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Tools for a scientifically rigorous and efficient monitoring of genetically modified organisms (GMOs) – VDI Guidelines to ensure high quality of GMO-monitoring data

Wiebke Züghart[1], Heike Beismann[2], Winfried Schröder[3]

1 *Bundesamt für Naturschutz, Konstantinstr. 110, 53179 Bonn* **2** *Westfälische Hochschule, Münsterstr. 265, 46397 Bocholt* **3** *Lehrstuhl für Landschaftsökologie, Universität Vechta, Driverstr. 23, 49377 Vechta*

Corresponding author: *Wiebke Züghart* (wiebke.zueghart@bfn.de)

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Abstract

The deliberate release of genetically modified organisms (GMOs) implies the potential occurrence of environmental impacts which are either unexpected or only partially predictable and, thus, necessitates development of appropriate monitoring methodology. Therefore, new challenges have to be met when implementing the post market environmental monitoring (PMEM) of genetically modified organisms (GMOs), which is mandatory according to the European legal framework. According to Directive 2001/18/EC PMEM has to follow standard methodologies, wherever available and appropriate. To provide all involved parties with appropriate standard monitoring methods, the so called VDI Guidelines are developed by working groups established by the Association of German Engineers (VDI). These working groups are composed by external experts participating on a voluntary basis. The VDI is an independent technical standardisation body. All Guidelines are published in German and English and can therefore be used throughout Europe. VDI Guidelines are available in the field of exposure of the environment to GM plants (e.g. standardised sampling of pollen, standardised observation of hybrids or ferals), biomolecular analyses (e.g. standardised extraction and detection of transgenes or their products in different environmental compartments), and the standardised monitoring of effects on non-target organisms (e.g. butterflies, wild bees, amphibians or soil organisms). The aim beyond this work is to facilitate generation of reliable and comparable monitoring data and enable an effective and efficient PMEM with high acceptability to the scientific community as well as the general public.

Keywords
Genetically modified organisms, post market environmental monitoring, VDI Guidelines, environmental effects, GMO monitoring, non-target organism

Introduction

According to the European legal framework it is mandatory to conduct a post market environmental monitoring (PMEM) when approval for the commercial release of genetically modified organisms (GMO) has been granted (EC 2001, EC 2003). The aim of PMEM is to identify potential adverse effects of the organism and its use to human health and the environment after placing on the market. It should serve as an early warning system and indicate the need of risk management measures and / or a reassessment of the released GMO. The monitoring results are the basis for subsequent regulatory decisions like the adaptation of monitoring plans or withdrawal of GMO approvals.

The Directive 2001/18/EC (EC 2001) distinguishes two parts of PMEM. *Case specific monitoring* (CSM) is closely linked to the outcome of the environmental risk assessment (ERA). The aim of CSM is to check the assumptions made during the ERA and to ensure that the ERA conclusions are valid as regards the authorised use of the genetically modified organisms (GMO) (EFSA 2011a). The focus of *General surveillance (GS)* is on impacts, which were not anticipated in the ERA as well as on long-term and cumulative effects (EC 2001).

Guidance on the general principles for PMEM and the development of an appropriate post-market monitoring plan is given in the Council Decision 2002/811/EC supplementing Annex VII to Directive 2001/18/EC (EC 2002). One focus of this guidance is laid on the monitoring methodology. Thus, the relevant parameters to be monitored have to be identified on a case by case basis and the methodology to monitor these parameters should be clearly identified and outlined, including techniques for sampling and analysis. The sampling methodology must be scientifically and statistically sound (EC 2002). A main requirement to monitoring methodology is to collect and analyse data in exact and unbiased manner. Besides comparability, further fundamental quality criteria are necessary such as correctness and reproducibility (Schröder et al. 1991, 2009). Technical data should be sufficiently reliable and able to stand up in court to serve as a basis for regulatory decision making. Accordingly, use of standardised methodology for PMEM is advisable wherever such methodology is available and appropriate (EC 2002, EFSA 2011a, BfN et al. 2011). Standardised methodology effectively represents these required high quality criteria, they create transparency and thus also acceptance (Plachter et al. 2002).

