assumed negligible under normal exposure conditions
- 2: Evidence effects can occur but at high doses or after prolonged exposure
- 3: Evidence effects can occur at moderate doses
- 4: Evidence effects can occur at low doses or after acute exposure
- ? Unknown: in situations where no judgement could be made because of lack of evidence, e.g. data unavailable
- P Probable: no accurate judgement could be made due to incomplete evidence, but data suggests a potential effect level above (1)

doi:10.1038/nature13531

# Declines in insectivorous birds are associated with high neonicotinoid concentrations

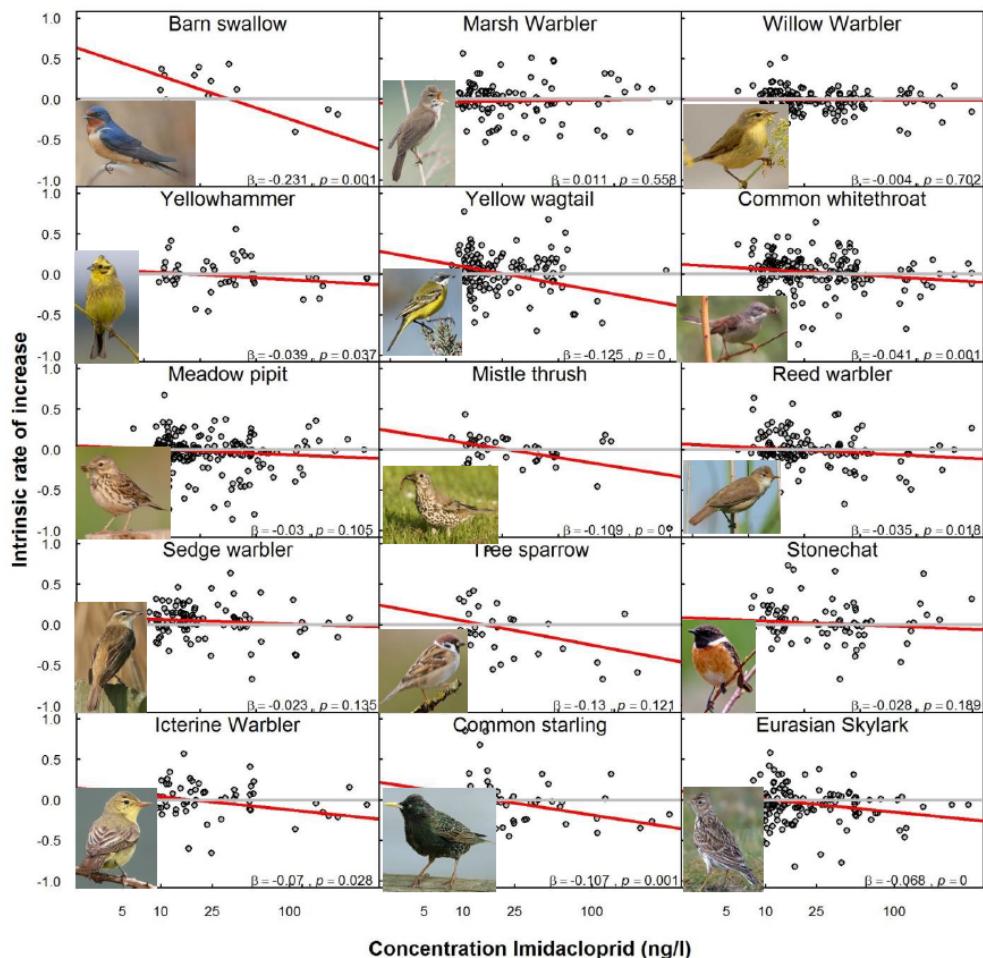
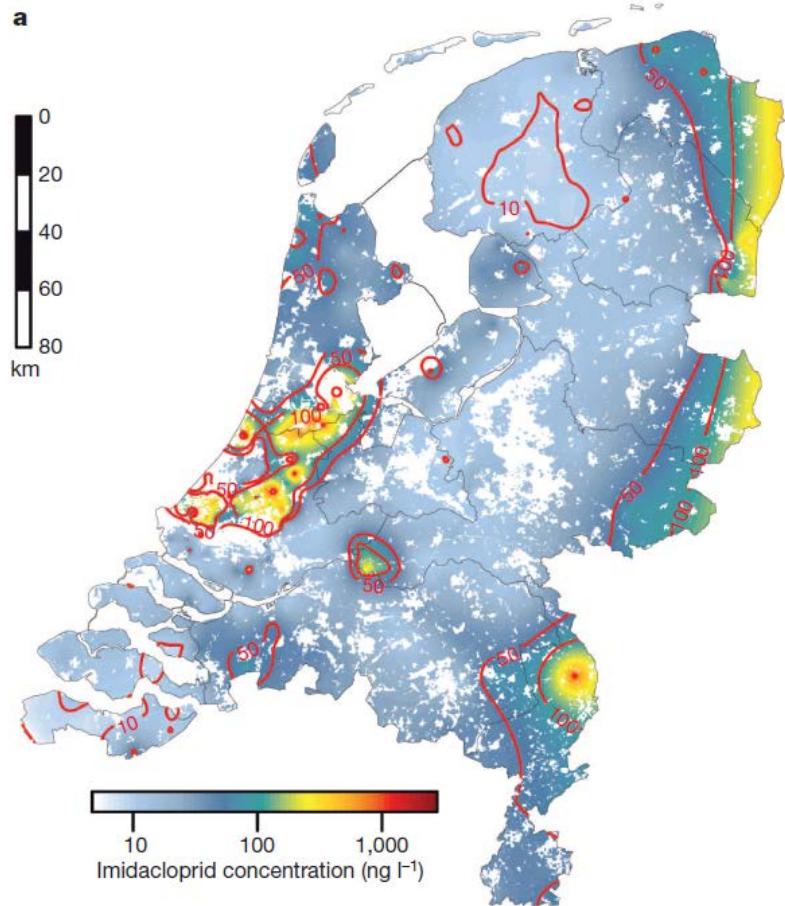
Caspar A. Hallmann<sup>1,2</sup>, Ruud P. B. Foppen<sup>2,3</sup>, Chris A. M. van Turnhout<sup>2</sup>, Hans de Kroon<sup>1</sup> & Eelke Jongejans<sup>1</sup>

