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D7.1 Report on existing monitoring strategies and experience

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This deliverable is made of:

- A short overview of monitoring strategies and experiences which have been implemented in Europe and other countries to accompany the deployment of GM crops (Part I);
- A description of existing butterfly monitoring schemes in Europe (Part II).

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PU	Public	**X**
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Abbreviations

ACRE: British Advisory Committee on Releases to the Environment
CSM: Case Specific Monitoring
DEFRA: Department for Environment Food and Rural Affairs (United Kingdom)
EFSA: European Food Safety Authority
EPA: Environmental Protection Act
ERA: Environmental Risk Assessment
EU: European Union
FSA: Food Standards Agency (United Kingdom)
GM: Genetically Modified
GS: General surveillance
PMEM: Post-Market Environmental Monitoring

Part I: Report on existing monitoring strategies

1. Introduction

Worldwide, in 2011 GM crops were grown by around 16.7 million farmers in 29 countries. The area grown has increased steadily year-on-year, reaching about 160 million hectares in 2011 (James, 2011).

Most current GM crops are insect-resistant or herbicide-tolerant and aim at making pest and weed control easier for farmers. The main crop species in which these GM traits have been introduced are soya, maize, cotton and oilseed rape. GM crops with different traits are currently being developed, e.g. drought-resistance, disease-resistance, and crops with enhanced nutritional attributes (Taverniers et al., 2008; Paul et al., 2012).

Regulatory systems have been put in place to assess the environmental impacts of GM crops. They usually include an *ex ante* Environmental Risk Assessment (ERA), which may lead to the implementation of risk management strategies, as well as *ex post* environmental monitoring provisions, to verify the assumptions made during the initial ERA (Sanvido, 2005). Different Post-Market Environmental Monitoring (PMEM) strategies have been implemented across countries, ranging from no specific PMEM systems to the European regulation, which closely links PMEM with the Environmental Risk Assessment rules.

This report analyses current strategies and experience on monitoring in Europe and overseas and discuss their relevance to support the objectives of the updated EFSA Environmental Risk Assessment and Post-Marketing Environmental Monitoring Guidance Documents (EFSA, 2010, 2011a). It also aims to help assess what can be learnt from existing monitoring strategies as well as to anticipate expected impacts from known experiences in GMO cultivating countries.

A short review of existing monitoring systems highlights similarities/differences between countries as well as the evolving and dynamical nature of such systems. The report also explores room for improvement as well as those monitoring-related issues which need further attention.

Special attention is paid to the existing butterfly monitoring systems to assess to what extent they could be used/adapted to fit with the requirements of PMEM guidelines for GM crops.

2. Current regulations and existing monitoring strategies in Europe

2.1 An overview of the EU regulation

Risk assessment is at the core of the legislative framework and of the regulatory process. A European-wide consensus has been found near 2001 and adopted. The common purpose is to ensure the protection of the natural resources, the biodiversity and the agro-ecological functions. Guidance on principles and procedures has been regularly updated.

Before a GM crop may be *imported*, *cultivated* or *placed in the market* in EU, and according to Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (EC, 2001), the applicant has to follow a step-by-step approach and submits an Environmental Risk Assessment as well as A Post-Market Environmental Monitoring plan.

The Environmental Risk Assessment (ERA) shall identify all potential risks that could arise from the *import* or *cultivation* of a GM crop. The principles and methodology of the risk assessment are set out in Annex II to Directive 2001/18.1 (EC, 2001): the risk assessment should identify and evaluate potential adverse effects of the GM crop, which could arise directly or indirectly from its release. The potential adverse effects of a GM crop or its use should always be compared with the effects of the non-modified crop (conventional counterpart) from which it is derived; this is the 'baseline'.

GM crops are only authorized in the EU once the environmental risk assessment has shown that their import or cultivation does not raise additional safety concerns with respect to the conventional crops they are likely to replace.

The Post-Market Environmental Monitoring (PMEM) plan aims at confirming the assumptions made during the ERA and at identifying the occurrence of any adverse effects which were not anticipated by the ERA, after the GM crop has been placed on the market. If it is confirmed that a GM crop causes an adverse effect, measures shall be undertaken to protect human health and the environment.

Post-market environmental monitoring consists of two separate parts (according to Annex VII of the aforementioned Directive and Council Decision 2002/811/EC) with different aims:

Case Specific Monitoring (CSM): it is designed to confirm, if there remain uncertainties, that any hypotheses regarding the occurrence and impact of potential adverse effects of the GM crop or its use in the environmental risk assessment are correct. CSM is therefore only necessary when the environmental risk assessment, on a case-by-case basis, gives reason for it. CSM is also an option to cover remaining, but acceptable, uncertainties that could not be investigated during the pre-market release assessment.

General Surveillance (GS): unlike the CSM, the GS is not based on specific assumptions. It is set up to determine whether the GM crop or its use leads to the occurrence of any unanticipated adverse effects (indirect, delayed and/or long term effects) as well as

cumulative effects which are difficult to predict during the initial ERA. Additionally, it covers direct and immediate effects as far as they were not anticipated in the ERA on human health or the environment. As stated in the EFSA PMEM guidance document (EFSA, 2011a), the major challenges in designing GS plans are:

- to detect a change in relevant endpoints whose values fall outside the normal range of variation due to changes in management practices, receiving environments and associated biota in the EU;
- to determine whether the change is causing an adverse effect (e.g. causing irreversible damage to a protection goal) and,
- to determine whether the adverse effect is associated with the release or cultivation of the GM crop.

The preparation (strategy and methodology), reporting and implementation of a GS plan are mandatory in all cases and are of the legal responsibility of the applicant. The GS plan is usually based on:

- the use of questionnaires to report unusual changes observed by farmers,
- the analysis of existing environmental monitoring networks,
- a review of relevant scientific literature.

From 2003 onwards, and according to the Regulation 1829/2003 (EC, 2003), PMEM plans are included in applications for placing on the market, and are assessed by EFSA as for their scientific quality as part of the opinion for authorisation of GM food and feed products.

In case of authorization by the EC, the final PMEM plan is implemented, together by risk managers and applicants. They must ensure that monitoring and reporting on it are carried out according to the conditions specified in the consent. A yearly report has to be delivered (Article 20 (1) of Directive 2001/18/EC).

The legislative requirements in the EU specify that "where unexpected changes in the environment are observed, further risk assessment may need to be considered to establish whether they have arisen as a consequence of the placing on the market of the GMO or as a result of other factors" (Council Decision 2002/811/EC).

To accompany the deployment of the overall PMEM strategy, Member States shall establish public national registers in which the location of the release of the GMOs is recorded.

The PMEM strategy for GMO cultivation is continuously evolving, based on interactions between the EC, EFSA and Member States authorities:

On July 13 2010, the EC adopted a comprehensive proposal (EC, 2010a & b) that proposes henceforth, more freedom and flexibility for GMO cultivation to the MS without affecting the authorization system (e.g. for adopting co-existence measures, the safeguard clause, etc.). Authorities can independently install control measures and

perform the GS and complementary PMEM which could ensure independence and reliability of the information;

In 2011, EFSA issued a revised PMEM guidance document (EFSA, 2011a) which proposes a holistic and integrative approach for monitoring GM plants in the EU that considers GS within a framework of general environmental protection monitoring; all parties (e.g. applicants, Member States) have to consider their respective roles in such an approach for environmental protection monitoring that embraces GS; in this context, GM crops are not considered in isolation but considered as one component of the overall production system.

In 2012, DG SANCO held a workshop to discuss challenges related to PMEM; it acknowledged that a broader framework should be implemented and that independent environmental monitoring should complement event-specific PMEM schemes (EC, 2012).

2.2 Examples of the EU regulation implementation

While EU regulations directly apply in all Member States, directives have to be translated into National laws and this may lead to specific provisions. Also, some Member States have implemented additional studies to support PMEM.

United Kingdom

No GM crops are being grown commercially in the UK, but imported GM products, especially soybean, are being used mainly for animal feed, and to a lesser extent in some food products.

The UK Legislation instituted the Environmental Protection Act (EPA, 1990) which is the primary legislation that gives the Department for Environment Food and Rural Affairs of the responsible ministry (DEFRA) general powers and responsibilities to control the deliberate release of GMOs in England, and to implement Directive 2001/18. It also considers wider issues surrounding the use of GM crop technology.

