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Identification of endpoints useful to characterize the impact of pesticides on amphibian aquatic stages

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Introduction

The EU legislation on Plant Protection Products does not require the generation of toxicity data from amphibian aquatic stages for risk assessment, being them supposedly covered by fish-derived data.

Amphibian vs. fish comparisons, which reach different conclusions on the convenience of using fish as surrogates[1,2], are generally restricted to lethal or common phenotypic responses (e.g. growth, development).

We aimed at determining which endpoints are better indicators of pesticide toxicity on aquatic amphibians and testing if fish are useful surrogates for those relevant endpoints.

Amphibian data review

Methods

•Literature review about effects of EU-approved active ingredients (a.i.) on aquatic stages of amphibians.

•Effect magnitude quantified as the proportional change in the response of exposed individuals relative to controls.

•Exposure concentrations relativized according to each a.i.'s lowest fish 96h-LC50 (amphibian LC50s commonly unavailable).

•Measured responses classified into major endpoint categories.

•PCA to check exposure and effect relationships with endpoint categories. GLMz to explore dose-response relationships for each endpoint category.

Results

1274 records analysed, covering 23 species and 11 pesticides.

The first three PCA axes explained 39.7% of the variance. PC1 was defined by exposure conditions (i.e. time and concentration). PC2 was defined by effect magnitude *(Fig. 1)*.

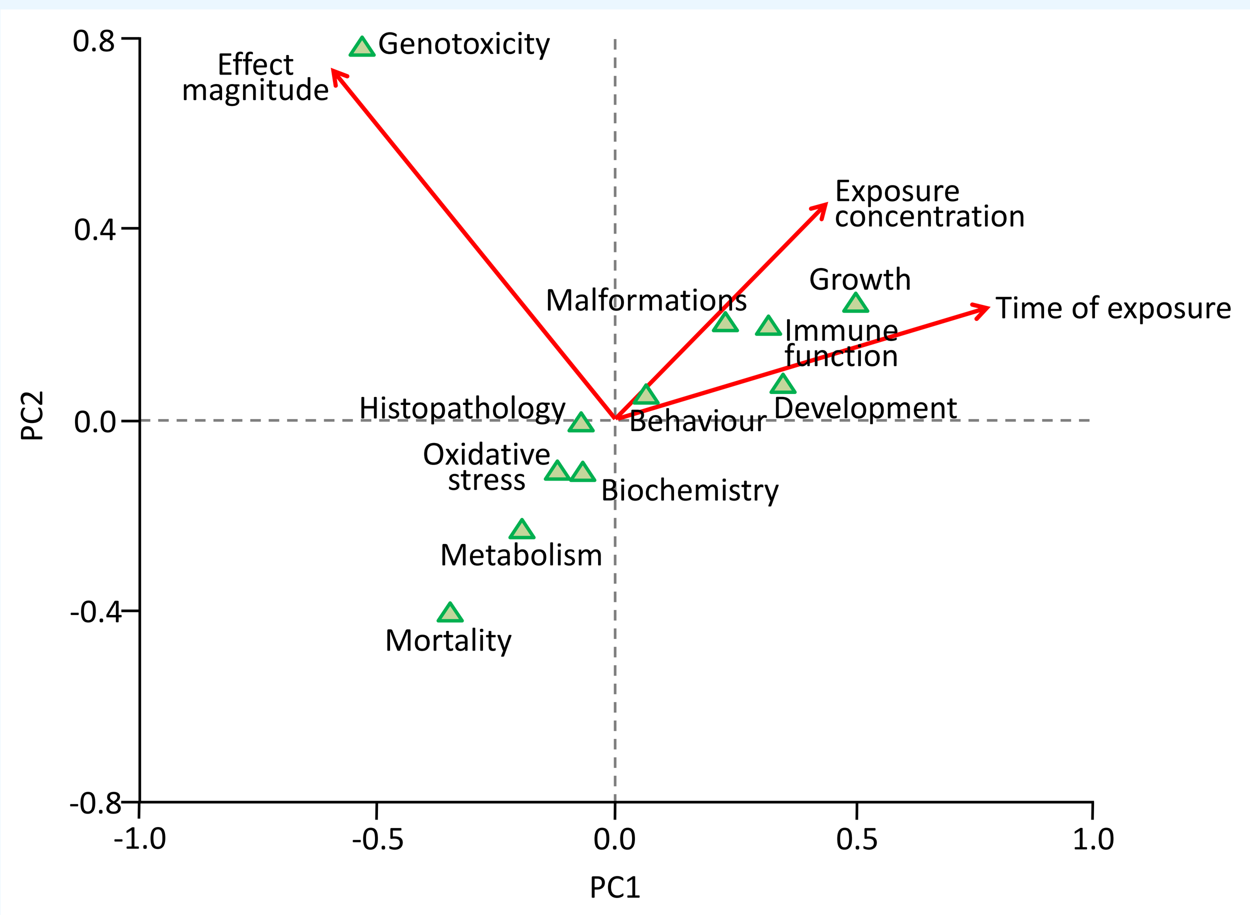

Fig. 1: Representation of the position of considered variables respect the two first axes of the PCA.

