Marion Dolezel, Marianne Miklau, Michael Eckerstorfer, Angelika Hilbeck, Andreas Heissenberger and Helmut Gaugitsch

Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU

Federal Agency for Nature Conservation

BfN – Skripten 259

2009

umweltbundesamt

Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU

Final Report for the Federal Agency for Nature Conservation (BfN) Germany

Wien, April 2009

Marion Dolezel

Marianne Miklau

Michael Eckerstorfer

Angelika Hilbeck

Andreas Heissenberger

Helmut Gaugitsch

BfN Federal Agency for Nature Conservation

Cover picture: M. Otto

Authors' addresses:

Dr. Marion. Dolezel
Marianne Miklau
Dr. Michael Eckersdorfer
Dr. Andreas Heissenberger
Dr. Helmut Gaugitsch

Main contractor:

Umweltbundesamt GmbH
Spittelauer Lände 5
1090 Wien
Austria
phone +43-(0)1-313 04)
fax +43-(0)1-313 04/5400

Subcontractor:

Dr. Angelika Hilbeck

Office Berlin
Friedensallee 21
15834 Rangsdorf
Germany
phone +49-(0)33 708939960
fax +49-(0)30 708939961

Office Zürich
Hottingerstraße 32
8032 Zürich
Switzerland
phone +41-(0)44-430 3060
fax +41-(0)44-430 3061

Scientific supervision at BfN:

Dr. Mathias Otto, Division „Biosafety – GMO Regulation"

This project (FKZ 806 64 007) was completed in April 2009

This publication is included in the literature database "***DNL-online***" (www.dnl-online.de)

BfN-Skripten are not available in book trade but can be downloaded in a pdf version from the internet at: http://www.bfn.de/0502_skripten.html

Publisher: Bundesamt für Naturschutz (BfN)
Federal Agency for Nature Conservation
Konstantinstrasse 110
53179 Bonn, Germany
URL: http://www.bfn.de

Printed by the printing office of the Federal Ministry of Environment, Nature Conservation and Nuclear Safety.

Printed on 100% recycled paper.

Bonn, Germany 2009

I CONTENTS

II ZUSAMMENFASSUNG

Hintergrund und Ziel der Studie

Viele Jahre nach der erstmaligen Kommerzialisierung von gentechnisch veränderten Organismen (GVO) und GVO-Produkten sowie nach der Umsetzung rechtlicher Vorschriften zur harmonisierten Risikoabschätzung, Kennzeichnung und Rückverfolgbarkeit von GVO auf EU-Ebene sind Kontroversen um potenzielle Umweltrisken von GVO noch immer Gegenstand von Diskussionen zwischen EU Mitgliedstaaten, Risikobewertern, Antragstellern und Wissenschaftern. Die Ursachen dieser Kontroversen liegen vor allem in den unterschiedlichen Auffassungen bezüglich Umweltrisiken. Nach Ansicht mancher werden diese durch ein striktes, regulatorisches System ausreichend geprüft, während andere der Meinung sind, dass diese Risiken in der derzeitigen Risikoabschätzungspraxis von GVO-Antragstellern nicht oder nicht ausreichend berücksichtigt werden.

Vor diesem Hintergrund wurde in diesem Bericht die derzeitige Praxis der Umweltrisikoabschätzung von GVO-Anträgen, die sich derzeit im EU-Zulassungsverfahren befinden, analysiert. GVO-Anträge gemäß Richtlinie 2001/18/EG oder Verordnung (EG) 1829/2003 wurden ausgewählt, wobei der Schwerpunkt auf jene Feldfrüchte gelegt wurde, die wirtschaftliche Relevanz für die Mitgliedstaaten der Europäischen Union besitzen (Mais, Raps und Kartoffel). Zudem wurde Augenmerk auf relevante gentechnisch veränderte Merkmale gelegt (Insektenresistenz, Herbizidtoleranz, Veränderung der Stärkezusammensetzung sowie „stacked event“ GVO). Alle ausgewählten Anträge sehen den Anbau der jeweiligen GVO in der EU vor. Für die Analyse der vorgelegten Umweltrisikoabschätzungen wurden allgemeine wissenschaftlichen Standards und die relevanten rechtlichen Anforderungen (RL 2001/18/EG mit den dazugehörigen Leitlinien) berücksichtigt.

Dieser Bericht stellt daher eine kritische Bewertung der Umweltrisikoabschätzung ausgewählter GVO-Anträge dar und identifiziert wesentliche Schwächen der derzeitigen Umweltrisikoabschätzungspraxis von GVOs. Die Analyse und Bewertung wird ergänzt durch Vorschläge zur Verbesserung der Methodik der Umweltrisikoabschätzung sowie durch Empfehlungen für weitere notwendige Richtlinien bzw. Standardisierung von Vorgaben. Die in dem Bericht formulierten Vorschläge und Empfehlungen richten sich gleichermaßen an Antragsteller, Risikobewerter und Entscheidungsträger.

Wesentliche Ergebnisse

Im Allgemeinen wird von GVO-Antragstellern ein sehr enger Ansatz der Umweltrisikoabschätzung verfolgt, der sich stark auf das Transgenprodukt konzentriert und dem GVO als Ganzes wenig Aufmerksamkeit schenkt. Die Antragsteller testen vor allem Transgene mit Pestizideigenschaften (v. a. *Bt* Proteine), während andere Transgene bzw. Proteine, die in herbizidtoleranten Pflanzen exprimiert werden (EPSPS oder PAT Proteine), einer weniger strengen Prüfung unterliegen. Dies ist darin begründet, dass für letztere angenommen wird, dass sie generell keine Wirkungen auf Ziel- oder Nichtzielorganismen besitzen. Ausgehend von der Annahme der Antragsteller, dass die neu eingefügten gentechnisch veränderten Eigenschaften die biologischen Charakteristika des GVO, abgesehen von den beabsichtigten, nicht verändern, wird die ganze gentechnisch veränderte Pflanze als sicher betrachtet. Diese Annahme der Umweltsicherheit der ganzen Pflanze wird häufig nicht oder nicht ausreichend mit relevanten und nachvollziehbaren wissenschaftlichen Da-

ten belegt. Zudem bleiben in der Risikoabschätzung auch indirekte Effekte des GVO, wie z.B. Biodiversitätseffekte durch das Komplementärherbizid, unberücksichtigt.

Dieser enge Ansatz in der Umweltrisikoabschätzung, der auf das Transgenprodukt fokussiert, zieht weitere wesentliche Mängel in der Bewertung von Umweltrisken nach sich. So werden umweltrelevante Pflanzeninhaltsstoffe oder Metabolite des neu eingebrachten Proteins oder Herbizidmetabolite der Komplementärherbizide nicht berücksichtigt. Auch Interaktionen mehrerer gentechnisch veränderter Merkmale in einer Pflanze (z. B. im Falle von „stacked event" GVO) oder Interaktionen von Pflanzeninhaltsstoffen und gentechnisch veränderten Merkmalen finden wenig oder keine Berücksichtigung.

Die Praxis der Umweltrisikoabschätzung zeigt weiterhin, dass das Fall-zu-Fall Prinzip in den analysierten GVO-Anträgen nicht umgesetzt wird. Da der Fokus der Umweltrisikoabschätzung auf den isolierten Transgenprodukten und nicht auf der ganzen Pflanze liegt, verweisen die Antragsteller häufig auf die Sicherheit anderer GVO mit gleichen oder ähnlichen gentechnisch veränderten Merkmalen (z. B. GVO, die das gleiche *Bt* Protein exprimieren), anstatt den relevanten GVO spezifisch zu testen, wenn Schlussfolgerungen bezüglich der Umweltsicherheit des GVO getroffen werden.

Der Fokus auf das Transgenprodukt in der Umweltrisikoabschätzung entspricht zudem nicht der ökologischen Realität des GVO in seiner Umwelt und ignoriert ökologische Wechselwirkungen, weil Einflussfaktoren der genetischen Veränderung und des Transgens (z. B. über Positionseffekte, epigenetische und pleiotrope Effekte), der ganzen Pflanze (z. B. über Sekundärmetaboliten) und der aufnehmenden Umwelt unberücksichtigt bleiben. Das ökologische Wissen über den GVO wird nicht mittels stufenweiser Freisetzung des GVO in die Umwelt (Labor – Glashaus – Feld) generiert. Die für die Bewertung vorgelegten Daten stammen i.d.R. aus Laborversuchen, die wiederum nur das Transgenprodukt und nicht den ganzen GVO zum Gegenstand der Prüfung haben. Die fehlende Integration von ökologisch relevanten Daten aus unterschiedlichen Freisetzungsebenen in die Risikoabschätzung von GVO-Anträgen misachtet damit das Stufenprinzips der Richtlinie 2001/18/EG.

Die Daten aus Feldversuchen, die für die phänotypische Charakterisierung des GVO, die Evaluierung seines agronomischen Verhaltens oder potenzieller Umwelteffekte vorgelegt werden, stammen häufig nicht aus der Europäischen Union oder decken relevante Umwelten innerhalb der EU nicht repräsentativ ab. Diese Daten sind daher oft nicht ausreichend, um Schlussfolgerungen bezüglich der Sicherheit des GVO in seiner aufnehmenden Umwelt zu treffen. Somit wird dem „Region-für-Region" Prinzip der Richtlinie 2001/18/EG in den analysierten GVO-Anträgen nicht ausreichend Rechnung getragen. Auch geschützte Organismen bzw. Arten oder Lebensräume, für die Erhaltungsmaßnahmen relevant sind, unterliegen keiner separaten Berücksichtigung in den analysierten GVO-Anträgen.

Die Analyse der GVO-Anträge in diesem Bericht zeigt weiters, dass die Schlussfolgerungen der Antragsteller über spezifische Umweltrisken von GVO oft auf unzureichenden Datengrundlagen basieren. Wesentliche Schwächen der vorgelegten experimentellen Untersuchungen beziehen sich sowohl auf Datenerhebung und -evaluierung als auch auf die Präsentation von Daten und Ergebnissen sowie auf die Nachvollziehbarkeit der Schlussfolgerungen der Antragsteller auf Basis

dieser Untersuchungen. Die wissenschaftlichen Methoden und experimentellen Ansätze sind häufig unzureichend in Bezug auf ihre Relevanz für die jeweilige Fragestellung und ihre allgemeine wissenschaftliche Qualität. Zudem ist eine beträchtliche Variation in den angewandten Methoden, den evaluierten Parametern und den gewählten Testorganismen für gleiche Fragestellungen zwischen den GVO-Anträgen festzustellen. Es werden daher insgesamt keine konsistenten Ansätze für die Evaluierung bestimmter Umweltrisken verfolgt. Dies vermittelt den Eindruck, dass es kein gemeinsames Verständnis zwischen den Antragstellern, aber auch zwischen Antragstellern, Risikobewertern und Entscheidungsträgern gibt, wie die Evaluierung spezifischer Umweltrisken durchzuführen ist.

In anderen Fällen erfolgen die Schlussfolgerungen bezüglich bestimmter Umweltrisken eines spezifischen GVO größtenteils annahmenbasiert oder ausschließlich mittels Querverweis zu Daten von Evaluierungen anderer Umweltrisken oder anderer GVO. Robuste wissenschaftliche Daten für den spezifischen GVO fehlen. Schwächen sind auch in der Zusammenstellung, Zitierung und Präsentation der Daten und Resultate zu beobachten, was dazu führt, dass die Risikoschlussfolgerungen der Antragsteller häufig nicht schlüssig und schwer nachvollziehbar sind. Häufig fehlen Informationen bezüglich der Quelle der Daten oder Studien (z. B. Autor, Institution), dem Status von Berichten (z. B. publiziert, vorläufige Ergebnisse etc.) und ihrer Relevanz für den spezifischen GVO (Angabe des verwendeten GVO in der Studie). Schlussfolgernd kann gesagt werden, dass die grundlegende Anforderung der Richtlinie 2001/18/EG, die Umweltrisikoabschätzung von GVO auf wissenschaftlichen und technischen Daten zu basieren, in vielen Bereichen der Umweltrisikoabschätzung nur unzureichend erfüllt wird.

Unabhängig von Qualität oder Quantität der vorgelegten Datenbasis und der daraus resultierenden Ergebnisse wurden die Umweltrisken eines spezifischen GVO von den Antragstellern generell als vernachlässigbar eingeschätzt. Die derzeitige Praxis der Umweltrisikoabschätzung von GVO basiert auf der Annahme, dass selbst kleine Effekte – im Vergleich zur großen Variabilität in der Umwelt – irrelevant sind und daher die Schlussfolgerung erlauben, dass der GVO sicher ist. Dazu werden häufig doppelte Standards in den Beurteilungsnormen möglicher Umweltrisiken von den Antragstellern angewandt. Als Beispiel sind die doppelten Standards bei der Auswahl von Kontrollbehandlungen, die für die Evaluierung der Wirksamkeit des GVO gegenüber Zielorganismen und der Evaluierung von möglichen Effekten des GVO auf Nichtzielorganismen verwendet werden, zu nennen.

