Development of Rynaxypyr®: Assessment of Impact to Non-target Organisms Including Pollinators in the Insecticide Discovery Process

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E. I. DuPont de Nemours & Co.

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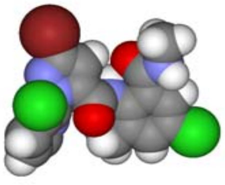
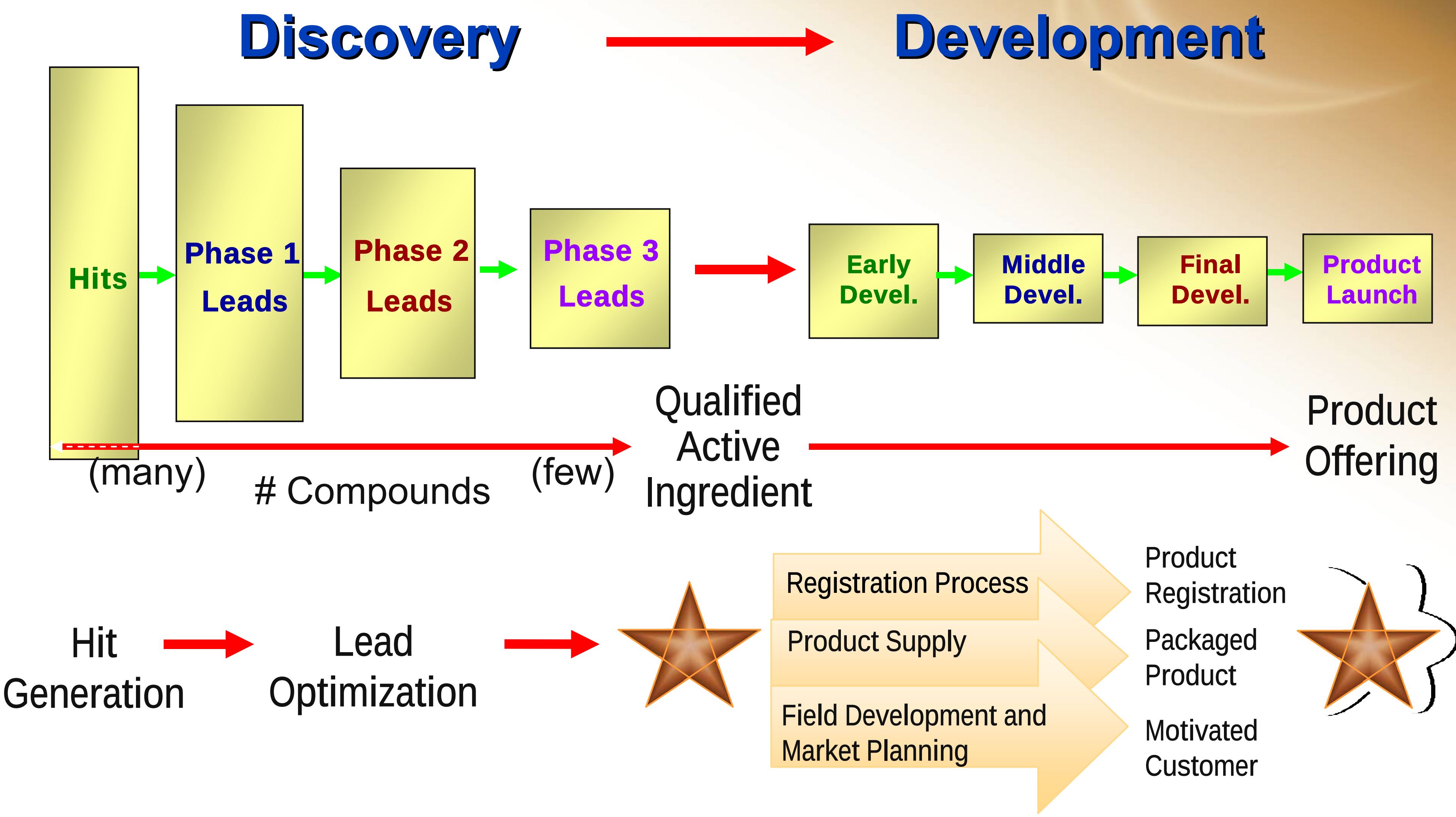


New Product R & D Process



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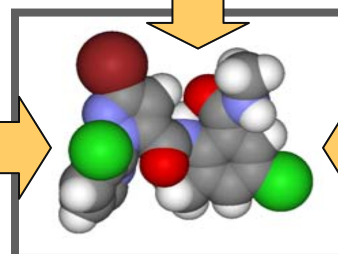
Previous Discovery Process

Nematode Control

Broad-spectrum, usable with precision application technology

Insect Control

Broad-spectrum, systemic activity



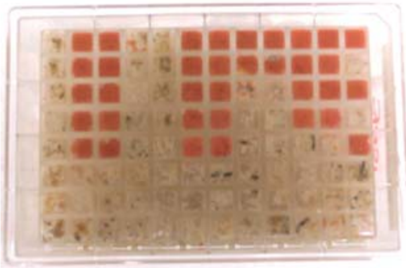
Disease Control

Protective, curative, systemic, broad-spectrum fungicides

Weed Control

Broad-spectrum, residual, pre/early/post-emergence herbicides

Evolving Discovery Process



Nematode Control

Broad-spectrum, usable with precision application technology

Safe

Insect Control

Broad-spectrum,

Disease Control

Protective, curative, eradicative, systemic, broad-spectrum fungicides

Toxicology

Acute, sub-chronic, developmental

Weed Control

Broad-spectrum, residual, pre-/early postemergence herbicides

Evolving Discovery Process

Nematode Control

Broad-spectrum, usable with precision application technology

Safe

"Green"