For pollution control, soil conservation or pest control standard monitoring methods are already available and widely applied. In contrast, the monitoring of adverse environmental effects of genetically modified organisms lacks such standardisation (Berhorn et al. 2005). To fill this gap, working groups composed of experts from relevant scientific disciplines and the administration started to develop technical stand-

ards (VDI Guidelines) for PMEM within the framework of the Association of German Engineers (VDI).

The VDI is an independent, politically unaffiliated and non-profit technical standardization body. The VDI covers a wide range of technical topics and communicates this knowledge through studies, technical discussions and congresses or the VDI Guidelines. The preparation of standards for environmental protection and environmental management has made a considerable contribution to today's high level of environmental protection, e.g. in the field of air pollution protection.

In the field of PMEM, external experts in cooperation with the VDI work on a body of regulations of specific methods for the monitoring of genetically modified plants (GMO Monitoring, VDI 4330 - VDI 4333, Table 2 and Table 3). This standardisation project was initiated by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the Federal Agency for Nature Conservation (BfN). The aim of this guideline work is to provide relevant standard methodology in order to enable and support a scientifically rigorous, harmonised and thus efficient PMEM.

Rules of guideline development

The development of VDI Guidelines is an open and transparent process according to defined rules. Important criteria for the development process are the participation of the public and an obligatory review after publication. Due to this open process VDI Guidelines are accepted as important for the German technical progress. VDI Guidelines also have a particular legal importance, especially when cited in legal acts, ordinances, decrees or regulations. This is comparable to the influence of ISO standards on an international level.

A project proposal can be made by any stakeholder to the VDI. Before starting a new project in the VDI, an evaluation is carried out by the responsible Advisory Board, in which the current need, the compliance with general criteria and the interest of the parties involved is inquired (Fig. 1). Furthermore, parallel work of other standardizing bodies (CEN, ISO, other national regulators) must be excluded. The involved external experts participate on a voluntary basis in their own capacity and not as representatives of their organizations. They are appointed personally to a corresponding working group after the project proposal has been checked and agreed by the responsible Advisory Board. The work of these working groups is organized and supported by the VDI.

The constituted working group for a new standardization project develops an internal preliminary working draft (Fig. 1). The draft is the result of broad discussions between the experts, based on their expert knowledge. After a detailed review within the responsible Advisory Board, the draft can be published as so called "green print". This draft is printed and available internationally at the Beuth publishing house (www.beuth.de) and its distribution network. The draft will be subjected to an approval procedure which is open to the general public. The time span to make objections

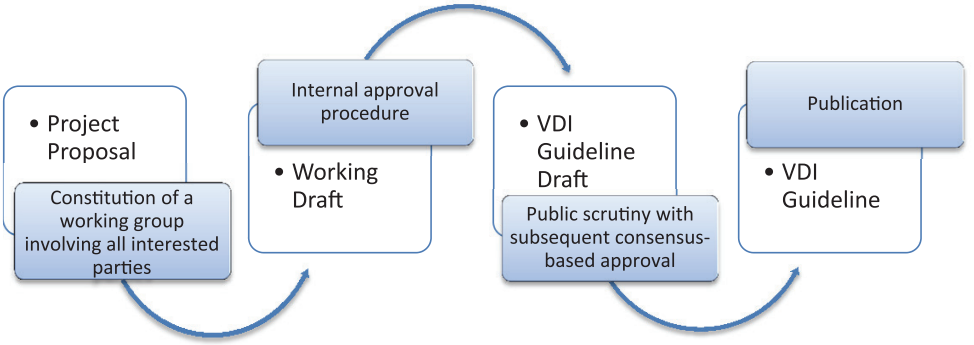

Figure 1. Simplified flowchart of the approval process of a technical standard.

is typically 3–4 months, and all received objections must be discussed and handled within the corresponding working group. After all objections have been dealt with and the draft is revised by the issuing working group and after report in the responsible Advisory Board, the final VDI Guideline can be passed and will be published as so called "white print". The aim of this procedure is to incorporate the publicity in a very transparent way and to reach a consensus as extensive as possible. At the latest five years after its publication, a VDI Guideline must be checked for its validity and, if necessary, revised or withdrawn.

