Beecome, Louvain la Neuve 9 Nov 2013

Neonicotinoids
risks to ecosystems and humans

Dr. Jeroen P. van der Sluijs, Utrecht University

IUCN CEM

SSC Species Survival Commission

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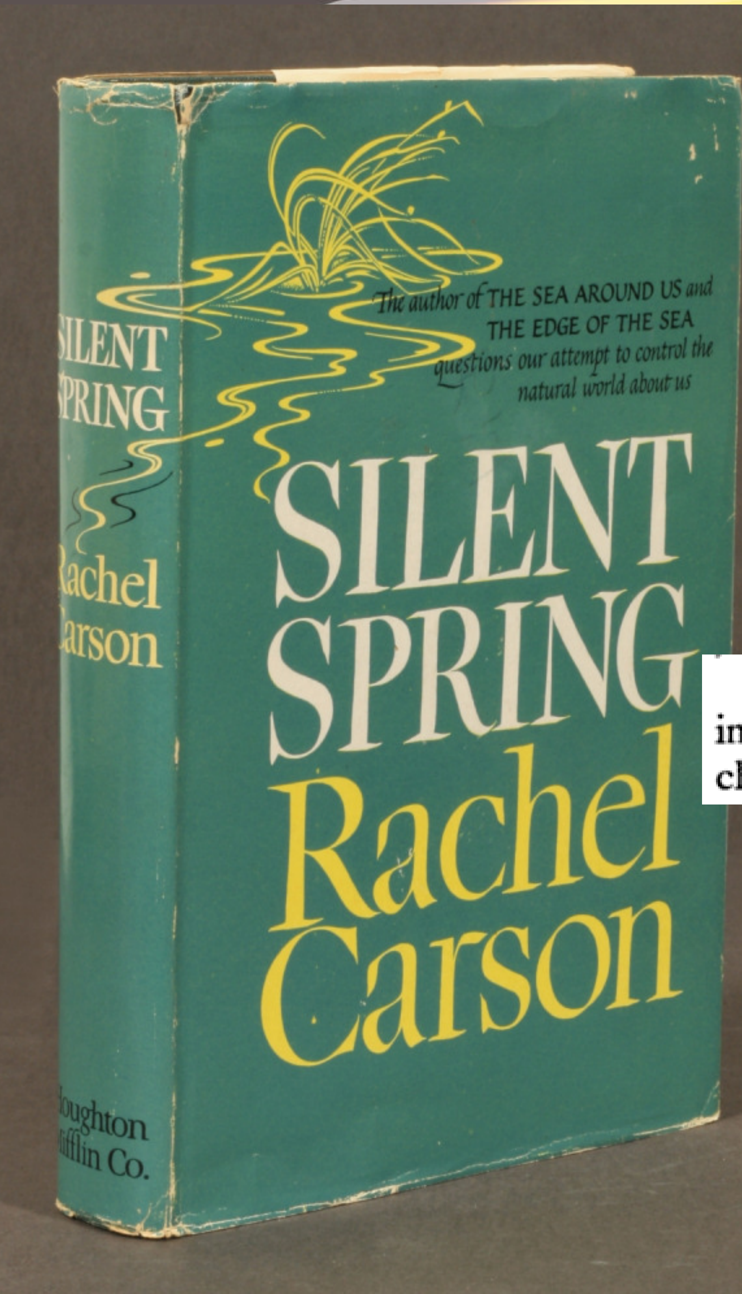
Triodos Foundation

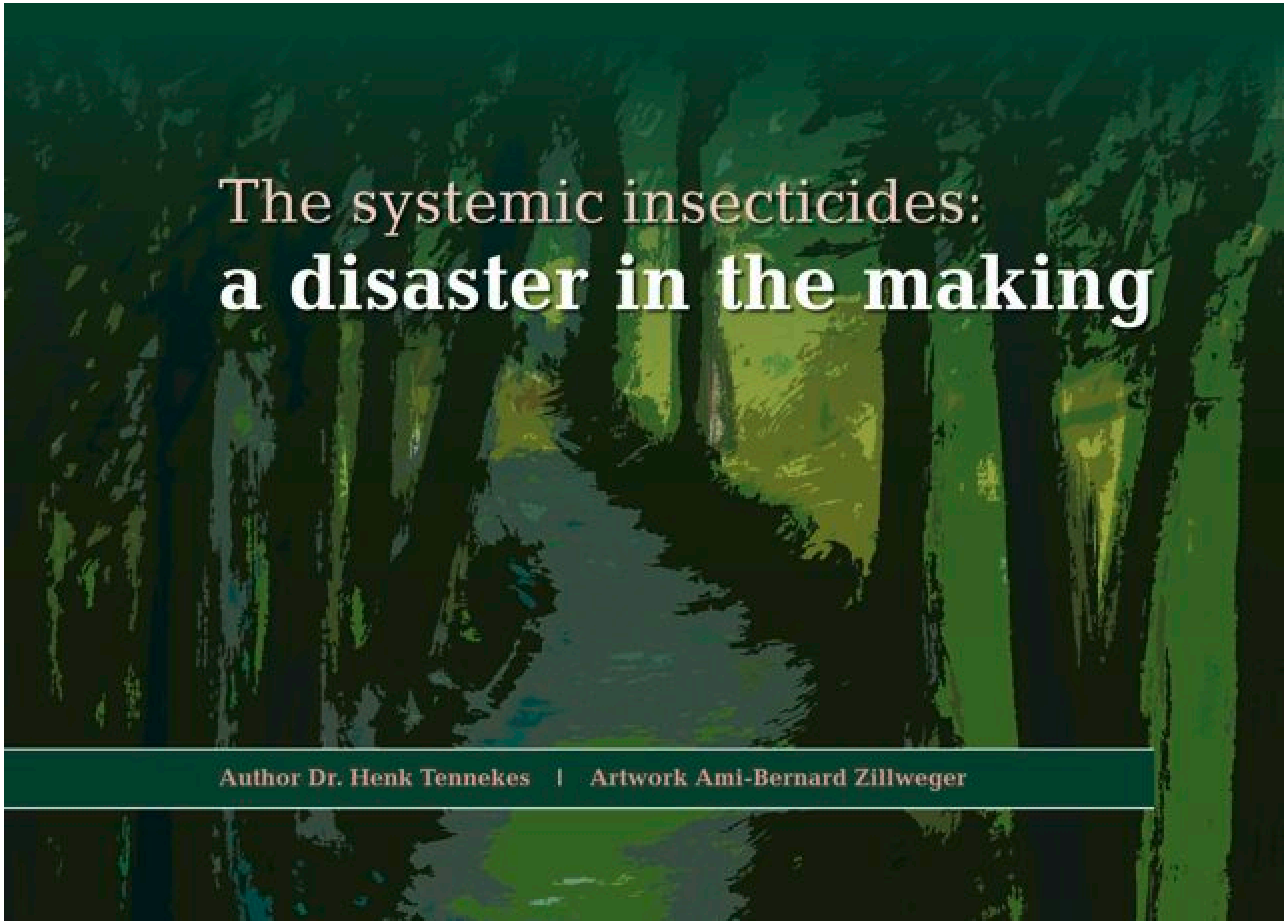


1962

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

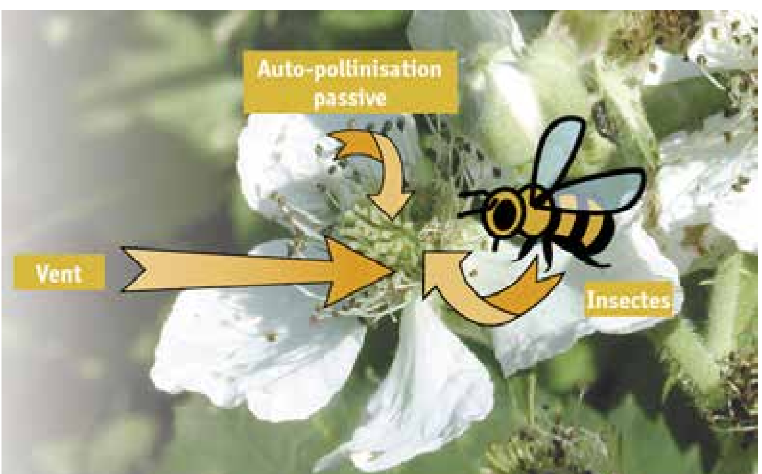
world where the enchanted forest of the fairy tales has become the poisonous forest in which an insect that chews a leaf or sucks the sap of a plant is doomed. It is a world where a flea bites a dog, and dies because the dog's blood has been made poisonous, where an insect may die from vapors emanating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

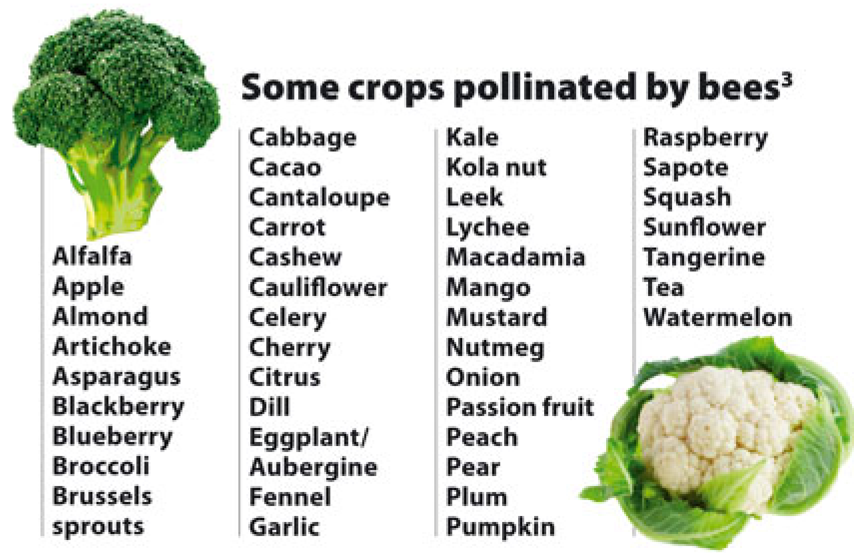


Book by dr Henk Tennekes
www.disasterinthemaking.com

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, β-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





