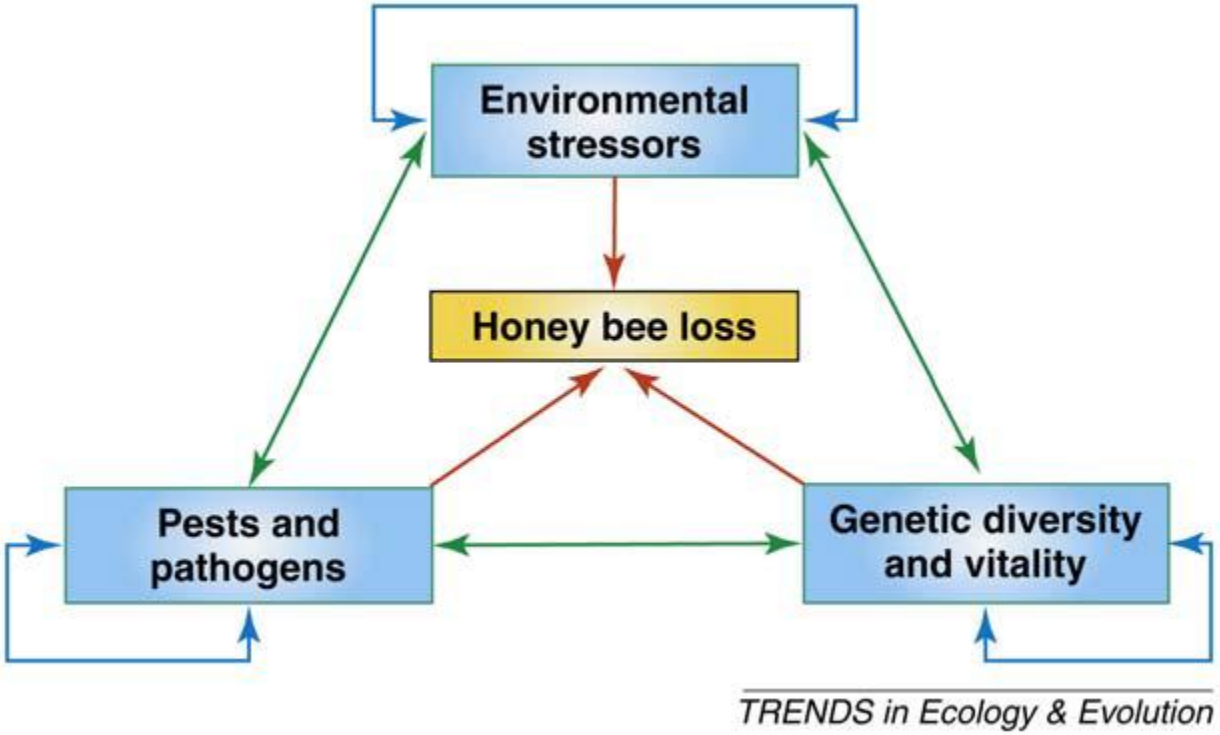
Pesticides and Pollinators

A look at modern neurotoxins

Pollinator losses - not one thing



It's Global

- Total managed honeybee losses in US running 25% per year since 2005.
- Monarch butterflies –only 3% of historical Mexican wintering area now has butterflies.
- Native bees under pressure
- Lower populations of mosquitoes, gnats, and midges impact birds and bats.

Look for Global Scale cause – Environmental toxins and pesticides?

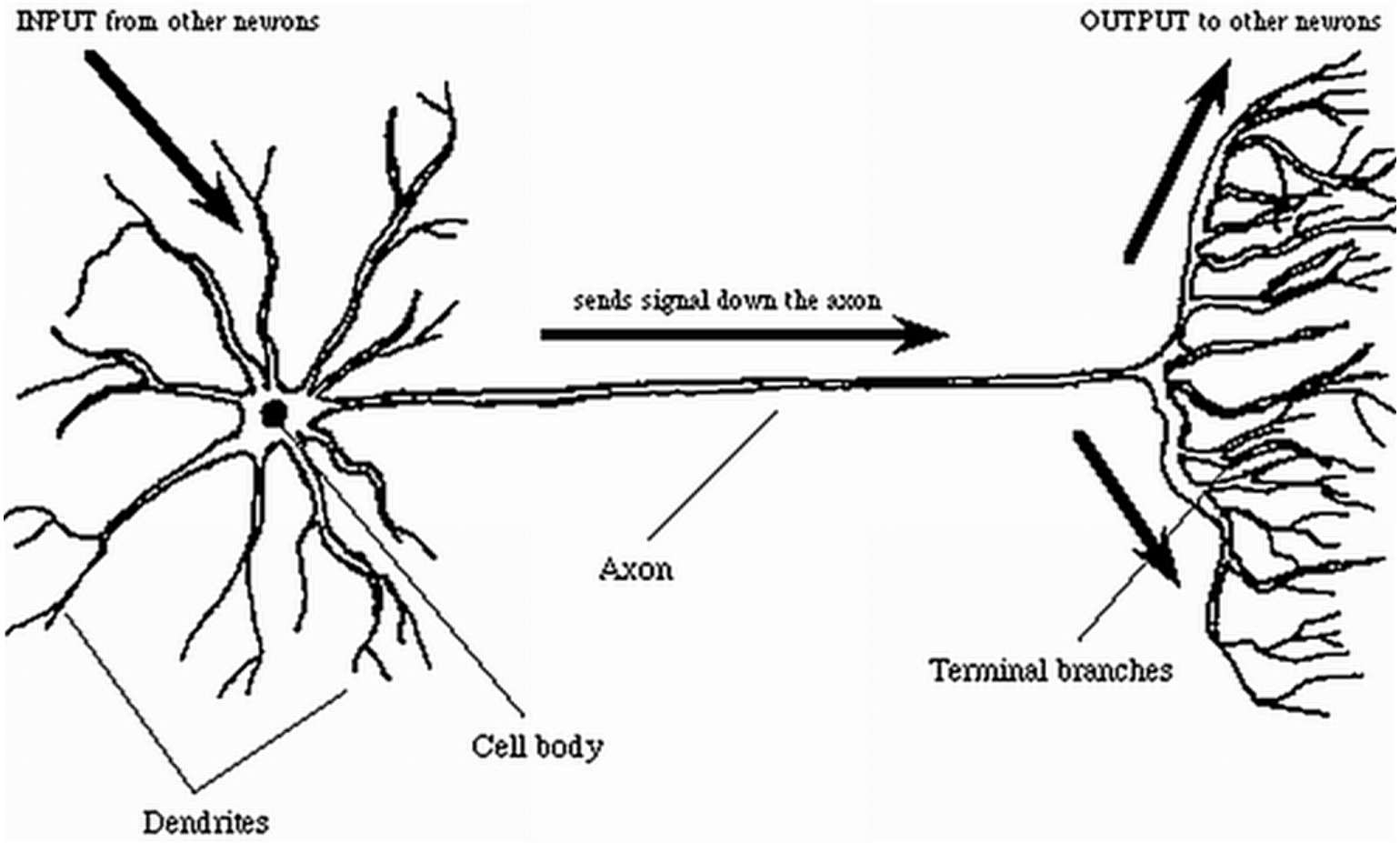
Pesticide Classes

- Organochlorines – DDT; Persistent in the environment; Now Mostly Banned
- Organophosphates – Malathion; Workhorse pesticides; AChE inhibitor – strong binding; Toxic to mammals; Quick acting, degrades in hours to days.
- Carbamates – Sevin; AChE inhibitor – weak binding; Degrades quickly.
- Neonicotinoids – Imidacloprid; NAChR agonist – strong binding; Slow to degrade; Used systemically.