Unsicherheiten in dem der Risikoabschätzung zugrunde liegenden Modell, in Methoden, Resultaten oder Interpretationen von Ergebnissen werden prinzipiell nicht berücksichtigt. Folglich werden auch Unsicherheiten aus der Risikoabschätzung nicht in die Entscheidung über ein fallspezifisches Monitoring miteinbezogen. Das Fehlen eines fundierten, wissenschaftlichen Ansatzes der Umweltrisikoabschätzung inklusive einer Unsicherheitsanalyse widerspricht nicht nur dem allgemeinen Konzept der Risikoabschätzung und somit der Intention der Richtlinie 2001/18/EG, sondern ignoriert auch, dass kleine, aber signifikante, Unterschiede oder regionale Unterschiede zwischen dem GVO und der Kontrollpflanze wesentliche Konsequenzen für die Umwelt besitzen können.

In keinem Fall wurden diese oder andere Schwächen in der Praxis der Umweltrisikoabschätzung der GVO Anträge durch das EFSA GVO Gremium in seinen Bewertungen aufgezeigt oder beach-

tet, obwohl selbst die Anforderungen, die durch die Leitlinien der EFSA vorgegeben sind, in vielen der analysierten GVO Anträge nicht oder nur unzureichend erfüllt werden.

Solange die Schlussfolgerungen der Umweltrisikoabschätzung auf Basis einer unzureichenden und schwer nachvollziehbaren Datenbasis getroffen werden, werden zukünftige Umweltprobleme nicht rechtzeitig erkannt werden und wird auch das öffentliche Vertrauen in die neue Technologie nicht hergestellt werden. Eine neuer Blick auf die Umweltrisikoabschätzung, wie sie von den GVO-Antragstellern vorgelegt wird, ist daher erforderlich und weitere Leitlinien und Standards zur Einhaltung einer wissenschafts- und vorsorgebasierten Umweltrisikoabschätzung sind notwendig.

Verbesserungsvorschläge und Empfehlungen für weitere Richtlinien und Standardisierung

Generell sollte ein breiterer Ansatz der Umweltrisikoabschätzung für GVO gewählt werden, der den Fokus der Prüfung von Umweltrisiken auf die ganze gentechnisch veränderte Pflanze und die damit verbundenen Managementtechniken für ihre kommerzielle Anwendung (z. B. die Herbizidanwendung) legt. Dies impliziert, dass in den Umweltrisikoabschätzungen in Zukunft der aufnehmenden Umwelt mehr Aufmerksamkeit geschenkt werden muss. Mit diesem Ansatz werden die rechtlichen Vorgaben und Prinzipien, wie z. B. das „Fall-zu-Fall“ Prinzip und das „Region-für-Region“ Prinzip der Richtlinie 2001/18/EG, entsprechend erfüllt. Zudem müssen in Zukunft die Daten der stufenweisen Freisetzung des GVO in die Umwelt (Stufenprinzip) in die Umweltrisikoabschätzung einbezogen werden. Dies bedarf weiterer Leitlinien und Vorgaben für die Umsetzung.

Die Verbesserungsvorschläge beziehen sich unter anderem auf die Einhaltung allgemeiner wissenschaftlicher Standards und der Vorlage von relevanten Daten aus der aufnehmenden Umwelt des GVO. Weitere Vorschläge beinhalten verfügbare Methoden zur Abgrenzung der Umweltrisikoabschätzung, die Auswahl von Testorganismen, die Formulierung testbarer Hypothesen nachteiliger Umwelteffekte sowie die Bestimmung von Effekten. In Kombination führen diese Vorschläge zu fundierten und wissenschaftsbasierten Schlussfolgerungen für spezifische Umweltrisiken, wobei der jeweilige GVO, die gentechnisch veränderten Merkmale und die aufnehmende Umwelt Berücksichtigung finden. Mit den vorgeschlagenen Konzepten können wesentliche Verbesserungen für alle Evaluierungskategorien der Umweltrisikoabschätzung, die in diesem Bericht analysiert wurden, erreicht werden. In diesem Zusammenhang muss auch geklärt werden, welche Daten vom Antragsteller spezifisch für einen GVO vorgelegt werden sollen und welchen Stellenwert zitierte Daten und Ergebnisse aus der publizierten wissenschaftlichen Literatur besitzen.

Zusätzlich sind strukturelle und formale Verbesserungen in der Zusammenstellung und Präsentation der Daten und Ergebnisse vonnöten, um die Nachvollziehbarkeit der daraus gewonnenen Schlussfolgerungen zu erhöhen.

Zudem sind Leitlinien für die Auswahl repräsentativer Standorte für die Evaluierung des agronomischen und des Umweltverhaltens eines spezifischen GVO notwendig. Diese Leitlinien sollten unter anderem Details zum Feldversuchsdesign, der Wahl von Vergleichslinien, den zu evaluierenden Parametern und der Dateninterpretation beinhalten. Ähnliche Leitlinien sind bereits in anderen legislativen Bereichen vorhanden, wie beispielsweise für Pflanzenschutzmittel. Diese könnten Ausgangspunkt für die Entwicklung vergleichbarer Leitlinien sein, die spezifisch auf die Anforderungen von GVO zugeschnitten werden müssen.

Leitlinien werden auch für jene Bereiche der Umweltrisikoabschätzung notwendig sein, die bisher in den GVO-Anträgen unberücksichtigt blieben, wie beispielsweise für die Abschätzung von Langzeit- und kumulativen Effekten von GVO, aber auch für die Berücksichtigung von Unsicherheiten in der Umweltrisikoabschätzung und für das Vorsorgeprinzip.

Einige Empfehlungen in diesem Bericht fordern einen wesentlich standardisierten Ansatz in der Umweltrisikoabschätzung von GVO, um eine aussagekräftige und nachvollziehbare Datenbasis für die Entscheidungsfindung zu garantieren. Andere Empfehlungen hingegen fokussieren auf die Entwicklung von Entscheidungskriterien, die – ausgehend von einer Fall-zu-Fall Bewertung – zu den relevanten Objekten, Organismen und Methoden führen und folgende Aspekte umfassen: die ganze gentechnisch veränderte Pflanze, das veränderte oder neu eingebrachte Merkmal und die aufnehmende Umwelt. Daher wird ein balancierter Ansatz vorgeschlagen, der die Fall-zu-Fall Bewertung nicht durch Standardisierung und die Vorgabe von Organismen und Methoden überdeckt, sondern eine Basis für ein gemeinsames Verständnis bezüglich wissenschaftlicher Modelle, Methoden, Inhalt und Form der Umweltrisikoabschätzung bietet.

Ein solches Konzept kann zu einem verbesserten und gemeinsamen Verständnis zwischen Antragstellern, Risikobewertern und Entscheidungsträgern sowie zu einem höheren Vertrauen in das Zulassungsverfahren von GVO auf EU-Ebene beitragen.

III SUMMARY

Background and aim of the study

The prevailing controversies on the potential environmental risks of genetically modified organisms (GMOs) still fuel ongoing discussions among EU member states, risk assessors, notifiers and scientists, even several years after the commercial introduction of GMOs and GMO products and after the implementation of several legal provisions for a harmonized risk assessment, labeling and traceability at the EU level. The disagreements mainly derive from differences in perceived environmental risks which – to the opinion of some – are covered by a strict regulatory system – while in the opinion of others – are not or not sufficiently addressed in current risk assessment practice of GMO notifiers.

Against this background the aim of this report was to scrutinize the current practice of environmental risk assessment of several GMO notifications currently pending for authorization in the EU. For this purpose representative GMO notifications submitted either according to Directive 2001/18/EC or Regulation (EC) 1829/2003 were chosen focusing on crops with commercial importance in the EU (maize, oilseed rape, potato) as well as balancing GM traits (insect resistance, herbicide tolerance, starch content, 'stacked event' GMOs). All notifications included cultivation in their scope of notification. The environmental risk assessments as carried out in these notifications were analyzed whether they fulfilled general scientific standards and requirements according to legal provisions and relevant guidance documents.

This report thus presents a critical appraisal of the environmental risk assessment of selected GMO notifications and identifies major shortcomings in the current practice of environmental risk assessment. Suggestions for improvements in the risk assessment methodology are made and needs for further guidance and standardization outlined. The suggestions for improvements and recommendations address likewise notifiers, risk assessors as well as decision makers.

Major findings

In general, a very narrow approach of the environmental risk assessment was applied in the GMO notifications reviewed focusing nearly exclusively on the transgene product instead of considering the GMO as a whole. Notifiers consider testing of the individual transgene product almost only necessary, if this has pesticidal properties (e.g. *Bt* proteins). Other transgene products such as non-pesticidal proteins or proteins expressed in herbicide tolerant plants (EPSPS or PAT proteins) are tested with less rigor as they are generally considered to have no effects on target or non-target organisms. The GM crop as a whole is regarded as safe resulting from the presumption of the notifiers that the introduced or modified GM traits do not change the biological characteristics of the GM crop beside the ones intended to. This assumption that the GM crop is safe is frequently not or not sufficiently supported with relevant and conclusive scientific data on the environmental safety of the whole GM crop. In addition also indirect effects of the GM crop in combination with any secondary stressors such as the complementary herbicide in herbicide-tolerant plants are often left unconsidered.

This narrow approach which focuses on the transgene product and does not consider the whole plant as well as any secondary stressors leads to further severe shortcomings in risk assessment. Environmentally relevant plant compounds, metabolites of the novel substance produced (e.g. the Bt toxin in insect resistant GM crops) or metabolites of the herbicide are left unconsidered as well as GM trait interactions or plant compound-GM trait interactions.

A further shortcoming is the non-compliance with the 'case-by-case' approach in the reviewed GMO notifications. As the focus of the risk assessment is on the isolated transgene product, notifiers frequently refer to other GMOs with identical or similar traits (e.g. other GMOs expressing the same *Bt* protein) when drawing conclusions on the safety of the GMO instead of testing the particular GM plant.

The narrow concept of environmental risk assessment focusing on the transgene products rather than the whole GMO lacks ecological realism of the GMO in its receiving environment and largely ignores ecological interactions as confounding and influencing factors of the genetic modification and the transgene (e.g. via position effects, epigenetic and pleiotrophic effects), the plant itself (e.g. via secondary metabolites), and the receiving environment are left unconsidered.

The relevant ecological knowledge of the GMO is not gained in a stepwise approach from increasing levels of release of the GMO into the environment (laboratory - greenhouse - field), as data are mostly derived from laboratory testings only, which – again – focus on the transgen product and not on the whole GM plant. This lack of integration of ecologically relevant data from different containement levels of the GMO into the environmental risk assessment of GMO notifications leads to the disregard of the 'step-by-step principle' of Directive 2001/18/EC.

In addition, the significance of data presented in the reviewed notifications is limited for EU environments and thus not sufficient to draw conclusions on the environmental safety of the GMOs in its receiving environment. The field data submitted for the phenotypical characterization, the agronomic behaviour or for the assessment of environmental effects are frequently not gained from EU environments or do not cover representative environments within the European Union. Notifications therefore do not fully comply with the 'region-by-region' principle of Directive 2001/18/EC. By this approach also species of conservation concern, either protected EU-wide or regionally, are not subject to a separate assessment in the analysed GMO notifications.

From the review of the GMO notifications in this report it became evident that conclusions drawn for particular environmental risks were often based on insufficient data. Severe shortcomings in the presented experimental assessments were identified with respect to the generation and evaluation of data as well as data presentation and comprehensiveness of conclusions drawn from these data. Scientific methods and experimental approaches are often flawed with respect to their significance for the questions asked and their general scientific quality. In addition, considerable variability in the methods, parameters and organisms chosen for a specific assessment were noticed between GMO notifications. No consistent approach is evident for a range of assessments of environmental risks. Apparently, there is no common understanding of how to conduct a risk assessment for specific assessment categories among notifiers but also between notifiers, risk assessors and decision makers.

In other cases the conclusions on environmental risks of a particular GM crop were largely assumption-based or exclusively based on cross-referencing using surrogate data from other assessments or other GMOs rather than on robust scientific data for the particular GMO. Shortcomings in the compilation, citation and presentation of data and results lead to inconclusive and incomprehensible risk conclusions. Moreover, Information and data referring to important aspects such as the source of the data or studies (authors, institutions), the status of reports (published, preliminary) and their relevance for the respective GMO (specification of the GMO used in the study) are frequently lacking. Hence, the basic requirement of Directive 2001/18/EC that the environmental risk assessment should be based on scientific and technical data is insufficiently fulfilled in in many areas of the environmental risk assessment.

Independent of the quality or quantity of the underlying data basis and the results obtained, conclusions on environmental risks of the respective GMO were generally estimated to be negligible by notifiers. Current environmental risk assessment practice of GMOs builds on the assumption that small effects compared to the large variability in the environment are irrelevant and, thus, permit the conclusion of safety of the GMO. To achieve this outcome, double standards are frequently applied in the assessment norms of potential environmental risks. As a prominent example the double standards applied to the choice of control treatments used for the evaluation of the efficacy of the GMO towards the target pests and the evaluation of effects of the GMO on non-target organisms can be highlighted.

Uncertainties in the underlying model of the risk assessment, in methods, results or interpretations are generally omitted from the assessments. As a consequence, uncertainties are also not incorporated in the decision on a case specific monitoring. This lack of a sound scientific risk assessment approach and an uncertainty analysis not only contradicts the concept of risk assessment in general and the intention of Directive 2001/18/EC in particular, but also ignores that even small, but significant, differences or regional differences between the GMO and the control may have severe consequences for the environment.

In no case these or other shortcomings in the environmental risk assessment practice of GMO notifiers were recognized by the EFSA GMO panel in its opinions, although the requirements as specified by the panel’s guidance were in many instances not or not completely fulfilled in the reviewed GMO notifications.