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

New pollinator emerging in China

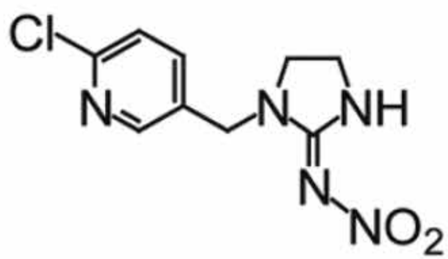
Pollinator decline: interaction of mutually reinforcing causes

PPPP

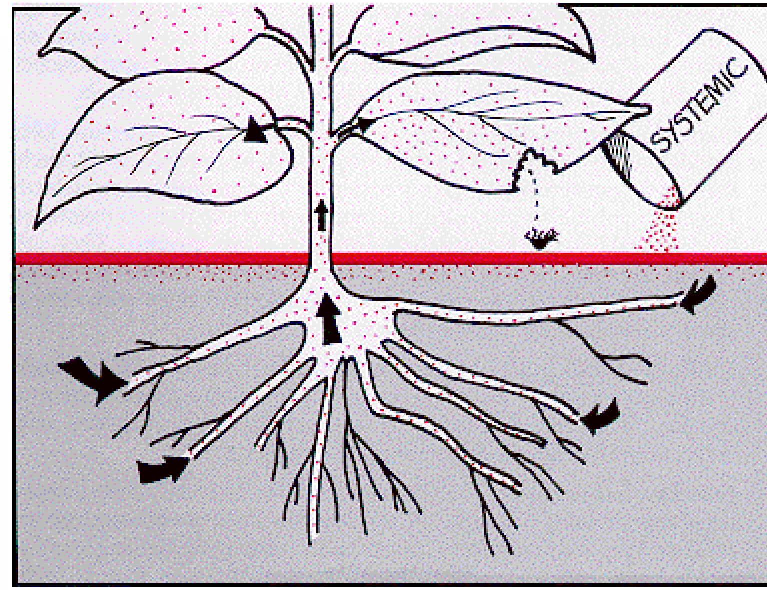
- **P**ollen
- **P**athogens
- **P**esticides
- **P**laces (for nesting)

Systemic insecticides: revolution in plant protection

Shinzo Kagabu



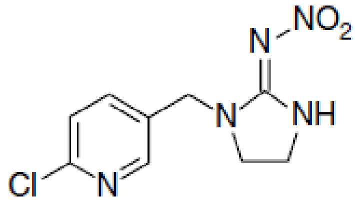
imidacloprid (1991)



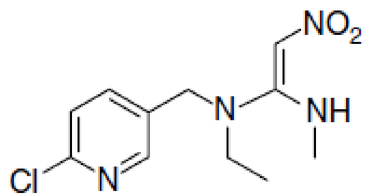
Systemic = crop takes it up into its plantsap: chemical makes plant toxic from inside

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**.

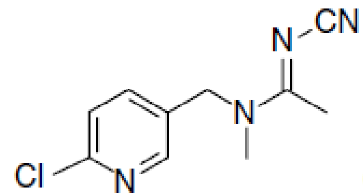
(Tomizawa & Casida, 2010, DOI:10.1021/jf103856c)



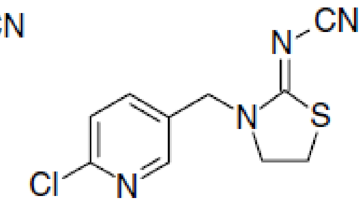
Imidacloprid (**1**)



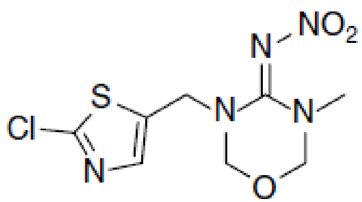
Nitenpyram (**2**)



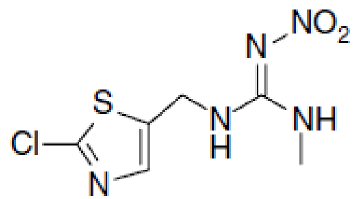
Acetamiprid (**3**)



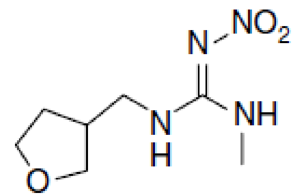
Thiacloprid (**4**)



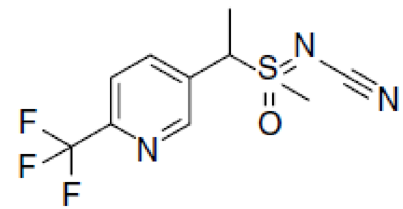
Thiamethoxam (**5**)



Clothianidin (**6**)



Dinotefuran (**7**)



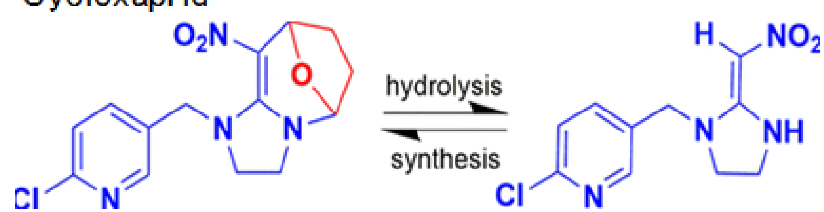
Sulfoxaflor (**8**)

Imidacloprid + Thiamethoxam + Clothianidin in EU:

>200 products

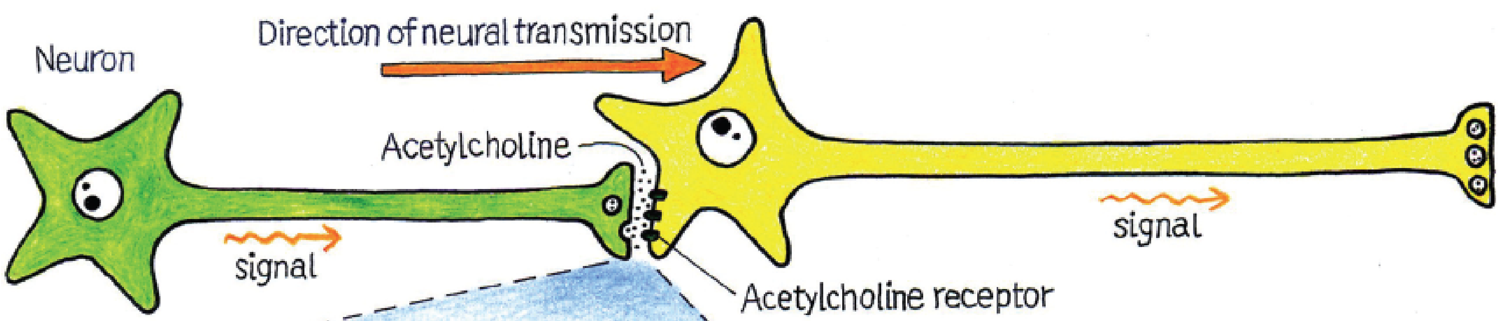
>1000 allowed applications

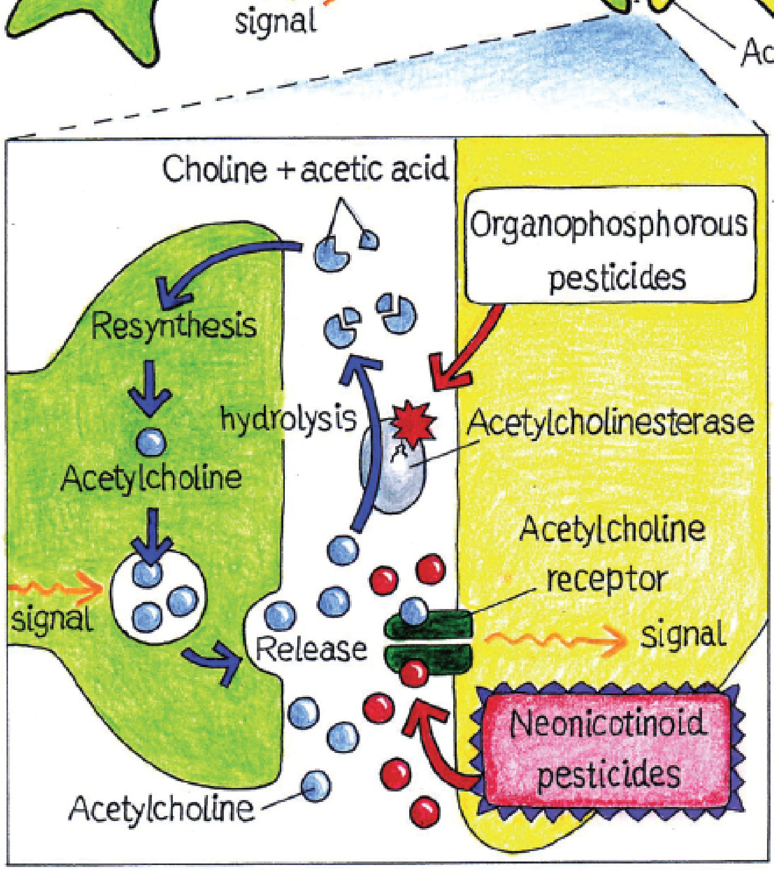
Cycloxaprid



Neonicotinoid / Organophosphorous pesticides disrupt the neural transmission

Neural transmission mechanism through acetylcholine





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 acetylcholin-esterase(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.