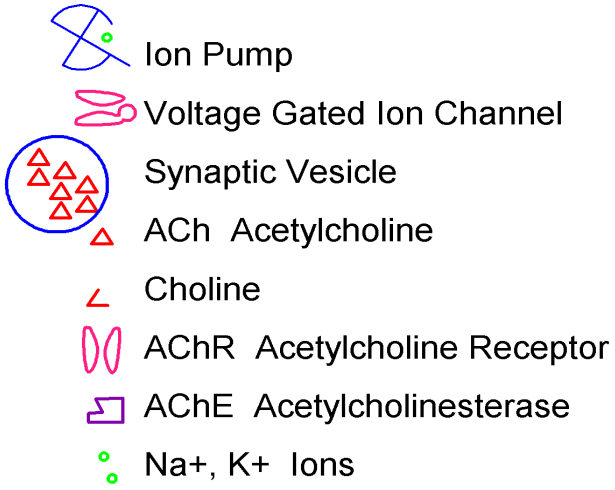
Pesticide Class	Example Chemical	Oral LD50 Honey-bees	Typical Soil half-life	Typical metabolic half-life	Typical binding dissociation time	Typical toxicity time-scaling exponent	Toxic Mechanism	Comment
Neonic-otinoids	imidacloprid	50 ng/bee	.5 – 3 yr.	4 hr.	>10 days	2	Synaptic nAChR agonist.	Often used as systemic insecticides
	Thiameth-oxam	20 ng/bee	30-300 days	2-6 hr. (rats)	?	2	Irreversible binding	Direct acting on nAChRs
Pyrethroids	Delta-methrin	60 ng/bee	11-72 days	2 hr.	Several seconds	2 ?	Keeps open voltage gated Na+ ion channels on axon	Direct acting on Na+ channels
Organo-chlorines	DDT	6190 ng/bee	2-15 yr.	6 yr.	Temperture dependant--suggests less than a second.	?	Keeps open voltage gated Na+ ion channels on axon	Most of these chemicals have been banned by international treaty as persistent organic pollutants
	dieldrin	133 ng/bee	5 yr.	9-12 mo. humans		?		
Organo-phosphate	diazinon	370 ng/bee	15-200 days	17 hr.	16 days	1 ?	Irreversible AChE inhibitor	AChE inhibitors have inherent "threshold" action since large fraction of AChE must be bound to have toxic
	malathion	720 ng/bee	1-15 days	12 hr.	? days	0.5 (fish)		
	Carbaryl	1540					Reversible AChE inhibitor	

The Neuron

Similar structure in insects and Humans



The Synapse – How it Works



0 In resting state Ion Pumps keep Na+ and K+ ion concentrations separated so interior of neuron is at negative electrical potential w.r.t. outside cell.

1 Membrane action potential, enhanced by Voltage Gated Ion channels along the axon, causes...

2 Neurotransmitter vesicles to release ACh into synaptic cleft.

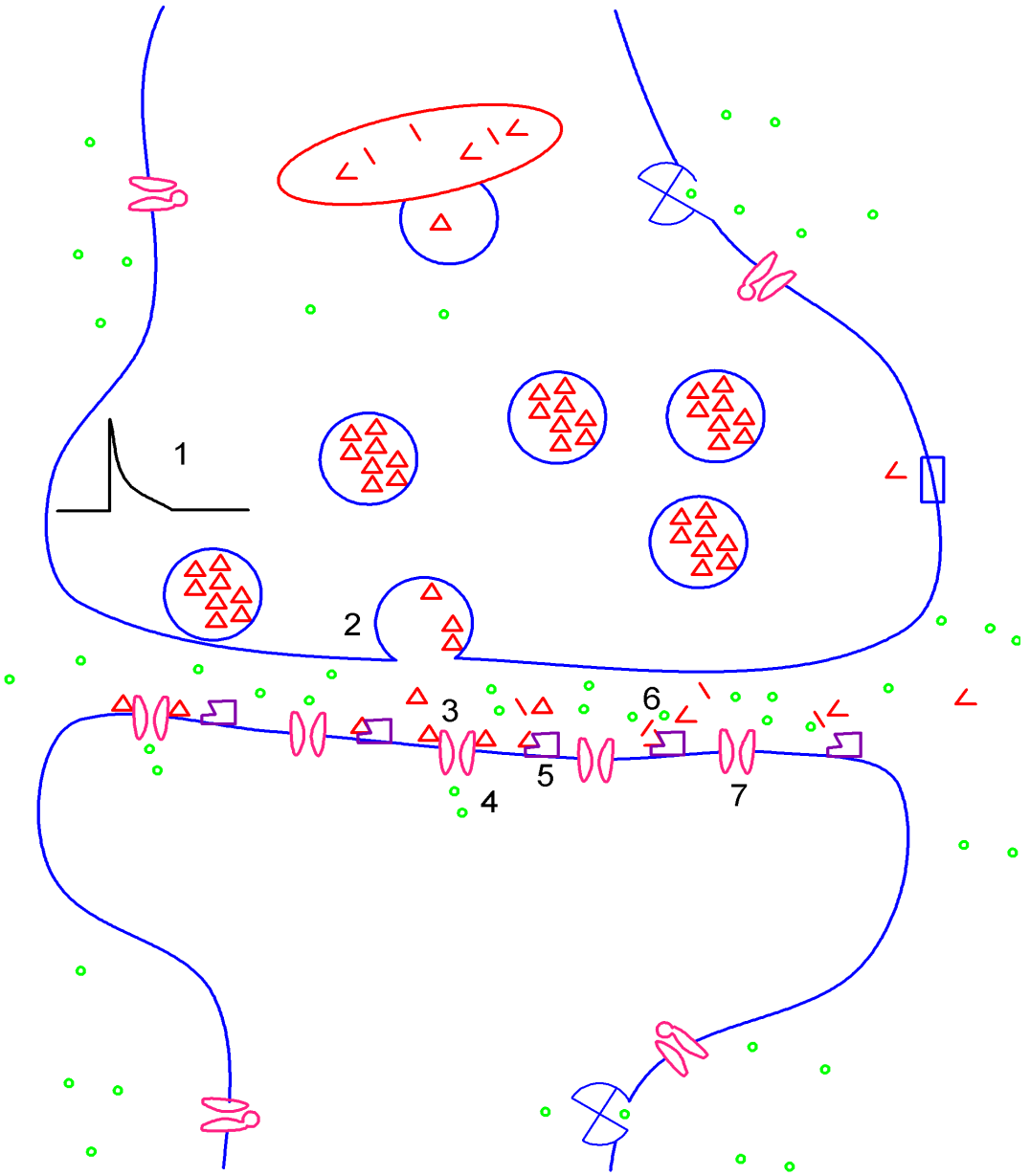
3 ACh diffuses quickly across the gap and binds to AChR.