As long as the conclusions in the environmental risk assessment are based on an insufficient or inconclusive data basis, future agro-ecological problems will not be timely anticipated and, last but not least, public confidence in this new technology not be established. This prompts for a novel view on the environmental risk assessment as provided by GMO notifiers and the need for more guidance and standardization for the compliance of a science-based, precautionary risk assessment.

Recommendations for improvement and need for standardization

A broader approach of the environmental risk assessment of GMOs needs to be applied, focusing on testing the GM plant and related crop management techniques for its commercial use. This implies that the receiving environment needs to gain considerably in importance in future risk as-

sessments. With such a broader environmental risk assessment concept several legal provisions and principles, such as the case-by-case and the region-by-region approach will be met accordingly. In addition the integration of data from stepwise introduction of the GMO into the environment (step-by-step principle) will have to be considered in future but will need guidance for its specific implementation.

The improvements suggested relate to the compliance to scientific standards but also to the generation of relevant data from the receiving environment. Methods are suggested on how to scope the risk assessment, choose test organisms, arrive at testable hypotheses of potential adverse effects, and determine the effect. In combination, these suggestions will lead to sound and scientifically-based conclusions for specific environmental risks, encompassing the particular GMO, its traits and the receiving environments. With the suggested concepts significant improvements can be achieved for all assessment categories evaluated in this report. In this context clarification is needed on the data requirements which need to be specifically generated by the notifier for a particular GMO and the role of published data and results cited.

In addition, structural and formal improvements for the compilation and presentation of the data in order to increase comprehensiveness of conclusions are needed.

Guidance is urgently needed for the selection of representative locations for the assessment of the agronomic and environmental behaviour of a particular GM crop. This guidance needs to include, among others, details on field trial design, choice of comparators, parameters assessed and data interpretation. Similar guidance is already available in other legislative areas such as the regulation of plant protection products and may be used as a starting point for the development of comparable guidance adapted to the specific needs for assessing GMOs.

Guidance is particularly needed for areas which have not been addressed so far in the environmental risk assessments of GMO notifications, such as long-term and cumulative effects but also for the consideration of uncertainty in the environmental risk assessment and the precautionary principle.

While several recommendations refer to a more standardized approach in the environmental risk assessment to provide a meaningful and conclusive data basis for decision making, others focus on the adoption of criteria which – based on a case-by-case evaluation – lead to the relevant objects, organisms, and methods and integrate all relevant aspects: the GMO, the trait and the receiving environment. Hence, a balanced approach is suggested, not overruling the case-by-case approach by standardization and simple prescription of objects and methods but providing a common basis of understanding with respect to scientific models and methods, content and form of the environmental risk assessment.

This approach will lead to an improved and common understanding among notifiers, risk assessors and decision makers and will increase confidence in the authorization procedure of GMOs at the EU level.

IV INDEX OF TABLES

Tables

V ACRONYMS

ERA environmental risk assessment

GMO genetically modified organism

GMP genetically modified plant

GM genetically modified

GMHT genetically modified herbicide tolerant

HT herbicide tolerant

IR insect resistant

IRM insect resistance management

Bt Bacillus thuringiensis

ECB European corn borer

PPP plant protection product

GS General Surveillance

CSM Case Specific Monitoring

EC_{50}/EC_{90} effect concentration; concentration where 50 % / 90 % of its effect is observed

LC_{50}/LC_{90} lethal concentration; concentration which kills 50 % / 90 % of the test organisms

DT_{50}/DT_{90} time required for 50 % / 90 % dissipation of the initial concentration

PEC predicted environmental concentration

PNEC predicted no effect concentration

n. st. notifier study (unpublished)

1 THIS REPORT

1.1 Background and aim of this report

In 2002, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, governing the placing on the market of genetically modified organisms (GMOs) for cultivation, import or processing came into effect replacing Directive 90/220/EC. However, until 2003 no new GMO products were approved as several EU member states blocked approval of GM crops unless labeling and safety regulations were further tightened. In 2004, new EU laws went into effect establishing new requirements for approval, labeling and traceability (Regulations (EC) 1829/2003 and 1820/2003) with the intention to streamline the approval process and to restore consumer confidence. Since then, several GM products have been authorized according to the new legislative provisions for import, food and feed use but none included commercial cultivation. Significant controversies on the presumed safety of GMO-products still remain among EU member states, becoming evident by the failure to reach the required qualified majorities for the EC's proposals and by marketing and import bans issued by several EU member states for specific GMOs or GMO products.

Despite the fundamental improvements in the GMO authorization procedures strengthening the environment and the precautionary approach in GMO authorization through the provisions of Directive 2001/18/EC and the new labeling and traceability provisions, no new genetically modified crops have been approved for cultivation since 2004. This lack of approval of GM crops at EU level has also been accused to be due to the now centralized authorization procedure of GM crops, with more power conferred to the European Food Safety Authority (EFSA) and less to individual EU member states. In addition, considerable controversies have arisen among EU member states on the scientific advice and opinions on food and feed safety as well as on environmental safety issued by the EFSA's GMO panel. Despite efforts to strengthen risk assessment guidance and scientific exchange between EFSA's and EU member states' scientific experts, increased acceptance and majorities of votes for an authorization of GMOs among EU member states have not been achieved.

The assessment of potentially adverse effects of a GMO on the environment is still one of the main controversies among risk assessors, decision makers and scientists and EU member states when the placing on the market of GMOs within the European Union is envisaged. There is an increasing number of product notifications of GMOs, mainly under the provisions of Regulation (EC) 1829/2003. Experience over the last years of product notifications within the EU has shown that the quality of these notifications is often low and varies considerably between notifications and GMOs as shown by several reports addressing shortcomings in the current risk assessment practice (Spök et al. 2002, Spök et al. 2003a, Spök et al. 2003b, Spök et al. 2008, Andow & Hilbeck 2004, Lövei & Arpaia 2005).

In order to achieve a high standard of environmental protection integrating the Precautionary Principle, the need for an improvement of the environmental risk assessment on the basis of common standards for the assessment of environmental effects has frequently been demanded by different stakeholders but has so far not been accomplished.

The standardization and harmonization of the environmental risk assessment constitutes a particular challenge as it needs to consider the case-by-case approach when addressing individual GMOs. In addition, harmonization efforts will be limited at some level if regional environmental aspects are to be taken into consideration. However, the need to arrive at a common understanding of the ERA among stakeholder is imperative to improve confidence in decision makings on GMOs at the EU level. Corresponding activities aiming at standardization and harmonization of risk assessment procedures have been recently launched also at international level (e.g. Environmental Consideration for Risk/Safety Assessment for the release of Transgenic Plants by the OECD) and a mandate for further development of guidelines by the EFSA GMO panel has currently been issued by the European Commission (Status: July 2008).

The aim of this report is to scrutinize the current practice of environmental risk assessment and to identify the major shortcomings. Those areas are highlighted for which improvements are considered urgently needed. The suggestions for standardization and improvement of the environmental risk assessment of GMOs made in this report address the basic needs of improvement from a science-based environmental point of view. It does neither claim to be final nor conclusive but intends to provide a basis for discussion and to fuel scientific but also political debate on the current practice of the ERA.

1.2 Structure of this report

This report is structured into three major chapters. The first chapter (Review of Notifications; chapter 2) constitutes the basis for the evaluation and the critical review of the selected GMP notifications notified either according to Directive 2001/18/EC or Regulation (EC) 1829/2003. In this chapter the data and results provided by the notifier in order to assess environmental risks of the respective GMP and the conclusions drawn by the notifiers on particular environmental risks are described and analyzed.

In the second chapter (Critical Appraisal; chapter 3) the risk assessment approach chosen, the data basis and the argumentations provided in the assessments of the ERAs presented in the selected GMP notifications, are evaluated whether they correspond to current legal provisions and scientific standards. Major shortcomings are identified and outlined.

In the fourth chapter (Recommendations for Standardization; chapter 4) suggestions are made for improvements of the ERA and the need for further guidance and standardization is outlined.

The Annex attached to this report contains several tables prepared for the analysis of GMP notifications (chapter 2). Several tables relevant for the analysis were shifted to the Annex for layout reasons and cross-referenced in the text. Tables in the text are consecutively numbered from Table 1 to 31, in the Annex from Table A1 to Table A14.

1.3 GMO notifications selected and documents analysed

Up to date market releases of GMP have been granted on the basis of three different regulations: Directive 90/220/EEC, replaced by Directive 2001/18/EC and, more recently by Regulation (EC) 1829/2003. This chapter describes the selection of the notifications of genetically modified organ-

isms (GMOs), the documents which were analysed and the approach chosen for the analysis of the environmental risk assessment (ERA) in this study.

By March 2001, 18 GMO products had been approved under Directive 90/220/EEC). By December 2007 seven notifications were authorised under Directive 2001/18/EC, while seven notifications were pending, and several were transferred to Regulation (EC) 1829/2003. In addition, several notifications were withdrawn by the notifiers. None of the notifications, so far authorised under Directive 2001/18/EC, includes cultivation.

Since 2003 46 notifications have been submitted according to Regulation (EC) 1829/2003 until December 2007. One of those applications was withdrawn by the notifier. Eight of the 46 notifications consider GMPs for cultivation, seven GM maize lines and one GM soybean. Considering notifications submitted under all provisions GMPs applied for cultivation in the EU include sugar beet (*Beta vulgaris*), potato (*Solanum tuberosum*), soybean (*Glycine max*), oilseed rape (*Brassica napus*), maize (*Zea mays*) as well as cotton (*Gossypium hirsutum*).

In this study the following criteria were applied during the selection of notifications for the analysis of the environmental risk assessment:

- The scope of the selected notifications should comprise cultivation.
- The selected notifications should be representative with respect to the crops/plant species generally notified or submitted.
- The selected notifications should be representative with respect to the traits currently used in GMPs.
- The selected notifications should either be ready for commercialisation or expected to be placed on the market within a short time.
- Some of the selected notifications should consider ‘stacked’ events, derived by crossing of the single events.

As a result of the above criteria most of the selected notifications are GM maize events, as maize constitutes the majority of notified crops within the European Union. The GM maize notifications selected for the analysis are of high relevance with respect to the inserted traits as they include insect resistant and herbicide tolerant GMP. Additionally, the respective notifications of GM maize are frequently used as parental lines in ‘stacked’ GMOs derived by traditional breeding of single GM varieties. One GM oilseed rape notification was selected for the analysis as this crop plant has wild relatives in Europes and thus is of particular relevance for the environmental risk assessment. One GM potato was included due to its distinctive trait and its foreseeable market relevance. Table 1 gives an overview of the notifications selected for the analysis of the environmental risk assessment.

Table 1. Overview of the notifications selected for the study and their status of approval within the European Union (Status: December 2007)

Dir = Directive; Reg = Regulation; HT = herbicide tolerance, IR = insect resistance; AC = altered composition; MS/MF = male sterility and restoration of male fertility; FO = food use; FE = feed use; I = import; P = processing; IP = industrial processing, industrial uses; CU = cultivation; Seed = seed production

GMP	Submitted under	Notification number	GM traits	Scope of notification	Current status
Oilseed rape Ms8xRf3	Dir 90/220/EEC – transferred to Dir 2001/18/EC	C/BE/96/01	MS/MF, HT	I, FO, FE, IP, CU	authorised
Potato EH92-527-1	Dir 90/220/EEC – transferred to Dir 2001/18/EC	C/SE/96/3501	AC	IP, FE, non-food use, seed, CU	pending
Maize MON810	Dir 90/220/EEC	C/F/95/12/02	IR	seed (CU, P)	authorised*
Maize Bt11	Dir 90/220/EEC – transferred to Dir 2001/18/EC	C/F/96/05/10	IR, HT	FO, FE, IP, CU	pending
Maize 1507	Dir 2001/18/EC	C/ES/01/01	IR, HT	CU, I, P (excluding food uses)	pending
Maize NK603	Reg (EC) 1829/2003	EFSA/GMO/NL/2005/22	HT	FO, FE, I, P, CU	pending
Maize 59122	Reg (EC) 1829/2003	EFSA/GMO/NL/2005/23	IR, HT	FO, FE, I, P, CU	pending
Maize 1507xNK603	Reg (EC) 1829/2003	EFSA/GMO/UK/2005/17	IR, HT	FO, FE, I, P, CU	pending
Maize NK603 x MON810	Reg (EC) 1829/2003	EFSA/GMO/NL/2005/26	IR, HT	CU only	pending

*this GMO is currently under re-evaluation according to Regulation (EC) 1829/2003.

The selected notifications contained the original notification as submitted to the Competent Authorities and - in several cases - also additional information, documents or notification updates supplied by the notifier at a later stage during the authorization procedure. In particular in response to questions raised by the Competent Authority or EFSA during the evaluation of the notification. Any information that was submitted until December 31st 2007 was considered in this study.

An overview of the documents submitted by the notifiers and exchanged between the notifier and the European Commission, the EFSA, or the lead Competent Authority during the notification procedure can be seen from Table A1 in the Annex. In this table the submitted documents are arranged in a chronological order. Documents and additional information submitted by the notifiers until December 2007 were considered in the review of the notifications in this report.