The process of determining a technical standard – especially the direct participation of the public during the consultation phase – guarantees a high level of transparence and acceptance. The results of the standardization are generally accepted as state-of-the-art and may be consulted for guidance during implementation of legal regulations. Thus, it is made possible to assess and thus increase the quality of tenders for measuring and monitoring programs and expert's reports considerably, to provide legal certainty for the user and to ensure comparability of the data.

PMEM strategy and determination of monitoring objectives

Appropriately addressing identified as well as unanticipated effects of GMOs to the environment during PMEM is highly challenging. Effects may occur on different ecological levels like organisms, biocoenosis, ecosystems or landscapes, and may appear in the different environmental compartments air, soil and water (Züghart and Breckling 2003). Furthermore, impacts may not be temporally and spatially limited. They may be direct or indirect and may happen immediately or after a long time of the onset of GMO release. When different GMOs are released concurrently in the same area, combined effects may occur. This indicates the need of a monitoring strategy that meets both, a targeted monitoring approach that takes into account specific causal interrelationships as well as a more general observation of the environment (Züghart et al. 2008). For GM plants (GMP) the monitoring approach will comprise a set of

mainly sectoral monitoring objectives and methodologies to complement one another accordingly, depending on plant species, inserted traits, intended use and the receiving environment.

The formulation of cause-effect hypotheses derived from the ERA, biosafety research results as well as from existing knowledge of ecology and ecosystem theory will be the main tool for the identification of potential adverse effects of a certain GMP and the determination of relevant monitoring objectives (BfN et al. 2011). Furthermore, the selection of indicators representing safeguard objects and protectin goals in the relevant environment could complement the PMEM approach. Table 1 gives an overview about a broad spectrum of possible effects of GM crops on items to be protected

Table 1. Protection goals, checkpoints and background data relevant for PMEM (source: VDI 4330 Part 1).

Items to be protected and protection targets	**Checkpoints / Assessment endpoints**
Terrestrial ecosystems *Flora and fauna* Conservation of the biological diversity • Genetic variability • Diversity of species and their functions • Diversity of habitats/ ecosystems Conservation of especially endangered or protected species, habitats and ecosystems	Dispersal and fate of the transgenes (e.g. pollen, dusts, plants, compost, sewage, sludge, stomach and intestinal contents or excretions from wild animals) Flora and fauna of farmland Flora and fauna of field margins Feralisation and dispersal behaviour of GMPs Occurrence of natural cross-breed partners Cross breeding with wild flora, establishment and ecological behaviour of hybrids and transgenic wild plants Toxic effects of specific GMP constituents (direct and indirect) on non-target organisms Herbicide/metabolite residues Changes in population density and behaviour of endangered and protected species and ecological key species of different stages of the trophic web Diversity, dominance structures and functions of ecosystems, in particular in protected habitats Alterations of landscape structures and properties
Soil Conservation of soil functions (soil fertility, biochemical and geochemical material and energy fluxes as well as filtering, buffering and cultivation properties, ecosystem function) Conservation of the soil biocoenosis Avoidance of erosion/compaction	Dispersal and fate of the transgenes (e.g. soil, compost, sewage sludge, silage) Feralisation and dispersal behaviour of GMPs Changes in chemical and physical parameters of the soil Horizontal gene transfer to microorganisms Detection of recombinant DNA Toxic effects of specific GMP constituents (direct and indirect) on non-target organisms Herbicide/metabolite residues Changes in population density and behaviour of endangered and protected species and ecological key species of different stages of the trophic web Soil microbiological parameters Diversity, dominance structures and function of soil organisms Degradation processes Soil erosion/compaction