4 This opens the AChR ion channel and ions enter the postsynaptic neuron.

5 AChE captures ACh and...

6 catalyzes its dissociation,

7 Without ACh, the ion channels close.



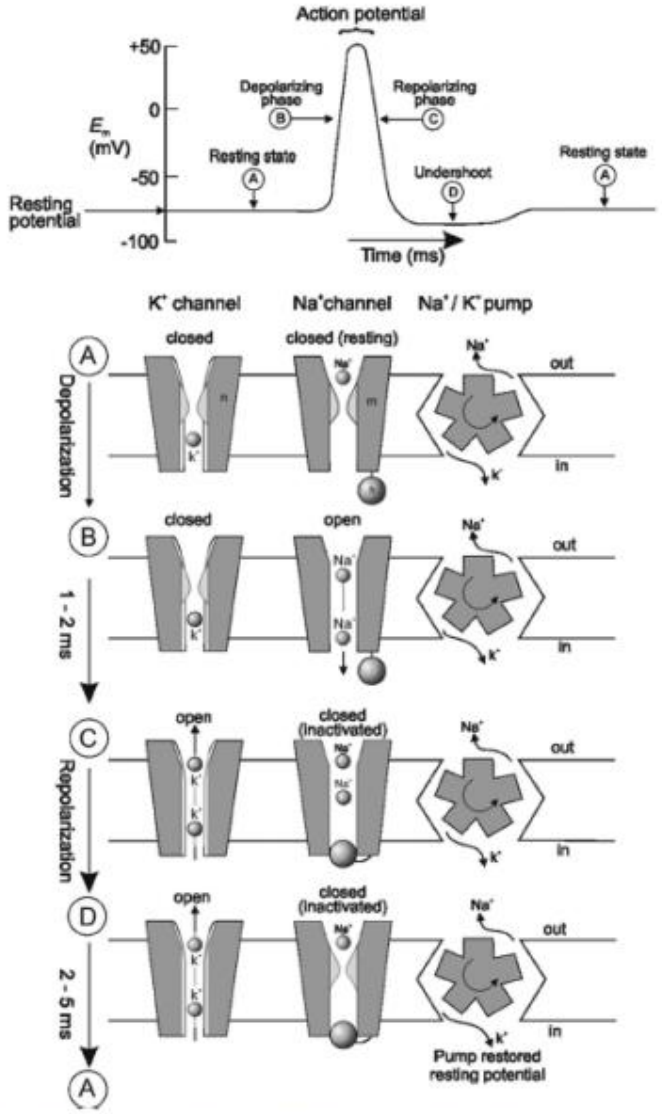
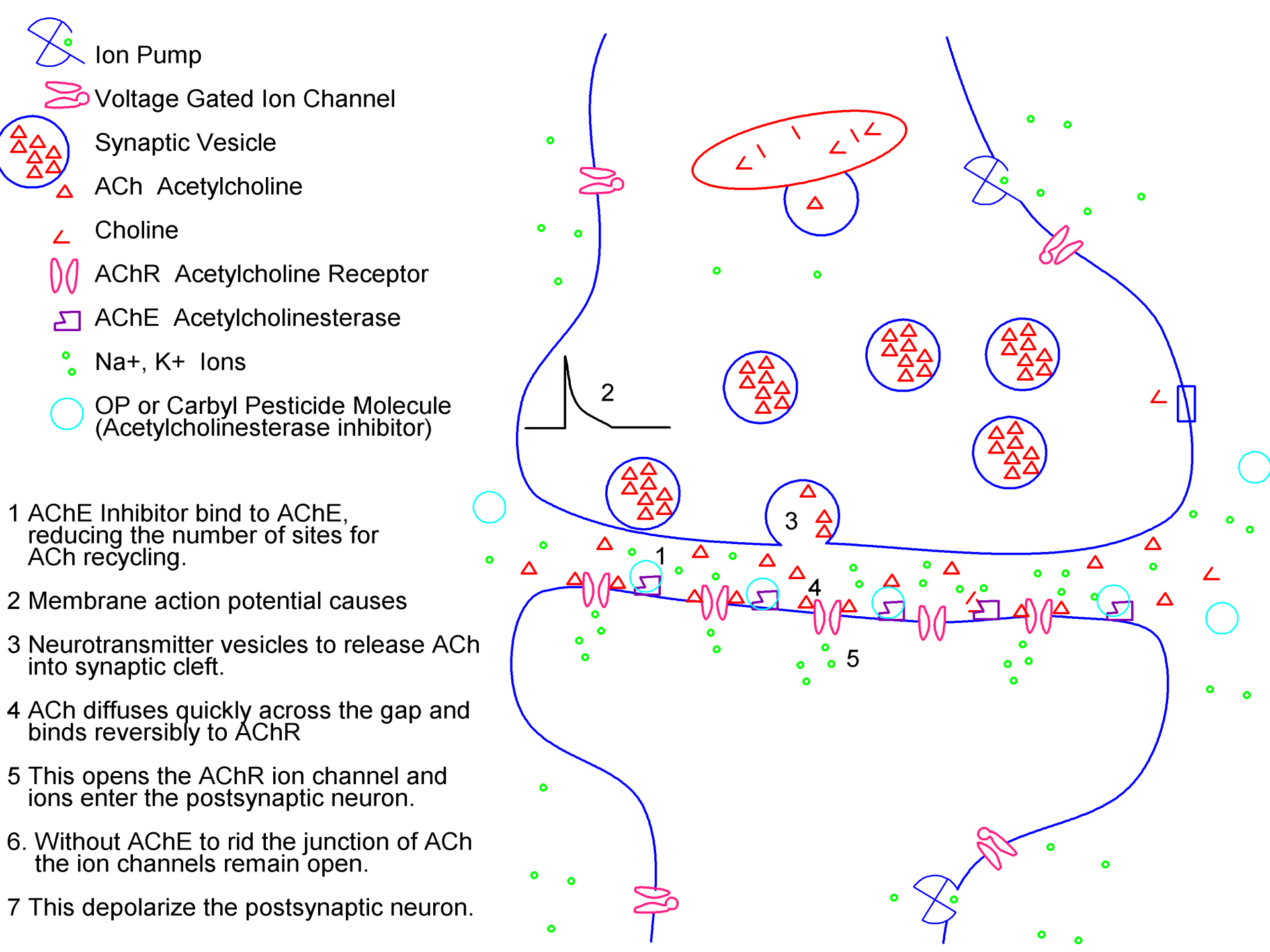


Figure 2. Generation of an action potential. The extracellular fluid surrounding the insect axonal membrane contains a high concentration of sodium ions (Na^+) and a low concentration of potassium ions (K^+), whilst the reverse is true for the inside of the nerve cell. At the resting potential (A) the axonal membrane is relatively permeable to K^+ but not Na^+. This makes the inside of the cell negative with respect to the outside, the difference in potential being around −60 mV. Nerve stimulation causes the axonal membrane to become permeable to Na^+ due to the sodium channel opening (B). This causes the inside of the axon to become transiently positive and generates the rising phase of the action potential. Sodium channel closure or inactivation (C) (usually within 1 ms) causes an efflux of K^+ as a result of opening of potassium channels and generates the falling phase of the action potential. The generation of the action potential results in sequential depolarization of neighbouring regions of the axon, resulting in a wave of depolarization along the axon. An ATP driven Na^+- K^+ pump maintains the ion gradient across the axonal membrane (D) and restores the resting potential. Continued transmission of the impulse across the synapse involves release of a chemical transmitter, which becomes attached to receptor sites at the postsynaptic membrane where it depolarizes the membrane to generate another action potential.