DEFRA leads on the directive 2001/18/EC legislation and applicants need to ensure that they comply with all the relevant regulations. In 2002, Regulations on the Deliberate Release of GMOs have been supplemented to the EPA by setting out detailed rules for the implementation of Directive 2001/18, including specific requirements for applications to release GMOs. Regulations 1829/2003 and 1830/2003 (EC, 2003a & b) are implemented in England by means of the GM Regulations 2004 of the Food, Animal Feed and the Traceability and Labelling. Similar Regulations have been implemented in Northern Ireland, Scotland and Wales.

The Food Standards Agency (FSA) leads on the safety of GM food and feed, and on applications to market GM food and feed products. Ministers are given expert scientific advice on the safety of proposals to cultivate GM crops or release other types of GMO by the independent scientific British Advisory Committee on Releases to the Environment (ACRE) in which a subgroup is dedicated to the PMEM.

ACRE has initiated a number of studies about GM monitoring issues, the design of monitoring strategies and guidance for the assessment of long-term environmental impact under commercial conditions. In addition to anticipated effects (investigated via CSM) and unanticipated effects (which can only be addressed by GS), ACRE considered a third category of adverse effects of GMOs that should be monitored (ACRE 2004): Interactive or cumulative effects, which are difficult or impossible to predict or assess comprehensively in the framework of the ERA for a single notification. These effects are considered as unanticipated because "within the ERA of an individual case, it may be difficult to predict what effects might arise due to an increase in the scale of cultivation, or the full effects of environmental interactions" (ACRE 2004).

Existing Environmental Surveillance Networks could be used considering their reliability and some protection goals. The challenge of the general surveillance is to meet the requirements of the regulations, be proportionate to the level of risk and be science-based.

ACRE recently issued an opinion on PMEM of GM crops (ACRE, 2013), which paid special attention to existing environmental surveillance networks (ESN) and their potential use for detecting unanticipated adverse effects of GM crops.

Although existing ESNs have not been implemented to investigate relationships between cause and effect, the report proposes to combine « reporting on the health of the farmed environment with searching for correlations with any drivers of change » and concludes that "with well chosen indicators and specific data analysis, some ESNs could be used to detect unanticipated adverse effects ». It also recommends that « GM crops are considered in the context of the wider impacts of agriculture on the environment and initiatives to identify drivers of change through use of existing ESNs ».

France

The first cultivation applications were initially assessed by France (Bt196 and MON810) under the former legislation (Directive 90/220 which had been translated into French law by the Act of 13 July 1992). No provision for PMEM was included in such a regulation but, when the first notification of maize cultivation occurred in 1998 (3 varieties of the Bt176 event were authorized by a decree of 5 February, JOCE, 1998), the decision made it mandatory to set up a mandatory environmental monitoring scheme which included:

- The verification of the efficacy of the trait on target species;
- The monitoring of resistance evolution;
- The effect on non-target organisms;
- The impact on soil bacteria (possible horizontal transfer of the *amp* antibiotic resistance gene);
- The potential evolution of bacteria population of animals fed GM maize.

No clear distinction between case-specific monitoring and general surveillance was made by then. A Bio-Monitoring Committee (Comité Provisoire de Biovigilance) was established in 1998 to set up adequate protocols and assess the monitoring results of large scale cultivation of GM maize.

Beside these studies on the unintended effects of Cry1Ab which took place until the implementation of a *de facto* moratorium, the Committee expanded its activities to set up a GM monitoring network ("Biovigilance" network) which would set up a baseline before the introduction of GM crops and consider other potential impacts of GM cultivation, such as weed shift, weed resistance, farmland diversity and impacts on mycotoxins (Delos et al., 2007; Fried, 2008).

The Bio-Monitoring Committee was composed of scientists, representatives of civil society and representatives of relevant Plant Protection Services. If unintended/adverse effects were identified, the Committee would send a warning or ask the Ministers of Agriculture and Environment to reassess the risk or to suspend the GM variety approval.

Regional Services of Plant Protection and Food inspection as well as Agriculture and Forestry and Fraud services were in charge to carry out inspections, sampling and analysis to ensure compliance with technical requirements.

The initial organization of risk assessment and monitoring changed when the new EU regulatory framework was adopted (Directive 2011/18 and regulation 2003/1829) and translated into National regulation in June 2008 (Act No. 2008-595 of 25 June 2008[1]). This National Act set up a new assessment committee, the High Council of Biotechnology, which includes a scientific committee as well as a socio-economic committee. Monitoring provisions have been included within a broader Biovigilance Surveillance framework. The Act also includes a legislative package on GMO/non-GMO co-existence.

Considering that GM crops cannot be monitored regardless of the other agricultural practices, the Act set up a Biological monitoring of the territory also named "National Land biological Surveillance", which aims at monitoring potential impacts of changing agricultural practices. Such changes may include low-pesticide practices (as currently implemented under the National Action Plan on pesticide use) or GM crops. This broader framework aligns on the EU PMEM provisions for GM crops and includes:

• a "specific surveillance" (similar to case-specific monitoring), based on specific queries of the biovigilance committee. It is limited in time (1 to 5 years), space and is based on specific hypotheses (e.g., comparing GM/ non GM crops for some endpoints).

• a general surveillance, which aims at identifying and monitoring the occurrence of unintended or adverse effects of GM crops on the environment or the agro-ecosystem.

[1] *Loi no 2008-595 du 25 juin 2008 relative aux organismes genetiquement modifies*

This network is supported by an advisory committee (CSBT[2], Comité de Surveillance Biologique du Territoire, Decree no. 2008-1282 of December 8, 2008), which proposes protocols and methodologies and assesses the monitoring results. It also develops recommendations on the directions to be given to the monitoring and alerts the administrative authority.

Farmers, industrials and stakeholders responsible for GM crops' release or placing on the market should be involved in the "biovigilance" providing all information useful for its implementation. Biovigilance also ensures traceability of GMO seed variety and helps ensure compliance with the requirements specified in the approval decision or with coexistence rules.

The results of the general surveillance are subject to an annual report to the government and the National Assembly and the Senate. When unintended effects are predicted and require specific management actions, CSBT alerts the administrative authority. Results are also made public.

Two monitoring programs are currently within the National Land Surveillance Network:

- **The pest monitoring network was** established in 2009 under the Directive 2009/128/EC on the sustainable use of pesticides. The implementation of such a national epidemio-monitoring network involves a range of public and agricultural actors (such as Chambers of Agriculture). Observation protocols and sampling schemes have been harmonized (field and plot selection, sampling management, data analyses and interpretation) in order to collect and build reliable data base leading to relevant conclusions. Numerous indicators of crop health are measured on about 12,000 fields and epidemiological models are used to predict pest incidence and evolution. Weekly Regional Plant Health Bulletins (BSV) are edited to help farmers decide whether to treat or not. Such a network might be of relevance to support PMEM of GM crops, should such crops be grown.
- **The "unintended effects monitoring network" was established in** 2012 to monitor unintended effects of agricultural practices on biodiversity. The organization of this network relies on the previous experience of the epidemio-monitoring network and harmonized protocols are implemented at a large scale (500 fields on 2012). Monitored endpoints include so far: flora diversity in field margins, common birds in rural zones ; field margin coleoptera or field earthworms. Such a monitoring network would be of specific relevance for PMEM of GM crops and might be extended to cover specific monitoring issues related to GM crops, such as possible development of resistance with Bt crops.

In addition to these ongoing monitoring networks, CSBT has proposed protocols for the implementation of a national monitoring of unintended effects of Bt or Herbicide-tolerant GM crops (CSBT, 2011).

[2] http://agriculture.gouv.fr/CSBT-missions-et-avis,1645

Spain

Since 1998, Spain has set up a regulatory framework for the registration of commercial varieties and adopted national monitoring plans designed and implemented by consent holders and public authorities (the National Biosafety Commission of the Ecology Department). Since 2003, its legislation is in line with the European Directive 2001/18 and involves a PMEM for Bt176 (1998-2005) and MON810, with:

- **Specific issues:** to monitor insect-resistance, to assess potential effects on non-target arthropods and soil microorganisms, unintended effects on bacterial microflora (only for Bt176);
- **General surveillance:** in addition to farmers' questionnaires (only for Mon810), a user's guide edited by the national association of farmers is intended for farmers for advice on their responsibility to comply with specific measurements and the relevant requirements for the pest resistance prevention plan.

The guides provide advices on the refuges plots as well as information on coexistence, traceability and labeling.