Documents for C-notifications (submitted according to Directives 90/220/EEC or 2001/18/EC) were only circulated as paper versions or on CD-ROM to the EU member states Competent Authorities. Since the coming into force of Regulation (EC) 1829/2003 notifiers of GMOs generally submit their notifications via this regulatory system, which can be accessed by an electronic system for designated Competent Authorities (EFSAnet). In some cases EFSA was consulted for C-notifications if EU member states objections could not be resolved (e.g. oilseed rape Ms8xRf3, potato EH92-527-1). In the case of the potato EH92-527-1 two notifications, one according to Directive 2001/18/EC and one according to Regulation (EC) 1829/2003 were simultaneously assessed by EFSA. EFSA apparently requested additional information on non-target organisms from the notifier on this GMO. It is unclear whether these additional studies delivered by the notifier were submitted for the EFSA notification or the C-notification. However, as cultivation is only included in the C-notification, these studies were included in the analysis.

Due to the fact that additional information or studies for a particular GMO are frequently submitted by the notifiers at a later time during the evaluation period or during several question-answer rounds between EU member states and notifiers, the comprehensiveness of the data submitted for a specific GMO notification can be severely hampered, in particular if the later submission of studies has not been well documented (relevant mostly for C-notifications). In addition, inconcise cross-referencing by notifiers to studies submitted earlier during the notification procedure (see also chapter 3.2.4) additionally complicates the comprehensiveness of the assessments.

1.4 Approach of the analysis of GMO notifications

1.4.1 Selection of assessment categories of potential environmental effects

The objective of Directive 2001/18/EC is to protect human health and the environment when a GMO is placed on the market as or in products (Article 1). The protection of human health and the environment requires due attention to be given to controlling risks from the deliberate release into the environment of genetically modified organisms (Preamble, point 5). The focus is therefore on the preventive action, due to the ability of living organisms to reproduce and the irreversibility of GMO releases in the environment. Thus the Directive requires a case-by-case environmental risk assessment (ERA) approach to be carried out prior to a release which should also take due account of potential cumulative long-term effects associated with the interaction with other GMOs and the environment (Preamble, point 19).

Annex II of the Directive defines the general principles of the ERA, the steps, the methodology and conclusions to be drawn on the potential environmental impact from the release or the placing on the market of GMOs. On the basis of an ERA carried out in accordance with these principles and methodology, information on several points listed in sections D1 or D2 of the Annex should be included in notifications with a view to assisting in drawing conclusions on the potential environmental impact of a GMO.

The information which may be necessary to carry out the environmental risk assessment is laid down in Annex III of the Directive and requires information on the recipient plant, the genetic modification and, most importantly, on the genetically modified plant. Thirteen information points are outlined in Annex III with respect to the GMP.

Similarly, Regulation (EC) 1829/2003 requires a technical dossier for the notification of a GMO supplying the information required by Annexes III and IV to Directive 2001/18/EC and information and conclusions about the risk assessment carried out in accordance with the principles set out in Annex II to Directive 2001/18/EC if the scope of the notification covers food or feed containing or consisting of GMOs (Regulation (EC) 1829/2003; Article 5). The information requirements as defined by Annex III in the Directive are also referred to in the EFSA guidance document (EFSA 2006a) although they differ to some extent in structure and detail.

In this report the analysis of the ERAs provided in selected GMO notifications was divided into assessment categories which cover individual aspects of the ERA. The division into these categories is based on the distinction as outlined in Directive 2001/18/EC and the EFSA Guidance Document (EFSA 2006a). However, certain aspects were grouped if they were considered to be related. Con-

sequently, the environmental risk assessment was distinguished into the following major assessment categories:

- Assessment of the molecular characterisation of the GMP
- Assessment of the expression of the new trait(s)of the GMP
- Assessment of agronomic behaviour of the GMP
- Assessment of composition of the GMP
- Assessment of dissemination and related processes
- Assessment of effects mediated via target organisms of the GMP
- Assessment of interactions of the GMP with non-target organisms and the biotic environment
- Assessment of effects of the GMP on biogeochemical processes and the abiotic environment
- Assessment of effects related to changes in land use or cultivation techniques
- Proposed risk management and monitoring plan

Each assessment category was subdivided into several sub-categories which were evaluated during the analysis. These sub-categories largely correspond to the information requirements as outlined in the Directive 2001/18/EC or the EFSA Guidance Document (EFSA 2006a). Table A2 in the Annex shows the structure of these assessment categories and the sub-categories as analysed in this report. The assessment of effects of the GMP on human health was omitted from the analysis due to the lack of direct relevance for environmental risks.

In the following the rationale for the individual assessment categories is presented on the basis of the legal requirements laid down in Directive 2001/18/EC and the Guidance Document on Risk Assessment issued by EFSA (EFSA 2006a). The scientific rationale and the reasons why specific assessments as presented in the GMP notifications were not considered sufficient are outlined in detail in the respective chapters of the Critical Appraisal (chapter 3) and the Recommendations for improvements (chapter 4).

1.4.1.1 Molecular characterisation of the GMP

The molecular characterisation of the genetic changes introduced into specific GMOs by the genetic modification can be considered as the starting point in the risk assessment of GMOs. Directive 2001/18/EC stipulates that relevant information on the molecular characteristics of a GMO has to be supplied by the notifier. Specifically details concerning the genetic modification by insertion or deletion as well as specifics about the used vectors and donor organisms have to be submitted. The EFSA guidance on risk assessment outlines which specific issues regarding the molecular characterisation of a GMO should be investigated (EFSA 2006a). The requirements for molecular data are the same for GMO notifications under Directive 2001/18/EC for the placing on the market (Part C) and for the assessment of GM food and feed according to Regulation (EC) 1829/2003. The molecular characterisation issues which were deemed relevant in this report as basic informa-

tion for the ERA are identical to those listed in the above mentioned guidelines (see Annex, Table A2).

Descriptions of the trait(s) and characteristics, which have been introduced or modified in the GMO, are required. Additionally concerning the sequences which are actually inserted (or deleted) in a GMO information is required on the size and copy number of all detectable inserts (both complete and partial), the organisation of the inserted genetic material at the insertion site(s) and methods used for the characterisation of insertion(s), and on the sub-cellular location(s) of insert(s) (including information on methods for determination). Furthermore sequence information of inserted elements and the respective 5' and 3' ends of the insert(s) is required. The determination of sequences should extend into the host plant genome to identify any insertions into known open reading frames (ORFs) or regulatory genomic regions, and any interruptions of such elements to assess the potential for insertions to produce novel chimeric proteins. Concerning genetic stability of the insert and phenotypic stability of the GMP, information is required for a representative number of generations to assess the inheritance pattern of the introduced trait(s) and the stability of the introduced trait(s).

The methods applied for the molecular characterisation of a GMP should be discussed with regard to their specificity and sensitivity. They should be complemented by bioinformatics analyses and an assessment of molecular interactions of the inserted traits. The latter requirement is relevant to address potential combined effects of modifications present in GMOs derived by a traditional crossing step ('stacked events').

1.4.1.2 Expression assessment of the GMP

The requirements for the submission of information on the expression of the insert(s) are outlined in Directive 2001/18/EC as well as by EFSA (2006a). Annex IIIB of Directive 2001/18/EC (item D.3.) requires that information on the expression of the insert is contained in notification of GMOs. Specifically information on the developmental expression during the life cycle and methods used for its characterisation shall be submitted as well as information on parts of the plant where the insert is expressed. According to EFSA (2006a) information of expression in certain parts of the plants is deemed relevant if tissue specific promotors are used but information is requested to demonstrate that the expression of the inserted gene(s) is like expected from the genetic construction. Also the stability of transgene expression in the targeted tissue(s) needs to be assessed. Expression data from any plant parts are only required if a potential risk is identified. Developmental expression data are relevant for parts of the plants used for food or feed purposes, but also with respect to exposure to non-target organisms (EFSA 2006a). Another important consideration is the assessment of differences of expression of the introduced trait(s) in different genetic backgrounds.

Additionally, EFSA (2006a) requires information on the expression of potential fusion proteins (section D, 3.c), suggesting a bioinformatic analysis. The investigation of newly expressed transcripts is considered necessary when a potential fusion protein is identified (EFSA 2006a). In case putative fusion proteins are identified, further investigations are warranted such as an analysis of transcription and translation of these proteins in the GMO (EFSA 2006a).

1.4.1.3 Assessment of agronomic behaviour of the GMP

The assessment of agronomic traits of a GMO is not specifically outlined in the ERA requirements of Directive 2001/18/EC. EFSA (2006) classifies the information required on agronomic traits under the information on any toxic, allergenic or other harmful effects on human or animal health (EFSA 2006a, point 7). It is argued that possible unintended effects may manifest themselves through changes in susceptibility to important pests and diseases, through morphological and developmental changes or through modified responses to agronomic and crop management regimes. Therefore EFSA (2006a) requires a comparison between the GMP and a comparator with respect to agronomic traits. With respect to the design of field trials, number of locations and seasons and statistics as well as baselines EFSA refers to the requirements specified for the comparative assessment (point 7.2, EFSA 2006a).

1.4.1.4 Assessment of composition of the GMP

Directive 2001/18/EC does not specifically address compositional aspects of a GMP to be assessed during the ERA. The relevance for the assessment of compositional aspects of a GMP derives from the guidance issued by EFSA, suggesting an assessment of compositional parameters of GMPs, termed as ‘comparative assessment’ (EFSA 2006a, section III D. 7.) with respect to the information that should be provided by the notifier on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food or feed. Since compositional changes in the GMP may also have environmental consequences, the evaluation of these has been included in this report.

1.4.1.5 Assessment of dissemination and related processes

Processes related to dissemination, survivability and establishment or persistence of a GMP are covered by Directive 2001/18/EC in the way that the notifier needs to provide information on how the GMP differs from the recipient plant in the mode(s) and/or rate of reproduction, dissemination, and survivability (Annex IIIB, D. 4). Furthermore, the notifier should include an assessment on the likelihood of the GMP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as well as an assessment on any selective advantage or disadvantage conferred to the GMP (Annex II, D.2.). In its guidance document EFSA foresees that the notifier should identify whether the GMP differs from the parental or near isogenic non-GMP in its biology, including information on biological features that affect fitness and environmental ‘sensitivity’ (EFSA 2006a, III D.4.). Additionally, persistence, invasiveness as well as the likely consequences of an increased persistence and any selective advantage or disadvantage conferred to the GMP should be assessed (EFSA 2006a, III, D.9.1. and D. 9.2.).

1.4.1.6 Assessment of target effects

According to Directive 2001/18/EC information on the potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMO and target organisms, should be included in the notification (Annex II, D.2.). According to Annex IIIB information on the mechanism of interaction between the GMP and target organisms is required. EFSA further specifies that data on the susceptibility of the GMP to pests and diseases compared with that of

the non-modified plants are useful indicators of effects, together with observations on agronomic performance during greenhouse and experimental field trials (EFSA 2006a).

1.4.1.7 Assessment of interactions of the GMO with non-target organisms and the biotic environment

Directive 2001/18/EC states that adverse effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations caused by GMOs need to be identified (Annex II, C.2.). Therefore, the environmental risk assessment should consider possible immediate and/or delayed environmental impacts resulting from direct and indirect interactions of the GMO with non-target organisms, including impacts on population levels of competitors, herbivores, symbionts, parasites and pathogens. Consequently, Annex IIIB of the Directive requires information on potential changes in the interactions of the GMP with non-target organisms resulting from the genetic modification.

EFSA further specifies that the risk assessment of non-target organisms should be subjected to a tiered approach, first identifying potential hazards in controlled tests, then evaluating exposure in the field in order to estimate potential risks (EFSA 2006a).

1.4.1.8 Assessment effects of the GMP on biogeochemical cycles and the abiotic environment

The assessment of possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s) is a requirement in the risk assessment procedure according to Directive 2001/18/EC (Annex II, D.2.). Annex II of Directive 2001/18/EC specifically mentions effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material as one of the potential adverse effects of a GMO (Annex II, 4.2.1, Step 1: Identification of characteristics which may cause adverse effects).

EFSA (2006a) suggests the assessment of soil processes such as CO_2 evolution, organic matter turnover or nitrogen fixation and emphasizes the importance of soil microbial communities and their associated functional activities for soil fertility and plant productivity. Exposure estimations to relevant soil biota such as earthworms and micro-organisms in relation to the impact on decomposition processes are required as well as an assessment of potential population shifts of deleterious organisms (EFSA 2006a).

Annex IIIB (point D.11) of Directive 2001/18/EC refers to the information required on potential interactions of the GMO with the abiotic environment. Abiotic environment refers to the non-living components of an ecosystem, such as light, temperature, wind, soil or atmospheric gases. EFSA (2006a) gives some ideas on potential interactions of the GMP and its abiotic environment such as the alteration of or the sensitivity to climatic conditions, altered sensitivity to or tolerance of abiotic soil fractions.

1.4.1.9 Assessment of effects related to changes in land use or cultivation techniques

The consideration of potential environmental effects of the GMP in combination with its herbicide or pesticide regime is a requirement of Directive 2001/18/EC (Annex II). According to the Guidance Notes supporting Annex II (EC 2002a), the relevance of changes in management procedures of the GM crop has to be assessed on the basis of existing procedures as it constitutes one of the mechanisms through which adverse effects may occur directly or indirectly (step 1 in the ERA). Also EFSA (2006a) states that the wider environmental impact of changes in management of the GMPs including changes in agricultural practices should be considered in the assessment under Directive 2001/18/EC while the risk assessment of the plant protection product itself is assigned to Directive 91/414/EEC (EFSA 2006a). EFSA requires the description of intended commercial management regimes for the GMP including changes in applications of plant protection products, rotations and other plant management measures where these are different from the equivalent non-GMP under representative conditions (EFSA 2006a, point 9.9.). Furthermore the assessment of effects of the management of the GMP including effects on biodiversity within the crop and in adjacent non-crop habitats is required and the need to compare the relative efficacy of different herbicides and their management programmes on weed species is emphasized in order to assess the impact on biodiversity.

