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Review

## Impact of genetically modified organisms on aquatic environments: Review of available data for the risk assessment



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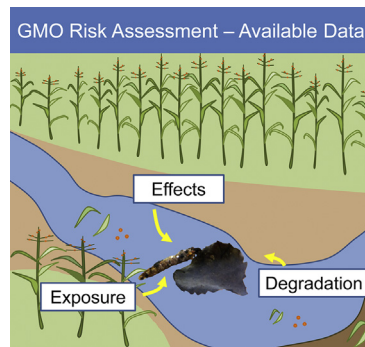
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HIGHLIGHTS

- Genetically modified (GM) crop material can enter aquatic environments.
- An analysis of available GM studies dealing with effects and fate is presented.
- Gaps should be addressed to improve risk assessment of GM crops.

GRAPHICAL ABSTRACT



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ABSTRACT

The aquatic environment is strongly connected to the surrounding agricultural landscapes, which regularly serve as sources of stressors such as agrochemicals. Genetically modified crops, which are cultivated on a large scale in many countries, may also act as stressors. Despite the commercial use of genetically modified organisms (GMOs) for over 20 years, their impact on the aquatic environment came into focus only 10 years ago. We present the status quo of the available scientific data in order to provide an input for informed aquatic risk assessment of GMOs. We could identify only 39 publications, including 84 studies, dealing with GMOs in the aquatic environment, and our analysis shows substantial knowledge gaps. The available information is restricted to a small number of crop plants, traits, events, and test organisms. The analysis of effect studies reveals that only a narrow range of organisms has been tested and that studies on combinatorial actions of stressors are virtually absent. The analysis of fate studies shows that many aspects, such as the fate of leached toxins, degradation of plant material, and distribution of crop residues in the aquatic habitat, are insufficiently investigated. Together with these research needs, we identify standardization of test methods as an issue of high priority, both for research and risk assessment needed for GMO regulation.

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Contents

1. Background . . . . .	688
2. Methods . . . . .	688

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3.	Results & discussion . . . . .	689
3.1.	Overall number of studies published . . . . .	689
3.2.	Effect studies. . . . .	689
3.2.1.	Effects on crustaceans. . . . .	689
3.2.2.	Effects on insects. . . . .	689
3.2.3.	Effects on other groups . . . . .	691
3.3.	Exposure studies . . . . .	692
3.4.	Degradation studies. . . . .	695
4.	Synthesis and outlook . . . . .	696
Appendix A.	Supplementary data . . . . .	696
References.	. . . . .	697

## 1. Background

Aquatic habitats are highly connected to the surrounding terrestrial ecosystems from which they receive minerals and organic input (Vannote et al., 1980). Agricultural ecosystems, comprising the largest terrestrial biome, contribute to this input into aquatic ecosystems and are substantial sources not only of phosphate and nitrogen but also of chemical stressors such as pesticides (Stehle and Schulz, 2015a). Stressors from agriculture, therefore, are the focus of efforts to limit damage to aquatic ecosystems such as rivers, lakes, or estuaries. With the Water Framework Directive (WFD) (EU, 2000), the European Union (EU) acknowledges the need for sustainable water management because of considerable pressures on the aquatic environment. Agriculture has been identified clearly as causing considerable problems for achieving the aim of good ecological status of all waterbodies (Vörösmarty et al., 2010).

In this review, we analyse the current knowledge related to risks posed to aquatic environments by the cultivation of genetically modified (GM) crops. GM crops have now been grown commercially for over 20 years, with increasing rates of adoption, especially in North and South America. In 2014, 82% of the worldwide production of soybean was transgenic, followed by 68% of cotton and 30% of maize (James, 2014). The increasing adoption is reflected in the increasing number of authorized single GM transformation events. Their numbers reached 102 in 2014 (Parisi et al., 2016). Only two traits dominate GM crops: herbicide resistance (HR) (45.1%) and insect resistance (IR) (34.6%) (Parisi et al., 2016). Whereas HR is achieved by the expression of enzymes breaking down herbicides, IR is realized by the expression of insect toxins derived from the soil bacterium *Bacillus thuringiensis* (Bt). The toxins are incorporated into plant tissues and trigger the lysis of gut membranes which is then followed by death in target organisms (Glare and O'Callaghan, 2000).