Insect Control

Broad-spectrum,

Disease Control

Protective, curative, eradicative,

Toxicology

Acute, sub-chronic, developmental

Environmental Fate

Physical properties, metabolism, photostability, soil dissipation

Weed Control

Broad-spectrum, residual, pre-/early postemergence herbicides

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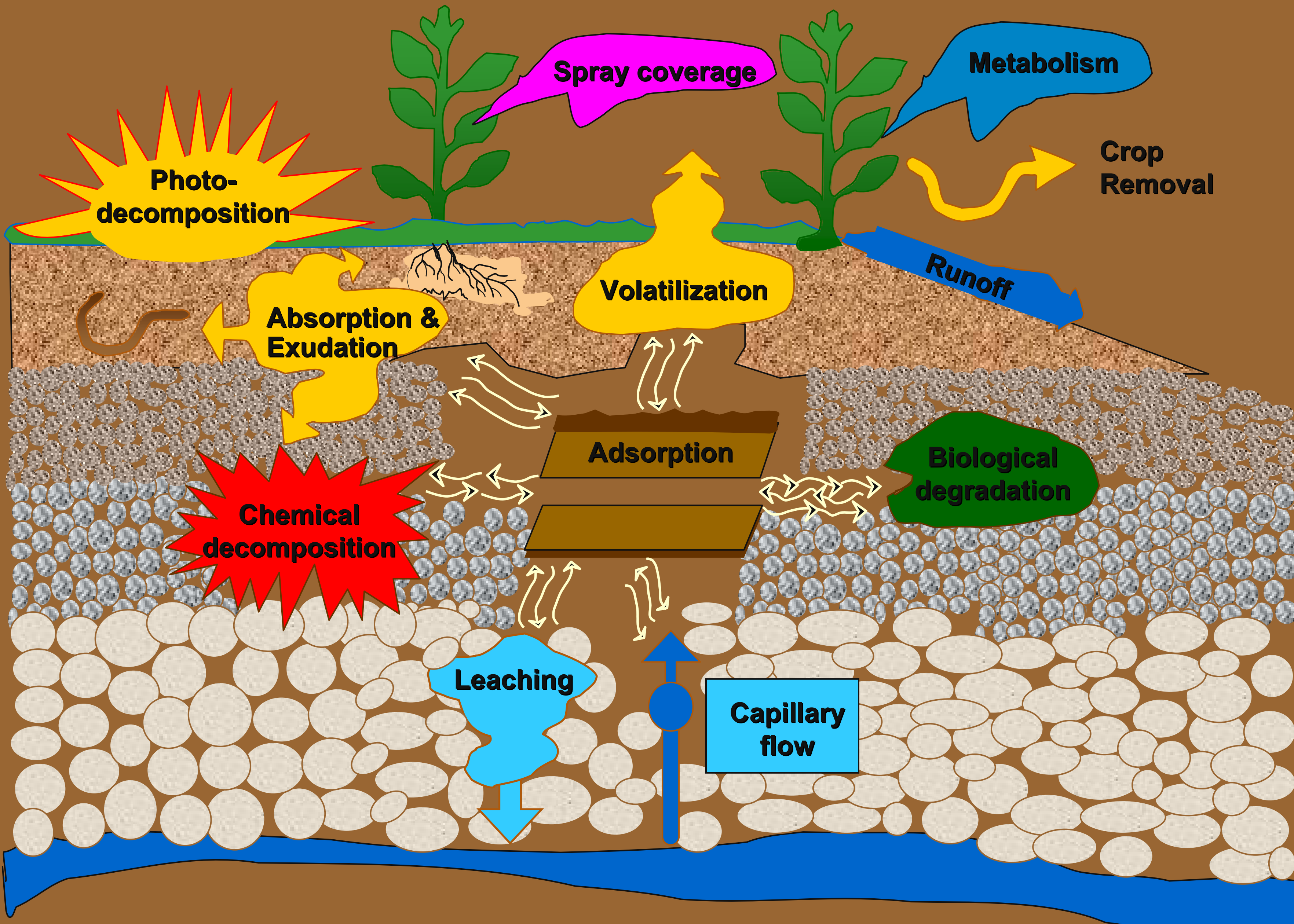
Environmental Fate of Agrochemicals



Evolving Discovery Process

Nematode Control

d-spectrum, usable
cision application
chnology

Mode of Action

Novelty desired, compatible with IPM & RM

Safe

"Green"