Items to be protected and protection targets	Checkpoints / Assessment endpoints
Aquatic ecosystems Conservation of the biological diversity • Genetic variability • Diversity of species and their functions • Diversity of habitats/ ecosystems Conservation of especially endangered or protected species, habitats and ecosystems Avoidance of adverse effects to bodies of water (ecosystem function, water quality)	Dispersal and fate of the transgenes (e.g. water body, sediments) Feralisation and dispersal behaviour of GMPs Occurrence of natural cross-breed partners Cross breeding with wild flora, establishment and ecological behaviour of hybrids and transgenic wild plants Changes in chemical and physical parameters of the water Toxic effects of specific GMP constituents (direct and indirect) on non-target organisms Herbicide/metabolite residues Changes in population density and behaviour of endangered and protected species and ecological key species of different stages of the trophic web Diversity, dominance structures and function of water biocoenosis especially in protected habitats
Air Protection of the atmosphere Air pollution prevention	Chemical composition (greenhouse gases, VOCs (volatile organic hydrocarbons)) Dispersal of transgenes (in particular pollen and aerosols)
Non-specific background data of the items to be protected Spatial and temporal dispersal of the GMP cultivation Climate and weather Data on the monitoring area (e.g. topographical data, soil class, characteristics of the cultivation area and the field margins, cultivation and landscape-care measures)	

and relevant checkpoints related to them. The checkpoints have been derived from the case studies herbicide-resistant rape, insect-resistant maize, virus-resistant sugar-beet and potato with modified starch content. The table can be used as a source for the conception of PMEM. If a checkpoint is rated as relevant, the next step would be to derive monitoring parameters and/or indicators (VDI 4330 Part 1, Table 2).

Availability of methodologies for PMEM

The methods applied in a monitoring program should be chosen according to their ability to generate appropriate data to address the selected monitoring objectives (McComb et al. 2010; Legg and Nagy 2006). In the case of PMEM of GM crops, for some key features already approved monitoring methods may be available, but for most parts of PMEM rather new developments or modifications are necessary (Berhorn et al. 2005). In the last decade, increased efforts were made to develop or optimise methods for the detection of dispersal of GM crops, inserted transgenes and transgene products as well as for the detection of environmental impacts caused by GM plants or their use. This includes methods for the detection of GM pollen dispersal and deposition (Hofmann et al. 2005, 2011), the quantification of Bt protein contents in plants (Nguyen and Jehle 2009), or the detection of adverse impacts on non-target organisms (Prasifka et al. 2005, Rauschen et al. 2008, Lang and Bühler 2012). At present, these novel methodologies are

mainly applied in research projects and experimental field studies, and their applicability for PMEM purposes often needs to be verified. Basically, the implementation of relevant and appropriate methods for PMEM into referenced standards like VDI Guidelines proved to be a suitable approach for promoting the use of harmonised and state-of-the-art methodologies, eventually enabling and facilitating an effective monitoring.

VDI Guidelines for PMEM

The VDI working groups discussed and decided on needs and priorities for standardised methods for PMEM. Table 2 gives an overview on the VDI Guidelines already published and Table 3 on guidelines still in progress.

All described methods focus on the detection of ecological effects of GM crops on the environment and do not address effects on human health. As a first step a general guideline on basic principles and strategies of PMEM was provided (Table 2, VDI 4330 Part 1). This framework guideline sets the context and supports the other guidelines which mainly describe detailed methods. A major topic of VDI 4330 Part 1 is the description of scientific requirements for a monitoring concept, that include planning and implementation criteria, protection goals and checkpoints that have to be taken into account (Table 1), criteria for the selection of methods and monitoring areas as well as requirements regarding quality assurance and data management (Schröder and Schmidt 2012, Schröder and Schmidt (2013), this issue).

The priorities for standardisations of detailed methods were set initially in the field of exposure of the environment to GM crops and bio-molecular analyses. The

Table 2. Standard methods for the environmental monitoring of genetically modified organisms (finalised).