Illustration: Saori Yasutomi

Toxicity of neonicotinoids

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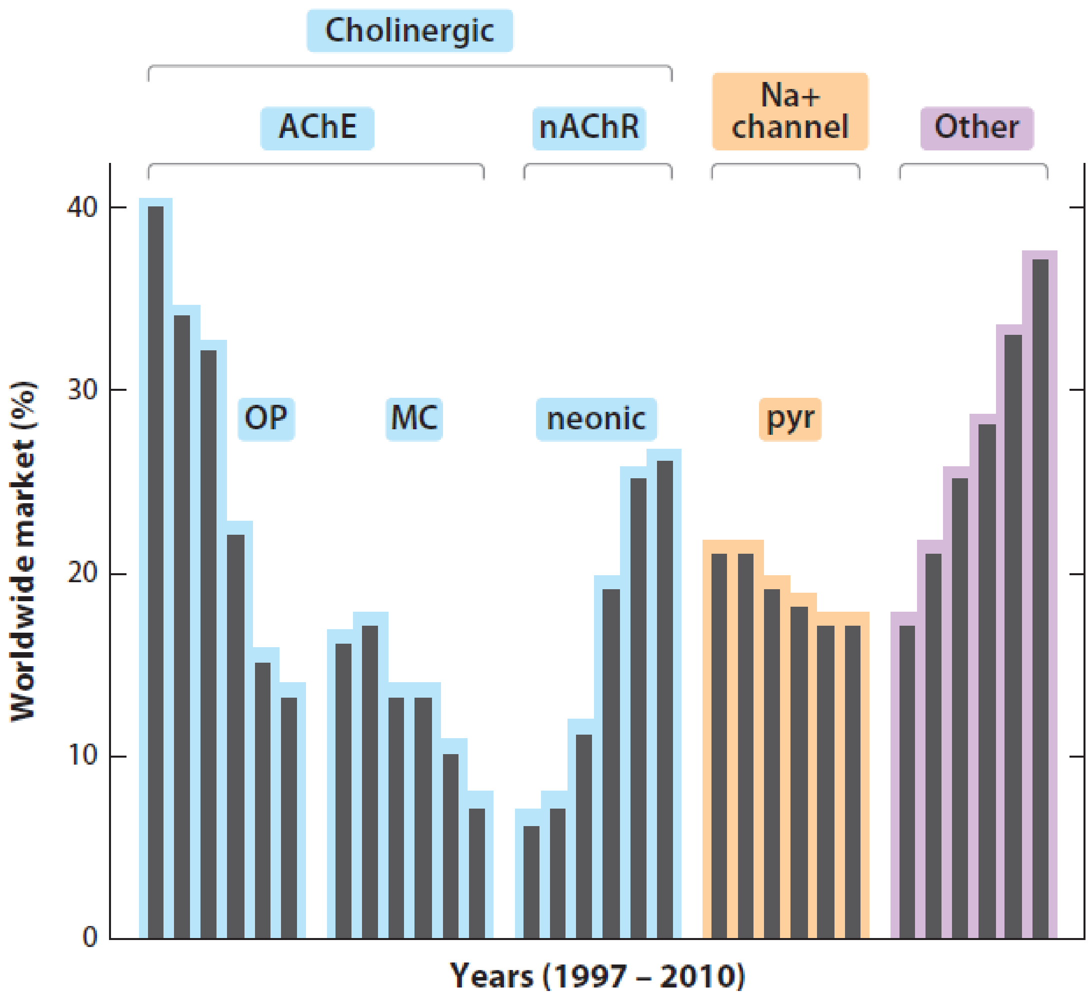
Toxicity of insecticides to honeybees compared to DDT. The final column expresses the toxicity relative to DDT. (Source: Bonmatin, 2009)

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

Corn coated with Gaucho (imidacloprid)

75	gram imidacloprid / ha corn
100000	seeds per ha
0.00075	gram imidacloprid/seed
3.7	nanogram = median lethal dose bee
202702	$LD50_{bee}$ per corn seed



Imidacloprid 2010 World production: 20,000 tonnes

(DDT peak-use 80,000 tonnes in 1959)

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

Figure 4

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%)

thiamethoxam	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,
clothianidin	potato, stone fruit, pome fruit
imidacloprid	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.
acetamiprid	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
thiacloprid	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,

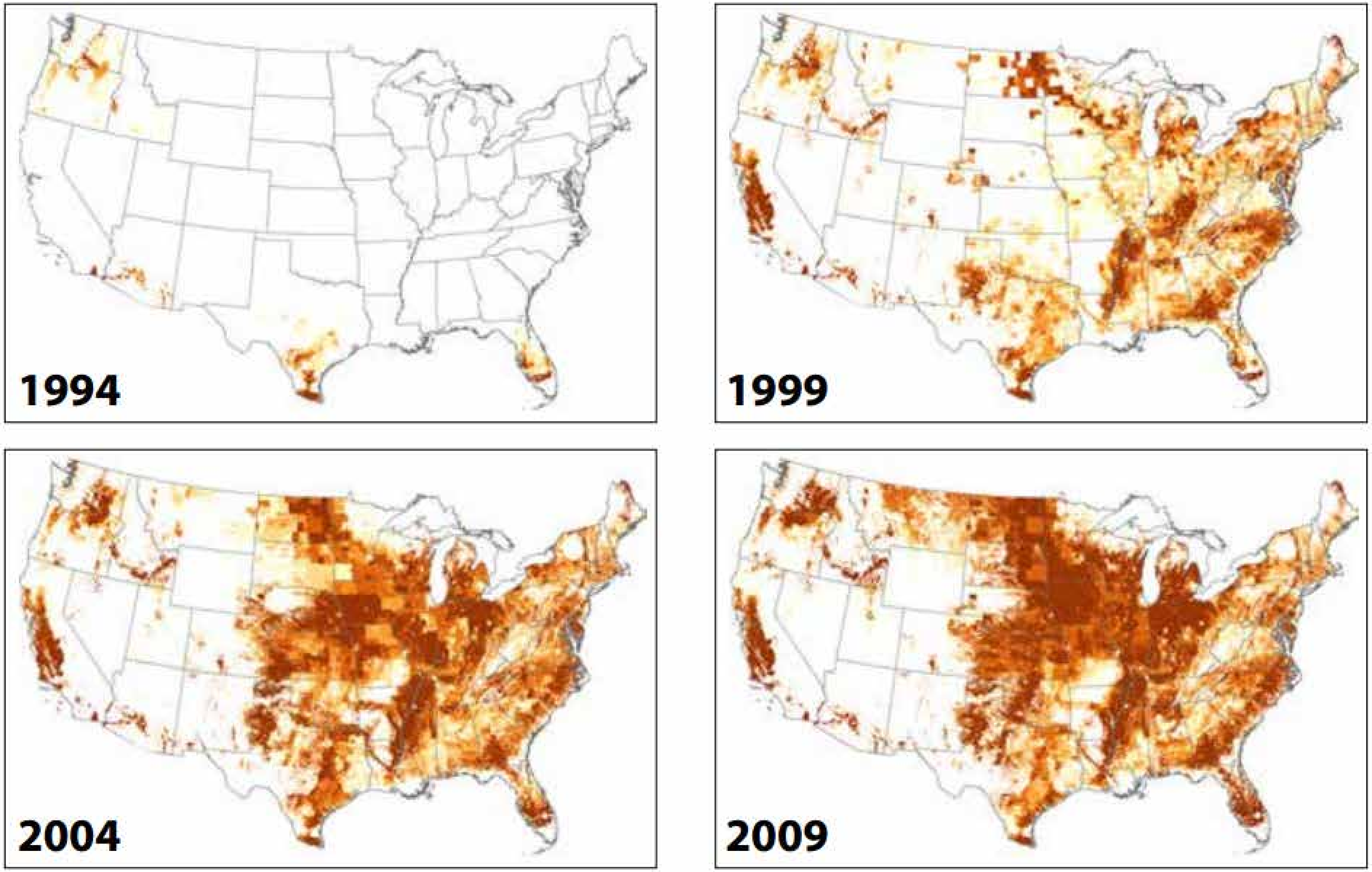
Seed treatment uses in Europe (20%)

thiamethoxam	beet seeds, cabbage, cauliflower, cotton, kale, lettuce, maize, mustard, peas, potato, oilseed rape, sorghum, sunflower, wheat, barley, rye, oat, triticale
clothianidin	beet seeds, cereals (wheat, barley, oat, rye, triticale), maize, oilseed rape, potato, sunflower
imidacloprid	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
thiacloprid	maize

Other uses in Europe

thiamethoxam	cucurbits, beans, brassicas, citrus fruit, salad, herbs, ornamentals, palm trees, peppers, tomato, flower bulbs, potato, house plants, pome fruit, forestry, citrus fruits, peppers,
clothianidin	maize, potato, sorghum, sorghum, poppy
imidacloprid	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,
acetamiprid	non fruiting trees and bushes
thiacloprid	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 (accessed 9/16/13).

