

# Determining indicators, methods and sites for monitoring potential adverse effects of genetically modified plants to the environment: the legal and conceptional framework for implementation

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**Abstract** According to Directive 2001/18/EC commercial cultivation of genetically modified plants (GMPs) have to be monitored. The aim of the monitoring is to identify potential adverse effects of the GMPs and their use on human health and the environment. There are few concepts showing how GMP monitoring may be implemented. This article indicates monitoring requirements with a focus on environmental issues. GMP monitoring has to be appropriate to detect direct and indirect, immediate and long-term as well as unforeseen effects. For choosing suitable monitoring indicators and methods, we propose a case-by-case approach, which is hypothesis-driven and related to specified protection targets. We present criteria for selecting suitable monitoring sites and demonstrate possibilities to integrate GMP monitoring with existing environmental monitoring programmes. To ensure comparability, interpretability and quality of GMP monitoring data a harmonisation on both national and international level is proposed.

**Keywords** GM crops · Monitoring · Environmental protection · Conceptual framework

## Introduction

Plant breeders that want to bring new genetically modified organisms (GMOs) on the market and into cultivation are requested, according to Directive 2001/18/EC (European Community 2001), to submit a dossier including an environmental risk assessment (e.r.a.) and a monitoring plan to the competent national and European authorities. This regulatory framework provides the precautionary principle and shall enable to handle uncertainties about potential adverse environmental effects still remaining after the e.r.a, which is primarily based on limited time and scale releases of the GMOs. Various studies, for instance on enhanced mortality of non-target organisms (Sears et al. 2001), hybridisation with related species (Lefol et al. 1996) or neighbouring non-GM crops (Rieger et al. 2002) and changing agricultural practice (Graef et al. 2007; Hayes et al. 2004; Relyea 2005) have been raising public and scientific concern about the environmental risks of the release of GM plants (GMPs). Based on the Directive (European Community 2001) and Supplementing Guidance Notes (European Community 2002) a number of specific GMP monitoring aspects have been developed (e.g. ACRE 2004; Bühler 2006; EFSA 2006; Graef et al. 2005; Sanvido et al.

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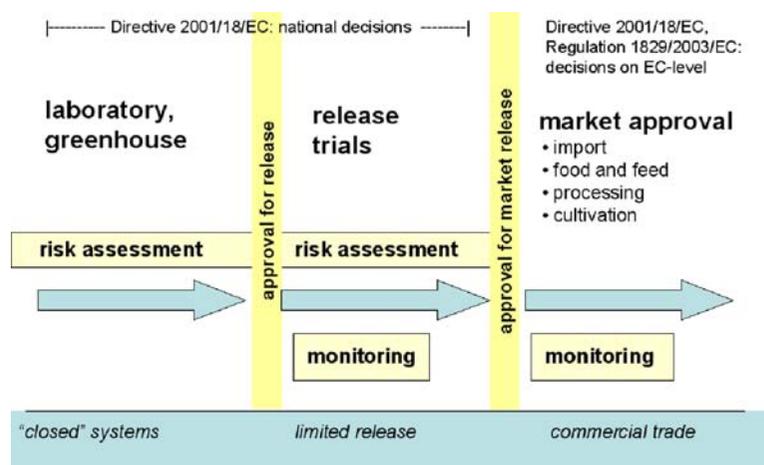
2005; Traxler et al. 2000; Züghart and Breckling 2003).

This paper presents a conceptual framework for the monitoring of cultivated and marketed GMPs with a focus on environmental issues. It identifies the legal requirements and displays a systematic monitoring concept including a case-by-case (a) identification of environmental protection targets, (b) selection of environmental indicators, (c) choice of methods for detection, (d) selection of monitoring regions and sites and (e) integration of existing agro-environmental monitoring programmes. We point out and discuss the necessity of harmonising, standardising and coordinating GMP monitoring data, which is a precondition for statistical data analysis and an effective good monitoring practice.