Guideline series	Monitoring the effects of genetically modified organisms
VDI 4330	
Part 1	Monitoring the ecological effects of genetically modified organisms; Genetically modified plants; Basic principles and strategies
Part 3	Pollen monitoring; Technical pollen sampling using pollen mass filter (PMF) and Sigma-2 sampler
Part 4	Biological sampling of pollen; Bee hives as biological pollen samplers
Part 5	Guidelines for the collection and preparation of plant samples for molecular biological analysis
Part 7	PCR-methods for the detection of genetically engineered nucleic acids in the environment
Part 9	Assessment of the diversity of ferns and flowering plants; Vegetation survey
Part 10	Floristic mapping of genetically modified plants, their crossing partners and their hybrid offspring
Part 11	Immunochemical detection of insecticidal Bt proteins from genetically modified crops in soil samples and plant residues
Part 13	Standardised monitoring of butterflies and moths (Lepidoptera); Transect method, light trap, and larval survey

Table 3. Standard methods for the environmental monitoring of genetically modified organisms (in progress).

Guideline series	Monitoring the effects of genetically modified organisms
VDI 4330	
Part 2	Sampling for pollen monitoring
VDI 4331	
Part 1	Effects of GMO on soil organisms
Part 2	Macroarthropods
Part 3	Microarthropods
Part 4	Lumbricina
Part 5	Enchytraeus
Part 6	Nematodes
Part 7	Microbial communities
VDI 4332	**Standardised detection of Wild bees**
VDI 4333	**Standardised detection of Amphibians**

focus was on standardised sampling of pollen, standardised extraction and detection of transgenes and their products, and on standardised observation of plant hybrids and ferals (Sukopp and Schmitz (2013), this issue).

With the increasing cultivation of Bt-maize in Europe, standard methodologies to monitor effects on non-target organisms like butterflies and moths (Lang et al. 2013, this issue) or soil organisms (Ruf et al. 2013, this issue) become more important. Furthermore, wild bees (Schindler et al. 2013, this issue) and amphibians (Böll et al. 2013, this issue) were identified as sensitive species and thus important indicators in agricultural landscapes (Lang 2007). The experts identified particularly with regard to the availability and appropriateness of PMEM methodologies for non-target organisms substantial gaps. Though monitoring methods may exist for those groups, approaches specifically adapted to PMEM monitoring of GMOs are missing and / or are not harmonised and standardised. This applies to species already covered by VDI Guidelines (Table 2, Table 3) as well as to further species groups with relevance for PMEM (Lang 2007).

The VDI Guidelines include detailed instructions on sampling methods, sampling strategies, sampling designs, detection methods, statistical evaluation, data management and quality control. For non target organisms like butterflies or amphibians, relevant species and developmental stages (e.g. adults, larvae) are determined to be monitored. In the case of soil organisms, a selection matrix that supports the choice of appropriate animal groups to be sampled is provided. The VDI Guidelines do not differentiate between *Case specific monitoring* and *General surveillance*, because the methodologies itself basically are applicable for both.

Conclusions

With the current set of VDI Guidelines for PMEM a first step was taken to address essential requirements to facilitate an effective PMEM. Initial PMEM reports of the cul-

tivation of MON810 maize (MON810 2010) and Amflora potatoes (Amflora 2011) in Europe have indicated the relevance of high quality scientific monitoring methodology for obtaining valid and reliable monitoring results (EFSA 2011b, EFSA 2012). Appropriate and standardized methodology is a key element to enable an effective and efficient PMEM. Preferably such methods should be available prior to the implementation of monitoring plans (EC 2002).

VDI Guidelines are published in German and English, and therefore accessible to all European stakeholders. Consideration of these standards by e.g. applicants, regulators, European Food Safety Authority (EFSA) and the European Commission would be a step forward in achieving a harmonised and reliable PMEM.

VDI Guidelines can thereby serve as basic documents in the European standardization process (Beismann et al. 2007). The technical standards on pollen monitoring (VDI 4330 Part 3 and Part 4) are the first VDI Guidelines for PMEM, which are transferred to the European Standardization Body (CEN) and are developed as European Technical Specifications for PMEM, to facilitate comparability of PMEM surveys at the European level (Peichl and Finck 2003).

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