1 AChE Inhibitor bind to AChE, reducing the number of sites for ACh recycling.

2 Membrane action potential causes

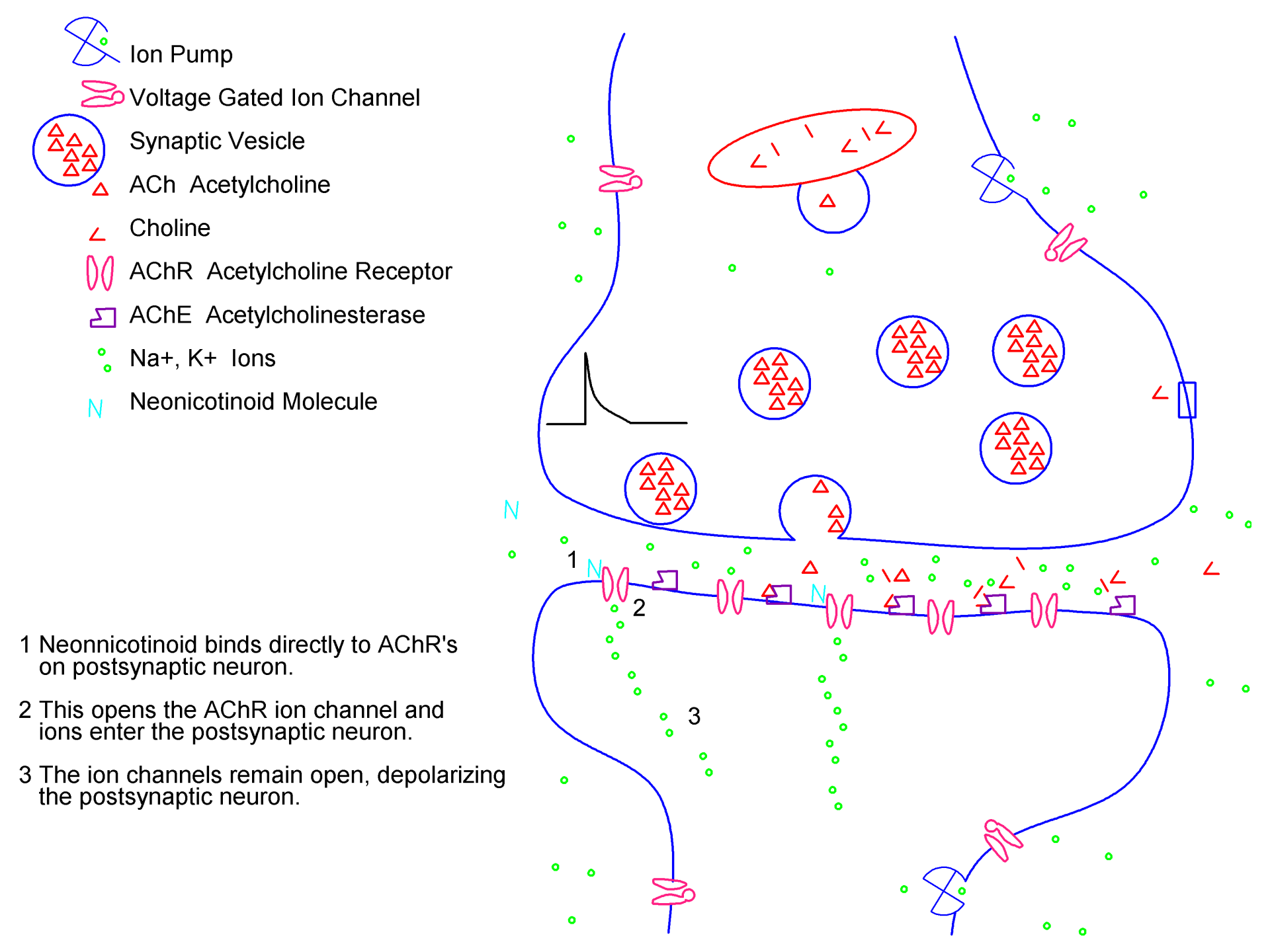
3 Neurotransmitter vesicles to release ACh into synaptic cleft.

4 ACh diffuses quickly across the gap and binds reversibly to AChR

5 This opens the AChR ion channel and ions enter the postsynaptic neuron.

6. Without AChE to rid the junction of ACh the ion channels remain open.

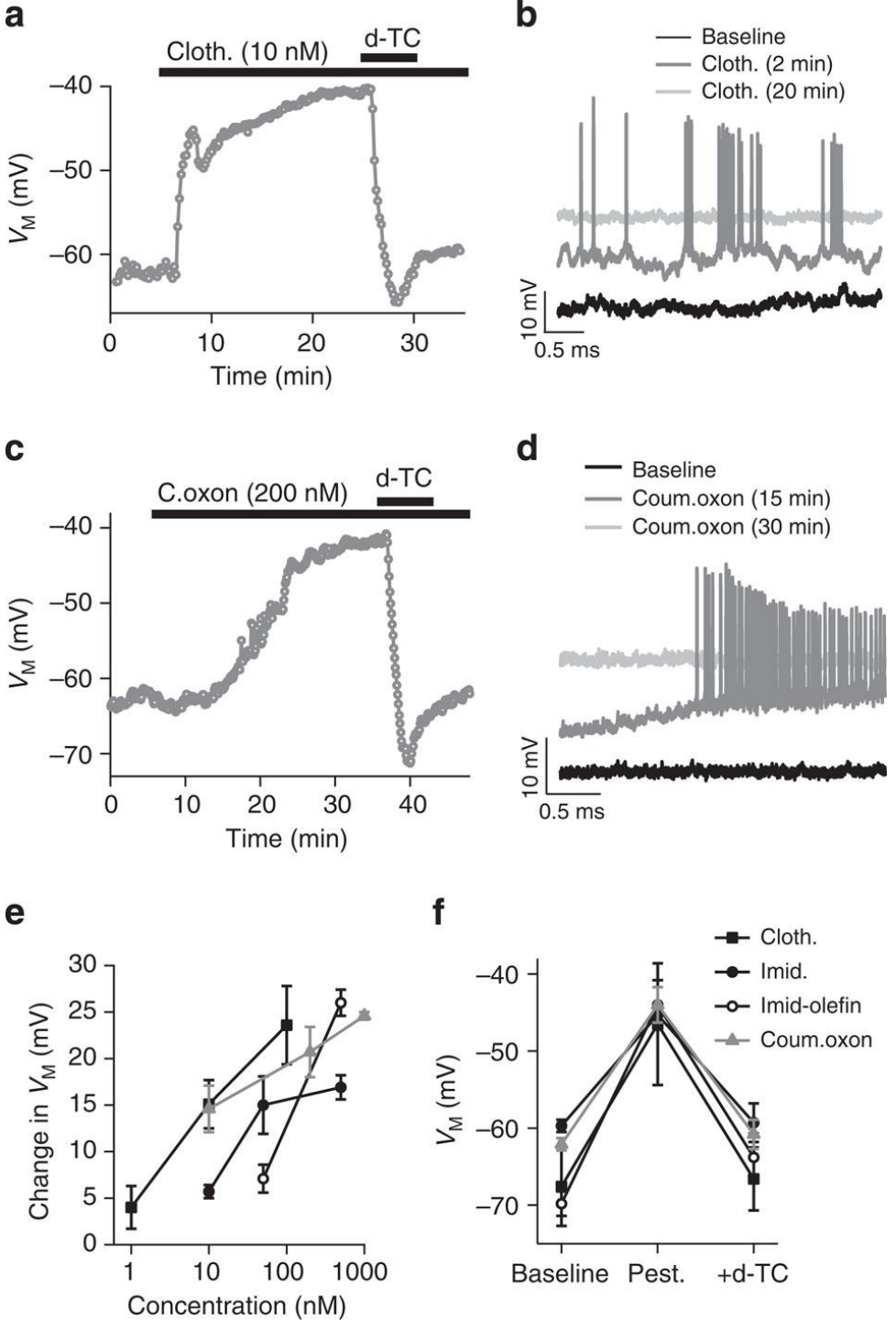
7 This depolarize the postsynaptic neuron.


Ion Pump
Voltage Gated Ion Channel
Synaptic Vesicle
ACh Acetylcholine
Choline
AChR Acetylcholine Receptor
AChE Acetylcholinesterase
Na+, K+ Ions
N Neonicotinoid Molecule
1
2
3
N
1 Neonnicotinoid binds directly to AChR's on postsynaptic neuron.
2 This opens the AChR ion channel and ions enter the postsynaptic neuron.
3 The ion channels remain open, depolarizing the postsynaptic neuron.

Electrophysiology of Honeybee brain neurons

Cells stimulated with bath of low concentration of clothianidin (neonic) and coumaphos oxon (organo-phosphate)

As neuron is depolarized action potentials are generated, followed by inactivity when sufficiently depolarized.