Genotoxicity *(Fig. 2)* was the endpoint type most related to amphibian sensitivity to pesticides. Growth, development, immune function and malformations were associated with increased dosage.

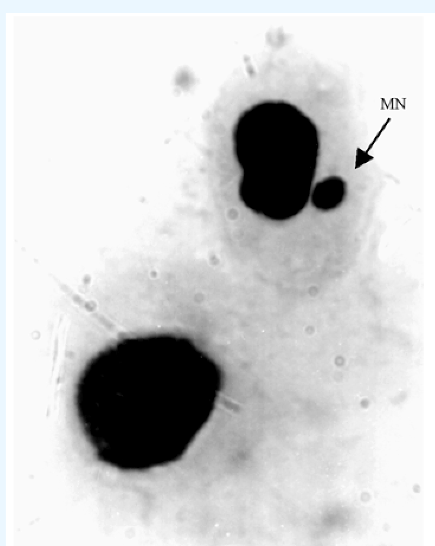

Fig. 2: Bullfrog micronucleated erythrocyte (top), a common genotoxicity indicator (retrieved from Campana et al. 2003[3])

Dose-response adjustments confirmed genotoxicity as the best indicator, with positive relationships of the effect magnitude with both exposure time and relativized concentration *(Fig. 3)*.

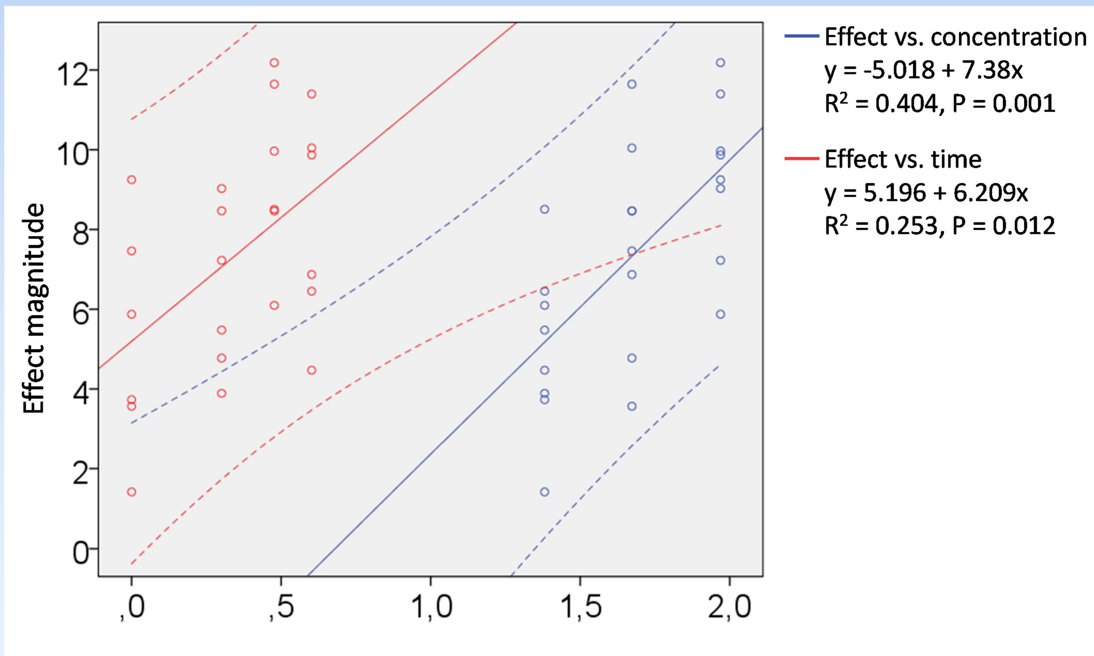

Fig. 3: Dose-response relationships based on studies quantifying genotoxic effects on amphibian aquatic stages.

Magnitude of immunotoxic effects (*Fig. 4*) was significantly explained by exposure time (ß = 9.795 ± 2.942; χ^2 = 11.081; P = 0.001).

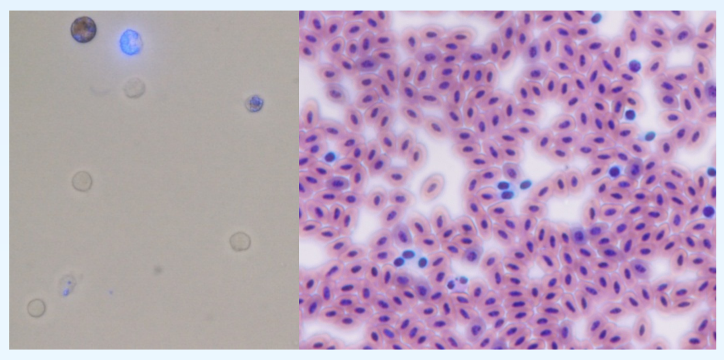

Fig. 4: Phagocytosis (left) and leukocyte counts (right) are two immune features commonly tested in amphibians.

