Non-invasive method to measure dermal exposure of amphibians to pesticides

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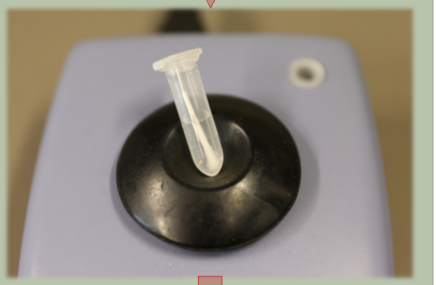
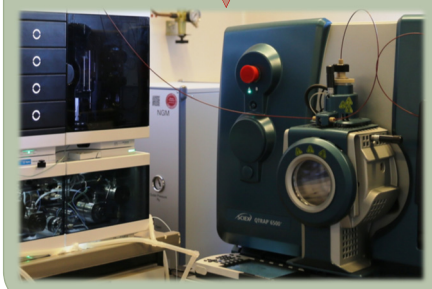
Background

Pesticide exposure of amphibians is difficult to assess due to the various possible exposure routes and the movement behavior between aquatic and terrestrial habitats (EFSA, 2018). In particular, little is known about the dermal uptake of pesticides in field, which appears to be very important for amphibian's exposure risk and survival (Quaranta et al., 2009; Llewelyn et al., 2019). We used swab samples to measure pesticides on the skin of amphibians which possibly originate from contact to contaminated soil, sediment, water, plants or air.

Material and Methods

We investigated adult amphibians, nine common toads (*Bufo bufo*) and one common frog (*Rana temporaria*), which were trapped in and around maize fields in North Rhine-Westphalia (Germany) in summer 2018. About 10 cm² of the ventral and the dorsal side of each animal were swabbed separately using individual sterile Dryswab™ with fine tip rayon buds.
The selection of pesticides based on a proposal for a representative monitoring in the framework of the "Implementation of the National Action Plan on the sustainable use of pesticides" (UBA, 2019).The substances were extracted with a solution of water/methanol (1:1, 1% formic acid) from the swabs. The amounts were measured with liquid and gas chromatography-mass spectrometry. (see supplement for methodical details).





Results

We detected terbuthylazine in samples of three animals (Tab.1). The highest amount of 75 pg /cm² was found on the ventral side of a toad. This amount corresponds to 0.001% of the maximum application rate permitted for maize fields.

Tab. 1: Content of terbuthylazine found in swab samples from five amphibians (all in stage >46 (Gosner, 1960) trapped in and around the same field (n. d. = not detected < 5 pg/cm²).

				Terbuthylazine [pg/cm²]	
No	**Sampling**	**Habitat**	**Species**	**ventral**	**dorsal**
1	26-06-18	Maize field (edge)	Common toad	**75.0**	n. d.
2	04-07-18	Roadside	Common toad	**57.8**	n. d.
5	10-07-18	Maize field	Common frog	**11.5**	**9.2**

Discussion

The detection of terbuthylazine in swab samples from animals in and around maize fields is an expression of the spatio-temporal behaviour of amphibians. The high values at the ventral sides originate possibly from contact of the individuals with residues on the ground after herbicide application.
Plant protection products containing terbuthylazine may be used in the pre- and post-emergence (BBCH 10-17) of maize with maximal 750 g/ha (BVL, 2018). The products contain further herbicidal active substances (i.e. bentazone, bromoxynil, dimethenamid-P, flufenacet and ethoxamid) which were not detected in the samples.

Perspectives

The method allows exposure assessment with an exact spatio-temporal resolution by non-invasive sampling of living amphibians at field sites. This is not possible by analysis of road-killed amphibian (Schenke et al., 2017). Method optimization and evaluation is necessary regarding the selection of other swab bud materials for analysis of other interesting substances.
The relations between environmental concentrations and measured pesticide on the swab bud as well as effects on animals are mostly unknown and requires further efforts.

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