New MOA

Insect Control

Broad-spectrum,

Disease Control

Protective, curative,

Toxicology

Acute, sub-chronic, developmental

Environmental Fate

Physical properties, photostability, metabolism, soil dissipation

Weed Control

Broad-spectrum, residual, pre-/early postemergence herbicides

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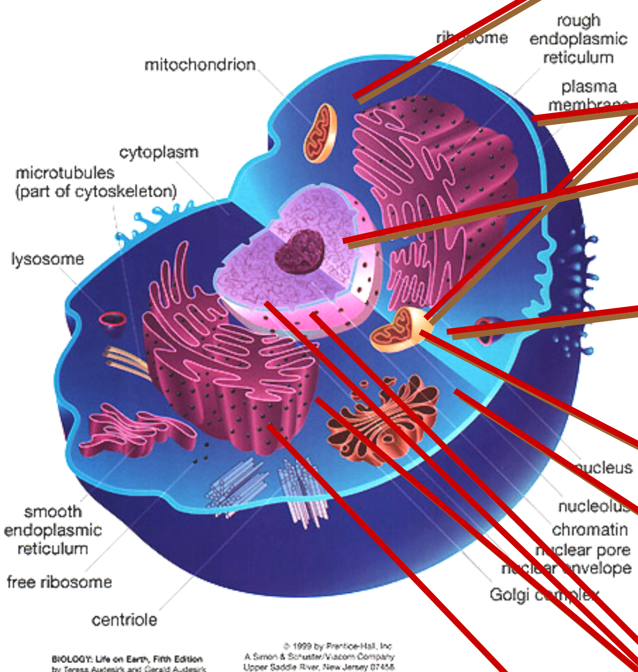
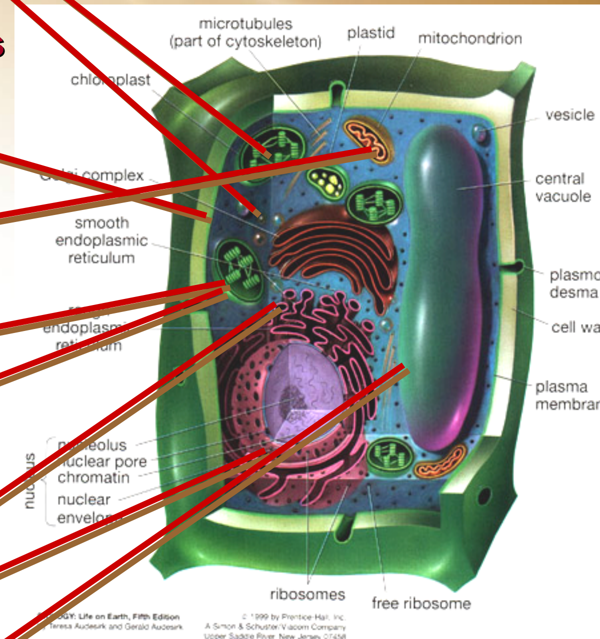
Biochemical Target Identification (SoA)





Amino acid biosynthesis

Cofactors, prosthetic groups

Cell envelope

Cell cycle

energy

Fatty acid, P-lipid, sterol

photosynthesis

respiration

purine, pyrimidine

regulation

transcription, translation

transport

nutrition

affinity labels

morphology

phenotype

metabolomics

biomarkers

proteomics

DNA microarrays

Current Discovery Process

Nematode Control

d-spectrum, usa
cision appli
chnology

Mode of Action

Novelty desired, compatible with IPM & RM

Ecotoxicology

Aquatic, birds & mammals, pollinators

Safe

"Green"