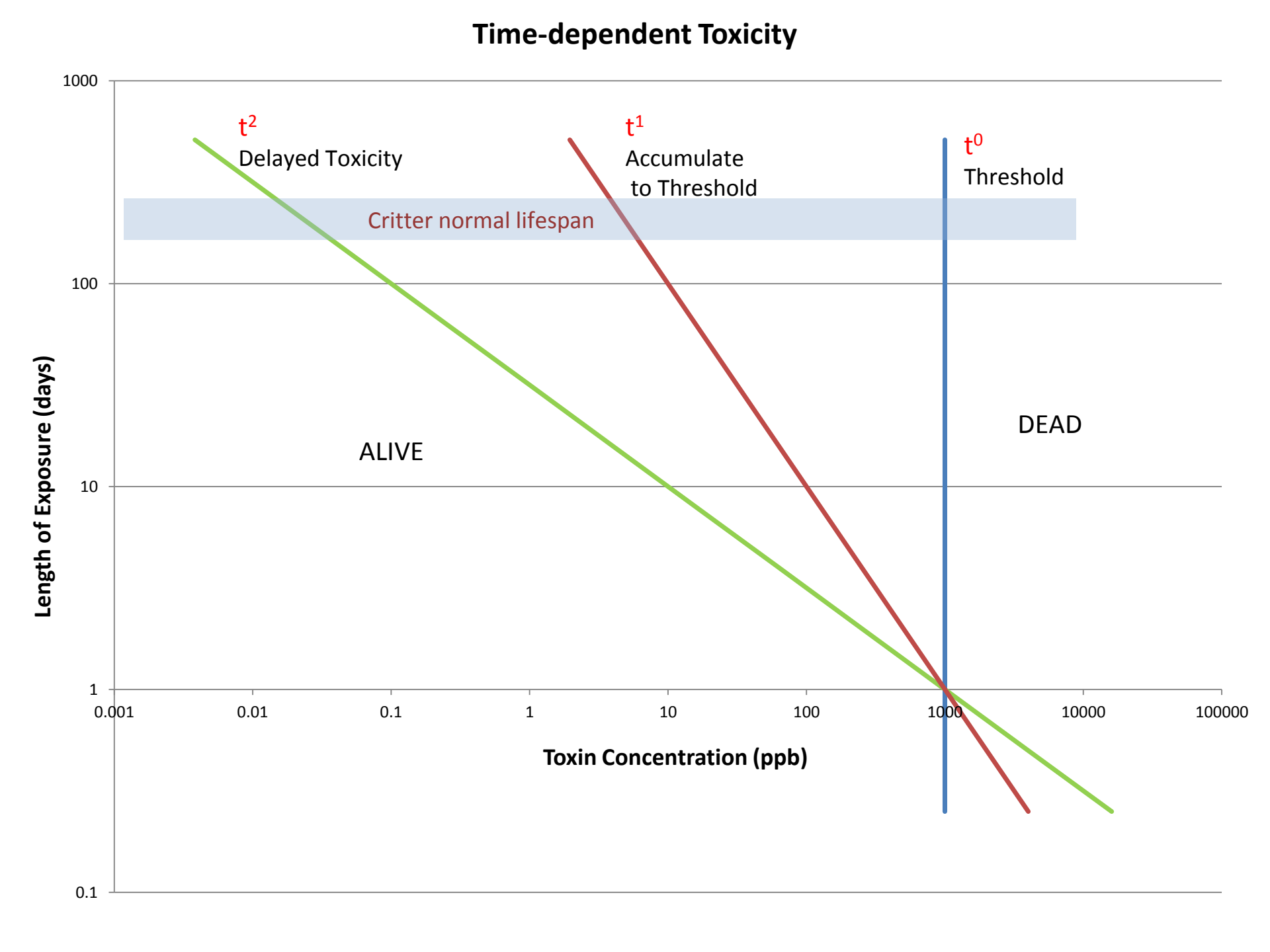
Time-dependent Toxicity

- Depends on the toxic mode of action
- t^0 Threshold action (time doesn't matter)

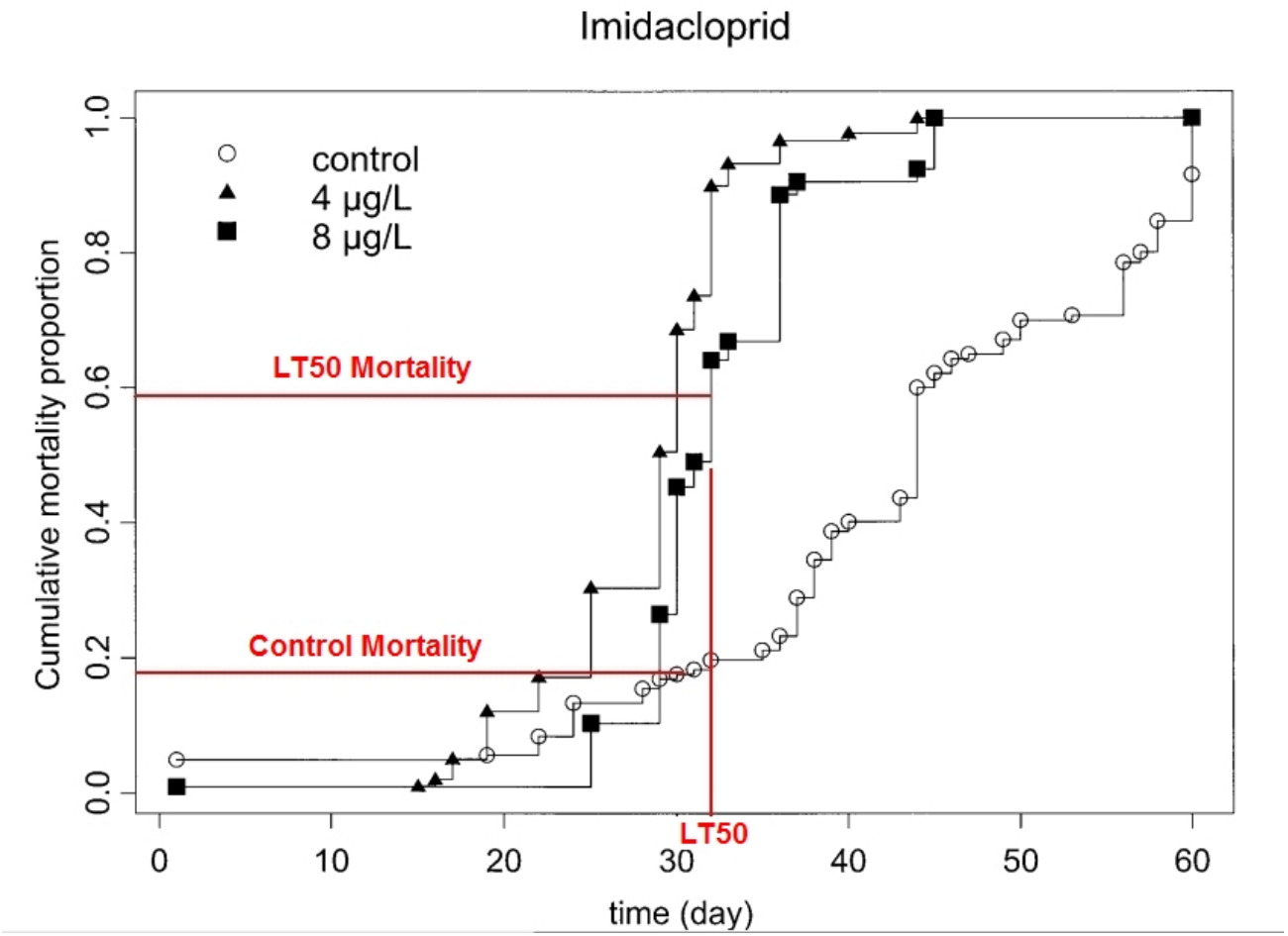
  CO_2 Suffocation; Carbaryl insecticides
- t^1 Accumulate to a threshold