As pesticides and GM crops contain biologically active compounds, and side effects have been recognized, both are subject to environmental risk assessment (ERA) and regulated in many countries. In the EU, the environmental risk assessment needs to identify potential adverse effects of the genetically modified organism (GMO) on the environment (EU, 2001). The relevance of insecticides, e.g. those sprayed as pesticides, for aquatic ecosystems is not questioned, and indeed is reflected in risk assessment (Stehle and Schulz, 2015b). However, risks from plant-incorporated insecticides via GM crops are considered poorly in risk assessments and biosafety research. For the first 10 years, assessment of risks of GMOs to organisms or ecosystems almost exclusively focused on terrestrial habitats. An influential study on the potential effects of GMOs on aquatic insect larvae (Rosi-Marshall et al., 2007), put impacts on aquatic environments into focus. The researchers also measured environmental exposure of headwater streams to Bt maize and the insecticidal Cry1Ab toxin from this GM crop (Tank et al., 2010). Besides experimental data showing a potential hazard to caddisflies (Trichoptera), an insect group with aquatic larval stages phylogenetically closely related to the Lepidoptera, and thus, to the target insects of Bt maize, Rosi-Marshall et al. (2007) also highlighted the input of GM

plant material into the aquatic environment and the potential risk of cultivation of GM crops to aquatic invertebrates. While some authors felt that the conclusions of Rosi-Marshall et al. were overstated (Beachy et al., 2008; Parrott, 2008), the issue of effects of Bt maize on aquatic ecosystems gained momentum and was addressed by other research groups and in another review (Venter and Bøhn, 2016). It was also picked up by regulatory bodies, such as the European Food Safety Authority (EFSA), dealing with the market release of GMOs. However, EU authorities concluded that the risks to aquatic ecosystems from the Bt crops analysed thus far were negligible (e.g. EFSA, 2011a, 2011b).

In this study, we analyse the available scientific literature on the hazard and fate of GMOs in the aquatic environment. Our objective is to describe the developments and the current state of knowledge of risk assessment of GMOs in the aquatic environment. As risk assessment in the EU is case specific, our analysis differentiates between crops, traits, and novel proteins.

## 2. Methods

The identification of relevant studies based on multiple sources. A literature search was carried out in CAB Abstracts (CABI Wallingford, UK) and in the databases ISI Web of Knowledge (Thomson Reuters, New York, USA), BIOSIS (Thomson Reuters, New York, USA), AGRICOLA (National Agricultural Library, Beltsville, USA), AGRIS (Food and Agricultural Organisation (FAO) of the United Nations, Rome, Italy), and BASE (Bielefeld University Library, Bielefeld, Germany). Two search strings were used for CAB Abstracts, i.e. [(*Bacillus thuringiensis*)] and [(*Bacillus thuringiensis*) and (aquatic\*)], and one for ISI Web of Knowledge, BIOSIS, AGRICOLA, AGRIS, and BASE, i.e. [(*Bacillus thuringiensis*) and (aquatic\*)]. Furthermore, the relevant publications identified were scanned for references not covered by the database searches. The literature search was performed up to December 2017 and all papers published online before this date were included.

We narrowed the results of the database search to the scope of these publications as follows: we included only publications that (1) investigated the fate of GM plants, the relevant novel protein, or relevant non-GM plants in the aquatic environment; or (2) investigated adverse effects of GM plants or the relevant novel protein on non-target aquatic invertebrates and protists in single-species tests. Only experiments that were peer-reviewed (3) and published in English (4) were included. By doing so, we excluded publications that included community studies, spray formulations of Bti (*Bacillus thuringiensis israelensis*), Bt genes, or vertebrates, and studies carried out in terrestrial or riparian habitats. As some publications contained different numbers of studies, five criteria were used to differentiate them: (1) every publication consisted of at least one study; (2) already published data were not classified as an own study; (3) several experiments with exactly the same test design were counted as one study; (4) every experiment with a different test design, e.g. a different test species, counted as one study; and (5) one study could investigate several endpoints, several treatments, several GMOs, several sampling sites, and several sampling dates and dates of

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