1.4.1.10 Risk management and monitoring plan

Directive 2001/18/EC introduced the necessity of the inclusion of a monitoring plan in the notification when a GMO or a combination of GMOs as or in products are placed on the market (Article 13, Article 20, Dir. 2001/18/EC). The specific objectives and general principles of this monitoring plan are laid down in Annex VII of the Directive. Council Decision 2002/811/EC (EC 2002b) establishing guidance notes supplements Annex VII of Directive 2001/18/EC and describes the objectives and general principles to be followed to design the monitoring plan. The Regulation (EC) 1829/2003 also introduces the obligation for applicants to include an Environmental Monitoring Plan according to Annex VII of Directive 2001/18/EC (Art. 5(5)b and Art 17(5)b) when a GMO is placed on the market.

According to the legal provisions the aim of the monitoring plan is to identify any direct or indirect, immediate and/or delayed adverse effects of GMOs to human health and the environment which were not anticipated in the risk assessment and to confirm if the assumptions in the risk assessment regarding the occurrence and impact of potential adverse effects of the GMO or its use are correct (Annex VII of Directive 2001/18/EC). Thus monitoring is a separate task from the ERA after a GMO is authorized at EU level.

According to Council Decision 2002/811/EC monitoring plans should be divided into case-specific monitoring (CSM) and general surveillance (GS). While CSM serves to confirm that scientifically sound assumptions in the environmental risk assessment regarding potential adverse effects arising from a GMO and its use are correct, GS should seek to identify and record any indirect, delayed and/or cumulative adverse effects that have not been anticipated in the risk assessment.

According to Directive 2001/18/EC risk management is a part of the ERA (Annex II). Thus a risk management strategy should be defined. During the risk assessment risks may be identified that

require management and the need for the definition of a risk management strategy (Annex II, C.2 of Directive 2001/18/EC). Risk management should control an identified risk and cover the uncertainties.

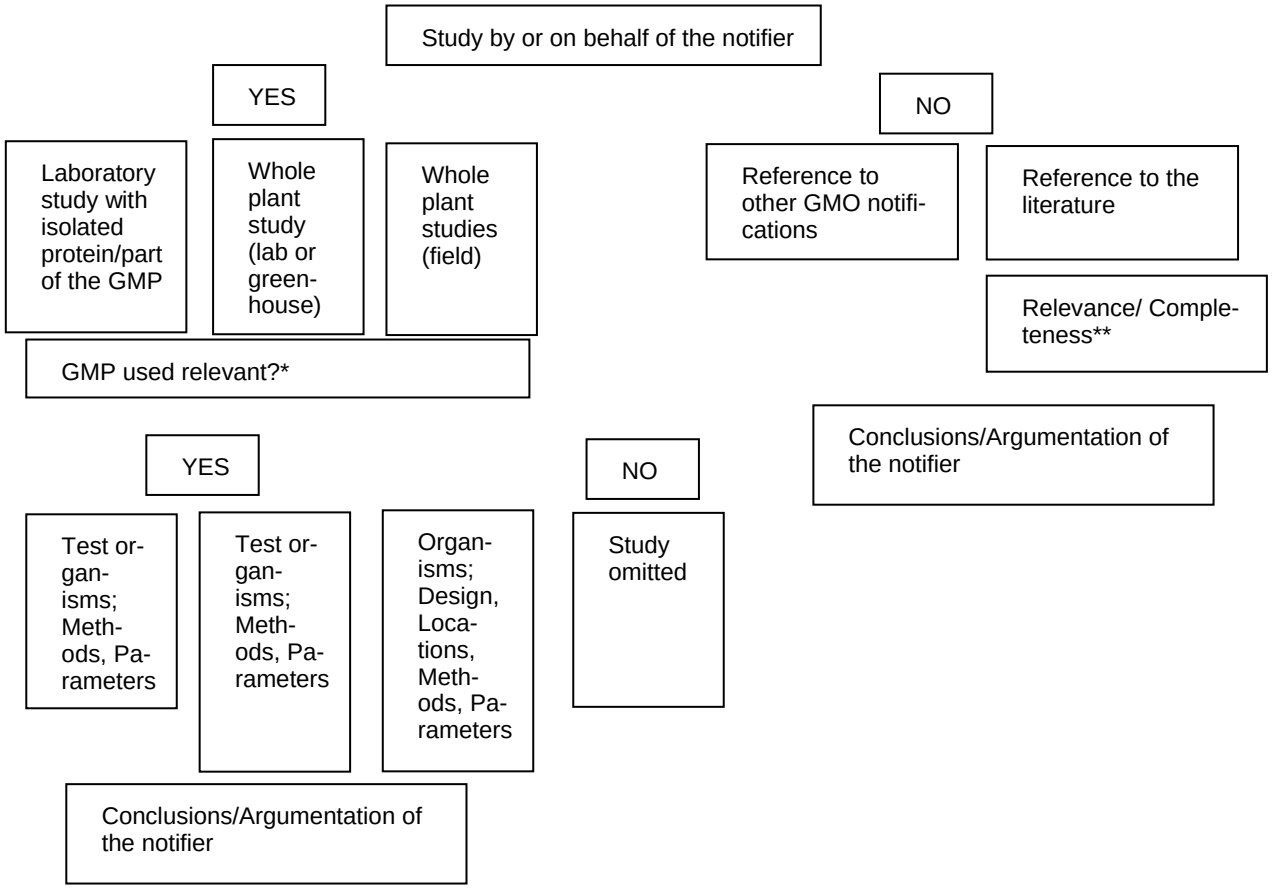
1.4.2 Criteria for the evaluation of assessment categories

The analysis of each assessment category followed a common approach depicted in the decision tree in Figure 1. Firstly, it was noted whether the notifier provided own studies or studies conducted on his behalf. If yes, laboratory studies using the introduced protein (e.g. Cry protein, EPSPS or PAT protein) or in some cases parts of the GMP (e.g. pollen fed to Daphnids or honey bees) were distinguished from whole plant studies under containment and whole plant studies in the field. With this approach, the step-by-step principle could be made transparent, whereby the containment of a GMO should be reduced and the scale of the release should increase gradually if the evaluation of the earlier steps indicates that the next step can be taken (Directive 2001/18/EC, Preamble, point 24). These studies were then further classified whether the GMO used corresponded to the GMO addressed in the notification. This approach mirrors the case-by-case evaluation of potential risks as outlined in Directive 2001/18/EC (Preamble, points 18 and 19). If the respective GMO was used in the studies the following information was evaluated:

- Exposure assessment (for non-target effects only)
- Locations of field trials
- Experimental design of the field trials
- Test organisms used/organisms assessed
- Methodology of assessment
- Parameters/toxicological endpoints assessed
- Statistics used
- Presentation of data
- Conclusions/argumentation of the notifier

A further distinction was made between reference made by the notifier to published studies in peer reviewed journals and reference made to other GMO notifications. In the assessment of non-target organisms, citations of published literature were checked for relevance, i.e. whether the GMO used in the respective publication corresponded with the GMO in the notification and whether the citations comprised all relevant studies at the time when the notification was submitted by the notifier, i.e. if the state of scientific knowledge was represented.

Fig. 1: Decision tree used in the review of the environmental risk assessment of GMO notifications according to Directive 2001/18/EC and Regulation (EC) 1829/2003.



* The GMP used in the studies was considered relevant if it matched the event of the respective notification (e.g. Oilseed rape Ms8xRf3, but not Oilseed rape Ms1xRf2, despite similar traits)

** Published studies were analysed for their relevance and completeness only for one assessment category: Effects of the GMP on interactions with non-target organisms and the biotic environement (see chapter 2.8).

The above mentioned criteria were applied to all assessment categories if applicable. For assessment categories which were supported by field trials conducted by the notifier (e.g. field trials for the agronomic evaluation of the GMP) the focus was laid on the methodological aspects of the field trials (locations, species, experimental design, and statistics). For assessment categories which did not include field trials but were based mostly on arguments, the focus of the analysis was laid on the argumentation of the notifier. In several assessments notifiers refer to studies published in peer reviewed journals. The extent of the cross-reference to the published literature varied significantly among notifications and assessment categories. As it was not feasible within the scope of this study to analyse every single publication individually that was submitted by the notifier, an analysis of the published studies was restricted to the published studies provided by the notifiers for the assessment of non-target organisms. For all other assessments the reference to published studies was noted only but not analysed in depth unless they happened to be known to the reviewing expert team.

2 REVIEW OF NOTIFICATIONS

In this chapter the risk assessment approach and the data provided in the ERA as presented in the notifications by the notifiers are reviewed. The review distinguishes between the data provided, the presentation of data and results and the argumentation of the notifiers to support a conclusion on a specific environmental risk.

This review considers the 10 assessment categories as outlined in chapter 1.4. For a specific assessment category data and results of the nine different notifications are also comparatively presented in tables in the corresponding subchapters. Some tables were transferred to the Annex. The data presented in three assessment categories (assessment of expression, agronomic behaviour and plant composition) were generally based on field trials. In some cases, data for all three assessment categories derived from identical field trials. Chapter 2.2 gives a comparative overview of these field trials. The data presented for the corresponding assessments are contained in chapters 2.3, 2.4 and 2.5.

2.1 Molecular characterisation

2.1.1 Information relating to the transgenic construct

In general, the relevant information necessary to identify the fragments of DNA, which are used in the modification process of the plant, and information on the sources of these fragments were included in all notifications. Sequence data for plasmids or DNA fragments used for transformation were supplied, and the source organisms for the sequences used to construct the transgenic inserts were documented.

For the stacked maize events 1507xNK603 and MON810xNK603 reference was made to the information submitted with the notifications of the respective parental, single event GMPs.

2.1.2 Characterisation of the genetic modification

The methods and strategies for the molecular characterisation of the respective GMPs were comparable for all notifications analysed in this report. Differences, however, existed in the extent and conclusiveness of the presented data as well as the quality of presentation. An overview of the methods used to analyse the number of integration sites, the copy number of inserts present in the GMP, the location of the insert(s) in the genome of the GMP and the stability of the modification(s) is shown in Table 2..

Table 2. Methods used for the molecular characterisation of GMPs.

n.d….no data presented/data missing; in brackets: methods or number of experiments conducted; Ph… Phenotypic data, G…Genetic data; Southern Blot experiment: data from hybridisation of one or several probes to genomic DNA digested with a specific restriction endonuclease (or a specific combination of restriction endonucleases)

GMP	Number of integration sites	Copy number of Inserts	Insert location	Genetic stability
Oilseed rape Ms8xRf3	Southern Blot (1 experiment)	Southern Blot (1 experiment)	Southern Blot (undigested genomic DNA) Pattern of inheritance (Ph) PCR-Analysis	Southern Blot (3 generations) Segregation analysis (Ph: Multiple generations)
Potato EH92-527-1	Southern Blot (3 experiments)	Southern Blot (3 experiments)	Southern Blot Sequence data	Segregation analysis (Ph: 9 seasons) Sequence data (different seasons)
Maize MON810	Southern Blot (1 experiment)	Southern Blot (2 experiments)	Pattern of inheritance (Ph/G)	Southern Blot (3 generations) Segregation analysis (Ph: over 7 generations)
Maize Bt11	Southern Blot (3 experiments)	Southern Blot (3 experiments)	Pattern of inheritance (Ph/G) RFLP analysis	Southern Blot (6 generations) Segregation data (Ph: 6 diff. generations)
Maize 1507	Southern Blot (4 experiments)	Southern Blot (4 experiments)	Pattern of inheritance (Ph./G)	Southern Blot (2 generations) Segregation analysis (Ph: 2 generations)
Maize NK603	Southern Blot (1 experiment)	Southern Blot (2 experiments)	Pattern of inheritance (Ph/G)	Southern Blot (2 generations) Segregation analysis (Ph: 9 generations)
Maize 59122	Southern Blot (3 experiments)	Southern Blot (4 experiments)	Pattern of inheritance (Ph/G) Sequence analysis	Southern Blot (segregation data 1 generation, additional data 3 generations)
Maize 1507xNK603	Southern Blot (4 experiments)	Southern Blot (4 experiments)	n.d.	Southern Blot (segregation data for 1 generation)
Maize NK603xMON810	Southern Blot (1 experiment/single event)	Southern Blot (1 experiment/single event)	n.d.	n.d. (reference to single events)

2.1.3 Number of integration sites and copy number of inserts

The assessment of the transgenic insertions present in the GMO was generally done by Southern Blot. For notifications available only as hardcopy (i.e. the notifications of oilseed rape Ms8xRf3, potato EH92-527-1, maize lines 1507, MON810 and Bt11) graphic data such as reproductions of figures of results of Southern Blot experiments were often of insufficient quality. For some notifications (maize 1507, maize NK603) the assessment was based on detection of DNA fragments of high molecular weight at ranges where different fragments are difficult to distinguish. In these

cases the conclusions by the notifiers were based on the identification of very large restriction fragments of approximately 23 Kb in size.