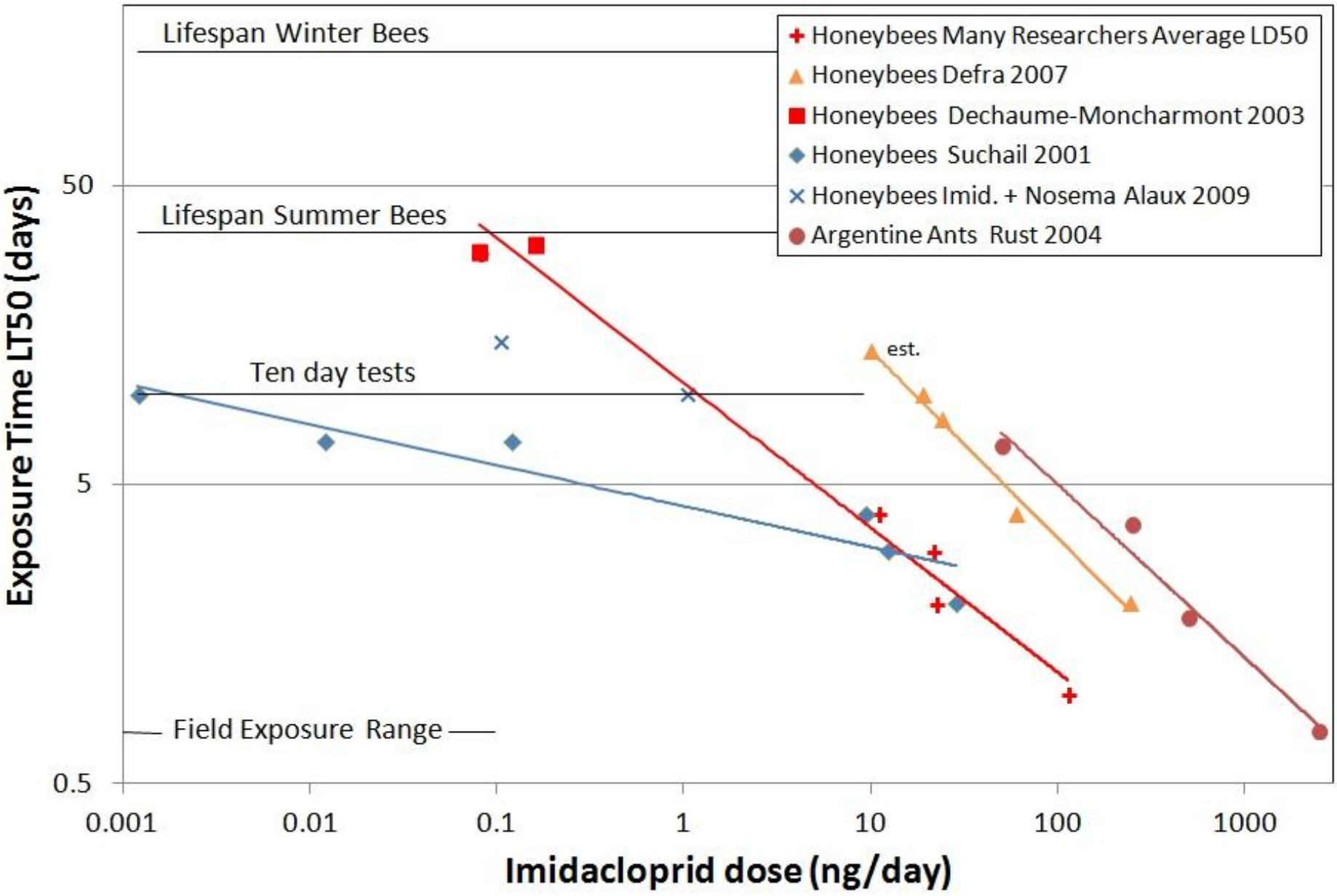
  Organophosphate insecticides
- t^2 Enhanced and Delayed Toxicity

  Carcinogens; Heavy Metals; Neonicotinoids


Time-dependent Toxicity
1000
100
10
1
0.1
Length of Exposure (days)
t2
Delayed Toxicity
t1
Accumulate
to Threshold
t0
Threshold
Critter normal lifespan
ALIVE
DEAD
0.001
0.01
0.1
1
10
100
1000
10000
100000
Toxin Concentration (ppb)

Toxicity Tests Need Enough Time




Lifespan Winter Bees
Lifespan Summer Bees
Ten day tests
Field Exposure Range
Honeybees Many Researchers Average LD50
Honeybees Defra 2007
Honeybees Dechaume-Moncharmont 2003
Honeybees Suchail 2001
Honeybees Imid. + Nosema Alaux 2009
Argentine Ants Rust 2004
est.
Exposure Time LT50 (days)
50
5
0.5
0.001
0.01
0.1
1
10
100
1000
Imidacloprid dose (ng/day)

Time Scaling & Safety Margin

Example: Target insect kill in 3 days; Pollinator protect for 100 days; Assume same intrinsic toxicity of pesticide.

Time Scaling	Description	Ratio	With x3 safety factor
t^0	Threshold	1 : 1	1 : 3
t^1	Accumulate to threshold	3 : 100	1 : 100
t^2	Enhanced & delayed toxicity	9 : 10000 = 1: 1100	3 : 10000 = 1 : 3300

Lowest Observed Effect Concentration (LOEC) for Imidacloprid

Researcher	LOEC for honey bees
1998 Bayer - lethality	100 ppb
2003 Maus, Bayer – survey	20ppb
2003 Dechaume-Moncharmont - lethality	<4 ppb, 30 days
2013 DiPrisco, Deformed Wing Virus replication	1 ppb, 1-3 days
2014 Feltham et.al., Bumblebees – pollen gathering	6 ppb
2014 Charpentier et al., Fruit fly – mating behavior	0.1 ppb
2001 Suchail	0.1 ppb, 10 days ?
2013 Rondeau – extrapolate t^2 scaling to 150 d	0.4 ppb

Reported Residues

Sunflowers – field	2 – 4 ppb
Canola – field	1 – 6 ppb
Pumpkins – field	4 -87 ppb
Linden trees - flowers	20 – >1000 ppb
Horse Chestnut – flowers	5 – 283 ppb
Serviceberry – flowers	1000-2800 ppb
Nursery plants (FOE)	11-1500 ppb

The Problems with Neonics

1) They are strongly binding and direct acting so they can and do show enhanced & delayed toxicity.
2) They have a long lifetime in the environment compared to the life time of non-target insects.
3) Are designed to end up in plant tissue, which includes nectar and pollen that are bee food.
4) Are water soluble so can move offsite into ground and surface waters.