Neonicotinoids – the problems

- Unprecedented scale of use: >1000 crops >120 countries
- High leaching potential: contamination of surface water
- High persistence in water & soil: build up in environment
- Broad spectrum toxicity - kills large range of non-target invertebrates
- Neurotoxic - bind irreversibly in nervous system causing cumulative and often delayed damage to non-target invertebrates (and vertebrates?)
- Ecosystem services provided by pollinators, soil decomposers, foodweb, etc. are at risk
- Most neonicotinoid use is unnecessary to reduce economic crop damage . Where action is needed, other strategies can be equally effective.

Exposure pathways:

- direct contact with spray drift and dust drift during application;
- intake of nectar, pollen, water, guttation, honeydew etc. that contain residues;
- residue in nesting material (resin, wax, etc.);
- contact with contaminated plants, soil, water;
- residue in cooling water used in the hive;
- inhalation of contaminated air

Field test in Padua

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

http://www.bijensterfte.nl/en/node/507

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.[1]

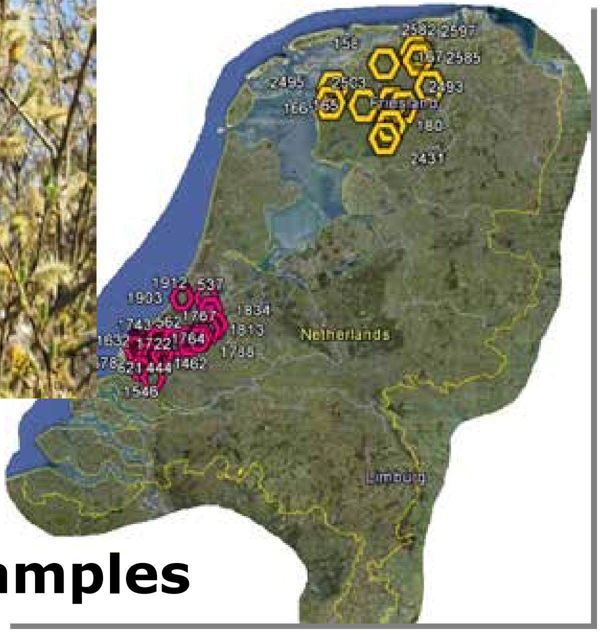
SAMPLE TYPE	Sample wt. (g)	THIAMETHOXAM LOD = 1.0	CLOTHIANIDIN LOD = 1.0	METOLACHLOR LOD = 0.5	ATRAZINE LOD = 0.2	AZOXYSTROBIN LOD = 0.2	COUMAPHOS LOD = 1.0
Soil, unplanted field 1, Soybeans 2010 (2 samples)	5.15, 5.01	ND	6.0±0.3	1014±14	771±170	0.2±0.1	ND
Soil, unplanted field 2, Soybeans 2010 (2 samples)	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.
[1]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

Wild trees translocate imidacloprid from surface water into pollen & nectar

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



13/27 = 48% samples Contaminated

5/27 = 19% two neonics

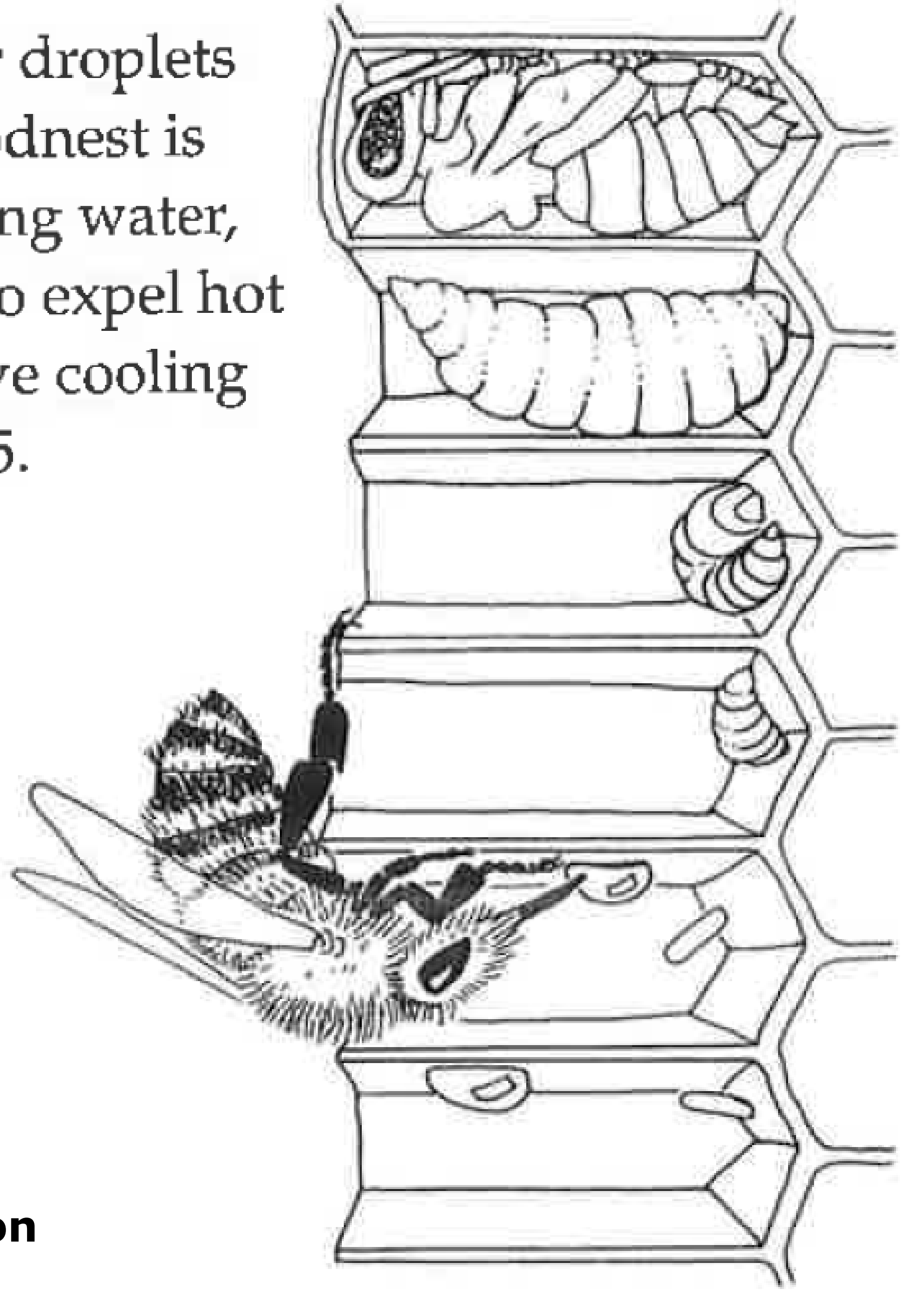


Figure 9.1 The spreading of water droplets by nurse bees when a colony's broodnest is threatened by overheating. Spreading water, combined with fanning the wings to expel hot air from the hive, causes evaporative cooling of the brood combs. After Park 1925.

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

Sublethal effects

- Navigation and orientation
- Feeding behaviour
- Memory and learning
- Neurophysiology
- Larval development
- Task differentiation in the colony
- Moulting
- Adult longevity
- Immunology
- Fecundity
- Sex ratio
- Mobility
- Oviposition behaviour
- Groomig and alloogrooming

Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production

Penelope R. Whitehorn,[1] Stephanie O'Connor,[1] Felix L. Wackers,[2] Dave Goulson[1*]

[1]School Natural Sciences, University of Stirling, Stirling FK9 4LA, UK. [2]Lancaster University, LEC, Lancaster LA1 4YQ, UK.

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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.