The conclusions for the assessment of the copy numbers of inserts presented by the notifiers were also based on the results of Southern Blot experiments, including the results of the experiments conducted for the assessment of the number of transgenic insertions referred to in the above paragraph. Most of these experiments were done with probes not covering the whole length of the DNA used for transformation. Generally, the notifiers did not state why they were confident that the chosen experimental design could identify also potential partial insertions at different sites. For all analysed notifications, the documentation of the Southern Blot experiments did not include information on the sensitivity of the methods and whether the chosen sensitivity was appropriate to detect small and/or complex additional insertions.

Different experimental designs were employed by the notifiers for the evaluation of the copy number of inserts. Conclusions were based on three or more different Southern Blot experiments using probes corresponding to the main structural elements in the inserts for potato EH92-527-1, maize lines Bt11, 1507, 1507xNK603, and 59122. For the other notifications (oilseed rape Ms8xRf3, maize lines MON810, NK603, and NK603xMON810) fewer data were supplied.

In the case of potato EH92-527-1, a reassessment by the notifier with additional data (Hofvander 2004, n.st.) was submitted in response to a request by the competent authorities. The additional data showed that more than a single copy of the insert was present, as concluded in the original notification. Further data from Southern Blots as well as PCR-experiments and sequencing demonstrated that indeed two copies of the insert were present in a tail-to-tail-configuration inserted in the potato genome at chromosome 5.

Different amount of data were established for the two analysed stacked maize events NK603xMON810 and 1507xNK603. In the case of maize NK603xMON810 the presence of the traits derived from the parental events NK603 (*epsps)* and MON810 (*cry1Ab*) was investigated by a single Southern Blot experiment each. The respective data aimed to demonstrate that similar hybridisation patterns were seen in the stacked GMPS and the respective single event, parental GMPs. Based upon these data the notifier concluded that the stacked event GMP is identical to the single event GMPs MON810 and NK603 regarding the molecular modifications. For all other issues of molecular characterisation reference was made to the data submitted in the previous notifications for the single events. Such reference to data submitted in previous notifications for single GM events was also made in the case of maize 1507xNK603, but complemented with additional data generated for the stacked event itself.

The data submitted for oilseed rape Ms8xRf3 for the molecular characterisation (number of integration sites, copy number of inserts, genetic stability, characterisation of insert and flanking sequences) were established for the parental events Ms8 and Rf3, respectively. No data demonstrating similarity of the transgenic insertions present in the stacked hybrid and the respective modifications in the parental events have been submitted by the notifier. The significance of the data submitted for the parental events with regard to the hybrid oilseed rape therefore cannot be assessed.

2.1.4 Localisation of inserts

A nuclear (chromosomal) location of the transgenic insertions was assumed by the notifiers of all notifications. This assumption was based on the segregation behaviour assessed by phenotypic analysis of the novel traits or segregation of genetic elements assessed by Southern Blots. The demonstration of Mendelian segregation behaviour was taken as an indication for chromosomal integration of the transgenic inserts.

Only for potato EH92-527-1, maize NK603xMON810 and maize Bt11 the location of inserts on specific chromosomes was specifically addressed. RFLP mapping was used in the case of maize Bt11. Sequence data for flanking sequences of the respective inserts were used for potato EH92-527-1 and maize NK603xMON810. For the latter two GMPs the homology of flanking sequences to sequences previously submitted to publicly available databases was used to identify the specific chromosomal location. For potato EH92-527-1 data from Southern Blot experiments using chloroplast DNA from the GMP were submitted showing that the insert was not located in the chloroplast. For the stacked maize events 1507xNK603 and NK603xMON810 reference was made to data from the parental, single event GMPs to conclude that inserts were located in the nuclear genome.

2.1.5 Stability of insertions

The assessment of genetic stability of the inserts in GMPs was usually based on the demonstration of a stable inheritance of the respective phenotypic traits (herbicide tolerance and insect resistance traits, or starch composition for potato EH92-527-1) and an assessment of the molecular identity of the inserted genetic elements in offspring of the GMP. Mostly, a combination of phenotypic (segregation) data for traits and data on genetic stability of the inserts as demonstrated by Southern Blot over a number of generations was submitted (see Table 2).

Genetic analysis of stability by Southern Blot was usually based on the analysis of few individual plants for each generation. Only for maize 59122 a segregation analysis at the genetic level was submitted analysing 79 offspring plants of a single generation by Southern Blot for the presence of the characterised transgenic insertion.

Segregation data were statistically analysed and data which were consistent with the expected segregation pattern regarded as indication for the stability of the genetic modification. Stability for potato EH92-527-1 was concluded from starch composition data of tubers from individuals from nine subsequent cycles of vegetative propagation. Additionally, sequence data of material derived from different vegetative generations which showed unchanged sequences was used to support this conclusion.

2.1.6 Characterisation of Insert(s)

Relevant for further analysis of the transgenic insertions are:

- the characterisation of size and organisation of insert(s), both complete and partial
- the assessment of integrity of the insert structure and methods used for the characterization
- the analysis of flanking regions at the integration site

- in the case of deletion(s), size and function of the deleted region(s)
- the demonstration that any vector DNA, which was not intended to be transferred into the GMP, is absent from the GMP

The following table summarises the respective information which was submitted by the notifiers on these issues (Table 3).

Table 3. Overview on details for the characterisation of insert(s).

n.d….no data presented/data missing; nt….nucleotides; bp….base pairs

	Size of the insert(s), organisation of the insert(s) at the insertion site	Integrity of insert	Flanking sequences, origin of flanking sequences	Absence of vector sequences
Oilseed rape Ms8xRf3	Southern Blot PCR-analysis Sequencing	Southern Blots PCR-analysis Sequencing (inserts and flanking sequences)	Sequencing (Left/Right Border) Detection of Sequence Homologies	Southern Blot (backbone fragments)
Potato EH92-527-1	Southern Blots PCR-analysis Sequencing	PCR-amplification/ Restriction (insert) Sequencing (borders)	Sequencing (Left/Right Border)	Southern Blot (entire backbone) & Assay sensitivity
Maize MON810	Southern Blots	n.d.	n.d.	Southern Blot (entire backbone)
Maize Bt 11	Southern Blots PCR-analysis Sequencing	Southern Blots PCR-analysis Sequencing	Sequencing (Left Border)	PCR (Amp-R, other backbone fragments)
Maize 1507	Southern Blots PCR-analysis Sequencing	Southern Blots PCR-analysis Sequencing	Southern Blots Sequencing (Left/Right Border)	Southern Blot (entire backbone, nptII)
Maize NK603	Southern Blots PCR-analysis Sequencing	Sequencing (insert and flanking sequences)	Sequencing (Left/Right Border) Detection of Sequence Homologies	Southern Blot (entire backbone)
Maize 59122	Southern Blots PCR Sequencing	Sequencing (insert and flanking sequences)	Sequencing (Left/Right Border) Detection of Sequence Homologies PCR (Non-GMO maize)	Southern Blot (backbone fragments)
Maize 1507xNK603	Southern Blot *	n.d. *	n.d. *	n.d. *
Maize NK603xMON810	Southern Blot *	n.d. *	n.d. *	n.d. *

* Reference is made to data in other notifications for the single event GMOs.

2.1.7 Size and organisation of the inserts and insert integrity

In all notifications size and organisation of the inserts were assessed with several methods. Size and gross structure of the inserts were demonstrated by Southern Blot experiments in all notifications. Usually, a number of probes corresponding to crucial elements of the insert (promoters, coding regions for introduced genes, termination regions) were used to demonstrate the presence and gross match of these elements in comparison with the sequences used for transformation.

In the case of the stacked events maize 1507xNK603 and NK603xMON810 only Southern Blot data were included to demonstrate gross similarity to the respective parental GM events. The conclusions with respect to the molecular organisation of the transgenic inserts present in the stacked events on a detailed level were solely based upon data which were submitted for the respective parental single events and not for the stacked events.

For all notifications except for the two stacked events and maize MON810 the analysis by Southern Blot was complemented with a further evaluation of the insert structure by PCR using primers corresponding to sequences in the DNA employed for transformation. The notifiers concluded the integrity of the insert from different PCR experiments yielding overlapping segments.

Generally, for the identification of flanking DNA sequences fragments of DNA spanning the insert junction and extending into genomic DNA were sequenced. Initially, for potato EH92-527-1 internal fragments were digested with restriction enzymes to assess their identity. Upon submission of additional information for this notification the entire insert sequence was submitted together with information on flanking sequences of different extent. Integrity of the insert was evaluated by comparing the generated sequence data with the respective sequence information available for the DNA used for transformation. Based on such a type of analysis small size sequences rearrangements like small deletions at border sequences and base substitutions in the insert sequence were assessed.

Single nucleotide substitutions in transgenic inserts were reported for potato EH92-527-1, maize NK603 and maize 59122. For maize 59122 the notifier stated that base changes found upon sequencing were possibly introduced during the experiment itself. However, since the notifier did not include information which types of PCR polymerases were employed in the experiment this conclusion cannot be assessed.

2.1.8 Information on flanking sequences

Generally flanking sequences were amplified by standard techniques (e.g. Genome Walking) and subjected to sequence analysis. Varying lengths of sequences of the flanking regions in the different GM plants were determined (see Table 3). The respective notifiers neither attempted to determine whether the transgenic inserts were integrated into known native genomic sequences of the recipient plant nor did they determine the (specific) chromosomal localisation of the insertion. These issues were usually not specifically addressed based on sequence data for flanking sequences. For notifications, which were supplemented with additional submissions by the notifier, updates typically contained more information regarding the sequence of DNA regions flanking the insert(s) than the original notifications (e. g. maize Bt11, oilseed rape Ms8xRf3, potato EH92-527-1).

In case of maize 59122, information on the flanking sequences was used to design primers for PCR experiments that demonstrated the presence of homologous sequences in the genome of non-modified maize. For the stacked maize lines 1507xNK603 and NK603xMON810 as well as for oilseed rape Ms8xRf3 no sequence data were provided for the stacked event itself. In case of these GMPs the respective notifiers referred to sequence data established for the parental events only.

2.1.9 Absence of vector backbone sequences from the GMO

Commonly data from Southern Blot experiments were submitted for the demonstration that no additional sequences derived from the transformation vector were present in the GMP. For most applications the entire plasmid backbone sequence was used as probe. For maize 59122 a number of smaller probes corresponding to different elements of the plasmid backbone but not ensuring a complete coverage were used. No vector backbone sequences were found in any of the notifications. However, only one application (notification for potato EH92-527-1) included information to assess the sensitivity of the assay used.

For maize 1507xNK603 and maize NK603xMON810 notifiers referred to data established the single event, parental lines to conclude absence of undesired vector sequences.

2.2 Field trials used for the assessment of expression, agronomic parameters and plant composition

Field trials generally provided the basis for the generation of data for the expression analysis (see chapter 2.3), for the agronomic assessment (see chapter 2.4) and for the compositional analysis of the GMP (see chapter 2.5). Depending on the notification the same field trials were used to generate data for one, two or all three assessment categories (expression, composition, agronomic; see Table A3 in the Annex). In case all three assessments derived from the same field trial(s), the field trial design usually contained four blocks with one block being used for the expression analysis and the other three blocks for the evaluation of agronomic parameters and the compositional analysis of the GMP.

In the following chapters only field trials conducted in Europe were analysed in depth with respect to the details on the field trial design used for these assessments, as these were considered of highest relevance when GM crops are placed on the European market for cultivation purposes.

The field trials conducted with a specific GMP often took place in different years, at different locations, and in different countries and/or continents. Usually, several field trials were presented together in one study which is separately attached to the notification (e. g. maize 1507: Pavely 2002, n.st.; maize 1507xNK603: Buffington 2004, n.st.). Throughout this report the field trials are indicated by the respective year(s) and country(ies) in which they took place.

2.3 Assessment of expression

2.3.1 Studies conducted for the expression assessment of the GMP

2.3.1.1 Field trials for the determination of expression of transgenes

The expression of the inserted traits of the GMPs was generally determined from samples taken from field trials carried out at different locations and different years (see Table 4). In some cases combined field trials were carried out for the assessment of expression, the evaluation of agronomic parameters and the composition of the GMP. In such cases four blocks of the specific GMP were grown at the respective locations, but only one block (replication) was designated to the evaluation of the protein expression (e.g. maize 59122, maize 1507xNK603).

Location of field trials

An overview of the field trials conducted for the assessment of expression of inserted traits is presented in the following table (Table 4), summarising the design of field trials at European locations. However, for two GMPs transgene expression was only determined by using samples of the respective GMP grown in contained facilities like greenhouses (potato EH92-527-1, oilseed rape Ms8xRf3). For these two cases no specific data on the design of the trials were available. For five notifications (maize lines MON810, Bt11, NK603, 1507, and 59122) data from field trials at non-European locations were included (see Table A3 in the Annex). For maize Bt11 only data from field trials in the USA were submitted.

As can be seen from Table 4, only six out of nine notifications included European field trials for the expression assessment. The evaluation of expression was based on data from 1-2 European countries and from 1-4 locations per country.

Table 4. Details of European field trials carried out for the assessment of expression of inserted traits

R…Replicates; "–"… not done/not relevant; n.i. …not indicated in notification; glu+…treatment with glufosinate; glu-… no treatment with glufosinate; gly+ …treatment with glyphosate; gly-…no treatment with glyphosate; fb…followed by; Develop. …Assessment of developmental expression of trait; Exposition… Assessment of exposition of humans and animals when consuming the GMP; tissues… Assessment of expression of trait in different plant tissues.