During the period 2006-2011, agreements between public and private research institutes are developed for studies at the territory scale that focus on **pre-market research** on the herbicide-tolerant maize (2006-2011) and cotton (2007-2011) for assessing potential indirect effects on non-target organisms due to the weed management.

Agreements with public and private research institutions are also developed to investigate **monitoring strategies**. Protocols are defined by Spanish and European panel of experts to be reproducible and comparable. Their conclusions are included in the monitoring plans. Monitoring at the long term, over a 10-year period and at a large field scale (Roda, 2010) , emphasized on the assessment of:

- Effects of the Bt176 (1998-2005) and MON810 (2003-2011) GM crops on the development of resistance or tolerance to the Bt toxin;
- Potential ecological effects of the exposure to Bt on non-target arthropods and their abundance and composition. Studies were also carried out in confined conditions in the laboratory for considering extreme scenarios;
- Potential effects on the soil micro-organisms;

These monitoring studies aim also at establishing a baseline to evaluate tolerance development over time and to implement insect-resistance management strategies (IRM). Spain plans to develop studies on the indirect effects of GM crops on the environment.

2.3 A monitoring case study: maize MON810 in the European Union

GM maize MON810 is the only GM crop currently grown in the EU. Commercialized by Monsanto (with the commercial name of YieldGard), it has been genetically modified by the introduction of the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki* (Bt), which is a molecule insecticide against lepidopteran target pests such as

the European corn borer (*Ostrinia nubilalis*) and the Mediterranean Corn borer (*Sesamia nonagrioides*), whose larvae are the main pest of maize crops.

In the EU, GM maize MON810 was approved under the former EU legislation in February 1998 for food and feed according to regulation procedure 258/97 (abrogated by regulation 1829/2003) for an unlimited period. In addition, GM maize MON810 was authorized in April 1998 for cultivation, under directive 90/220/EEC (abrogated by directive 2001/18/CE). This consent of MON810 maize for import and use (including cultivation) in the EU was notified by the French Competent Authorities on 3 August 1998. It included an insect resistance management plan (based on the high dose/refuge strategy) to minimize/delay the onset of resistance in the target pests. No requirements for environmental monitoring after placing on the market apply with Directive 90/220/EC and, in particular, no General Surveillance was set up.

However, the notifier, France, required for Cr1Ab-based Bt maize crops (including Bt176 as well), the establishment of further monitoring, including the occurrence of unintended effects on the maize pests' populations, and the evolution of entomofauna, soil bacterial population and intestinal animal flora (decree of 5 February; JOCE, 1998). This PMEM scheme included for MON810:

- The verification of the efficacy of the trait on target species;
- The monitoring of resistance evolution;
- The effect on non-target organisms;
- The impact on soil bacteria;
- The potential evolution of bacteria population of animals fed GM maize.

A Bio-Monitoring Committee (Comité Provisoire de Biovigilance) as described above was established to set up adequate protocols and assess monitoring results of the large scale cultivation of GM crops.

In 2007, Monsanto submitted an application for the authorization renewal after nine years since the initial marketing authorization, under Regulation (EC) 1829/2003.

The EFSA GMO Panel assessed the renewal application of MON810 with reference to the intended uses and appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed. The scientific assessment tackled molecular characterization and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the PMEM plan were also undertaken.

EFSA issued an opinion in June 2009. The renewal decision by the EU is still pending. According to the legal framework, these authorized products remain lawfully on the market until a decision on re-authorization is taken.

The PMEM plan submitted by the applicant now includes:

- case-specific monitoring, of insect resistance evolution to the Cry1Ab toxin expressed by MON 810 maize among populations of target insects, provide the results of the IRM strategy aiming at preventing/delaying the development of insect resistance (such as refuge zones establishment, insect sensitivity);
- general surveillance, aiming at identifying the occurrence of unexpected adverse effects of MON810 maize on human health or the environment; it focused on the geographical regions within the EU where the GM crop is grown. This surveillance is being undertaken through questionnaires of MON 810 growers (250 per year), scientific literature analysis and data collection, and support measures (such as alert systems by authorities, through existing networks and the press).

As no decision has been made on the renewal so far, only the initial PMEM plan is legally binding. However, when Monsanto notified in 2004 its different MON810 products placed on the market for food, feed and cultivation, as existing products, under Regulation (EC) n° 1829/2003, and inscribed them on the Community Register in April 2005, it started to submit to the EC and MS, annual reports comprising the results of the insect resistance monitoring along with the results of a voluntary general surveillance (complying with PMEM provision of Regulation (EC) n° 1829/2003 and Directive 2001/18/EC).

Since 2010, the European Commission has asked EFSA to evaluate these MON810 monitoring reports on cultivation in Europe. The EFSA GMO Panel has assessed the 2009 and 2010 reports (EFSA 2011, 2012).

As for the implementation of the CSM plan, and particularly the IRM plan, it concludes that « there is no evidence of resistance evolution in target pests based on the available information but, in light of the shortcomings identified during the evaluation of the methodology, advises the applicant to reconsider its IRM plan ». Recommendations include

- to further educate farmers on the need to comply with refugia implementation and to inform them about the situations which increase the probability that resistance to the Cry1Ab protein may evolve in the target pests and other regionally important lepidopteran pests, and thus threaten the efficacy of maize MON810.

- to focus the sampling of target lepidopteran pests in « hotspot areas » over time (e.g., high adoption rate and frequency of maize MON810 and multivoltine populations) to increase the likelihood of detecting resistance evolution.

- to consider regionally important lepidopteran pests (other than ECB and MCB) of maize MON810 in the context of CSM for IRM strategy (EFSA, 2011b) and, where appropriate, adjust the design and implementation of the IRM plan accordingly;

- in « hotspot areas » (i.e., regions with high uptake of maize MON810 and multivoltine populations), to revise the monitoring protocol aiming at a detecting resistance allele frequency between 1% and 3%.

As for general surveillance, recommendations include a better definition of the

comparator used to compare MON810 performances with, a more explicit sampling scheme to select farms.

Based on these recommendations, it is foreseen that the PMEM plan of MON810, as well as other Cry1 protein expressed maize crops will be amended, illustrating the dynamic nature of PMEM and its interplay with the Environmental Risk Assessment.

3. Monitoring schemes implemented worldwide

While in Europe the precautionary principle has early been chosen and adopted, in other regions, including those where GM crops are widely grown such as USA, PMEM is not considered mandatory. The substantial equivalence principle is followed and PMEM activities are limited to specific areas of concern, such as insect resistance monitoring of Bt maize.

3.1 USA

Between 1996 and 1998, crop area using GM seeds had increased 15 fold in the USA: one third of the American maize and cotton crop and more than half of the soybean crop are now grown from GM seeds - representing among the most rapid adoption of a new technology in the history of agriculture. USA used regulations implemented for products obtained with conventional methods and existing bodies to address GM crops. Thus, unchanged since 1986, The *Coordinated Framework for Regulation of Biotechnology* is the key US government document providing statutory authority for biotechnology regulation. No new regulations were deemed necessary to cover the many issues related to risks to human health, non-target organisms and the environment. This framework established a biotechnology working group - Biotechnology Science Coordinating Committee - and specified three primary regulatory agencies[3] for regulating biotechnology with specific roles:

- **EPA**[4] **(Environmental Protection Agency)**, to ensure that no adverse effects on the health or the environment would occur. EPA acts only on behalf of pesticide law (The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA reports N°6(a) 2)) and therefore only deal with Plants-Incorporated Pesticides (PIPs);
- **USDA-APHIS (Animal and Plant Health Inspection Service of the US Department of Agriculture)**, to ensure among others that GM products have no negative effects or risk on conventional ones, such that called “regulated articles”;
- **FDA (Food and Drug Agency)**, to ensure that GM products are as healthy as conventional ones. It also became responsible for biotechnologically-derived medical products.

The act NEPA (National Environmental Policy Act) requires that before any authorization to GMOs, an environmental risk analysis is conducted. Depending on the outcome, the administration may decide to approve the GMOs or to request more in-depth environmental studies.

[3] http://usbiotechreg.epa.gov/usbiotechreg/
[4] http://www.epa.gov/oppbppd1/biopesticides/pips/index.htm

There is no requirement for PMEM after deregulation, according to Code of Federal Regulation (7 CFR 340). However, EPA set up specific provisions to prevent and monitor the development of insect resistance for Bt crops.