***Science*, 29 Mar 2012 http://dx.doi.org/10.1126/science.1215025**

Yamada 2012

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

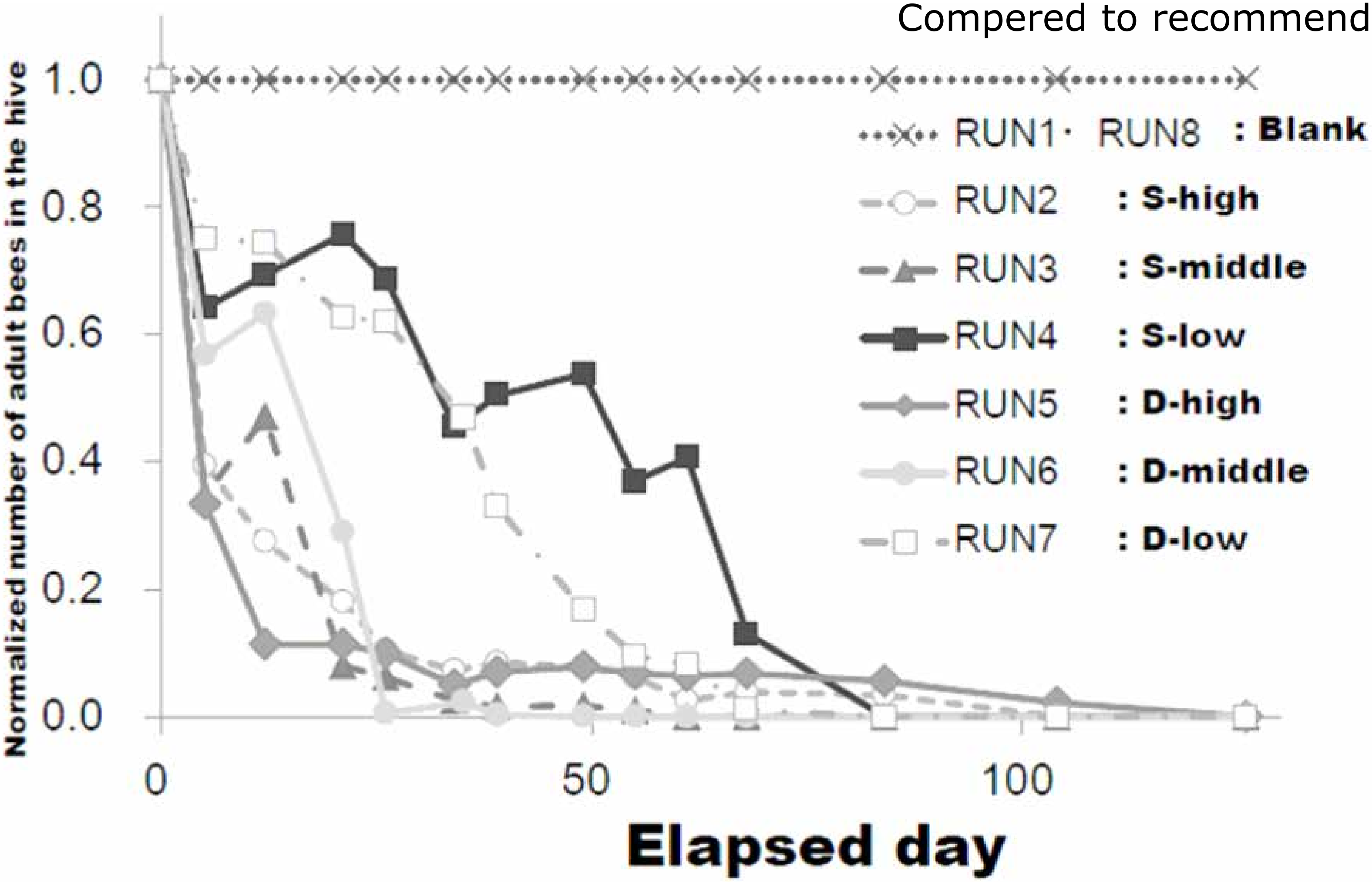
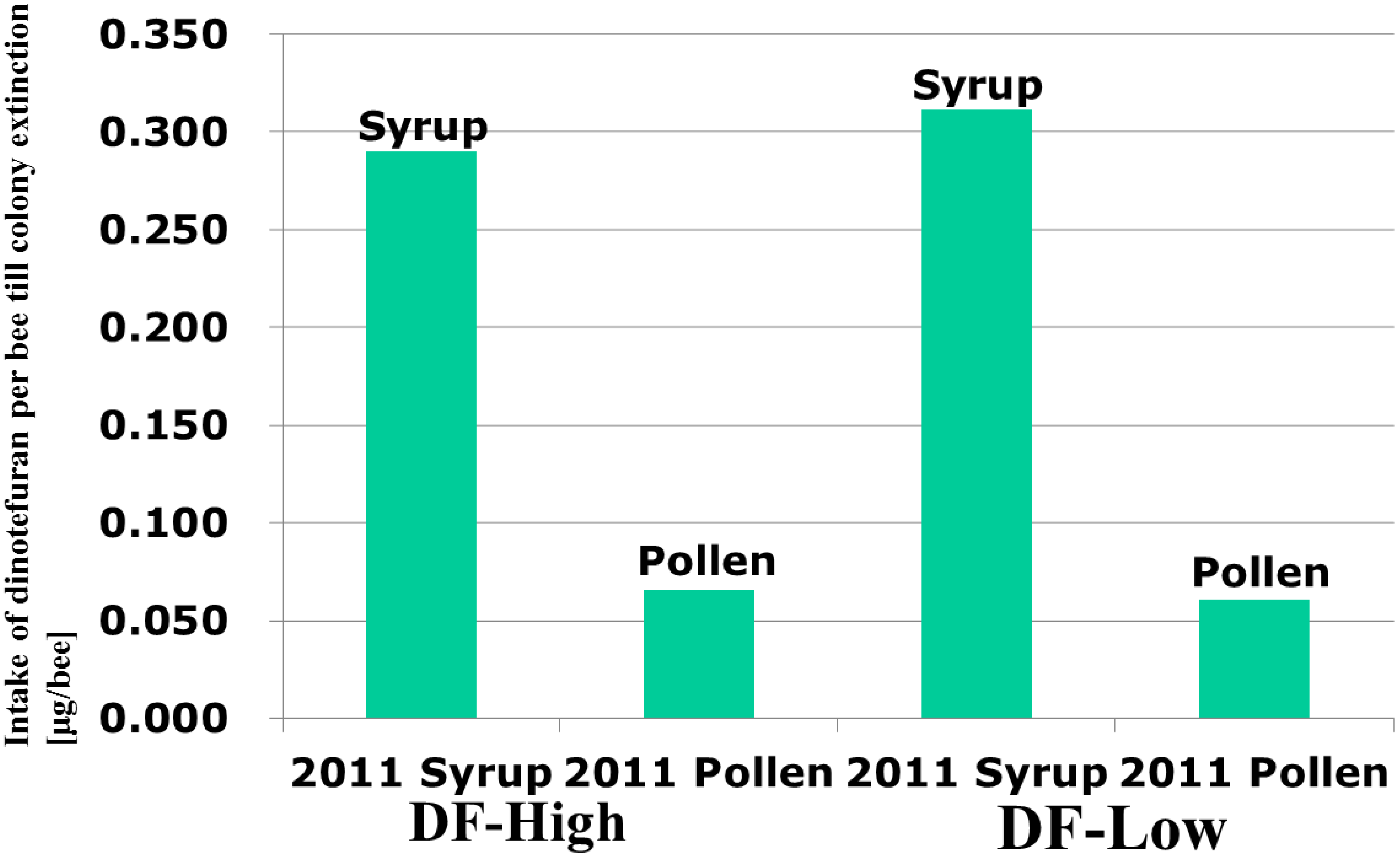


Figure 1 Normalized number of adult bees in the hive with the elapsed days

Required dose of neonicotinoid per bee till colony extinction



(Yamada, 2013)