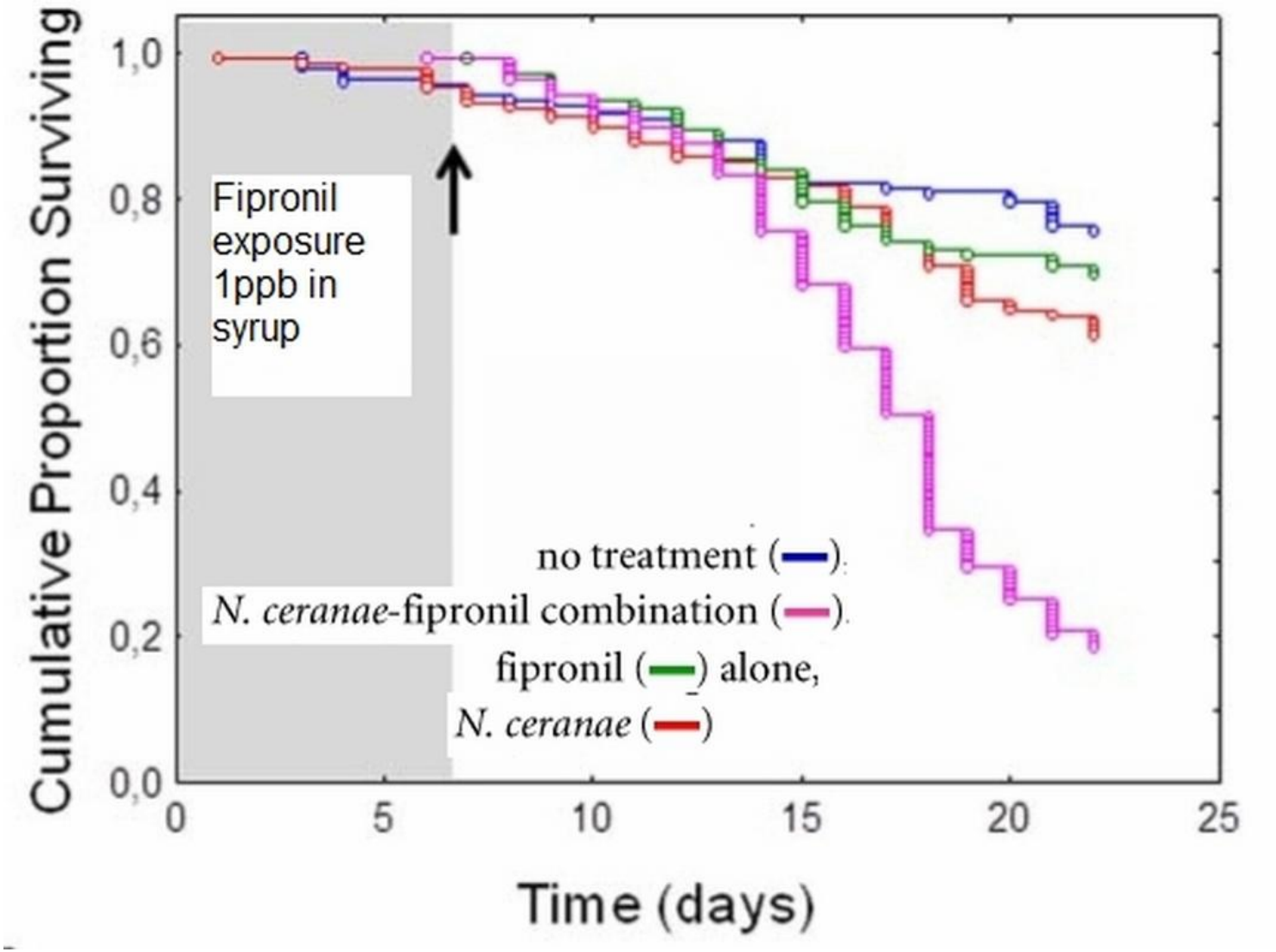
What no one saw coming

Immune suppression from low residual concentrations of neonics – don’t typically see this with OP pesticides.

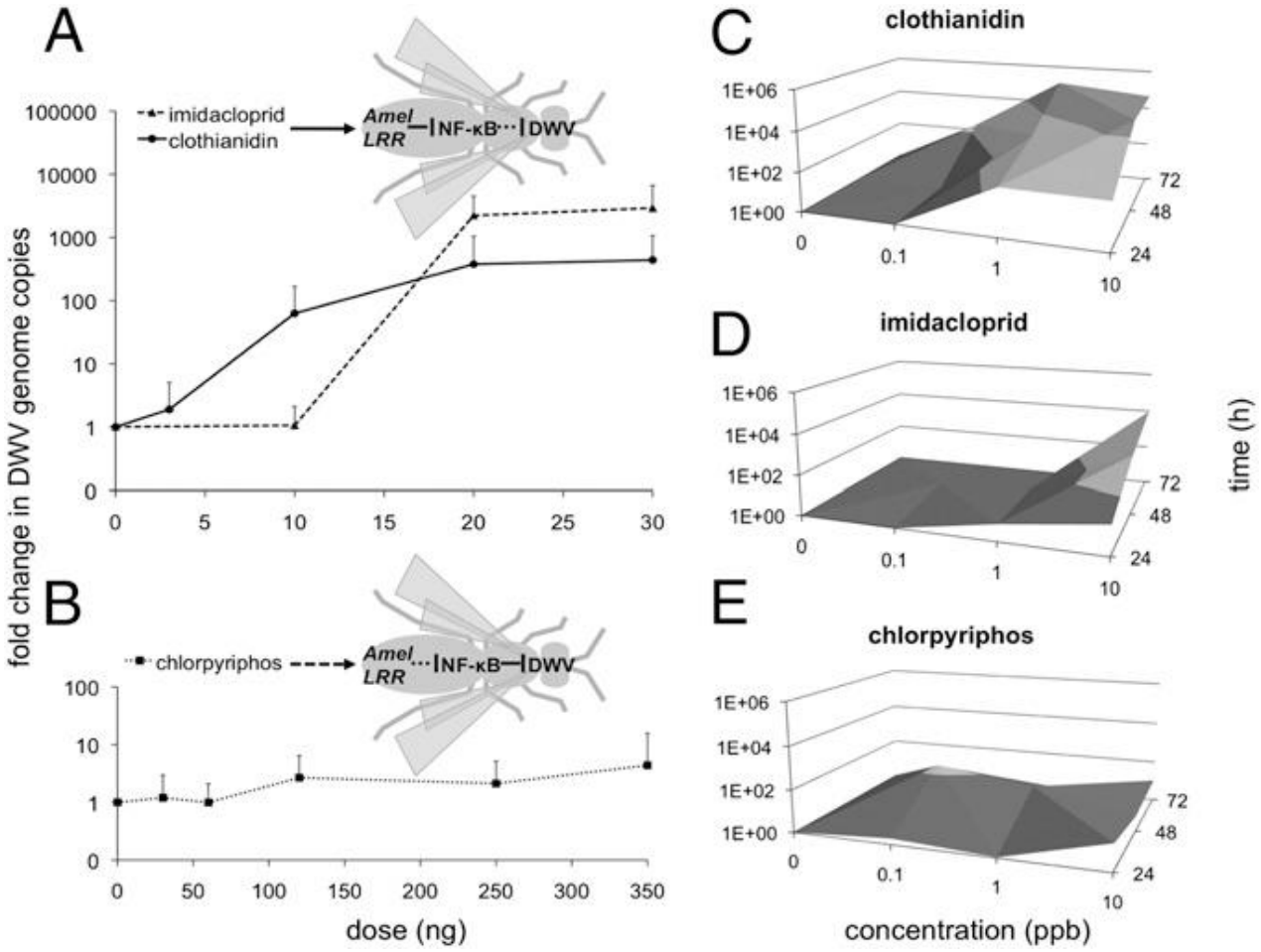
Pesticide – pathogen interactions

- Hint with Suchail et al. – unrepeatable experiment with extraordinarily high sensitivity to imidacloprid – 10 days.
- Pettis et al. 2012 – Chronic colony exposure 5ppb imidacloprid makes newly emerged workers more susceptible to Nosema pathogen.