Pathogen-induced mortality of juveniles often occurs after larval exposure[4], pointing the need for linking aquatic and terrestrial assessments.

Oxidative stress biomarkers responded significantly to the relativized exposure concentration (ß = 4.695 ± 1.453; χ^2 = 10.443; P = 0.001).

Comparison with fish data

Methods

•Information on genotoxicity, immunotoxicity and oxidative stress of malathion (most common a.i.) on freshwater fish processed as for amphibians. 801 records analysed (353 for amphibians, 448 for fish).

•Amphibians and fish compared through GLMz parameter estimates of taxon*exposure interaction influence on effect magnitude.

Results

Relationship between genotoxic effects and exposure time was clearly stronger in amphibians (ß = 1.240) than in fish (ß = -0.161) *(Fig. 5)*.

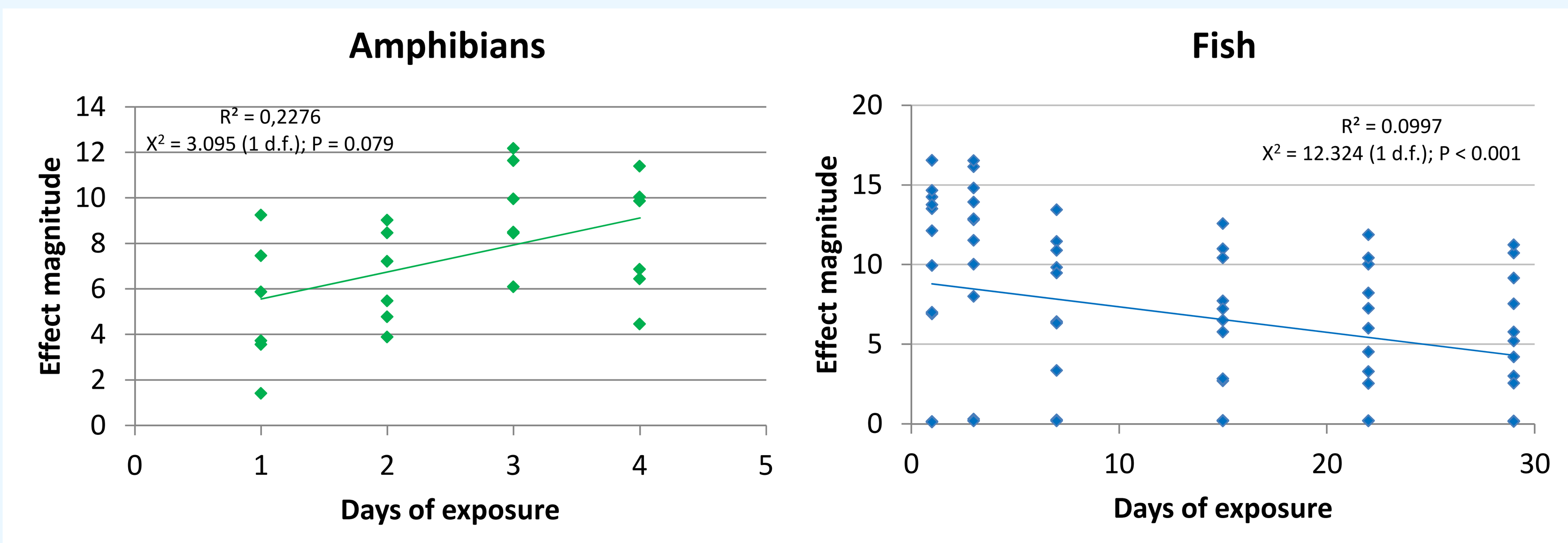

Fig. 5: Influence of exposure time on genotoxic effects of malathion on amphibians (left) and fish (right).

The interactions between taxa and relativized exposure concentrations significantly explained genotoxic effect magnitude (Amphibian: ß = 8.098 ± 2.243; χ^2 = 13.443; P < 0.001. Fish: ß = 11.358 ± 2.255; χ^2 = 25.381; P < 0.001.). We did not find any significant taxon*exposure interaction explaining the immunotoxic or oxidative stress-observed effects.

Conclusions

➤Effects at the physiological or molecular levels reflect dose-response impact of pesticides on aquatic amphibians better than lethality or phenotypic variables commonly used in standard tests.

➤Fish-derived toxicity data may not be relevant to amphibians when exposed to pesticides with genotoxic potential.

➤Immunocompetence can be a valid indicator of chronic toxicity of pesticides on amphibians.

➤A high-tier integration of aquatic and terrestrial assessments is desirable to efficiently evaluate chronic toxicity of pesticides on amphibians.

References

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