EPA required a crop risk management for Bt maize and cotton and directed companies marketing Bt maize to request that farmers voluntarily plant a buffer zone of conventional maize to provide a reservoir of insects that remain susceptible to the Bt toxin. EPA also required a monitoring of its efficacy. Approval of the renewed registrations of Bt maize and cotton is also determined by the implementation of an IRM (Insect Resistance Management) plan required for all Bt crops to prevent insects from developing resistance to Bt proteins. Here, contracts between registrants and educated growers are planned. As a protection for Monarch butterflies despite negligible risk (ABST Committee, 2001), EPA required companies an assessment of the risk of potential harm that Bt maize pollen poses to Monarch butterfly larvae and other butterfly species.. As a result of these studies, environmental concerns have raised about effects to unintended species and reduction in biodiversity.

3.2 Argentina

Argentina has regulated activities connected with agricultural GMOs since 1991, once biotechnology activities began. The **National Advisory Commission on Agricultural Biotechnology** (**CONABIA** for its name in Spanish) was created within the scope of the **SAGyP** (**Secretariat of Agriculture, Livestock and Fisheries**). As the biotechnology activity increased, it was the responsibility of the CONABIA and the **Biotechnology Directorate** (established in November 2008) to conduct the assessment of applications submitted to develop GMO-related activities: development, seed production, release into the environment (confined conditions, laboratories or field trials) and commercialization for which permits are requested.

According to the Resolution SAGyP N°701/11, the regulation establishes a comprehensive **risk assessment** as the method to ensure the safe use of agricultural GMOs cultivated at large scale for the agroecosystem and to ensure that GM crops behave similarly to the same non-GMO.

The risk assessment is carried out following a case-by-case analysis and is based on scientific information, technical criteria, quantitative data and relevant literature. It is divided into three stages: risk assessment (is performed before a GM crop is commercially released) risk management and risk communication. The PMEM is not considered necessary and would only be advisable if the information that can be obtained via PMEM could provide added value to the data used in the regulatory ERA.

The two competent organisms (CONABIA and Biotechnology Directorate) jointly deliver a report and submit it to the SAGyP, the Competent Authority which takes decision on

GMOs and related activities, giving or not the authorization for releasing into the environment[5].

3.3 Brazil

Brazil has developed a **regulatory framework** to address GMOs. Since 1995, it is governed by a Biosafety Law, a decree and 'normative' instructions. In 2005, this regulatory framework was revised to support both research activities and commercial releases of GMOs for safety norms (Melo et al, 2011).

The competent authorities of the GMOs biosafety system are made of Government Agencies: the National Biosafety Technical commission (CTNBio), the National Biosafety Council (composed by ministry of states), Registration and Inspection Bodies, as well as the Biosafety Internal Commission, which is the most important body, responsible for the **risk assessment** of GMOs and their **post-market monitoring**.

The GMO biosafety evaluation in Brazil is based on sound scientific basis, a case-by-case approach and multidisciplinary approach.

The **registration** of GMOs products does not differ from conventional products. After the mandatory risk assessment, done by the CTNBio, a GM plant can be approved for commercial release after which there is registration by the ministry of Agriculture (MAPA) if the product is for agriculture.

Recently, in 2008 and then in 2011, new resolutions (N°5/2008, N°9/2011) set up a mandatory Post-Market Monitoring. Since then, there had been specific provisions for Roundup Ready soybean (1998) and for maize (2007).

The purpose of PMEM consists of obtaining information that may indicate any adverse effects after commercial release of GMOs on the environment or on human or animal health, in line with the scope of the application. It also provides general technical information, assesses **the benefits of the technology** and proposes **mitigation actions**. Applicants can ask for exemption if no measurable risk was found in the risk assessment. Upon request by the technical commission, a general surveillance may be required anyway. If any potential significant alterations to biosafety are detected or no mitigation measures are possible, it may result in the immediate suspension and/or revocation of the technical decision and of the commercialization of the product.

In Brazil, a specific case study was carried out on GM Roundup Ready soybean, the first commercial Agriculture Biotechnology product that was harvested in 2005. This monitoring study was set to assess the efficiency of the new biosafety framework and to implement a **precautionary approach**. No evidence of potential risks on the environment and the human and animal health was found during the risk assessment. Nevertheless, CTNBio developed a comprehensive case specific post-market monitoring of commercial production areas of soybean for a period of 5 years through "comparison

[5] *For further details about the legal framework, please connect to the website of the Argentina Agriculture Ministry:* ***http://64.76.123.202/site/agregado_de_valor/biotechnology/30-REGULATORY%20FRAMEWORK/index.php***

studies" about the plant species, insects and microorganisms present in the fields and environmental indicators. The **consent holder** (Monsanto) had to assess many specific parameters, organize, implement and execute this monitoring scheme. A confidential yearly report had to be submitted. A farmer guide was also provided to the field team.

The soybean was considered as safe as the conventional one.

The specific case of monitoring GM maize was subjected to the **Normative Resolution No. 03 of August 16th, 2007.** The monitoring plan should be approved by CTNBio, based on scientific methodologies and address risk assumptions raised during the assessment. The commercial release requests applicants to be responsible for implementing the monitoring plan, which may be executed through hiring services from institutions able of executing them independently, .

The new **Resolution CTNBio nº 09/11** specifies that the purpose of monitoring post-commercial release consists of information that may indicate adverse effects arising from the commercial release of GMOs on the environment or on human or animal health, in line with the scope of the application. The monitoring plan includes:

- a **case-specific monitoring** (a set of processes for assessment of adverse effects observed in the overall monitoring or anticipated in the risk assessment of CTNBio, arising from the commercial release of GMOs on the environment or human or animal health),
- a **general surveillance** (it is a set of processes for detection and identification of unanticipated adverse effects on risk assessment of GMOs, arising from the commercial release of this on the environment or human or animal health).

Its preparation, submission and subsequent implementation after commercial release is under the responsibility of the applicant. No official controls from the Minister of Agriculture exist for the post market monitoring, just field trials and risk assessment are inspected. First reports from the notifiers are submitted to the National Biosafety Technical Commission CTNBio. At present, improvements of PMEM requirements are envisaged[6].

3.4 South Africa

With the rise of biotechnology and GM planting crops since 1999 in South Africa, a **Biosafety Regulatory System** has been developed since 2001 to minimize disturbance to the environment.

There are numerous pieces of legislation related to GMOs in South Africa, and implementation of these is shared by various government departments (Bohn et al., 2010). The **GMOs Act** (Act No. 15, 1997, Act No. 23, 2006, Amendment of 2011) regulates research, development, production (general release activities), import and export, transport, use and application of GMOs and ensures minimum potential risks to

[6] *For further details about the legal framework, please connect to the website of the Brazil Science, Technology and Innovation Ministry:http://www.ctnbio.gov.br/index.php/content/view/12857.html*

the environment (provision for the post-market monitoring of GMOs is included) and to human and animal health. It is implemented by the Directorate Biosafety of the Department of Agriculture, Forestry and Fisheries (DAFF).

The Advisory Committee (AC) composed of independent scientists, assesses the potential risks associated to individual applications and makes recommendations to the Executive Council (EC), composed of representatives of government departments, which takes decision on GMOs and related activities. The submitted AC report may include risk management strategies (i.e. conditions for post-market monitoring to be implemented by notifiers if the application is approved). Monitoring for compliance to permit conditions is carried out by inspectors within the DAFF who should submit inspection reports (detailing the risk management measures, adverse effects, etc.).

Within terms of the GMO Act, notifiers are required to develop a monitoring plan and evaluate the impact of the GM crop on the environment. They submit annually a report describing the field locations, the agricultural practices and the observed resistance development. The applicant should also undertake grower educational programs and monitor farmer compliance.

Other stakeholders are also involved in monitoring related activities in South Africa in addition to the DAFF. The South African National Seed Organization (SANSOR) plays an active role in permit holder compliance monitoring through the SANSOR GMO Seed Standing Committee (ensure industry compliance with regulations, IRM).

There are two acts that are directly related to the issue of **monitoring** of GMOs:

- **The National Environmental Management Act, NEMA** (Act No. 107, 1998, Act No. 14, 2009)**:** it provides general principles for decision making with regards to the environment. General guidance has been provided by the Department of Environmental Affairs (DEA) regarding the objectives of ERA (pre-release risk assessment) for GM plants, including the criteria that may trigger an Environmental Impact Assessment (**EIA**).

- **The National Environmental Management Biodiversity Act** (Act No. 10 of 2004, **NEMBA**)**:** it confers to the South African National Biodiversity Institute (SANBI), the responsibility to monitor and report on the environmental unintended effects of GMOs released into the environment on biodiversity (General surveillance).