### Legal framework

To detect potential adverse effects of releases, imports and cultivation of GMPs the regulatory framework 2001/18/EC (European Community 2001) specifies an approval process in a case-by-case assessment of the risks to human health and to the environment (Fig. 1). Preamble 24 of the Directive 2001/18/EC regulates that “the introduction of GMOs into the environment should be carried out according to the ‘step by step’ principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.”

**Fig. 1** ‘Step by step’ principle for release of GMOs



In terms of duration and scales of release (Fig. 1) the Directive (European Community 2001) distinguishes between (a) experimental risk research carried out in laboratories and greenhouses, (b) release-related research and monitoring, which extends the trials to field plots limited in time, number and space. For market-approved GMOs the Directive distinguishes (c) a case-specific monitoring (CSM) which focuses on direct and indirect, immediate and delayed potential effects on human health and the environment, identified in the preceding e.r.a. process and which is limited to a specified time period in which to obtain results and (d) a general surveillance (GS), which aims to identify and record indirect, delayed and/or cumulative adverse effects that have NOT been anticipated in a preceding e.r.a. In contrast to CSM general surveillance should aim at the identification of unforeseen and long-term effects and therefore be carried out over a longer time period and possibly wider area. However, some effects (e.g. cumulative effects) may be either anticipated (then inducing CSM) or unforeseen (leading to GS). Hence, the general definitions of CSM and GS leave some room for interpretation. Therefore ACRE (2004) proposed a more precise distinction and introduced an additional category of “*Interactive or cumulative effects that are difficult or impossible to predict*”. Thus, they differentiate to monitor (a) *Anticipated effects* based on potential risks identified in the e.r.a. worth to investigate via CSM as well as those effects assessed as being extremely unlikely to occur and to cause harm, (b) *Interactive or cumulative effects that are difficult or impossible to predict* or assess fully in a single dossier and its e.r.a. These are for instance

effects that might arise as a result of an increase in the scale of cultivation or interaction between the GM crop, future varieties (GM and non-GM) and the environment and (c) *Unanticipated effects* that are completely unknown potential effects not identified in the ERA, which can only be addressed by GS. According to the Directive 2001/18/EC and Council Decision 2002/811/EC all categories of effects should be within the scope of monitoring. There are gradual differences in predictability among the effects. For instance, local effects on cropland can be more easily assessed than effects beyond cropland and on larger scales. It is therefore necessary to include the options of collecting parameters in CSM, in GS or in both simultaneously. This would have to be decided on a case-by-case basis.

The monitoring results of marketed GMOs contribute to decisions, for instance, on approval or additional precautions, and can enhance the certitude of prognosis for future risk assessment. The GMO monitoring provides the basis for an early warning system (Fig. 2) to react at an early stage in case of reported adverse effects and decide upon counter measures.

Once environmental changes are identified it is essential to determine whether they are harmful or not (Fig. 2). Adverse changes cannot always be attributed to a GMP, since there are numerous influencing environmental and agricultural practice covariables (Graef et al. 2007; Hails 2002; Stein and Ettema 2003). If harmful, more in depth studies are envisaged in order to detect causal relationships. In case of a relationship between a GMP and an adverse

effect measures to avoid or minimise effects have to be taken. At the same time a new e.r.a. is required. The subsequent results are the basis for decisions upon extending GMP approvals, new applications and adaptations of the monitoring plan.

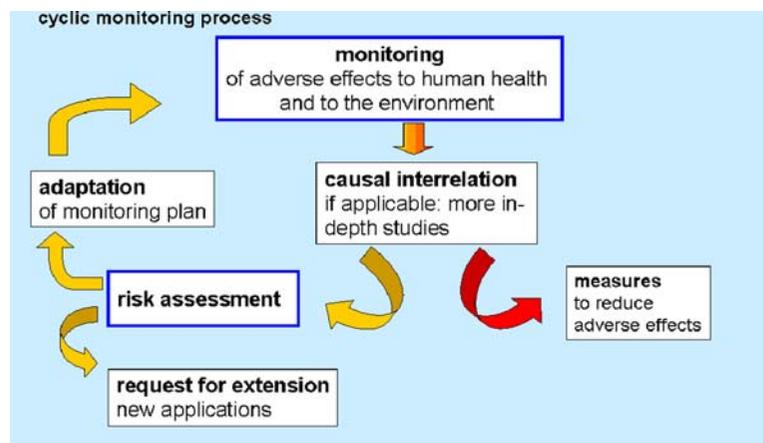
### GMP monitoring case-by-case

Conceptual framework and assessment of potentially affected environmental protection targets