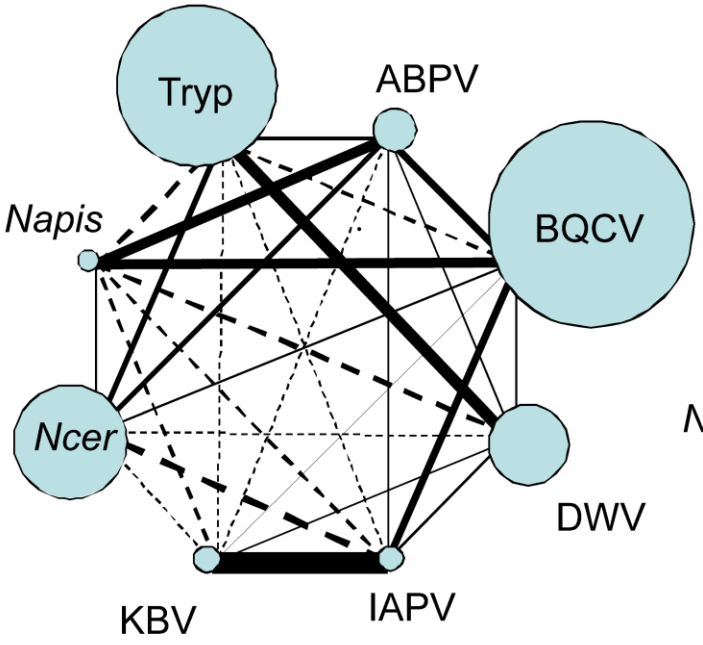
Fipronil Nosema Interaction (Aufauvre)

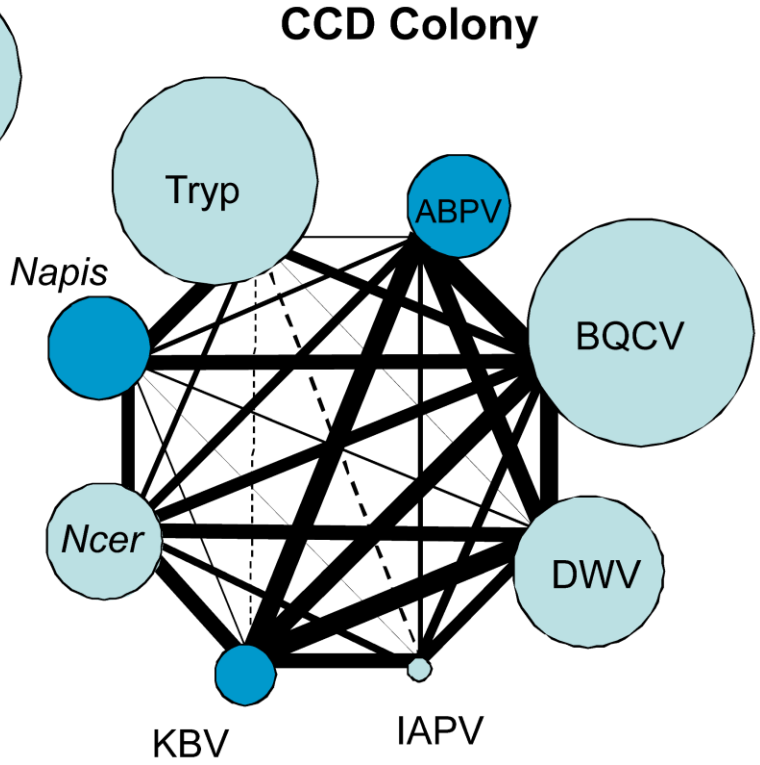


Neonics & DWV (Di Prisco)



Pathogen Interaction Web (Cornman)





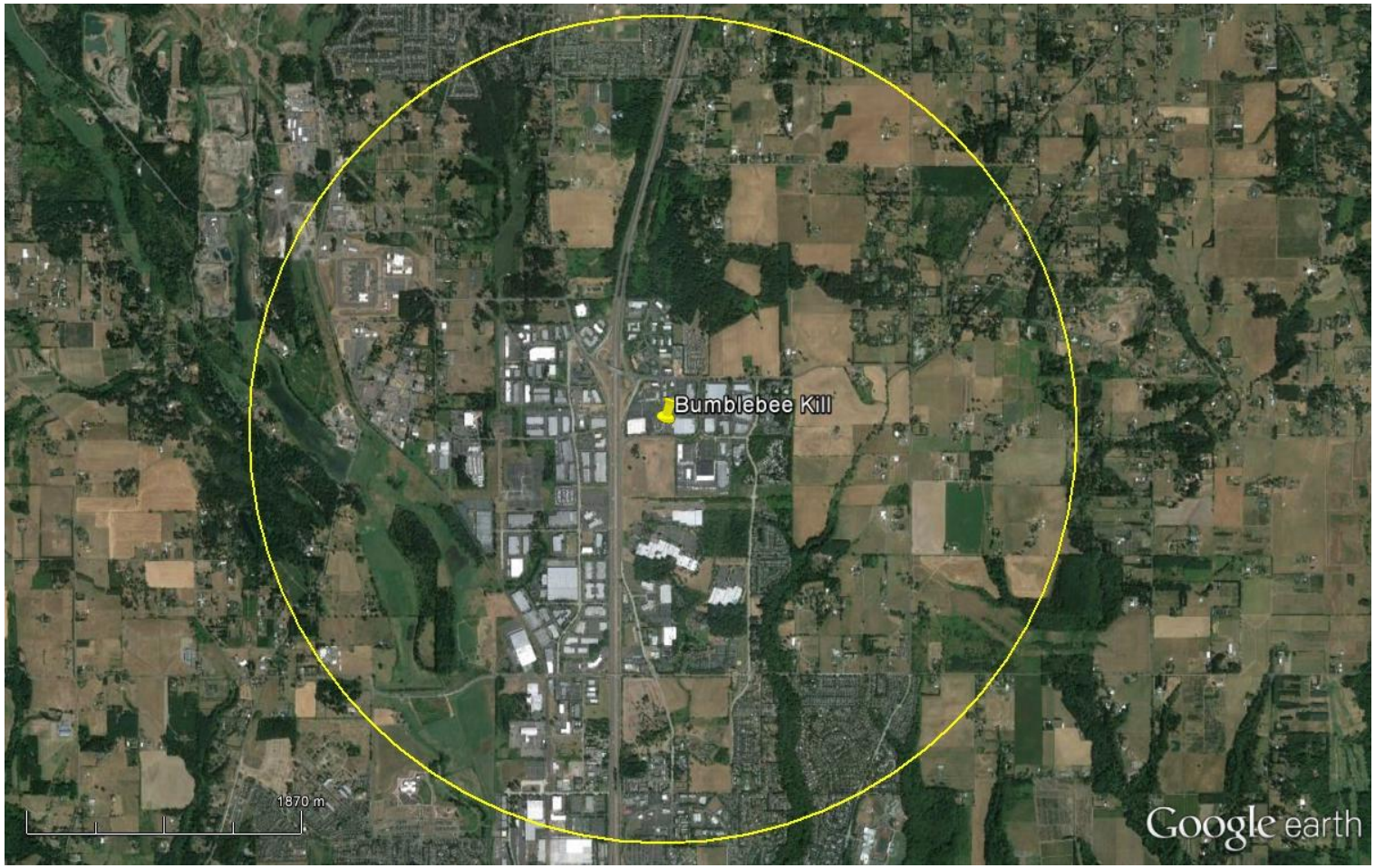
Conclusions

- Neonics have the potential to do damage at virtually undetectable doses <0.1 ppb when interacting with pathogens.
- Time-of-exposure matters! Chronic exposure at sublethal levels will kill and weaken bees.
- Finding a dose that kills target insects yet does not harm bees – can’t happen with most neonics.
- Ban them!

Wilsonville Bee Kill

- >50,000 Bumblebees died
- Dinotefuran, a neonicotinoid sprayed while Linden tree was blooming.
- VERY high toxicity – killed bees immediately 930 ppb in bees; 10,000 ppb in flowers!
- Was not applied according to label so pesticide applicator was fined.

Wilsonville Bumblebee Range



Hillsborough and other small bee kills

- Not so dramatic – hundreds of dead bees.
- Dinotefuran and Imidacloprid (both neonics) were to blame.
- Applications at least 6 weeks prior to blooming were according to label instructions or nearly so.
- Typical residual toxin tested 40 ppb blossoms killed some bees while they foraged.