The approach to be implemented for monitoring is quite similar to that of the European regulatory system[7]. Notifiers have the same tasks, and the methodological monitoring plan must meet the same endpoints: hypothesis based research using a non-GMO comparator, early detection and warning instruments, surveillance related to protection goals and a biosafety assessment.

[7] *For more information, please connect to the website of the Department of Environmental Affairs of the Republic of South Africa:* http://www.environment.gov.za

A complementary independent post commercial monitoring to be implemented under NEMBA in the future will mostly be a system of global oversight of biodiversity to identify trends in populations.

Until 2010, no EIA for a GMO has been required under NEMBA and consequently no EIA under NEMA has been conducted. But, Independent 'biosafety research' is part of a current global trend in South Africa essential to establish independent (non-developer associated), risk-based research (Bohn et al., 2010).

A collaborative project between South Africa and Norway, the **EBCP** project (Environmental Biosafety Cooperation Project, 2008-2010), implemented a long-term comprehensive monitoring system to assess the impact of Bt maize MON810 on the environment.

Studies were conducted under controlled conditions in glasshouses, in laboratory, or under field conditions to assess the effects of Bt maize on specific parameters/ biodiversity indicators: soil microbial diversity, mycorrhizal fungi, earthworms, non-target pests, non-target insects, non-target Lepidoptera. The role of refuge zones strategy was also tested on resistance development through measures of insects and parameters of susceptible and/or resistant strains.

This experience would serve to enlarge databases, fill scientific gaps, strengthen and support the regulatory framework governing GMOs in South Africa.

4. Conclusion

The short overview of monitoring strategies worldwide indicates that the overall objectives and principles of PMEM plans are rather similar across countries even if their practical implementation and contents may differ. By nature, PMEM is dynamic and its efficiency can only be assessed during large-scale cultivation. While the European Union has set up a rather extensive framework for PMEM, the development of GM cultivation remains limited. To date, there is only limited experience with monitoring of unexpected environmental effects of GM crops and practicable monitoring protocols indicating how PMEM programs of GM crops could be implemented are still lacking.

Due to the high diversity of receiving environments, as well as of management and production systems, the Environmental Risk Assesment considers a range of representative scenarios but cannot cover all possible situations. In addition, long-term effects may occur, either because of a delayed response (or detection) of some effects, or as the result of an inevitable increase in spatial and temporal complexity for large-scale cultivation. Also, receiving environments and management systems are continuously evolving. In this context, the Post Market Environmental Monitoring (PMEM) is of major importance, not only to address those risks or critical uncertainties which would have been identified during the ERA, but also to check the hypotheses made during the ERA, to assess the efficacy of proposed risk management measures, to ensure that the deployment of the GM plant still "falls within the domain of validity of the conclusions of the ERA", to detect any unexpected adverse effects, and to help mitigate risks through

crop management systems which are fine tuned for specific crops, regions and agronomic practices.

This implies that boundaries between CSM and GS cannot be strict and that their implementation should be further investigated. Major challenges are related to general surveillance :

- How to detect biologically relevant changes in environmental monitoring? Which spatial scale should be considered? How many « points » should be monitored? How long should we monitor endpoints overtime? As responses depend on the specific endpoints, their natural variation and the effect size which might be considered harmful, a thorough problem formulation should be conducted (Oberhauser et al., 2009);
- How significant changes can be related to causes, namely the introduction of GM crops, as other changing factors (climate, cropping systems might have higher impacts ?

Various contributions to these challenges have been produced (ACRE, 2013 ; COGEM, 2011; EC, 2012, Van den Brink, 2010; Züghart et al., 2008, 2010). In both cases, modelling could help setting up monitoring strategies by (i) carrying out sensitivity analyses based on a range of assumptions concerning the variability of parameters and (ii) identifying those receiving environments for which impacts of GM crops are likely to occur. AMIGA will further assess the potential of modelling approaches to support the implementation of PMEM strategies.

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Appendix

Summary from the Executive Report of the Evaluation of the EU Legislative Framework.

Evaluation of the EU Legislative Framework in the Field of Cultivation of GMOs Under Directive 2001/18/EC and Regulation (EC) No 1829/2003, and the Placing on the Market of GMOs as or in Products under Directive 2001/18/EC.

The evaluation has investigated both the decision-making process and the practical risk management issues associated with GMOs cultivation. It was concluded that adjustments to the procedure and the authorization process and its financing could be helpful by creating a more efficient system, time-limited and transparent. But these would be unlikely to be sufficient to remove the blockages. There is a need for more fundamental and difficult reforms strengthening the infrastructure needed to support the legislation's requirements for monitoring and surveillance. In addition, targeted changes to the rules should be introduced into the decision-making mechanisms and criteria, at both EU and MS levels, with new flexibility, freedoms and confidence of those involved, without distorting the authorization process.

The Commission's proposals of July 2010, which present options to allow more choice to MS in deciding whether to cultivate GMOs, are an attempt to meet that challenge (EC, 2010a, b,c).

Changes that result in more efficient and transparent institutional decision-making could help to prevent the 'misuse' of national safeguard and emergency measures. Special effort should be made to explain the differences of EFSA/MS interpretation of the science being used to justify existing bans. Differences, especially in application, between the Regulation's emergency measure and the Directive's safeguard clause should be made clearer.

The decision-making framework could also be modified to give MS greater freedom to use non-scientific factors in setting national rules and regulations that affect GMO cultivation (e.g. Co-existence measures)

The scope of the information that can legitimately be used to inform the authorization decision could be expanded through more explicit consideration of socio-economic factors. The geographic scope of an application could be defined by the notifier either at the outset or after a final scientific Opinion from EFSA that contains a favorable assessment in cases where a MS then declares reservations about the GMO.

Pressure to update the legislation's scope arises as innovations lead researchers and industry to adopt new techniques. Principles that should define the scope of the legislation in the future must be considered (do they focus on the techniques used or the characteristics of the final products).

Involving risk managers from MS in the determination of the procedures boundaries and assumptions, and asking them to formally accept the guidance could help to align otherwise diverse MS concerns and the EFSA-mediated process under the Regulation.
Thus, there is a need of measures that: enhance dialogue amongst notifiers, MS and EFSA; streamline the process and promote engagement through system reforms; address the scale and flow of financial resources in the system; and improve predictability and efficiency through greater harmonization of practice among MS and notifiers.

Part II: Evaluation of existing butterfly monitoring schemes in Europe for their feasibility to be used for GMO monitoring purposes

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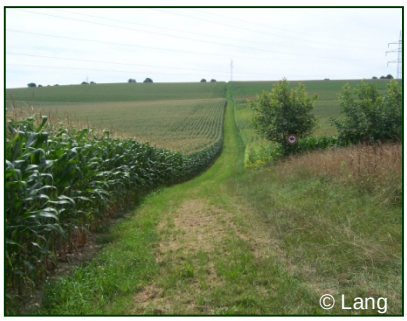

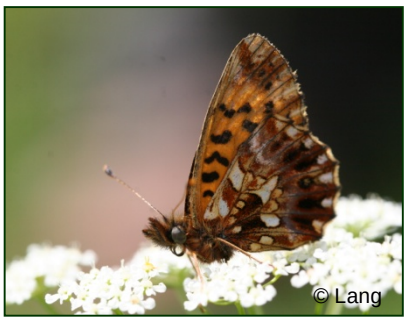

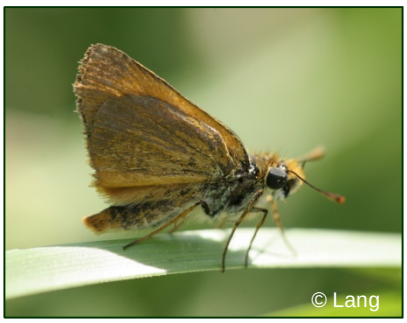

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Abstract

Butterflies (Lepidoptera) have often been suggested as a major component of an environmental monitoring of genetically modified (GM) crops, because they are proven and tested bio-indicators, represent relevant protection goals, and adverse effects of GM crops on Lepidoptera have already been reported. Therefore, one of the objectives of the AMIGA project is to develop, suggest and evaluate a monitoring concept for butterflies within the framework of post-market environmental monitoring of GM plants.