New MOA

Insect Control

Broad-spectrum,

Disease Control

Protective, curative,

Toxicology

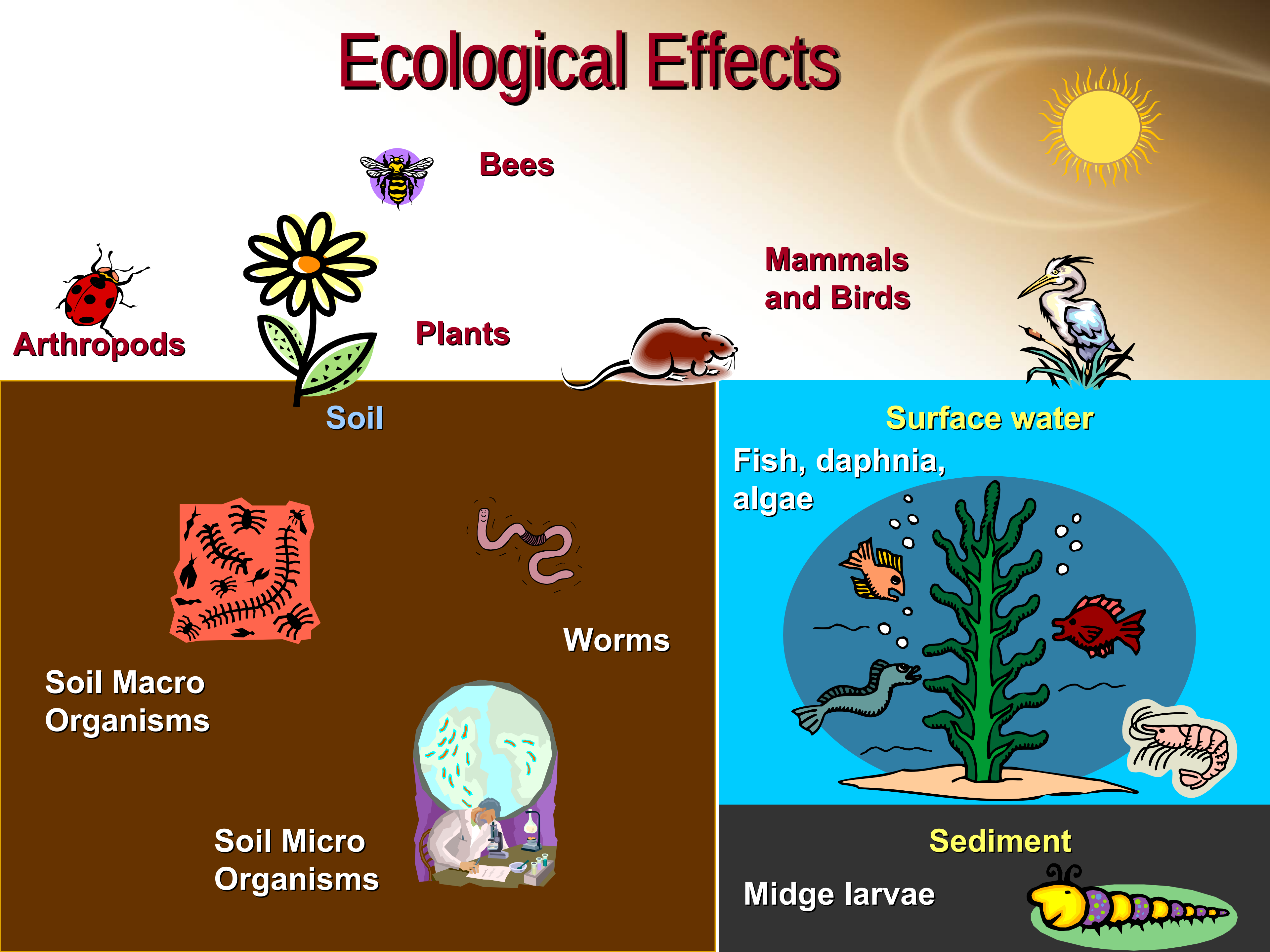
Acute, sub-chronic, developmental

Environmental Fate

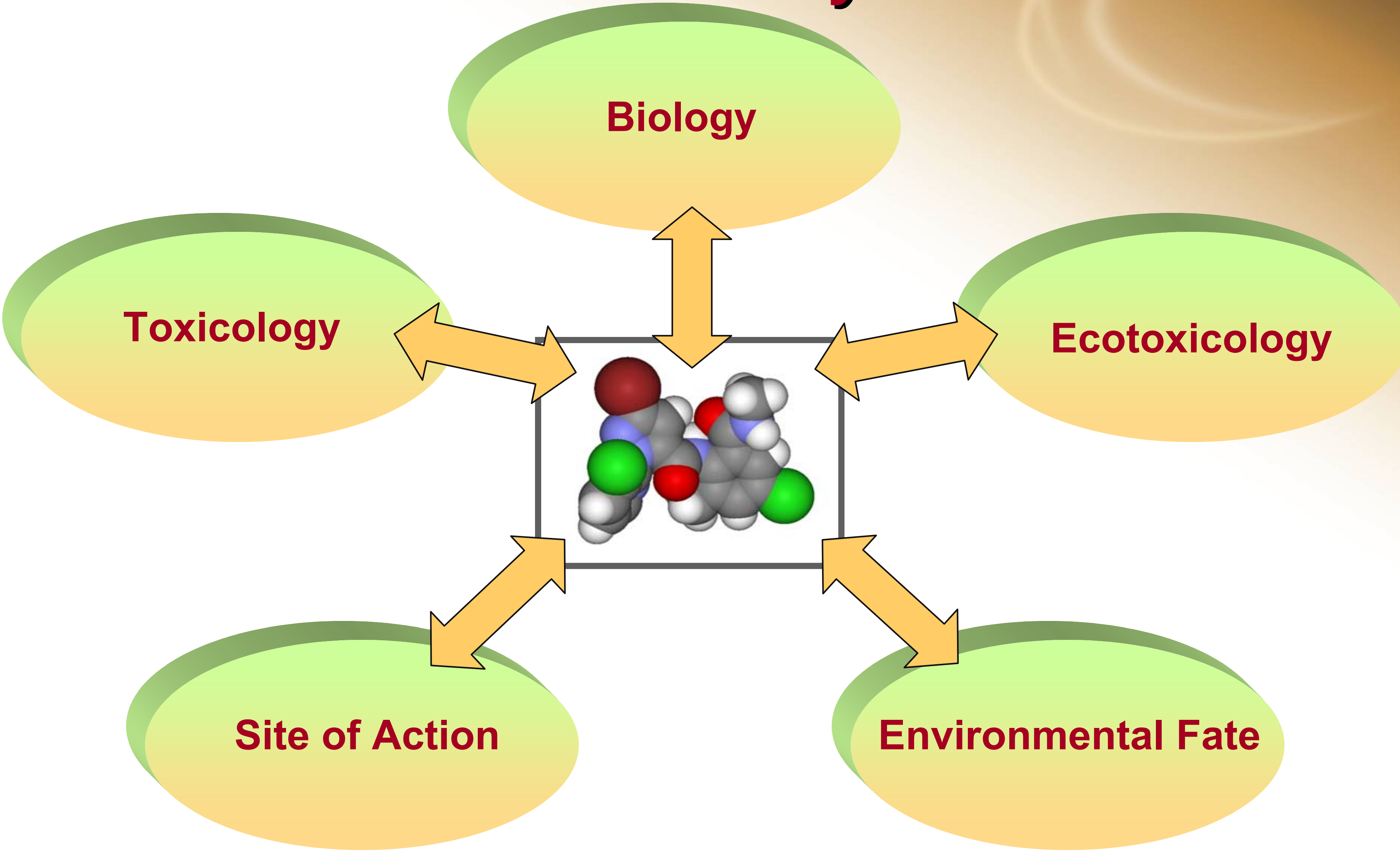
Physical properties, photostability, metabolism, soil dissipation

Weed Control

Broad-spectrum, residual, pre-/early postemergence herbicides


Ecological Effects
Bees
Arthropods
Plants
Mammals
and Birds
Soil
Surface water
Fish, daphnia,
algae
Worms
Soil Macro
Organisms
Soil Micro
Organisms
Sediment
Midge larvae

Current Discovery Process



Drivers for Early Pollinator Testing

- Regulatory requirements
  - Directive 91/414/EEC
  - Regulation (EC) 1107/2009
  - US Reduced Risk
- Early identification of potential regulatory issues
- Save time in planning for appropriate higher tier testing
- Determine extent of Integrated Pest Management (IPM) fit
- "Customer" expectations around stewardship

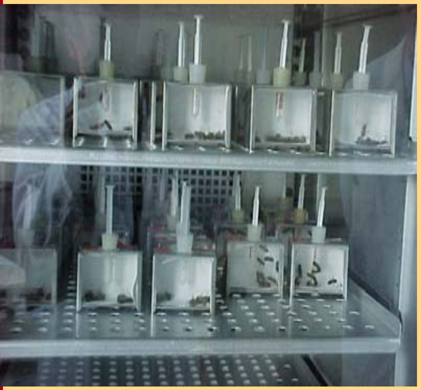
Choose the best candidate molecule

Factors facilitating Early Testing

- Testing should require minimal amounts of compound
- Testing should allow for more compounds to be screened
- Testing results need to be available in a useful timeframe
- Testing should be predictive/reliable indicator
  (standardized, regulatory protocols)