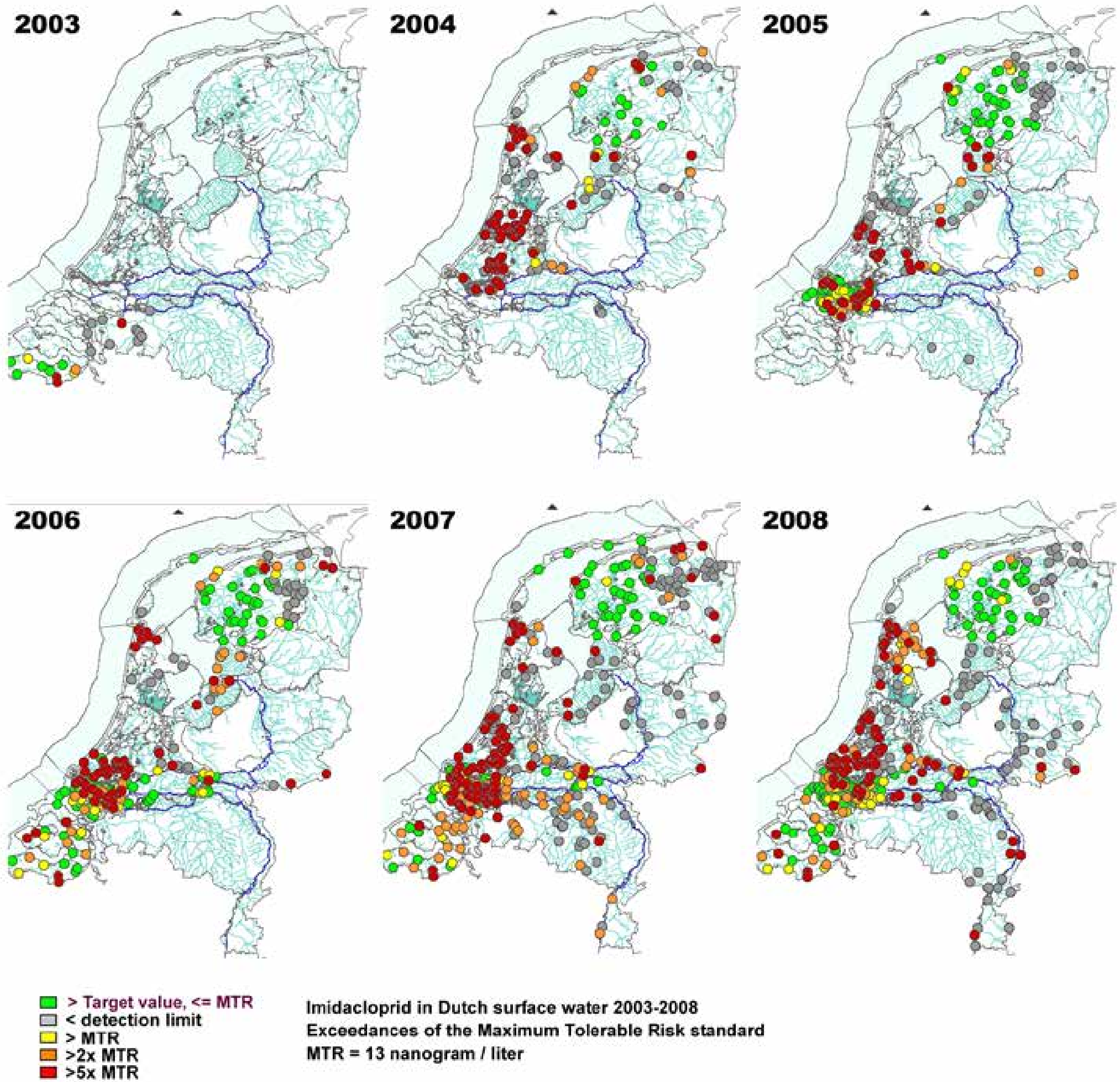
Conclusions on pollinators

- At field realistic concentrations, neonicotinoids produce wide range of adverse sublethal effects in bees, affecting colony performance through impairment of foraging success, brood, larval development, memory and learning, susceptibility to diseases, hive hygiene, etc.
- Neonicotinoids synergistically reinforce infectious agents such as *Nosema ceranae*.
- 85% reduction in bumblebee queen production could be a key factor explaining global trends of bumblebee decline.
- Few studies assessed toxicity to other wild pollinators. Available data suggest that they are likely to exhibit similar toxicity to all wild insect pollinators.
- Pollination is of vital importance both natural ecosystems and farming.
- Insect pollinators require a high level of protection.
- Transition to pollinator-friendly alternatives to neonicotinoids is urgently needed

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!

Since 2004, Netherlands surface water is heavily polluted with Imidacloprid

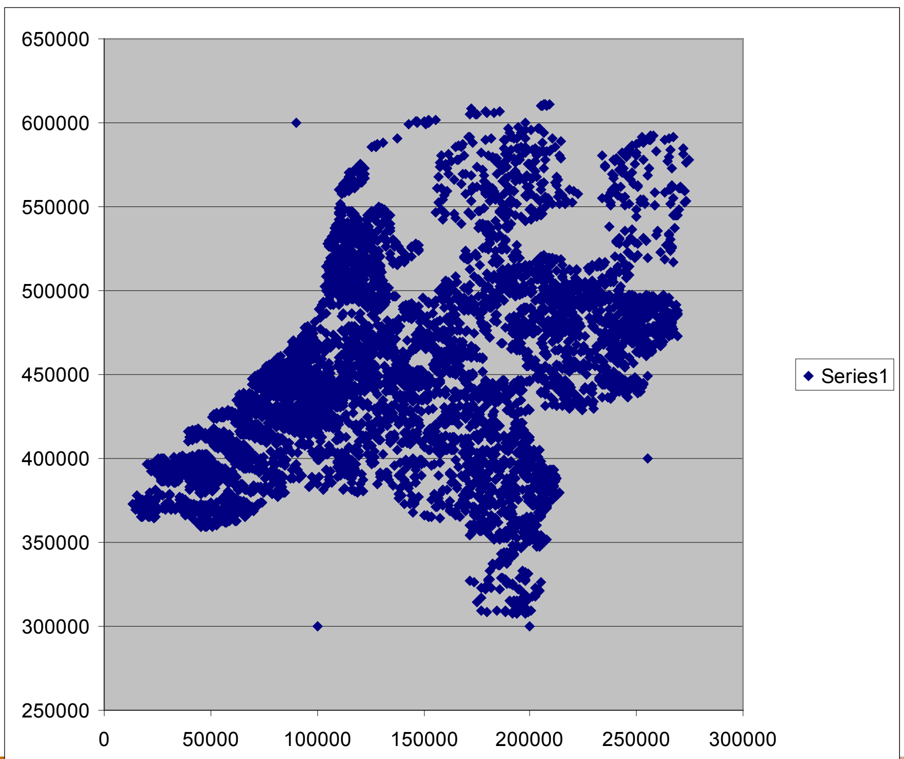
www.bestrijdingsmiddelenatlas.nl

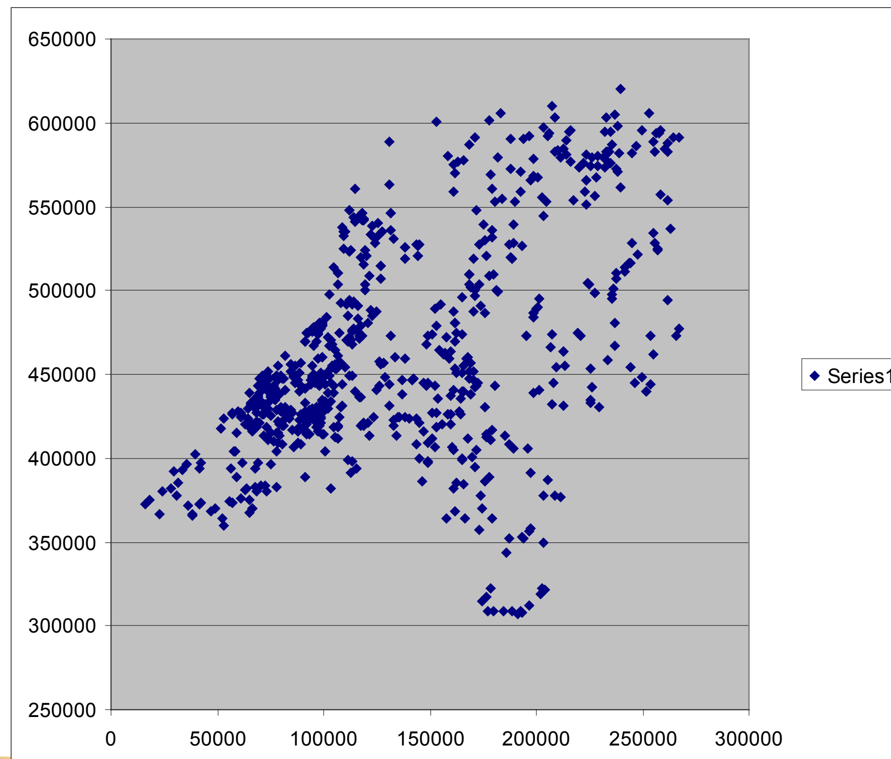


Imidacloprid in Dutch surface water 2003-2008
Exceedances of the Maximum Tolerable Risk standard
MTR = 13 nanogram / liter

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

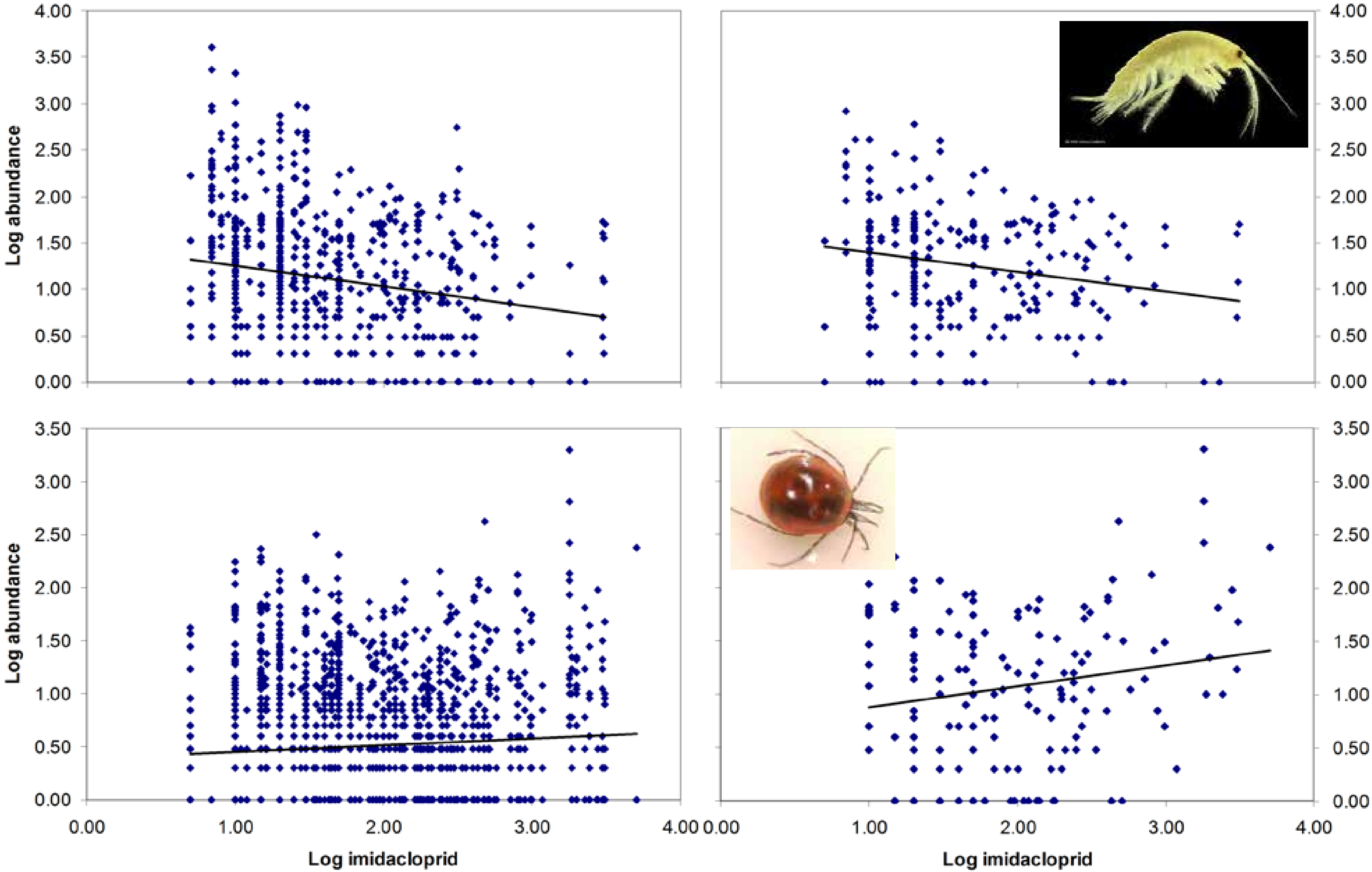




Significant negative relationship between species abundance and imidacloprid concentration found for:

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

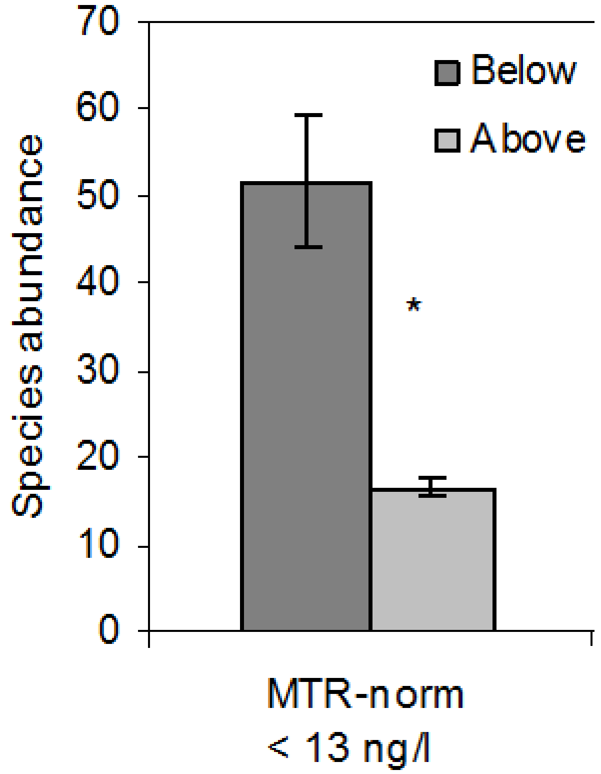
For one order we found significant positive relation: Actinedida

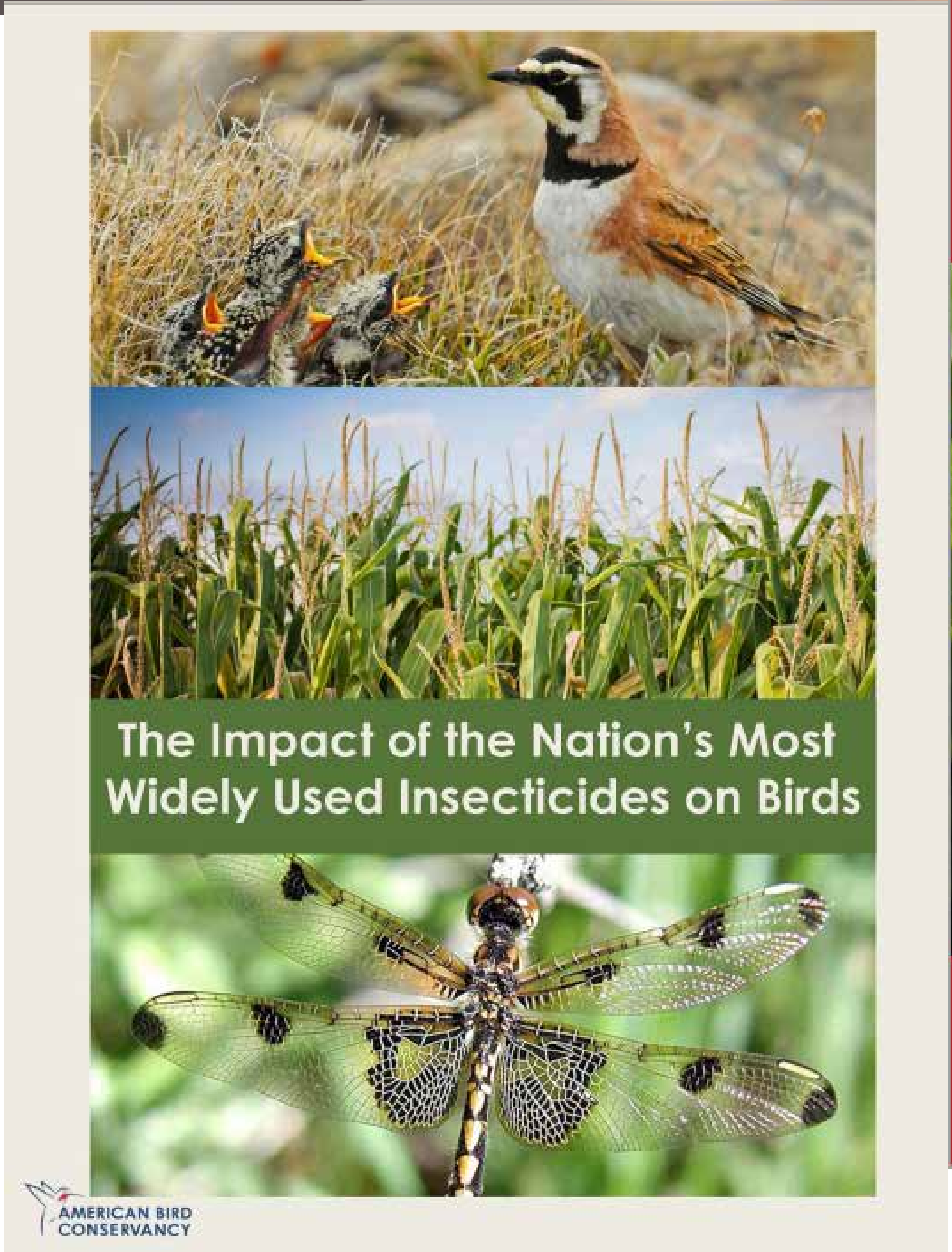


log10 imidacloprid concentration (ng/l) versus log10 macro-invertebrate species abundance in surface water for a) Amphipoda, b) its most abundant species *Gammarus tigrinus*, c) Actinedida and d) its most abundant species *Limnesia undulata*.

Findings on aquatic ecosystems

- 45% of all samples (*n*=9037) on 801 locations: imidacloprid exceeds MTR (>13 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%





http://www.abcbirds.org/abcprograms/policy/toxins/Neonic_FINAL.pdf

http://www.xerces.org/wp-content/uploads/2013/09/XercesSociety_CBCneonics_sep2013.pdf

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, Schizofrenia, ADHD??
- invivo versus invitro
- Parkinson (?)

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

OPEN ACCESS Freely available online PLoS one

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

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Abstract

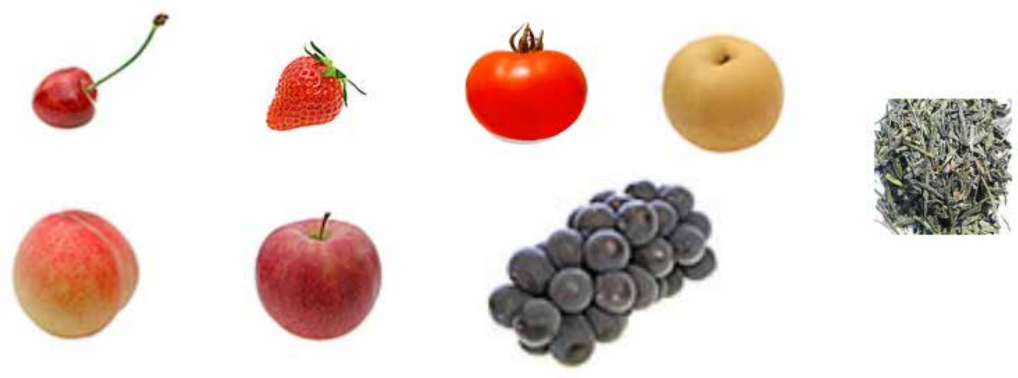
Background: Acetamiprid (ACE) and imidacloprid (IMI) belong to a new, widely used class of pesticide, the neonicotinoids. With similar chemical structures to nicotine, neonicotinoids also share agonist activity at nicotinic acetylcholine receptors (nAChRs). Although their toxicities against insects are 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^{2+}-influx assay was employed as an indicator of neural physiological activity. Significant excitatory Ca^{2+} 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^{2+} 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**

http://www.actbeyondtrust.org/wp-content/uploads/2012/10/taira.pdf

Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees

Gennaro Di Prisco[a], Valeria Cavaliere[b], Desiderato Annoscia[c], Paola Varricchio[a], Emilio Caprio[a], Francesco Nazzi[c], Giuseppe Gargiulo[b], and Francesco Pennacchio[a,1]

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Edited by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved October 1, 2013 (received for review August 8, 2013)