In this report, the existing volunteer butterfly schemes in Europa were collected and a first evaluation carried out with regard to their suitability to be incorporated in general surveillance of GM plants. This was done, because the Council Decision 2002/81/EC recommends making use of established routine surveillance programmes where appropriate. All in all, 16 butterfly monitoring schemes were identified in the member states of the European Community, plus another 5 schemes in non-member countries in Europe. All butterfly monitoring schemes use the common line transect counts for recording the butterflies. As most of the monitoring programmes have just started in the last decade, long-term time series larger than ten years are rare. A considerable part of the transects are located in arable land, which would make them suitable for GM crop monitoring. However, the scope of the surveys differ greatly among the various countries in number of transects, transect length and counts per years. It appears that only four countries have sufficient large and comprehensive butterfly schemes, which could render them possible for general surveillance of GM crops to some extent, i.e. Finland, France, Ireland and Switzerland (and perhaps the UK).

I. Background

In the European community, the Directive 2001/18/EC on the Deliberate Release into the Environment of Genetically Modified Organisms (GMOs) stipulates a monitoring plan in order to trace and identify any harmful effects on human health or the environment of GMOs after they have been placed on the market (EC 2001). Guidelines with regard to the requirements for monitoring design, sampling methods and analysis techniques are outlined in further documents of the European Community (EC 2002, EFSA 2011). The Directive 2001/18/EC distinguishes two parts of post-market environmental monitoring (PMEM): g*eneral surveillance* and *case-specific monitoring* (EC 2001). Case-specific monitoring should, when included in the monitoring plan, focus on potential adverse effects of GMOs that have been identified in the previous environmental risk assessment (ERA). Thus, a case-specific monitoring plan would serve to confirm or reject the assumptions of the ERA, and case-specific monitoring should address specific hypotheses associated with identified potential effects of the GM crop (EC 2002). In contrast, general surveillance should focus on unanticipated and unforeseen as well as on possible delayed and long-term effects that were not predicted in the risk assessment, and if unexpected changes in the environment have been observed, further risk assessment may need to be considered to establish whether they have arisen as a consequence of GMO cultivation (EC 2002). General surveillance should, where compatible, make use of established routine surveillance practices such as ecological monitoring, environmental observation and nature conservation programmes (EC 2002).

Butterflies and moths are often suggested as a relevant parameter to be recorded in a GMO monitoring plan (e.g. Lang 2004; Sanvido et al. 2004). In general, Lepidoptera are considered relevant and valuable bio-indicators, because they can indicate various states and changes in the environment such as conditions of climate, vegetation, habitat or the landscape (Aviron et al. 2007a; Settele et al. 2009; but see Fleishman and Murphy 2009 for a critical evaluation of the use of Lepidoptera as indicators). This includes the assessment of agri-environmental schemes (Aviron et al. 2007b, Roth et al. 2008), the detection of effects on biodiversity (Wenzel et al. 2006, Nilsson et al. 2008), the record of management effects in arable land (Field et al. 2005, 2007, Dover et al. 2010) or adverse effects of pesticide use (e.g. Johnson et al. 1995, Russell and Schultz 2010), and the impact of land use change (e.g. Stefanescu et al. 2009, van Dyck et al. 2009). The features contributing to the value of Lepidoptera as environmental indicators further include the good knowledge on their faunistics, ecology and conservation biology, relatively easy identification of species and the presence of field guides, existence of sound and widely accepted monitoring methods, the establishment of many volunteer monitoring schemes in Europe and the wider public acceptance of Lepidoptera as valuable protection goals (Skinner 1998, Bachellard et al. 2007, VanSwaay et al. 2008, Settele et al. 2009). In addition, Lepidoptera fulfill important ecological key roles as herbivores, pollinators and prey organisms in many terrestrial ecosystems, and, depending on the specific circumstances, can be representative for general biodiversity, thus potentially indicating

changes in other animal groups and plants (Thomas 2005, Thomas et al.2004) or habitats (van Swaay et al. 2010).

Most important, adverse effects of genetically modified (GM) plants on Lepidoptera have already been reported, which strongly supports their quality and significance for an appropriate GMO monitoring (Graef et al. 2005). Currently, the major events of GM plants developed and being cropped worldwide are insect-resistant and herbicide-tolerant crops (Kvakkestad 2009). Pollen of insect-resistent *Bt* (*Bacillus thuringiensis*) maize toxic to pest Lepidoptera may be drifted by wind onto host plants of non-target lepidopteran larvae growing nearby (Pleasants et al. 2001, Lang et al. 2004). Non-target lepidopteran larvae may be affected adversely by consuming this pollen attached to their host plants (e.g., Dolezel et al. 2005, Lang and Vojtech 2006, Lang and Otto 2010). The combination of transgenic, herbicide-tolerant crops together with the application of broad-spectrum herbicides, such as glyphosate or glufosinate-ammonium, is likely to change the herbicide regime, which can reduce the weed community within fields and in field margins, in turn affecting larval and adult butterflies associated with such food plants (e.g., Haughton et al. 2003, Roy et al. 2003). Direct toxic effects of the complementary broad-spectrum herbicides on non-target Lepidoptera have received less attention, but have been reported for glufosinate (El-Ghar 1994, Kutlesa and Caveney 2001). Potentially, cultivation of the above transgenic events put at risk non-target butterflies and moths occurring in agro-ecosystems as well as protected species living in habitats near the GMO fields (Traxler et al. 2005, Hofmann and Schlechtriemen 2009).

II. Objectives

The objectives of T.7.1.1 “Evaluation of existing butterfly monitoring schemes in Europe for their feasibility to be used for GMO monitoring purposes” are:
- Collection and compilation of volunteer butterfly monitoring existing in Europe;
- First assessment of the suitability of butterfly volunteer schemes for GMO monitoring.

Below, the major technical details are summarised for the monitoring of day-active Lepidoptera, describing the existing methodologies and some of their limits (see chapter “Methods to monitor butterflies”). This is followed by the presentation of the current volunteer butterfly monitoring schemes implemented across Europe (see chapter “Volunteer butterfly monitoring schemes in Europe”).

III. Methods to monitor butterflies

There exist various methods to count and monitor butterflies, for example standardised line-transect counts, point counts, distance-sampling, or mark-release-recapture methods (e.g., Hermann 1992, Pollard and Yates 1993, Mühlhofer 1999, Sutherland 2006, Nowicki et al. 2008, vanSwaay et al. 2012). Lang et al. (2012) have published a concise summary of the methodologies with regard to the environmental monitoring of

the effects of transgenic plants, including the monitoring of night-active Lepidoptera and lepidopteran larvae. The below paragraphs refer to the description of the methodologies as given by Lang et al. (2012).

The most commonly approach is the transect count method, often called standard Pollard walks (Pollard and Yates 1993, VanSwaay et al. 2012). Here, a fixed route is placed in the landscape, walked under standardised conditions, and all observed specimens are recorded within a defined observation area. The various advantages of the method include the fact that line-transect counts are highly adaptable, cheap and quite efficient in terms of recorded quantity and quality of data, and easily employable by non-professional volunteers. Also, transect counts can be used to count individuals, species or groups of species, and various indices can be derived from the recorded data (Pollard and Yates 1993, Sutherland 2006, Nowicki et al. 2008). Often, the transects are divided in 50 m sections and butterflies are recorded separately for these sections (VanSwaay et al. 2012). This allows for assigning species occurrence and possible adverse effects locally, which is especially important for GMO monitoring where recordings should be referred to the sites of transgenic crop fields (Lang et al. 2012). Transect counts of butterflies is also the most applied monitoring approach in the European volunteer butterfly monitoring schemes (VanSwaay et al. 2008). However, transect counts provide indices of relative abundance rather than precise estimates of population densities, though transect count data are often correlated with population sizes (Haddad et al. 2008, Nowicki et al. 2008). For example, detection probability of the species, region, habitat types, year and season as well as bias through observers can affect the data (e.g., Kery and Plattner 2007). A common approach to minimise this variation is through standardisation of the field monitoring approach and increased sampling effort. Ideally, variables causing much variation are recorded additionally and introduced as co-variables in the analysis of the monitoring results, e.g. habitat types adjacent to the transects or estimates of the density of nectar plants (VDI 2010). Further, it may sometimes be helpful to have information on species detectability in order to correct monitoring results, and not to erroneously confound changes and differences in detectability with abundance trends, at least for rare species as routine transect counts might not always be the appropriate method to track changes in the abundance of rare species (Kery and Plattner 2007). For example, distance sampling can be used to calculate detection probabilities (Isaac et al. 2011). Distance sampling is a common line-transect count, where the distances of the observed butterflies to the observer are noted. However, Isaac et al. (2011) concluded that the variation in detection probability among species is small compared with the variation in true abundance, and that the method would be too laborious for routine schemes by volunteer recorders. Point counts are another monitoring method (VanSwaay et al. 2012). For this, points are marked, the observer stays stationary on these points, and counts all observed butterflies within a defined time slot and area. This approach is often applied when longer transect lines cannot be established and walked, e.g. in very small plots or in some wetlands (VanSwaay et al. 2012). Another method frequently applied in studies of butterfly populations are mark-release-recaptures (MRR) methods (e.g., Haddad et al.

