have a statistically significant difference in percentage of DNA in the head and tail of the spermatozoa compared to the control group and American bullfrogs injected with 12.5 or 25 mg/kg. These preliminary results imply that the sperm is fragmented, and sterility is induced with an injection of 50 mg/kg bisazir.

The question rises if this DNA damage is sufficient to induce 100% sterility in bullfrogs in order to use the organisms in a sterile-male-release program to control the widely spread exotic invasive populations. In humans, DNA fragmentation detected through an alkalic comet assay is reported to be responsible for a decrease in fertilisation rate and it showed a strong negative relationship with embryo quality (Simon et al. 2011). Future research on amphibians in this matter is needed.

The results in the ecotoxicity test battery show that bisazir has inherent ecotoxic properties. The compound is genotoxic and the ecotoxicity data presented here show that bisazir is also toxic for the aquatic environment with long term effects at rather low concentrations (1–2 mg/l) with derived PNEC value as low as 20 µg/l. This implies that the environmental risk for bisazir must be defined before it can be used in the field.

Data are needed to model the fate and behaviour in the environment of bisazir. Toxicokinetic data on bisazir are however scarce and it is not clear how residual bisazir and/or its metabolites are released from treated organisms to the aquatic ecosystem during their life and during decomposition after death. It is yet unsure if bisazir is metabolised in the living animal and is excreted as metabolites or as original molecule. The elimination of ^{14}C-residues in adult sea lamprey was investigated and showed that most radioactive residues that remained after 48 h in the injected animals were tissue-bound and evenly distributed in subcellular fractions of the liver. It was not clear if it was bisazir or its metabolites that were found in the liver (Allen and Dawson 1987). Some preliminary results indicate that the compound is not really biodegradable. K_{ow} values predict low bioaccumulating potential.

The ecological risk of this chemical needs to be further documented by investigating the exposure (toxicokinetics, the biodegradation pathways and bioaccumulation potential in the aquatic environment, adsorption behaviour…). When relevant the acute and chronic toxicity of possible residues and metabolites need to be established and the safe values for unwanted genotoxic effects need to be documented.

Taking into account the inherent properties of bisazir it is important to assess extensively the long-term implications of metabolites and residues to prevent unexpected and unwanted environmental risks when the product is used in the field. Furthermore, Europe has set out a legislation (European parliament and Council of the European Union 2009) on the sustainable use of pesticides where the use in aquatic ecosystems must be reduced and possibly banned. Therefore, alternatives should be investigated to avoid chemical control of invasive species, especially in aquatic habitats.

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Authors' contribution

S.D. designed and performed the experiments and analysed the data. S.D. wrote the manuscript in consultation with A.D.V.

Ethics and permits

All applicable international, national and institutional guidelines for the care and use of laboratory animals were followed (approval number 201024). Supervision was performed by the Ethical Commission of Hasselt University and the Flemish Authority on Animal welfare.

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