GMP notifications have to be prepared by the notifier and assessed by the authorities on a case-by-case basis. Thus, monitoring plans have to be specific on GMP characteristics, for instance with respect to potential lifetime (annual or long-living crops and trees), modified traits (herbicide-tolerance, inclusion of an insecticide, altered substances of content) and the intended use of the GMP (import only, processing only or cultivation). The GMP monitoring requires an adapted and dynamic concept.

A systematic GMP monitoring has not yet been realised. However, there are few indications of environmental effects of GMPs, for instance enhanced mortality of non-target organisms (Sears et al. 2001; Relyea 2005), hybridisation with related species (Lefol et al. 1996) or neighbouring non-GM crops (Rieger et al. 2002) and changing agricultural practice (Graef et al. 2007; Hayes et al. 2004) which show the necessity of monitoring GMP effects. Effects may occur on the level of cells, organisms,

**Fig. 2** Monitoring as an early warning system (2002/811/EC)



populations, biocenosis, ecosystems and landscapes (Hayes et al. 2004; VDI 2006; Züghart and Breckling 2003) as well as on different trophic levels (Birch et al. 1999; Hawes et al. 2003). Since GMPs are living organisms their effects may not be temporally and spatially limited. They may appear immediately or only after decades (Hails 2002) and extend on the cultivated field only or to some kilometres distance (Rieger et al. 2002; Züghart and Breckling 2003). Furthermore they may occur in the different environmental compartments air, soil and water (Devaux et al. 2005; Relyea 2005; Douville et al. 2006). Therefore a monitoring concept is required that builds upon causal interrelationships and also includes general observations of the environment.

In doing so it should be primarily directed towards environmental protection targets, for instance conservation of the biological diversity in terrestrial and aquatic ecosystems, conservation of especially endangered or protected species, habitats or ecosystems, conservation of soil functions and soil biocenosis or human health. Terrestrial ecosystems, aquatic ecosystems and air are strongly merged on the one hand and on the other hand they are also closely linked with other items such as human health, cultural and real assets, utilisation of the environment or the natural landscape. Conservation of biodiversity and respective ecosystem functions are among the most essential protection targets. Hence, if exposed to GMP the monitoring should also be performed in protected areas, national parks, biosphere reserves and sites of the European NATURA 2000 network.

#### Identifying environmental indicators and detection methods to monitor

The selection of environmental indicators depends on the characteristics and intended use of the GMP. An overview of potential topics and indicator groups relevant to potential adverse effects of different GMPs is presented in Table 1. A more detailed list of potential adverse effects and protectable items is provided for instance by Hayes et al. (2004) and VDI (2006). An important criterion for indicator selection is their potential to indicate GMP-induced changes. This potential depends inter alia on (a) direct and/or indirect interrelationships with the GMP, (b) a preferably widespread distribution of the indicator, (c) sufficiently high abundance, (d) importance for

ecological processes and ecosystem functions and (e) their suitability to indicate protectable items (Andow and Hilbeck 2004). To make selection criteria transparent they can be systematically assessed within a matrix containing criteria and scores (Meier and Hilbeck 2005). For GS also the environmental exposure of the GMPs or its products non-specific items is important to monitor, for instance the persistence and accumulation of living DNA or toxins. This may indicate long-term pathways for GMP spread and persistence (VDI 2006). The role of persistence of living DNA in the environment and the potential of horizontal gene transfer is difficult to assess (Heinemann and Traavik 2004, Nielsen et al. 2001).