2008). However, MRR methods are time-consuming and costly, which makes them quite unsuitable for large-scale routine monitoring programmes. Potentially, MRR could be applied in special instances, e.g. for the recording of rare and protected species, and simplified MRR methods have been described reducing the required effort in sampling time (Nowicki et al. 2005).

Due to the above described advantages, the common transect count method is considered the best approach with regard to invested effort and recorded data, and is also suggested for the butterfly monitoring in the AMIGA project. The transect length itself and the number of visits to the transects per season mostly determine the sampling costs of line transect counts. Longer transects and a higher number of visits records and detects more species (Pollard and Yates 1993, Sutherland 2006, Pellet 2008), thus is sampling more information, which can be crucial in order to detect an adverse GMO effect on specific species. However, reduced number of visits can still produce reliable data, although this has to be balanced by a higher number of transects (see Roy et al. 2007, Brereton et al. 2011). In the Swiss biodiversity monitoring scheme, 4 – 5 visits per season still recorded about 90% of the present species on 2.5 km long transects situated in agricultural land (Lang, Bühler and Dolek, unpublished results), as long as the samplings included sufficient counts during summer time. However, species numbers in agricultural settings may be poor, and especially on shorter transect (e.g. 250 m long) more visits are recommended (Lang and Bühler 2012). In particular, the variance of the recorded data determines the required sampling effort, i.e. the more variable the data are the more transects must be sampled to detect a given effect (Nakagawa and Foster 2004). In a case example in Switzerland, the coefficient of variance for the average species number on transects was not different when visiting the transects 7, 5, 4 or 3 times (Lang, Bühler and Dolek, unpublished results). This means that from a pure statistical point of view no power is lost for the detection of a difference in species richness with lower numbers of visits; however, it has to be noted that less species are recorded when visits to the transects are reduced.

Considering the variable 'species number', the variance of the recorded data drops significantly on transects longer than 1.5 km (Lang, Bühler and Dolek, unpublished results). This means that shorter transects not only record less species, but the recorded data are more variable, thus a higher number of sampled transects is necessary to detect a given effect (Lang and Bühler 2012). On the other hand, it requires less time to walk shorter transects, and there will be an optimal economic combination between transect length and required sampling time. It appears that transect lengths between 1.5 km and 2 km provide the highest efficiency in determining butterfly species number, i.e. the best ratio of invested effort and resulting data (Lang, Bühler and Dolek, unpublished results).

Often, day-active moths are also recorded within the framework of butterfly monitoring schemes. For example, the VDI guidelines for the monitoring of GMO effects (VDI 2010), recommend the recording of Burnet Moths (Lepidoptera: Zygaenidae) and Crambid Snout Moths (Lepidoptera: Pyralidae, Crambinae) in addition to butterflies. Burnet moths are already recorded in many other monitoring programmes, while

recording Snout Moths is a new approach. The Crambinae are a prominent subfamily of the Pyralidae of about 80 species occurring in Central Europe with a few abundant species that can regularly be expected in grassy field margins in agricultural land (Küppers 2008, Slamka 2010). Crambid Snout Moths are a promising addition to transect counts of butterflies, because they are wide-spread, common and abundant in agricultural land, easy to catch and the species can be identified well (Slamka 2010). Especially in intensively managed agricultural habitats, the Crambinae might be more prevalent than other Lepidoptera species, thus supporting the monitoring results in butterfly-species poor areas (Lang et al. 2011).

IV. Volunteer butterfly monitoring schemes in Europe (T7.1.1)

The EC (2002) suggests using existing routine environmental observation programmes, where compatible, for general surveillance of transgenic crops. Therefore, a list was compiled of all the butterfly monitoring schemes in existence in Europe, including the relevant characteristics and features of these schemes.

In a first step, the current butterfly monitoring schemes in Europe were identified by internet research, enquiries with colleagues, and available publications (e.g. Van Swaay et al. 2008). The list was completed through the personal support by Chris van Swaay from the Dutch Butterfly Conservation and Butterfly Conservation Europe (BCE). On behalf of BCE, Chris van Swaay sent a questionnaire to all European butterfly schemes asking for the characteristics of these schemes. The questionnaire was complemented by questions particularly relevant for the AMIGA project and GMO monitoring.

In 15 countries of the European Community a butterfly monitoring scheme is running at the moment (Table 1), and in one country (Romania) one is planned to start in 2013, however, the scopes of the various programmes differ considerably (see Table 2). Outside the European Community, further schemes have been established or started in Norway, Jersey, Switzerland, Russia and the Ukraine (Tables 1 and 2).

Table 1. Current butterfly monitoring schemes in Europe (December 2012).

Country	Name	Internet
Andorra	Programa de seguiment de ropalòcers d'Andorra (Butterfly Monitoring Scheme d'Andorra, BMSAnd)	http://www.iea.ad/index.php?option=com_content&view=article&id=186&Itemid=70
Belgium - Flanders	Vlaamse Vlinderwerkgroep (Flandern Butterfly Working Group)	http://www.inbo.be/content/page.asp?pid=MON_VL_start
Estonia	National Butterfly Monitoring Estonia	?
Finland	Monitoring butterflies in Finnish agricultural landscapes	http://www.environment.fi/butterflymonitoring
France	Suivi Temporel des Rhopalocères de France (STERF)	http://vigienature.mnhn.fr/page/suivi-temporel-des-rhopaloc-res-de-france
Germany	Tagfaltermonitoring Deutschland	http://www.tagfalter-monitoring.de/
Ireland	Irish Butterfly Monitoring Scheme	http://butterflies.biodiversityireland.ie/
Jersey	Jersey Butterfly Monitoring Scheme	http://www.gov.je/ENVIRONMENT/LANDMARINEWILDLIFE/INSECTS/Pages/Butterflies.aspx
Lithuania	Butterfly Monitoring Lithuania	http://www.entomologai.lt/
Luxemburg	Butterly Monitoring Scheme Luxembourg	http://data.mnhn.lu/node/543
Norway	Butterfly and Bumblebee Monitoring in Norway	http://www.nina.no
Portugal	Portuguese Butterfly Monitoring Scheme	http://www.tagis.org/
Romania	to be started in 2013	https://sites.google.com/site/monitorizareafluturilor
Slovenia	Butterfly Monitoring Slovenia	http://www.metulji.biologija.org/
Spain - Catalonia	Pla de Seguiment de Ropalòcers de Catalunya (Catalan Butterfly Monitoring Scheme)	http://www.catalanbms.org
Slovakia	Slovakian Butterly Monitoring	http://www.lepidoptera.sk

Sweden	Svensk Dagfjärilsövervakning (Swedish Butterfly Monitoring Scheme)	http://www.dagfjarilar.lu.se/
Switzerland	BDM (Biodiversitätsmonitoring)	www.biodiversitymonitoring.ch
Switzerland - Aargau	LANAG (Langfristbeobachtung der Artenvielfalt in der Normallandschaft des Kanton Aargau)	www.ag.ch/alg/de/pub/naturlandschaft/erfolgskontrolle/lanag.php
The Netherlands	De Vlinderstichting (Dutch Butterfly Conservation)	http://www.vlinderstichting.nl
Ukraine – Carpathians and adjacent parts	Butterfly Monitoring in the West-Ukraine	http://www.alexanor.uzhgorod.ua/
United Kingdom	UK Butterfly Monitoring Scheme	http://www.ukbms.org
United Kingdom	Wider Countryside Butterfly Survey	http://www.ukbms.org/wcbs.htm

All butterfly monitoring schemes use the common line transect counts for recording the specimens. In Sweden, additional point count sites have been implemented (Pettersson et al. 2011). Most of the programmes have been started just recently during the last decade (Table 2). Exceptions are for example the schemes of the Netherlands (already started in 1990), Belgium (1991), of Catalonia in Spain (1994), of Aargau in Switzerland (1998), Finland (1999), the oldest one running in the United Kingdom since 1973 – 1976.The average transect length is often fairly long (≥ 1 km), and the number of counts per season is also relatively high, i.e. more than 5 visits per season to each transect. Exceptions are found in Sweden (4 counts), Norway (3 counts) and Slovakia (2 counts). Although most of the sites are freely chosen by the recording persons themselves, this does not seem to result in an overrepresentation of protected areas being sampled (which are more attractive to butterfly lovers). In those cases, where the organisers reported back on the proportion of transects situated in arable land, often a considerable part of the sites were located in agriculturally managed areas (Table 2). However, it has to be noted that the overall number of transects walked per year varied greatly among the different schemes, and is sometimes extremely low like in Belgium, Estonia, Lithuania, Norway, Slovenia or Slovakia. In many butterflies schemes the habitat types adjacent to the transects are characterised, which will help in the analysis of recorded effects on butterfly abundance.