Honey Bee Testing (*Apis mellifera*)

- **Acute contact (topical application)**
  - Screening test: adapted OECD 214
  - Technical material or formulation
  - Dose response testing (0.1 - 100 µg a.i./bee)
  - Measured parameters: mortality and behavior
  - Endpoints: 48-96h LD_{50} (µg a.i./bee)

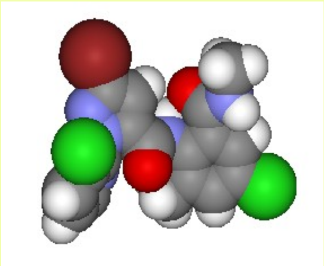


- **Acute oral (dietary exposure)**
  - Screening test: adapted OECD 213
  - Technical material or formulation
  - Dose response testing (0.1 -100 µg a.i./bee)
  - Measured parameters: mortality and behavior
  - Endpoint: 48-96h LD_{50} (µg a.i./bee)

Summary

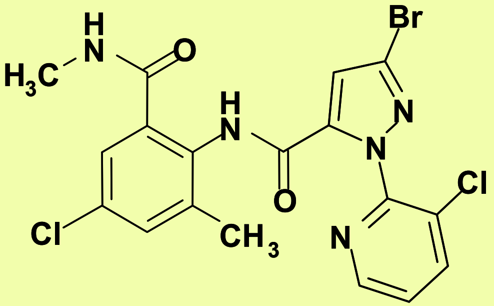
- Regulatory test designs (OECD methods) can be adapted to meet needs of a Discovery process (amount of test material needed, feasibility for testing numerous analogs)
- Discovery level Ecotox testing is contributing to the optimization/selection of new candidate molecules
- Challenges - potential bee brood effects, prediction of effects on other pollinators, etc.

Nomenclature and General Properties



Trade name:	**Rynaxypyr®**
Common name:	**Chlorantraniliprole**
Chemical class:	**Anthranilic diamide**
Code number:	**DPX-E2Y45**
Molecular formula:	**$C_{18}H_{14}BrCl_2N_5O_2$**

Structural formula:

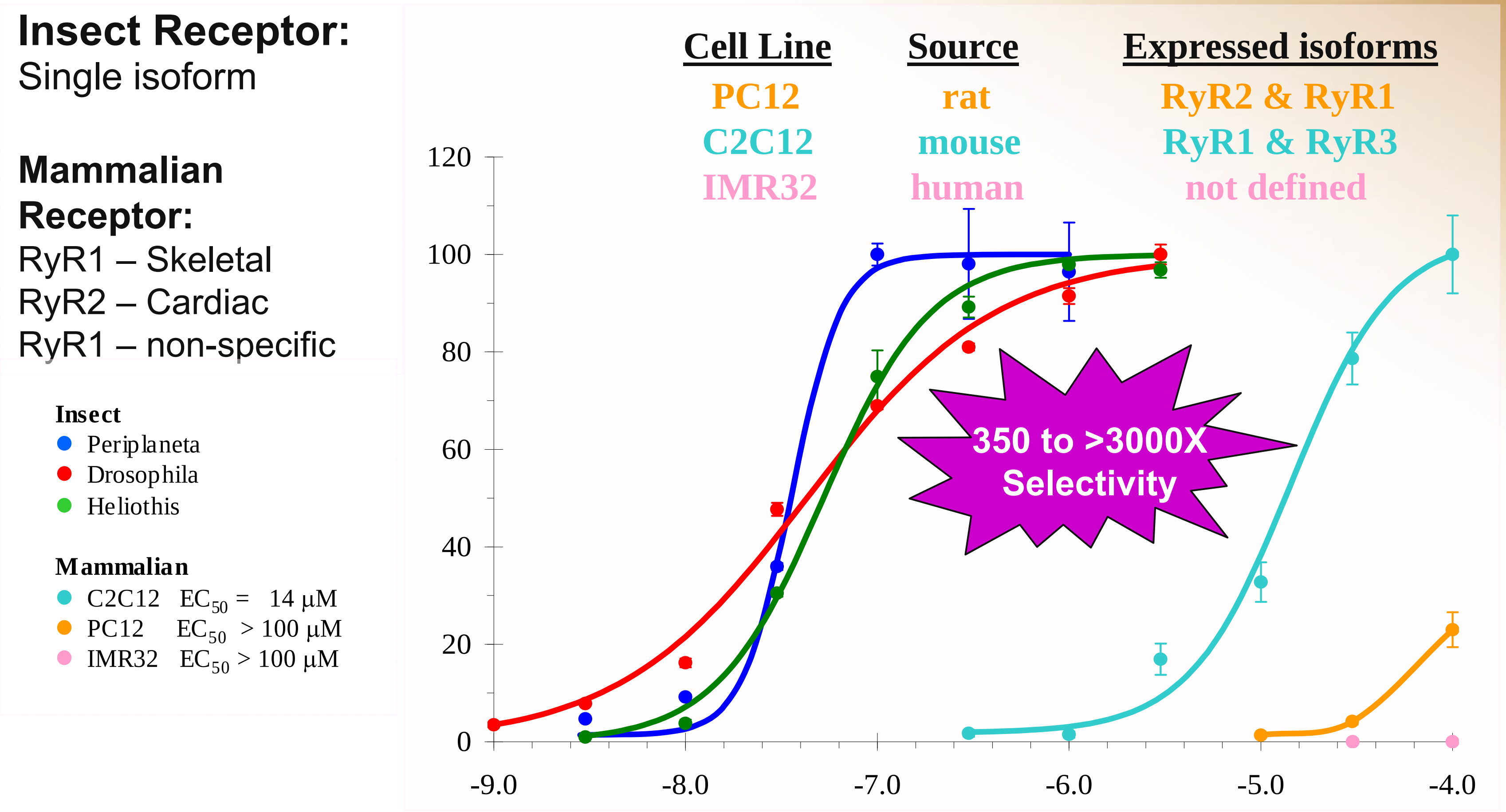


Molecular weight:	483.15 g/mole
Physical state (pure a.i.):	fine crystalline off-white powder
Melting point (pure a.i.):	208-210°C [(200-202°C (technical)]
Vapour pressure (20 °C):	6.3×10^{-12} Pa
Part. Coeff. oct/w (Log P_{ow}) (20 °C):	2.76 (distilled water)
Dissociation Constant (pK_a) (20 °C):	10.88
Solubility in water (20 °C):	1.0 mg/L

Excellent Insect Receptor Selectivity

Insect Receptor:
Single isoform

Mammalian Receptor:
RyR1 – Skeletal
RyR2 – Cardiac
RyR1 – non-specific



Honey Bee Risk Assessment

EU - Hazard Quotient Approach

- **HQ = max. application rate (g a.i./ha) / LD_{50} (µg a.i./bee)**
- **If HQoral and HQcontact are < 50, then acute safety to bees can be assumed based on historical comparison of lab data and field data with numerous different test substances. Otherwise higher tier testing is possible.**
- **If LD_{50} >100 µg a.i./bee, product is considered non-toxic.**

US categorizes hazard to honeybees as follows:

- **LD_{50} < 2 µg a.i./bee: highly toxic**
- **$LD_{50} \geq$ 2-10.99 µg a.i./bee: moderately toxic**
- **LD_{50} > 11 µg a.i./bee: practically non-toxic**

DUPONT

Rynaxypyr®
Safety to Honey Bees - Acute Exposure

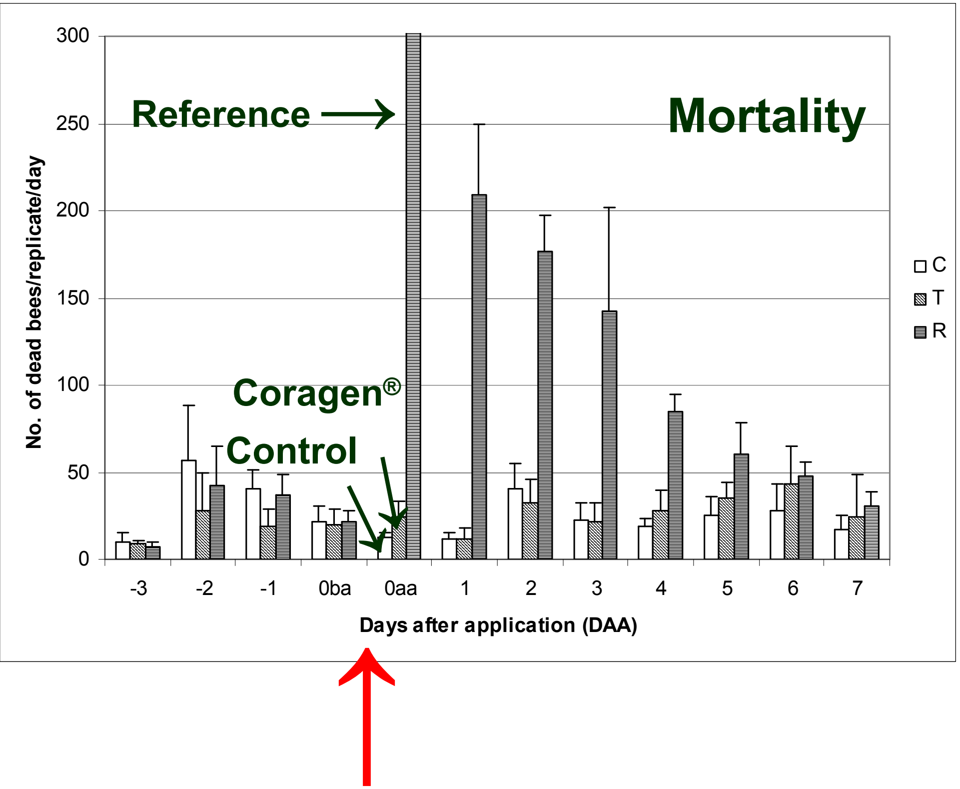
Test material	Oral (µg Rynaxypyr® /bee)	Contact (µg Rynaxypyr® /bee)
Coragen® 20SC	LD_{50} = 117.8 EU HQ = 0.5	LD_{50} = 81.5 EU HQ = 0.7 USA = PNT
Altacor® 35WG	LD_{50} > 119.2 EU HQ < 0.5	LD_{50} = 100 EU HQ = 0.6 USA = PNT