A basic prerequisite for comparing monitoring data is the utilisation of appropriate and standard detection or analytical methods. Many of these methods exist already and have been standardised for other environmental safety contexts such as plant protection (BfR 2007) or for the detection of irradiated foods (European Community 2007). Other methods more specific to GMO monitoring still have to be developed and/or standardised. The Association of German Engineers VDI (2006) is developing such standards, among them molecular-biological detection methods, vegetation surveys, faunal relieves and insect-resistance monitoring—and aims to establish them on a broader level together with the European Committee for Standardisation (CEN) (Fink et al. 2006).

#### The spatial monitoring design: selecting monitoring sites and regions

GMP monitoring has to take place in exposed areas, preferably cultivated fields and their environment. On a case-by-case basis depending on the GMP characteristics, the selected indicators and analytical methods may consider different spatial and temporal levels (Graef et al. 2005; VDI 2006). The number of monitoring sites and regions needs to be sufficient to support statistical analysis of results based on good scientific practice (Bühler 2006; Leigh and Johnston 1994; Stein and Ettema 2003). For every GMP the monitoring design and data analyses should be based on a specific scale, quality and quantity of data to be representative and interpretable. This requires flexibility with the monitoring design. Monitoring every

**Table 1** Potential topics and indicator groups relevant to monitoring of potential adverse effects of different GMPs\* (Züghart and Breckling 2003, complemented with Hayes et al. 2004, VDI 2006)

\* based on four case studies: cropping of Bt-Maize (*Zea mays* L.), herbicide-tolerant (HT) winter oilseed rape (*Brassica napus* L.), HT sugar beet (*Beta vulgaris* L.), and starch-modified potato (*Solanum tuberosum* L.)

- Spread and und escape of GMP into the environment
- Volunteers in subsequent crops
- Hybridization and introgression with wild relatives and feral crop plants, establishment of hybrids
- Effects on non-target flora and fauna in cultivated areas and non-target environments
- Secondary infestation of crops and hybrids with bacterial, fungal and viral phytopathogens
- Consequences of altered farming practice
- Effects of herbicide tolerance technique
- Development of crop and weed resistance
- Effects on phytophagous invertebrates and their antagonists
- Effects on interrelations of the food web
- Effects on grain- und plant-feeding mammals and birds
- Effects on soil functions
- Effects on soil fauna und flora
- Horizontal gene transfer on microorganisms
- Effects on water bodies und water organisms
- Effects on species biodiversity and habitat diversity
- Unexpected gene expression
- Unexpected physiological and biochemical plant properties

GMP everywhere is neither necessary nor feasible and data analysis may also lead to adjustment of the overall number of representative monitoring sites. If the sites and regions are adequately distributed an intelligent systematic monitoring design can be representative for large areas (Graef et al. 2005). Criteria for selecting monitoring sites and regions include inter alia (a) representativeness of sites cultivated with specific GMPs with an emphasis on regions repeatedly cultivated with GMP, (b) representativeness of ecological regions containing the spectrum of selected indicators, (c) availability of sites already monitored within cultivated other agro-environmental programmes (Schröder and Schmidt 2001), and (d) areas with favourable environmental conditions facilitating spread or survival of GMOs (Wilkinson et al. 2000; Züghart and Breckling 2003). This requires availability of thematic GIS data containing this information like ecoregion maps (Metzger et al. 2005), CORINE Land cover data, agricultural census data and the precise knowledge of the cultivated fields, which have to be indicated via a national register (e.g. [http://www.194.95.226.237/stareg\\_web/showflaechen.do](http://www.194.95.226.237/stareg_web/showflaechen.do)).

Monitoring site selection includes aspects of both flexibility and stability: the design needs to be flexibly adapted to the specific GMP, its environment and the annually changing cultivated fields. On the

other hand cumulative and long-term effects can only be monitored on sites which remain over years.