GMP	field trials Europe	locations/EU country	R.	Treatment with herbicide	Traits (quant. anal. EU)	Purpose of the trials
Oilseed rape Ms8xRf3	-	-	-	(n.i.)[3]	PAT	Exposition, Tissues, Develop.
Potato EH92-527-1	-	-	-	-	NPTII	Exposition, Tissues
Maize MON810	1995/FR, IT	4/FR, 1/IT	n.i.	-	Cry1Ab	Exposition, Tissues, Develop.
Maize Bt11	-	-	-	(n.i.)[4]	Cry1Ab, PAT	Exposition, Tissues, Develop. (Cry1Ab)
Maize 1507	2000/FR, IT	3/F, 3/I,	1	glu- (FR) glu+/glu- (IT)	Cry1F, PAT	Exposition, Tissues, Develop.
Maize NK603	1999/FR, IT	3/FR, 1/IT	4	n.i.	EPSPS	Exposition, (Tissues/USA, Develop./USA)
Maize 59122	2003/BG	3/BG 3/ES,	4	glu+/glu-	Cry34Ab1 Cry35Ab1	Exposition, Tissues, De-

	2004/ES, BG	3/BG[1]			PAT	velop.
Maize 1507 x NK603	2003/ES, BG 2004/ES 2005/ES	3/ES, 2/BG 2/ES[2] 4/ES[2]	1	gly+ glu+ gly fb glu+ (2003, 2004) gly+/gly- glu+/glu- gly fb glu+/gly fb glu- (2005)	Cry1F, PAT, EPSPS	Exposition, Tissues, Develop.
Maize NK603 x MON810	2000/FR	3/FR	4	n.i.	Cry1Ab, EPSPS	Exposition

[1] 2004 trials were conducted at the same locations in Bulgaria as for 2003 growing season. [2] 2004 and 2005 trials were conducted at different locations as in 2003. [3] expression values only from greenhouse; [4] only data from USA.

Number of replications for determination of expression levels

Generally, expression levels were determined for pooled samples. Samples from a number of individual GM plants were taken from a specific field trial block and analysed. The number of replications, i.e. blocks from which such a pooled sample was taken, was only indicated in some notifications (maize lines NK603, 1507, NK603xMON810, 59122, 1507xNK603) while it was not indicated in others (oilseed rape Ms8xRf3, potato EH-92-527-1, maize lines MON810 and Bt11). For maize lines 1507 and 1507xNK603 only one replicate from the block design of the field trials was used for the analysis of expression of the inserted traits.

Number of growing seasons

The field trials conducted at European locations were commonly restricted to a single growing season for most notifications (see Table 4). For some notifications data were compared which derived from locations on different continents (e.g. maize MON810, NK603).

In two cases only expression levels were evaluated from more than one and consecutive years at European sites (maize 59122, maize 1507xNK603). However, for maize 1507xNK603 the tests conducted in Spain in 2004 and 2005 were conducted at different locations than in 2003. Data for the additional seasons were only submitted following requests for additional information. For maize 59122 field trials were conducted over two consecutive seasons (2003 and 2004) only at the three Bulgarian locations but not at all test sites.

In the notification of maize Bt11 the number of growing seasons in the USA was not fully indicated. The growing season was only indicated for the assessment of the PAT protein levels. Data on the expression of the Cry1Ab protein were obviously derived from different samples than data on the expression of the PAT protein. For the expression of the Cry1Ab protein plants grown in the greenhouse were analysed beside plants grown in the field at two locations in the USA.

Treatment of plots

In four out of seven notifications of GMPs with herbicide tolerance trait(s) the herbicide application was not indicated for the expression assessment (maize lines NK603, NK603xMON810, Bt11, oil-

seed rape Ms8xRf3). In the case of maize NK603xMON810 the evaluation of compositional parameters was conducted in the same year at the same locations. For these trials the respective information was included and the non-selective herbicide (glyphosate) used in these trials. However, it remains unclear whether the same plots were used also for the assessment of expression for this maize.

For the other three notifications (maize lines 1507, 1507xNK603 and 59122) the herbicides used were indicated in the dossiers. In all three cases the respective non-selective herbicides were applied in the field trials but either at different locations, in different years or in different combinations (for details see Table 4). An appropriate comparison of expression levels in treated versus untreated GMPs in European field trials was not presented in any of the notifications.

In field trials with maize 1507 the respective herbicide, glufosinate ammonium, was only applied at Italian but not at the French locations. Expression data from the locations in France and Italy were pooled and mean expression values across the six locations presented. Hence, no comparison between sprayed and unsprayed GMPs was carried out for this GMP.

Different herbicide regimes were applied in the field trials with the stacked maize 1507xNK603. The notifier used three different herbicide regimes. Non-treated GMPs were not included in the years 2003 and 2004. Expression values were analysed for samples from non-treated maize 1507xNK603 in the year 2005 only. Although means of expression values for treated and untreated plants were presented (pooled across the locations) no statistical evaluation was conducted whether there were any differences between the treated and the untreated plants.

In the European field trials with maize 59122 sprayed as well as unsprayed GMPs were included. The expression values were presented as means across locations. However, a statistical evaluation for differences between sprayed and non-sprayed GMPs was not included in the notification. However, the notifier concluded that expression levels were comparable regardless of the herbicide treatment.

2.3.1.2 Tissues analysed

All reviewed notifications contained information on the expression of the inserted transgenes in several tissues. The types of tissues examined varied considerably among notifications (for comprehensive information see Table A4 in the Annex).

In many cases tissues analysed in field trials differed depending on the location where the field trials were carried out. For example, for maize NK603 from European field trials only forage and grain were analysed, while more tissues were studied for samples from trials conducted in the USA. Also, in other notifications expression data for specific tissues were derived from selected sites only (see Table A4 in the Annex). For maize MON810 European data for expression in forage and grain were derived from plants collected from French but not from Italian locations. In contrast, the European data for expression in leaves for MON810 maize were derived from Italian locations only. The lack of comprehensive data on similar tissues from different locations complicates the comparison of expression of the GM trait in certain plant tissues. This is a major drawback with regard to the evaluation of the efficacy of the GMP for target organisms (e.g. in case of Bt maize) or the assessment of potential effects on non-target organisms.

Leaf expression levels were indicated for all maize lines except maize NK603 and NK603xMON810. However, in several notifications the notifiers did not indicate the specific stage at which the expression of the leaf was analysed (e.g. oilseed rape Ms8xRf3, potato EH92-527-1, maize MON810 and for maize Bt11).

In several maize notifications expression of the inserted trait(s) in the whole plant was determined (maize lines 1507, MON810, 1507xNK603 and 59122). However, generally no explanation was available which plant parts were analysed for the 'whole plant' samples. The other maize notifications contained information on the expression levels in forage (maize events NK603, 59122, 1507xNK603, MON810 and MON810xNK603). No information on expression in forage or whole plant was presented in the notification of maize Bt11. The difference between forage and whole plants is not evident as it is generally unclear which parts of the plants were analysed as 'forage'. In one case (maize MON810) data for the whole plant were also labelled as 'forage'. In other notifications, expression levels of forage and of the whole plant were differentiated (maize lines 59122, 1507xNK603).

For four out of seven GM maize events expression data for root tissue were included (maize lines NK603, 1507xNK603, 59122, Bt11). For maize Bt11 the roots were only analysed for PAT expression levels. For maize NK603 root samples were only analysed from US locations but not from European locations. For maize lines 59122 and 1507xNK603 roots were analysed for expression levels of all traits at 5 and 4 growth stages of the GMP, respectively.

Expression levels in maize stalks were also submitted in four notifications only (maize lines 1507, 1507xNK603, 59122, Bt11).

Generative tissues analysed in GM maize lines are pollen, silk and grain. For all GM maize lines data on the expression of the respective transgenes in grains were included. Pollen was analysed in five out of seven GM maize events (not MON810 and MON810xNK603). Expression levels for silk were only contained in the notifications of maize lines 1507 and Bt11.

Expression analysis in potato EH92-527-1 was carried out for samples from the leaves, tubers and root tips as well as immature buds (pollen) and stamens. The trait of this GMP is correlated with a decrease of a protein which is native for potato plants and not with the presence of a novel protein. Therefore data for the amount of the GBSS protein in the amylose fraction of the starch were submitted by the notifier. Determination of the reduction of amylose content in other plant tissues such as root tips, buds, stamina and tubers was done with iodine staining. Additionally the change in starch structure (indicated by the degree of branching) was photospectrometrically analysed in tubers and potato leaves. Potato EH92-527-1 also contains the *nptII* gene under control of a constitutive promotor. Expression of the APH(2')II protein was determined only in fresh leaves. The notifier concluded that expression in other parts of the plant might be possible, but assumed that the protein would be expressed in smaller amounts in the tubers than in leaves.

Data on different expression parameters (e.g. mRNA levels and protein levels) were submitted in different updates of the notification of oilseed rape Ms8xRf3. This GM crop expresses an enzyme (BARNASE, a specific type of RNAse) together with a transgenic protein, which inhibits this enzyme (BARSTAR). The use of a promotor sequence specific to the tapetum cell layers of the an-

thers suggested that expression is restricted to this tissue. The submitted data determined with different methods supported this assumption. Surprisingly, the RNAse was not detected in the male-sterile line (Ms8), which was explained by the notifier with rapid breakdown of the BARNASE gene product in the Ms8 tapetum cells, while in the hybrid the BARNASE and BARSTAR proteins are assumed to be complexed and are therefore detectable.

For maize NK603x MON810 the notifier referred to the single event notifications and submitted only forage and grain expression values for the stacked event. In contrast, for the second stacked event (maize 1507xNK603) expression data for several tissues were submitted (see Table A4 in the Annex).

2.3.1.3 Developmental expression

Developmental expression of inserted traits was differently analysed for the GMPs. Developmental expression in GM maize leaves was analysed for the maize lines 1507xNK603, NK603 (US locations only), 59122 and Bt11 (greenhouse data only). In the first three GMPs four different leaf stages were analysed. For the remaining GMPs the expression in the leaves was recorded at the stages V9, R1, R4 and R6. For a single notification (maize NK603) leaf expression was analysed at the growth stages V2-3, V6-8, V10-13 and at pollination[1]. In the case of maize Bt11 cotyledons, 2nd leaf, 5th leaf, 10th leaf and 15th leaf were analysed separately. For maize MON810 the specific developmental stage used to assay developmental expression was not specified. For oilseed rape Ms8xRf3 the analysed stages were only classified as ‘young’ and ‘mature’.

The analysis of whole plants was carried out using material from different growth stages for maize lines 1507, 1507xNK603 and 59122. Expression in different stages of roots was evaluated for maize lines 1507xNK603, NK603, 59122 and Bt11 (see also above).

2.3.1.4 Expression over several generations

Expression data over several generations were provided for two out of nine notifications. For oilseed rape Ms8xRf3 segregation patterns were evaluated for progeny of Ms8 and Rf3 lines up to the third generation on the basis of flower segregation or herbicide tolerance segregation. In the notification for NK603 maize segregation data were qualitatively evaluated for nine different generations based on glyphosate sprays.

2.3.1.5 Expression in different genetic backgrounds

The expression of the targeted traits was analysed in different varieties to assess whether the introduced traits are stably inherited when the GM trait is crossed into different commercial hybrids. Such an assessment was made in five out of nine notifications (oilseed rape Ms8xRf3, maize lines MON810, Bt11, 1507 and 59122, see Table A4 in the Annex). Only for two GMPs (maize lines

1 For a description of developmental stages see: Hanway, J.J. and S.W. Ritchie. (1984): How a Corn Plant Develops: Special Report No. 48, Iowa State University. http://www.extension.iastate.edu/hancock/info/corn.html.

Bt11 and 59122) the varieties were specified which were used to produce the test hybrids (Table A4 in the Annex). For the majority of notifications (maize lines 1507, NK603, 1507xNK603 and NK603xMON810, potato EH92-527-1) no specific information on the varieties used to generate the test hybrids was included. In some cases (maize MON810 and oilseed rape MS8xRF3) it was indicated that different hybrids derived from different genetic backgrounds were analysed, but without giving details on the specific varieties used.

For maize MON810 the plants tested were either derived from backcrosses with the parental line or derived from crosses with unrelated inbred lines (Mo17 and unspecified commercial inbred lines for the use in Europe). Further details on the breeding history or the commercial hybrids used in crosses were not included. For maize 59122 different backcross hybrids (BC1 and BC4 hybrids) were used for the expression assessment. These lines were derived from crosses with different inbred lines (e.g. Chile: maize line 581; USA and Canada: maize lines 1W2 and 3KP, Europe: inbred maize lines 05F and 581). For the oilseed rape Ms8xRf3 the individual Ms8 and Rf3 lines were bred into different winter or summer oilseed rape lines.

2.3.1.6 Presentation and statistical analysis of expression data

In all notifications the levels of expression of the respective GM traits were not analysed beyond descriptive statistics. Generally, mean expression values were indicated, often across locations of field trials. Specific statistical tests to evaluate potential differences in expression between locations or treatments were generally not applied. Consequently, the variability of expression due to environmental factors, both biotic and abiotic, or due to different herbicide treatments could not be assessed.