Large-scale losses of honey bee colonies represent a poorly understood problem of global importance. Both biotic and abiotic factors are involved in this phenomenon that is often associated with high loads of parasites and pathogens. A stronger impact of pathogens in honey bees exposed to neonicotinoid insecticides has been reported, but the causal link between in
sure and the possible immune alteration
elusive. Here, we demonstrate that the n
clothianidin negatively modulates NF-κB
insects and adversely affects honey bee
trolled by this transcription factor. We ha
a negative modulator of NF-κB activation,
repeat protein. Exposure to clothianidin, b
scription of the gene encoding this inhib
defenses and promotes the replication of the
in honey bees bearing covert infections. Thi
suppression is similarly induced by a differe
dacloprid, but not by the organophosphate
does not affect NF-κB signaling. The occurren
of this insecticide-induced viral proliferatio
studied neonicotinoids might have a negativ
level. Our experiments uncover a further level
immune response in insects and set the stage f
modulation of immunity in animals. Furtherm
implications for the conservation of bees, as it
the definition of more appropriate guidelines fo
sublethal effects of pesticides used in agricultu

Apis mellifera | DWV | NLR (CLR) | neuroimmunity | to

attention. In particular, neonicotinoid insecticides are currently the subject of intense debate (13). Over the last few years, several countries have restricted their use in agricul
are currently under the
...
), subsequently
...ases, as "Nucleotide-binding domain
...Leucine-rich Repeat" (NLR) (26). These proteins play an

REVIEW

Journal of Environmental Immunology and Toxicology 1:1, 3-12; March/April 2013; © 2013 STM Publishing

Immune Suppression by Neonicotinoid Insecticides at the Root of Global Wildlife Declines

Rosemary Mason[1], Henk Tennekes[2], Francisco Sánchez-Bayo[3], Palle Uhd Jepsen[1]

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Abstract

Outbreaks of infectious diseases in honey bees, fish, amphibians, bats and birds in the past two decades have coincided with the increasing use of systemic insecticides, notably the neonicotinoids and fipronil. A link between insecticides and such diseases is hypothesised. Firstly, the disease outbreaks started in countries and regions where systemic insecticides were used for the first time and later they spread to other countries. Secondly, recent evidence of immune suppression in bees and fish caused by neonicotinoid has provided an important clue to understand the sub-lethal impact of these insecticides not only on these organisms, but probabl on other wildlife affected by emerging infectious diseases. While this is occurring, environmental authorities in developed countri ignore the calls of apiarists (who are most affected) and do not target neonicotinoids in their regular monitoring schedules. Equal scientists looking for answers to the problem are unaware of the new threat that systemic insecticides have introduced in terrestr and aquatic ecosystems.

Journal of Environmental Immunology and Toxicology 2013; 1:3-12

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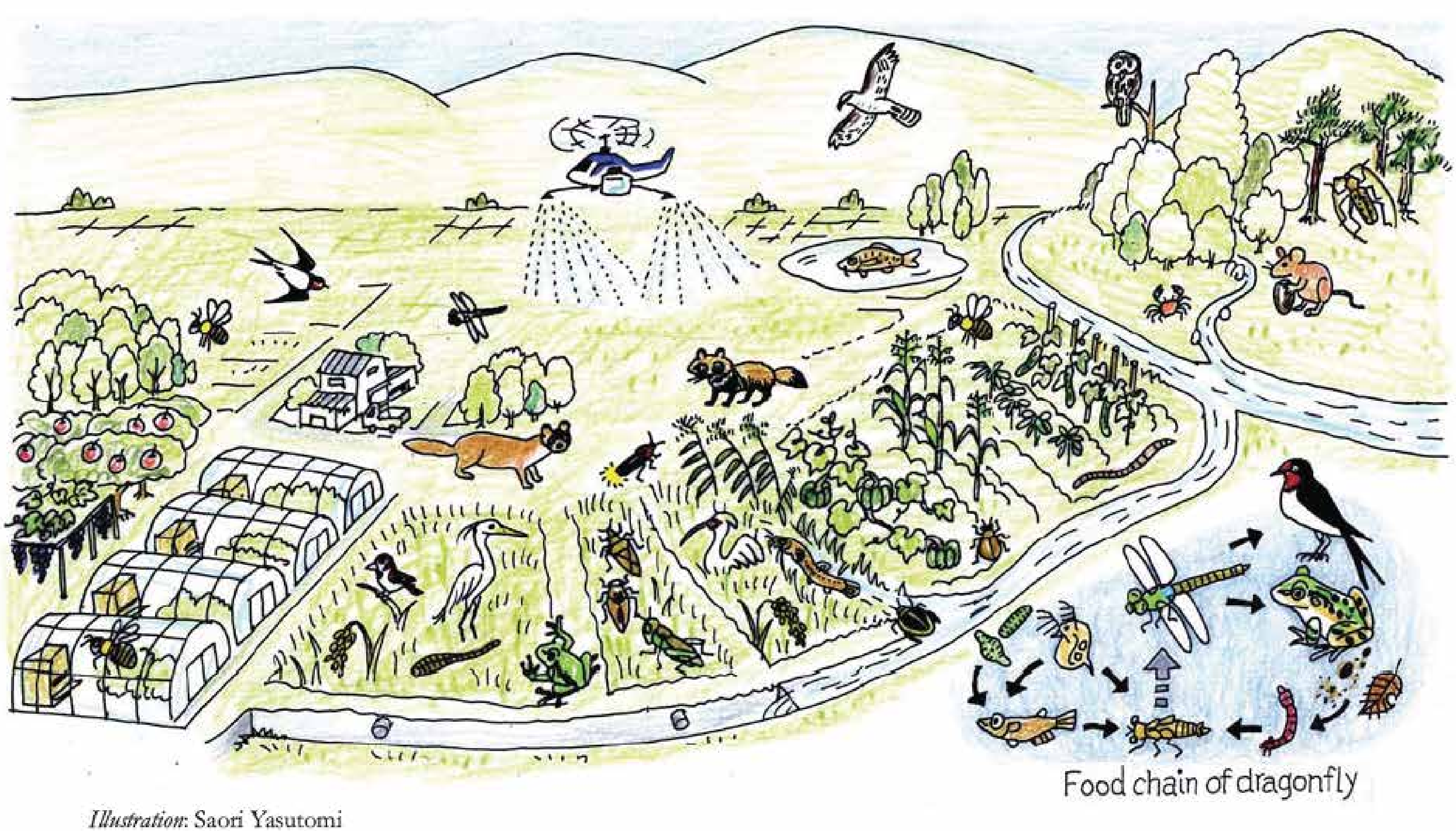
Accelerating the collapse of the ecosystem

(Picture from: Japan Endocrine-disruptor Preventive Action)

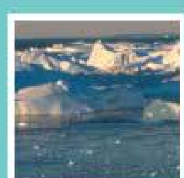


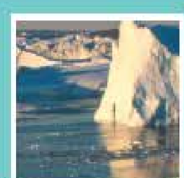
EEA Report | No 1/2013

Late lessons from early warnings: science, precaution, innovation

Summary

ISSN 1725-9177





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Current Opinion in Environmental Sustainability

Neonicotinoids, bee disorders and the sustainability of pollinator services☆

Jeroen P van der Sluijs[1], Noa Simon-Delso[1], Dave Goulson[2], Laura Maxim[3], Jean-Marc Bonmatin[4] and Luc P Belzunces[5]

In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. For pollinators, this has transformed the agrochemical landscape. These chemicals mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects. Their systemic mode of ac... plants means ... phloemic and xylemic transport th... to pollen and nectar. Their wide... soil and water and potential for ... and wild plants make neonicot... pollinators at sublethal concer... This results in the frequent p... honeybee hives. At field reali... a wide range of adverse su... bumblebee colonies, affec... impairment of foraging su... development, memory a... nervous system, suscep... Neonicotinoids exhibit... various other agroche... reinforce infectious a... together can produc... data suggest that t... virtually all other v... production of nec... transition to polli... is urgently need... pollinator ecos...

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[4] Centre d...
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Current Opinion in Environmental Sustainability 2013, 5:xx–yy

This review comes from a themed issue on Open issue 2013

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...org/10.1016/j.cosust.2013.05.007

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PLOS | ONE

Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid

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Environmental Sciences, Utrecht University, Utrecht, The Netherlands

Abstract

Imidacloprid is one of the most widely used insecticides in the world. Its concentration in surface water exceeds the water quality norms in many parts of the Netherlands. Several studies have demonstrated harmful effects of this neonicotinoid to a wide range of non-target species. Therefore we expected that surface water pollution with imidacloprid would negatively impact aquatic ecosystems. Availability of extensive monitoring data on the abundance of aquatic macro-invertebrate species, and on imidacloprid concentrations in surface water in the Netherlands enabled us to test this hypothesis. Our regression analysis showed a significant negative relationship ($P<0.001$) between macro-invertebrate abundance and imidacloprid concentration for all species pooled. A significant negative relationship was also found for the orders Amphipoda, Basommatophora, Diptera, Ephemeroptera and Isopoda, and for several species separately. The order Odonata had a negative relationship very close to the significance threshold of 0.05 ($P = 0.051$). However, in accordance with previous research, a positive relationship was found for the order Actinedida. We used the monitoring field data to test whether the existing three water quality norms for imidacloprid in the Netherlands are protective in real conditions. Our data show that macrofauna abundance drops sharply between 13 and 67 ng l^{-1}. For aquatic ecosystem protection, two of the norms are not protective at all while the strictest norm of 13 ng l^{-1} (MTR) seems somewhat protective. In addition to the existing experimental evidence on the negative effects of imidacloprid on invertebrate life, our study, based on data from large-scale field monitoring during multiple years, shows that serious concern about the far-reaching consequences of the abundant use of imidacloprid for aquatic ecosystems is justified.

Citation: Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. PLoS ONE 8(5): e62374. doi:10.1371/journal.pone.0062374

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