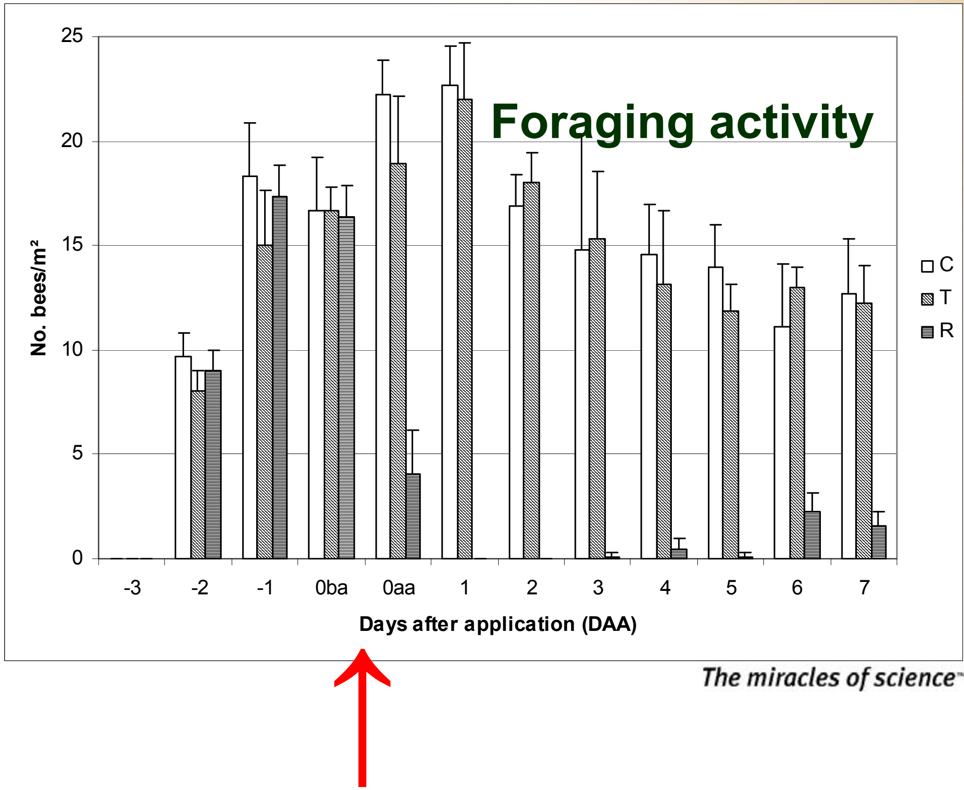
EU Higher Tier Studies
EPPO 170-3 *Phacelia* Tunnel Tests

- **GLP tunnel tests – Germany, Spain, France**
- **Coragen® – Spray application during foraging activity**

EPPO 170-3 *Phacelia* Tunnel Tests Results – France 2006

- Coragen® tested at up to 60 g a.i./ha had no negative impact on the honey bee, *Apis mellifera* in several semi-field tunnel tests with flowering *Phacelia tanacetifolia*, when directly over-sprayed during foraging activity of honey bees (e.g. France 2006)





EPPO 170-3 *Phacelia* Tunnel Tests

Test	Mortality	Behavioural effects	Impact on colony
Germany, 2004 52.5 g a.i./ha *A. m. Carnica*	**No significant increase in mortality compared to control**	**No inhibition of flight intensity or changes in individual behaviour compared to control**	**Colony strength not affected** **All brood stages present at on day +8**
Spain, 2004 52.5 g a.i./ha *A. m. Mellifera*			**Colony strength not affected** **All brood stages present at on day +22**
France, 2006 60 g a.i./ha *A. m. Carnica*			**Colony strength not affected** **All brood stages present at on day +7, +14, +22, +28**

Rynaxypyr® – Exposure Assessment

- **EPPO 170-3 tunnel design, 3 treatments:**
  1.) untreated control
  2.) soil application at Phacelia planting at 314 g Rynaxypyr®/ha (6 weeks before flowering)
  3.) spray application during Phacelia flowering at 60 g Rynaxypyr®/ha

- **Bees of all treatments exposed in parallel to flowering Phacelia**

- **Residue sampling dates**
  DAA -1, +1, +3, +7; (no DAA+3 inside hive)

- **Residue samples taken**
  pollen & nectar from forager bees, outside hive
  pollen, nectar and wax samples, inside hive

Rynaxypyr® – Exposure Assessment

Rynaxypyr® – Exposure Assessment

		Nectar from Forager Bee Stomachs	Pollen from Forager Bee Legs	Nectar from Bee combs inside hive	Pollen from Bee Combs inside hive	Wax from Bee Combs inside hive
Treatment	Collection Date	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
1.) Control - water	-1	**0**	**0**	**0**	**0**	**0**
sprayed onto	+1	**0**	**0**	**0**	**0**	**0**
foraging bees in	+3	**0**	**0**	**--**	**--**	**--**
flowering Phacelia	+7	**0**	**0**	**0**	**0**	**0**
2.) 314 g ai/ha	-1	**0**	**0**	**0**	**0**	**0**
soil application	+1	**0**	**0**	**0**	**0**	**0**
at Phacelia	+3	0.0032	0.0010	**--**	**--**	**--**
planting	+7	**0**	0.0018	**0**	**0**	**0**
3.) 60 g ai/ha spray	-1	**0**	**0**	**0**	**0**	**0**
onto flowering	+1	0.0330	2.601	0.0472	2.348	0.0105
Phacelia during	+3	0.0096	0.7633	**--**	**--**	**--**
foraging activity	+7	0.0036	0.2643	0.0013	0.1080	0.0757

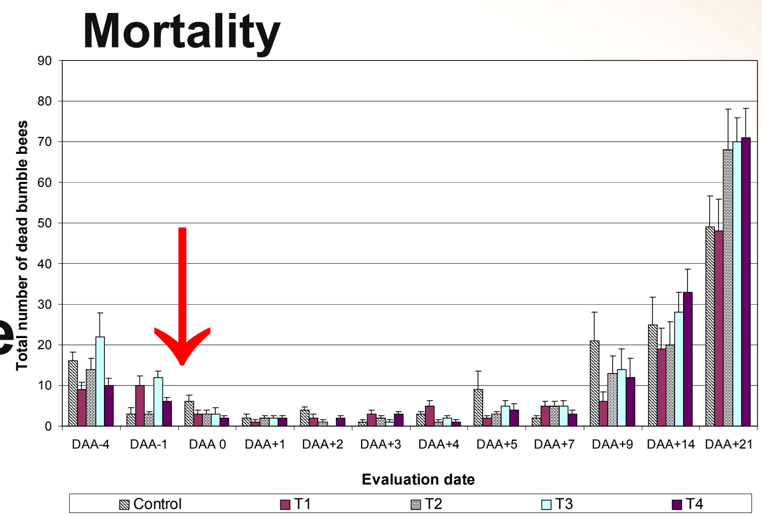
Low risk for honey bees via systemic uptake

