Characterizing the risk of pesticide use on amphibian and reptile populations based on multiple, ecologically relevant responses

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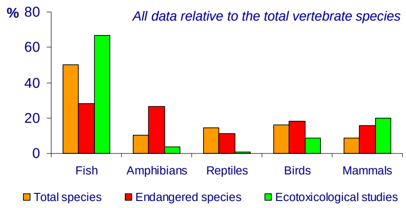
State of the art

Amphibians and reptiles are **the two less studied vertebrate groups** in ecotoxicology, which is inconsistent with their species richness or global conservation status



Traditionally excluded from pesticide risk assessment procedures, which is attributable to:

- Lack of information and consequent scarcity of standardized guidelines for toxicity assessment
- Assumption that toxicity data based on fish cover amphibian aquatic stages[1]
- Assumption that toxicity data based on birds and mammals cover amphibian terrestrial stages and reptiles [not supported by published literature]

Questions to be answered

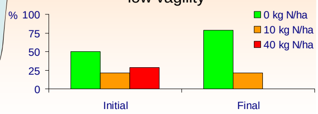
Regarding exposure

▪Oral exposures in the aquatic environment not considered in fish tests

▪Worst-case scenarios based on applications on fish-present environments, neglecting shallow ponds used by amphibians

▪Dermal exposures on land not considered in bird or mammal tests

▪Limited avoidance ability because of low vagility



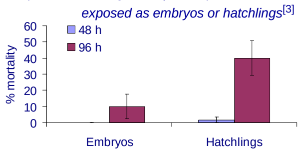
Selection of terrestrial patches by Iberian newts as a function of nitrogen fertilizer load application[2]

Regarding effects

▪Incomplete information on sensitivity: high inter-species and inter-stages variability

▪General lack of regard on **sublethal effects**

Comparison of mortality rates after short-term exposure to 46 mg N-NH_4^+/L of spadefoot toads exposed as embryos or hatchlings[3]



Selection of study species

	Palmate newt *Lissotriton helveticus*	Common frog *Rana temporaria*	Sand lizard *Lacerta agilis*
			
Family	Salamandridae	Ranidae	Lacertidae
IUCN Cat.	LC	LC	LC
Range			

- Belonging to three of the four more diverse families of European amphibians and reptiles (excl. Colubridae)
- No conservation problems
- Present in agricultural areas
- Present in at least two of the three EU regions (Annex I EC/1107/2009)

Objectives and development

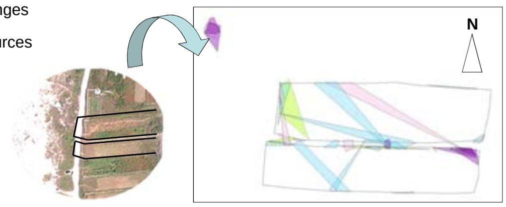
I) Analysis of the risk of exposure of amphibians and reptiles to pesticides on the basis of spatial and behavioural studies

I.a. Oviposition site selection

- In the water (newts)
- On land (lizards)

I.b. Terrestrial habitat use by adult frogs and lizards as a function of pesticide applications

- Characterization of home ranges
- Identification of pesticide sources in space and time
- Analysis of potential shifts in space use through spatial statistics (ArcGis, GeoDa)



II) Comparison of the relative importance of different ways of exposure to pesticides in aquatic and terrestrial environments

II.a. Oral vs. dermal exposure in tadpoles

II.b. Oral vs. dermal exposure in frogs and lizards

NOAEC/LOAC (Oral) + EEC (Prey/food) → TER (Oral)
NOAEC/LOAC (Dermal) + EEC (Soil/water) → TER (Dermal)
TER (Oral) + TER (Dermal) → Relative exposure risk

III) Assessment of effectiveness of toxicity data based on fish, birds or mammals to cover amphibians and reptiles

III.a. Establishment of a toxicity database, including non-standardized tests and sublethal effects

- Standardization of effects relative to each control organism
- Comparison per active ingredient among taxa
- Implementation of life stages, types of responses and conservation status to identify major areas to focus research efforts

→

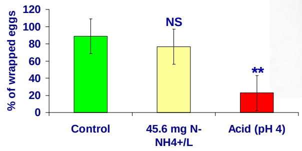
- Sensitive taxa, stages and endpoints
- Hazardous compounds
- Conservation threats

IV) Implementation of ecologically relevant responses specific to amphibians and reptiles as endpoints for studying pesticide effects

IV.a. Newt oviposition behaviour

- Wrapping eggs with plants protect them from damage, predation and UV radiation[4]
- Chemical pollution may impair this behaviour and increase embryo mortality

Oviposition (wrapping) behaviour of pygmy newt and alteration by water acidification[5]



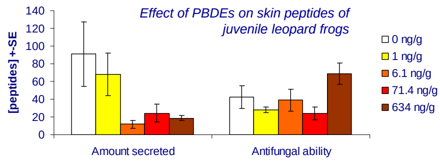
IV.b. Lizard thermoregulation behaviour

- As ectothermic animals, temperature is essential to determine their physiological processes
- Increased temperature helps animals in detoxification
- Reduced thermoregulation efficacy (e.g. wrong selection of basking sites) increases exposure to predators and soil chemicals

IV.c. Constitutive immunity in frogs

- Skin secretions are the main defense against the deadly fungus *Batrachochytrium dendrobatidis*, responsible for amphibian declines worldwide[6]
- Chemical stress can reduce antifungal efficacy (e.g., carbaryl[7], PBDE)

Effect of PBDEs on skin peptides of juvenile leopard frogs



Timeline	2013	2014		2015	
	III.a	I.b / IV.c	II.a / II.b	I.a / IV.a	IV.b

Practical advantages

Government
Identifying mechanisms useful to be developed in guidelines for assessing chemical risks on amphibians and reptiles

Academia
Advancing knowledge on amphibian and reptile ecotoxicology

Industry
Ensuring that mechanisms to test product safety are useful for amphibians and reptiles

Inter-sectorial communication is a must

Let's share experiences

References: [1]Weltje et al (2013) *Environ Toxicol Chem* 32, 984-994. [2]Ortiz-Santaliestra et al (2005) *Bull Environ Contam Toxicol* 75, 662-669. [3]Ortiz-Santaliestra et al (2006) *Environ Toxicol Chem* 25, 105-111. [4]Miaud (1994) *Copeia* 1994, 535-537. [5]Ortiz-Santaliestra et al (2007) *Aquat Toxicol* 85, 251-257. [6]Woodhams et al (2007) *Anim Conserv* 10, 409-417. [7]Davidson et al (2007) *Environ Sci Technol* 41, 1771–1776.

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