#### Integrating existing agro-environmental monitoring programmes

For GS the Directive 2001/18/EC recommends to make use of existing agricultural and environmental monitoring programmes. They can provide useful baseline data over many years and different sites. These data are therefore very useful to interpret GMP monitoring data. The selection of GMP monitoring sites can be based on the programmes spatial design and make use of their infrastructure (Graef et al. 2005). Suitability criteria of the programmes to GMP monitoring are (a) spatial representativeness of the agro-environment in terms of site numbers and spatial distribution, (b) amount and relevance for GMP monitoring of measured biotic and abiotic indicators, (c) frequency and methodology of monitoring activities, (d) adaptability to GMP monitoring indicators, (e) long-term continuity.

For Germany a systematic investigation showed that there are options to connect GS to those programmes (Schröder and Schmidt 2001; Züghart and Breckling 2003). But they cannot fully cover all aspects of GM crop monitoring. Ultimately their suitability needs to be assessed on a case-by-case

basis depending on the specific GMP and the criteria above.

### Baseline data and parallel observations

Determining the baseline status of the field and its environment exposed to GMP as well as the parallel monitoring of GMP areas is a prerequisite for identifying adverse changes via monitoring and is provided for by the Council Decision 2002/811/EC (European Community 2002). An alternative is the split field design and comparable long-term GMP-free reference areas (Stein and Ettema 2003), which has been done for instance in the Farm Scale Evaluations (Perry et al. 2003). We consider this a must in order to reduce the background noise data induced by the high dynamic in agricultural practice and landscapes (Graef et al 2007; Hails 2002). As for every scientific field trial, it depends on the parameter to be monitored whether the baseline status of the field, its environment and reference areas without GMPs have to be analysed prior to GMP cultivation or in parallel (Leigh and Johnston 1994). Both long-term time series monitoring after baseline analysis and parallel monitoring of GMP areas and GMP-free areas can ideally complement one another.

### Monitoring data: harmonisation, coordination and quality

Once GMPs are monitored, different types of data will accumulate, which need to be centrally collected and efficiently processed. GMP monitoring data may originate from (a) the applicants using own surveys or contracted third parties and (b) existing agro-environmental monitoring programmes. For data coordination a structured information system is preferred (Reuter et al. 2006) with the potential to store, exchange, visualise and analyse numerical and geographical data. The data need to be compared to each other including baseline data and statistically analysed to assess potential adverse effects. This requires a minimum common standard for data quality, for instance on data pre-processing stage, number of replications and statistical power (Leigh and Johnston 1994; Perry et al. 2003; Stein and Ettema 2003), and they

must be based on standardised methods to be comparable (Fink et al. 2006). Comparability and good quality of data require a broad-scale harmonisation. Currently there is no legal obligation to harmonise and coordinate GMP monitoring data on the national or the EU-wide level. There is merely an obligation to analyse generated data based on good scientific practice (European Community 2002). However, there are efforts to develop regulations of standardised methods specific for GMO-monitoring (VDI 2006). GMP monitoring in routine operation should ultimately use standardised data formats, storage media and und database interfaces to enable automatic data transfer. Since the GMPs are commonly approved for EC the monitoring results should be coordinated on both national and EC level to provide for the early warning aspect (Fig. 2). Data interoperability and analysis step-by-step will have to be enhanced and adapted to national and EC circumstances during the GMP monitoring process.

### Conclusions and perspectives

Many details of GMP monitoring and its implementation are still at an initial stage. However, a number of GMPs for cultivation are pending in the approval process. We have pointed out the key issues and potential solutions for GMP monitoring of adverse environmental effects. They should be targeted and agreed upon prior to the commercial GMP cultivation to ensure a harmonised and scientifically-based approach.

The principle of stepwise increase of release scale based on results from a gradually enhanced data base is required by the Directive 2001/18/EC, however, it is not yet solved in a satisfactory manner. The essential basis to ensure this principle is to collect sufficient data during pre-commercial releases, which enables a solid e.r.a. and design for the subsequent monitoring.

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