Figure 2 | Comparison of the effect of agricultural land-use changes and the effect of imidacloprid on bird population trends. **a**, The marginal variance

# Risks for humans?

- Neonicotinoids are not less toxic than organophosphates
- Subacute poisoning of humans in Japan
- ADI norms outdated (IMI 0.06 mg/kg = knowledge of 2005)
- Japanese research Kimura-Kuroda 2012: damage to developing brain in rats at 1173x lower dose imidacloprid than the thyroid effect on which present ADI is based.
- Autism, Schizophrenia, ADHD??
- invivo versus invitro
- Parkinson (?)

<http://www.actbeyondtrust.org/wp-content/uploads/2012/10/kuroda.pdf>

# Nicotine-Like Effects of the Neonicotinoid Insecticides Acetamiprid and Imidacloprid on Cerebellar Neurons from Neonatal Rats

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## Abstract

**Background:** Acetamiprid (ACE) and imidacloprid (IMI) belong to a new, widely used class of pesticide, the neonicotinoids. With similar chemical structures to nicotine, neonicotinoids also share agonist activity at nicotinic acetylcholine receptors (nAChRs). Although their toxicities against insects are well established, their precise effects on mammalian nAChRs remain to be elucidated. Because of the importance of nAChRs for mammalian brain function, especially brain development, detailed investigation of the neonicotinoids is needed to protect the health of human children. We aimed to determine the effects of neonicotinoids on the nAChRs of developing mammalian neurons and compare their effects with nicotine, a neurotoxin of brain development.

**Methodology/Principal Findings:** Primary cultures of cerebellar neurons from neonatal rats allow for examinations of the developmental neurotoxicity of chemicals because the various stages of neurodevelopment—including proliferation, migration, differentiation, and morphological and functional maturation—can be observed *in vitro*. Using these cultures, an excitatory Ca<sup>2+</sup>-influx assay was employed as an indicator of neural physiological activity. Significant excitatory Ca<sup>2+</sup> influxes were evoked by ACE, IMI, and nicotine at concentrations greater than 1 μM in small neurons in cerebellar cultures that expressed the mRNA of the α3, α4, and α7 nAChR subunits. The firing patterns, proportion of excited neurons, and peak excitatory Ca<sup>2+</sup> influxes induced by ACE and IMI showed differences from those induced by nicotine. However, ACE and IMI had greater effects on mammalian neurons than those previously reported in binding assay studies. Furthermore, the effects of the neonicotinoids were significantly inhibited by the nAChR antagonists mecamylamine, α-bungarotoxin, and dihydro-β-erythroidine.

**Conclusions/Significance:** This study is the first to show that ACE, IMI, and nicotine exert similar excitatory effects on mammalian nAChRs at concentrations greater than 1 μM. Therefore, the neonicotinoids may adversely affect human health, especially the developing brain.

Research by dr. Kumiko Taira

# Subacute neonicotinoid poisoning humans



MRL Imidacloprid  
5 mg/kg (berries)  
= **5000 ppb !!!**  
1 mg/kg (citrus)

1111 patients 2006/2007; 549 high fruit & tea diet

- Most sensitive group: non-smoking females
- Trembling fingers
- Abnormal ECG (Heartrate abnormality tachycardia >100, bradycardia <60)
- Loss of memory
- Headache, shoulder pain, chest pain, muscle crump
- **6-chloronicotinic acid (6CNA) in urine**