Lang and Bühler (2012) concluded that a potential adverse effect of GMO cultivation on species richness and overall abundance of butterflies could be detected with about 60 – 70 transects located in arable land. This conclusion refers to the detection of the loss of 10% of the species and a decline of 30% in total numbers. Only few volunteer butterfly schemes appear to be appropriate for GMO monitoring in the current state under the premise that about 60 – 70 transects are walked in arable land, that transects are at least 1 – 1.5 km long and are visited at least four times a season (see chapter “Methods to monitor butterflies” for criteria of transect length and frequency of visits). Finland, France, Ireland and Switzerland would meet the above criteria, possibly also the United Kingdom (but number of transects in arable land is not known for the UK). All other butterfly schemes seem to have too few transects, or too few transects in agriculturally managed land, or too short transects, or too few counts per seasons (Table 2). These deficient programmes would need to be upgraded before becoming suitable for a possible GMO monitoring.

Within the AMIGA project, butterfly counts are planned in Spain, Slovakia, Romania, and possibly in Sweden. The region Catalonia in Spain appears quite suitable for AMIGA purposes as the butterfly scheme includes about 70 transects of which 66% lie in arable land, the transects having an appropriate length and being visited quite frequently. Likewise, the Swedish scheme covers many transects, also in arable land, however the transect lengths are relatively short. In Slovakia the butterfly programme is relatively restricted, with only 10 short transects, which are only counted twice a year. In Romania, no butterfly monitoring scheme is in existence at the moment. However, a butterfly monitoring is planned to start 2013 in Romania, for which the recruitment of

personnel has just begun (J. Loos, personal communication). This means that for AMIGA additional transects have to be implemented and walked.

In addition, all national authorities competent for GMO monitoring of the 28 member states of the European Community were contacted and asked, if they plan to consider volunteer programmes such as butterfly schemes for general surveillance of GMO cultivation. Of the 28 member states plus Switzerland nineteen countries responded, and the following refers to these responding countries. Except for Spain, no country was currently planning to establish a GMO monitoring at all, because field cultivation of transgenic crops does not exist and is not expected in the near future in Europe. So far, most of the countries (89%) have not planned to include volunteer butterfly schemes in a potential GMO monitoring programme. The two exceptions are The Netherlands and the United Kingdom. In the Netherlands, first considerations and concepts have been developed for building up a general surveillance programme on existing volunteer monitoring systems (Glandorf 2012). In the UK, a current research project is evaluating the suitability of volunteer monitoring schemes to be used for general surveillance (DEFRA 2012). The final DEFRA report is to be published in 2013. In Germany, Monsanto used published data for surveying the environmental effects of cultivation of Bt maize MON810 (Monsanto 2009). However, it turned out that the German volunteer butterfly monitoring scheme was not suitable in its current state for monitoring possible effects of transgenic crops, partly because too few transects were running in arable land (UFZ 2009). Other countries would not rule out the possibility to use environmental volunteer programmes, but have just started and not completed their evaluation of the respective schemes (e.g., Spain, Belgium, Czech Republic, Sweden, Italy). Two countries, France and Switzerland, do not intend to use volunteer schemes, but want to implemented and use professional monitoring programmes.

Table 2. Characteristics of the butterfly monitoring schemes in Europe (December 2012).

Country	Starting year	Area represented	Area represented (km2)	Average transect length (km)	Number of transects per year 2009-2011 (average or range)	Number of counts on a transect per year (average or range)	Counts by (v=volunteers, p=professionals)	Method to choose sites	Nature reserves overrepresented	Habitat characteristics known?	If yes, to which level	Percentage of transects in agriculturally managed land
Andorra	2004	whole country	468	1,5	6	20-30	v	free	no	yes	section level, with cover (in %) of main plant communities	30%
Belgium - Flanders	1991	region	13500	0,8	10	15-20	v	free	no	no, but could be done fairly easily if needed		0
Estonia	2004	whole country	45100	1,8	11	7	p	by co-ordinator	no	no information	no information	no information
Finland	1999	whole country	338000	3	65-67	ca 11	v ~70%, p ~30%	free for volunteers, but co-ordinated for professionals	no	yes	margin habitats between or surrounding arable fields are predominant on most sites (ca.30-40%); semi-natural grasslands are mostly abandoned and of rather poor quality (ca.20-30%), completely lacking on many sites; few sections actually within closed woodlands but instead ca.20-30% are located on forest edges or forest road margins; urban areas on very few transects (<5%); heathlands, coastal dunes, wetlands not included.	90%
France	2005	whole country	551000	1	611-723	4,4 (1-15)	v	half random, half free	no	Yes	Grasslands/arable lands/orchards; with or without trees/hedgerows; fallows/pastures/crops	48%
France - Doubs	2001-2004	region	5000	1	0	10-15	p	by co-ordinator	no	no information	no information	no information
Germany	2005	whole country	357000	0,5	400	15-20	v	free	yes	yes	EUNIS - I., II. or III. level	no information
Germany – Pfalz (Phengaris nausithous	1989	region	3000	0,5	50-87	1	p	by co-ordinator	no	no information	no information	no information
Ireland	2007	whole country	70000	1,5	190	16.3	v	free	no	yes	basic habitat level	most
Jersey	2004	whole country	116	1	0	15-25	v	free	no	no information	no information	no information
Lithuania	2009	whole country	65000	1,3	14	6-9	v	free	no	no information	no information	no information
Luxemburg	2010	whole country	2600	0,34	30	8.2 (3-11)	v ~10%, p ~90%	random	no	yes	crops, bushes, broadleaved forest, heathland, dry grassland, agricultural grassland, urban areas, wetland	54% (Crops (18%), agricultural grassland (35%), dry grassland (0.5%))
Norway	2009	region		1	9-18	3	v ~42%, p ~58%	grid	no	yes	no information	no information
Portugal	1998-2006	whole country	92000	1	0	3-5	v	free	no	no information	no information	no information
Romania	starting up	not clear yet										
Russia - Bryansk area	2009	region	30000	1,2	2-14	3-5	v ~90%, p ~10%	free	no	yes	agriculture - semi-natural grassland - woodland - water meadow- urban	30%
Slovenia	2007	whole country	20000	1,3	9-14	6.25 - 7.53	v	by co-ordinator	no	yes	CORINE - II. or III. level	74%
Slovakia	2008	whole country	49000	0,5	10	2	v ~90%, p ~10%	free	yes	mostly no	basic habitat level	~10%
Spain - Catalonia	1994	region	32000	1	60-70	30	v	free	no	yes	section level, with cover (in %) of main plant communities	66%
Sweden	2010	whole country	450000	0,65	90	4	v	free	no	yes	agriculture - semi-natural grassland - woodland - heathland - urban - wetlands - mountains	~25%
Switzerland	2003	whole country	41000	2 x 2,5 (away and back)	90-95	7 (4 alpine region)	p	grid	no	no	CORINE	~12%
Switzerland - Aargau	1998	region	1400	2 x 0,25 (away and back)	101-107	10	people from civil service	grid	no	yes	grassland-agricultural land-forests-settlements	~42%
The Netherlands	1990	whole country	41500	0,7	430	17 (15-20)	v	free	no	yes	agriculture - semi-natural grassland - woodland - heathland - coastal dunes - urban - wetlands	12%
Ukraine – Carpathians and adjacent parts	1990	region	70000	1	158	5 (2-10)	p	free	yes	yes	CORINE - II. or III. level	no information
United Kingdom	1973 (1976)	whole country	243000	2,7	819-977	19	v	free	yes	yes	EUNIS - I., II. or III. level	no information

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