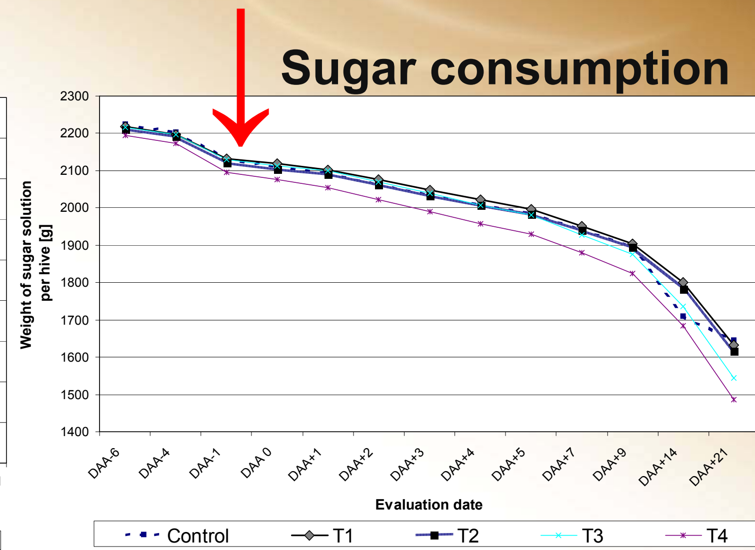
Rynaxypyr® Bumble Bee Greenhouse Trial

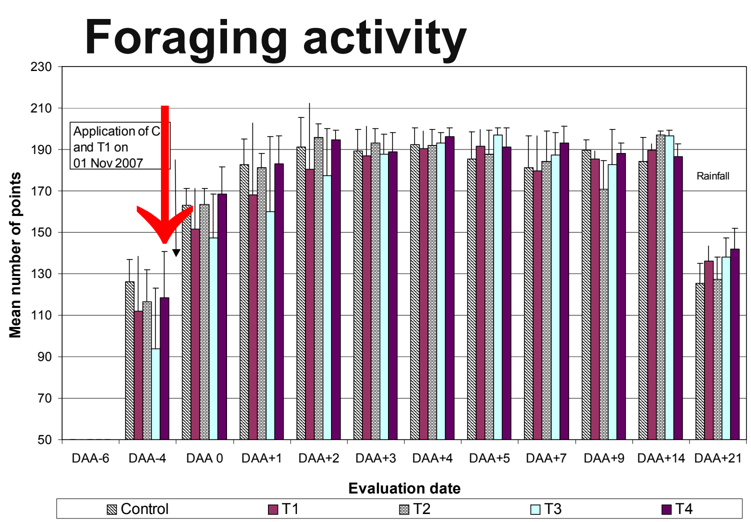
Rynaxypyr® Bumble Bee Greenhouse Trial

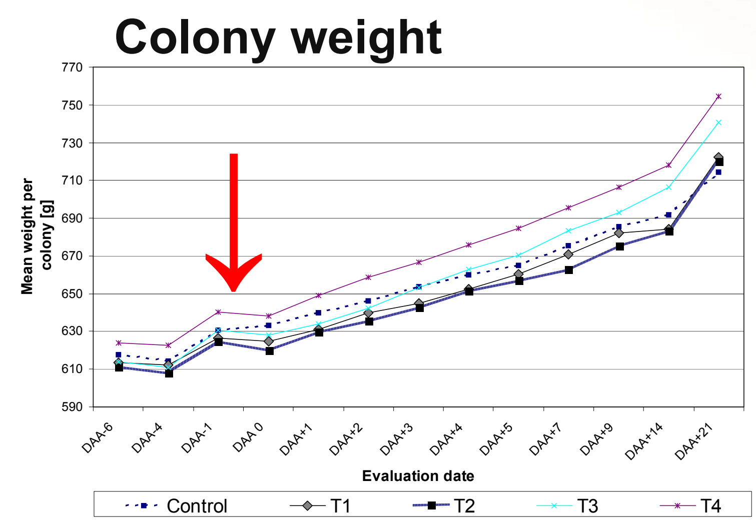
Rynaxypyr® Bumble Bee Safety

- **40 g a.i./ha**
- **Exposure during foraging activity and to 1, 2 & 3 day old residues**









Conclusions – Pollinator Safety

Rynaxypyr® and its formulated products – Coragen® and Altacor® – are expected to present low risk for honey bees and bumble bees, because

- Low intrinsic honey bee toxicity
- Safety demonstrated in worst-case honey bee EPPO 170-3 & CEB 230 tunnel tests:
  - No increased mortality
  - No behavioral effects
  - No effects on honey bee brood
- Safety shown in worst-case bumble bee greenhouse tomato test

Also, low impact to beneficial arthropods

Excellent IPM profile!

Dinter *et al.* 2008. Rynaxypyr & Beneficials. IOBC Bulletin 35: 128-136

Dinter et *al.* 2009. Rynaxypyr: Honeybees & Bumblebees. Julius-Kühn-Archiv 423: 84-96

Gradish *et al.* 2010. Rynaxypyr & *Bombus impatiens*. Pest Manag Sci 66: 142-146

Brugger et al. 2010 online. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Manag Sci 66

DU PONT®