In two cases, oilseed rape Ms8xRf3 and potato EH92-527-1, the influence of environmental factors on expression could not be evaluated because no field data were presented. Also in these cases no statistical analysis of expression data was included in the dossiers (see Table 5).

Only in the case of maize 59122 field trials were conducted for more than a single growing season at the Bulgarian locations (see also Table 4). For other notifications (maize lines MON810, 1507, NK603, 59122 and 1507xNK603) data from more than a growing season were submitted, however not from consecutive growing seasons at the same locations, but mostly from trials at different European locations or sites located in different continents. Therefore comparisons of expression values from specific locations over successive growing seasons and hence assessments of the environmental variation at a specific location were not made in any of the notifications.

For all GM events except maize Bt11 the expression values were presented as mean values across locations. Only for maize Bt11 expression data from single locations were presented. Therefore the variability of expression levels between individual plants at a specific site and between locations was not determined.

With regard to herbicide tolerant GMPs data for the assessment of differences in the expression of new traits between GMPs treated or untreated with the respective herbicide were not submitted for most notifications (see also above: ‘treatment of plots’). No indication whether herbicides were applied or a lack of inclusion of either a treated or an untreated variant in the field trials impeded a comparison of treated and untreated GMP plants. A statistical comparison of expression levels in

treated versus untreated GMPs in European field trials was not presented in any of the notifications of GMHT plants.

For stacked GM events a direct comparison between expression of traits in the stacked event and its parental events was only made for one of the stacked events. In case of maize NK603xMON810 the parental GM events were included as control lines during the field trials. Hence, the expression of the transgene expression could be compared to the expression of the respective trait in the parental single event GMOs. However no statistical analysis was employed for this comparison.

2.3.1.7 Expression of potential fusion proteins

Not all notifications included specific data regarding analysis of potential fusion proteins (see Table 5). In the case of the maize lines MON810 and Bt11 the necessary data for a bioinformatics analysis were not included in the notification as adequate sequence information concerning flanking regions was not provided. In case of maize 1507xNK603 and maize MON810xNK603 only references were made to the respective assessments for the individual parental lines.

Generally, the bioinformatics analysis of potential fusion proteins included an assessment of the presence of potential open reading frames (ORFs) at the junction of insert and flanking regions. The sequence information at these junctions was translated into all possible reading frames and investigated for the presence of ORFs of a certain length and the presence of potential regulatory sequences for transcription and translation.
Only in the case of two notifications (potato EH92-527-1, maize 1507) the same method was also applied to the sequence data of the whole transgenic insert. For identified ORFs homologies to known proteins (including toxins and allergens) were assessed.

However, different criteria for the relevance of potential ORFs and the assessment of the results of the bioinformatics analysis were used in the different notifications. For potato EH92-527-1, oilseed rape Ms8xRf3 and maize 1507 reference was made to the guidelines by FAO/WHO (/FAO/WHO, 2001). This guidance document contains guidance for two different tests to assess the potential for allergenicity of specific proteins by homology comparisons with sequences of previously identified allergens. According to the guidance cross-reactivity between the expressed protein and a known allergen has to be considered when there is: 1) more than 35 % identity in the amino acid sequence of the expressed protein using a window of 80 amino acids and a suitable gap penalty (using Clustal-type alignment programs or equivalent alignment programs), or 2) identity of 6 contiguous amino acids. However, the referenced guidelines were not applied for the assessment of potential homologies to allergenic epitopes of short length. In the notifications identical matches to sequences of eight linear contiguous amino acids were scored instead of analysing windows of six amino acids as specified in the FAO/WHO guidance.

Some notifications contained additional data on the possible expression of potential fusion proteins identified by bioinformatics analysis (see Table 5). In the case of potato EH92-527-1 the expression of the identified ORF4 was investigated by immunological analysis of expressed proteins with antisera specific for translated ORF4 sequences. For maize 1507 the transcription of ORFs (ORF3

and ORF4) was analysed by Northern Blots with probes specific for the respective ORFs and by RT-PCR (reverse transcription-PCR).

For two other notifications (maize NK603 and maize 59122) the potential expression of protein variants of the inserted transgenes was assessed in detail. Such fusion proteins can be generated in case of incomplete termination of transcription at the inserted transgenes. For these GMOs Western Blot data were submitted to determine the expression of potential variants of the EPSPS-protein and Cry34Ab1/Cry35Ab1, and PAT proteins respectively.

As can be seen in Table 5 only for certain notifications (oilseed rape Ms8xRf3, potato EH92-527-1 as well as maize lines 1507 and NK603) the detection limit of the methods to identify expression of potential fusion proteins was included.

Table 5. Overview of the data provided necessary for the assessment of potential fusion proteins.

x…submitted in a study conducted by the notifier or by order of the notifier ; N…reference to assessment in other notifications; n.d….no data presented/data missing

GMP	Bioinformatics Analysis	Assessment criteria	Expression Analysis of fusion proteins	Detection Limit
Oilseed rape Ms8xRf3	x (add info 2003)	FAO/WHO, 2001 (overall homologies) Other (epitopes)	Northern Blot	x
Potato EH92-527-1	x	FAO/WHO, 2001 (overall homologies) Other (epitopes)	Western Blot (ORF4)	x
Maize MON810	n.d.	n.d.	n.d.	n.d.
Maize Bt11	n.d.	n.d.	n.d.	n.d.
Maize 1507	x	FAO/WHO, 2001 (overall homologies) Other (epitopes)	Northern Blot, RT-PCR (ORF3+4)	x
Maize NK603	x	n.d.	Western Blot (EPSPS-fusion pr.)	x
Maize 59122	x	n.d.	SDS-PAGE, Western Blot (Cry34Ab1/35Ab1, PAT-fusion pr.)	n.d.
Maize 1507xNK603	N	n.d.	n.d.	n.d.
Maize NK603xMON810	N	n.d.	n.d.	n.d.

2.4 Assessment of agronomic parameters

2.4.1 Studies conducted for the assessment of agronomic parameters

The assessment of agronomic traits of a specific GMP is usually based on field trials in which the GMP is cultivated together with one or several control lines. Field trials for the agronomic assessment are analysed in detail in this report. Emphasis of the analysis is put on field trials and their design conducted in European countries as the focus was put on data relevant for European conditions.

2.4.1.1 Field trials conducted for the assessment of agronomic parameters

In the following certain aspects of the field trials, such as the field trial locations, the characterisation of these locations, the number of growing seasons, the field trial design, the plot sizes used in field trials, the non-GM comparators chosen and the agronomic parameters assessed in these trials are analysed and discussed.

Field trial locations

The notifiers generally refer to ‘locations’ or ‘sites’ when indicating where a field trial was carried out. However, the terms ‘location’ or ‘site’ are not consistently used across notifications. In this report both terms are used synonymously and indicate where a field trial was set up (see also below: ‘characterisation of locations’).

Table 6 gives an overview of the field trials carried out in European countries and shows details on how these field trials were set up.

In most cases field trials for a specific notification were conducted at different locations either in one country or in different European countries or even in different continents. Generally, no rationale or criteria were given for the selection of sites (see below). Field trials in Europe were carried out in one (potato EH92-527-1, maize Bt11), two (maize NK603, 1507xNK603, 59122) or four (oilseed rape Ms8xRf3, maize 1507) European countries. In two notifications no field trials were carried out in European countries (maize MON810, maize NK603xMON810).

Agronomic data from field trials from non-European countries were contained in four notifications (maize NK603, maize NK603xMON810, maize 59122 and oilseed rape Ms8xRf3). Non-European trials included in the notifications were mostly carried out in Canada or the US, but also in Chile (see Table A3 in the Annex)

Table 6. Details of European field trials carried out for the evaluation of agronomic data of GMPs.

n. r. … not relevant;, “-“… no data provided; glu+…treatment with glufosinate; glu-…no treatment with glufosinate; gly+ …treatment with glyphosate; gly-…no treatment with glyphosate; fb…followed by

GMP	Field trials Europe	Locations/ European country	Herbicide applied	Replication	Plot size
Oilseed rape Ms8xRf3	1994/BE	1	glu+	2	6 x 1,8 m (6 rows)
	1995/BE, FR,	1/BE, 1/FR,	glu+	3-4	-

	SE, UK	1/SE, 2/UK			
	2000-1/BE 2001-2/BE	6 (in both years)	glu+/glu-	4	5 x 2 m (6 rows)
Potato EH92-527-1	1993 (-97)[1] /SE	1993: 1	n.r.	-	-
Maize MON810	none	n.r.	n.r.	n.r.	n.r.
Maize Bt11	1995/FR	-	glu-	-	-
Maize 1507	2000/FR, IT, BG	3/FR, 2/IT, 1/BG	glu+/glu-	3	each row 5,2 – 6 m (24-30 seeds); row width 0.75 - 0.8 m
	2002/ES[4]	3	glu-	3	-
Maize NK603	2000-2001/DE	5 (4)[2]	gly-	4	3 x 8 m, 0,75 m row width
	2002/FR	4	gly-	4	
Maize 59122	2003/BG	3	glu-[3]	3 (out of 4)	2-row plots; each row 6 - 7 m length (30 seeds)
	2004/ES, BG	3/BG, 3/ES			
Maize 1507 x NK603	2003/ES, BG	3/ES, 2/BG	gly+ fb glu+	3 (out of 4)	2-row plots; each row 7 m length (30 seeds)
Maize NK603 x MON810	none	n.r.	n.r.	n.r.	n.r.

[1] Field trials from 1993-1997 are cited but data from field trials are only given for the 1993 trials and data for one parameter from the 1996/97 trials (frost sensitivity of tubers), [2] according to the notifier at one location 2 field trials were situated, [3] glufosinate-treated and non-treated GM maize was used in the field trials, but agronomic parameters were assessed in non-treated GM maize plants only, [4]The notifier mentioned two set of field trials in Spain in 2002, but no details on the field trial design is provided. The impression is that at two of the three locations another field trial was set up with two replications, in which the efficacy of maize 1507 was investigated.

Characterisation of locations

Information on where a field trial took place varied significantly between notifications, especially with respect to the regional or local level (e.g. country, region, district or exact location of the field). Some notifiers indicated the name of the town most closely to the field (e.g. Aberdeen, Scotland). Others provided the name of a geographical region without further specification (e.g. Aragon, Spain). Sometimes names for locations were mentioned without indicating whether these names referred to a town or a regional classification and without specifying the geographical position of these locations. Thus it was often not possible to figure out the positions of several field trial locations to each other or distances between them. In several cases where a map of the field trial locations was attached it could be inferred that for instance different locations were actually situated very close to each other. In some notifications the locations of the field trials were not characterised at all (e.g. maize MON 810, maize Bt11, maize NK603 and maize NK603xMON810).

Generally, for the characterisation of field trials the notifiers did not indicate whether different biogeographic, climatic or agricultural regions or certain agronomic or environmental conditions were represented by the locations chosen for the field trials. Only for maize NK603 the notifier stated that field trials conducted in France and Germany represented the ‘northern and southern region of Europe’, respectively.

Except for the name of the location where the field trials took place information provided was mainly restricted to climatic data (see Table 7). In most cases a table was provided indicating the

monthly mean of minimum, maximum and average temperatures as well as average precipitation of the year of the trial. Additionally 'historical' weather data for temperature and precipitation, i. e. the average over a range of years was included. For example, in the notification of maize 1507xNK603 the time period from which the 'monthly mean' was calculated, was indicated for the two locations in Spain (30 years) and Bulgaria (8 years). For the field trial with maize 1507 the 'monthly mean' of the weather stations closest to the respective field site was indicated. The period for the collection of weather data ranged from 5 to 30 years depending on the location. The weather data provided for the agronomic evaluation of oilseed rape Ms8xRf3 (update 2004) was derived from three weather stations and contained minimum and maximum temperatures, precipitation and information on wind for the indicated period (2000-2001). Information on the origin of the climatic data and their measurement period was in some cases not indicated (e. g. EU trials 2003 of maize 59122). Only in two notifications information on the soil type of the field trial locations was included (maize 59122, maize 1507xNK603).

Table 7: Information on the characterisation of EU field trials included in the notifications.

"-"=not data provided; "x"=data provided

GMP	Soil type	Temperature*	Rainfall*	other	Sources
Oilseed rape Ms8xRf3					
1994-1995	-	-	-	-	Technical dossier, Annex (Doc C, Part I)
2000-2002	-	x	x	Air pressure, wind speed, snowfall	Additional info 2004, Annex 5, Oberdöfer 2003 (Appendix B)
Potato EH92-527-1	-	-	-	-	Annexes 18-23, Annex 40
Maize MON810	-	-	-	-	-
Maize Bt11	-	-	-	-	Appendix 11
Maize 1507					
EU 2000	-	x	x	-	Pavely 2002 (Annex 19)
EU 2002	-	-	-	-	Technical dossier
USA 1999	-	-	-	-	Technical dossier
Maize NK603	-	-	-	-	Jacobs et al. 2005
Maize 59122	x	x	x	Map of locations	Buffington 2004, 2005 (Annex 3, 4)
Maize 1507xNK603	x	x	x	Map of locations	Buffington 2004 (Annex 3)
Maize NK603xMON810	-	-	-	-	-

*refers to 'historical' weather data, i.e. average temperature and rainfall data for a specific location over a range of years

Number of growing seasons

In several cases field trials which took place in different European countries were carried out also in different years (e.g. maize NK603, maize 1507). Field trials conducted in the same European country and in consecutive growing seasons were restricted to the notification of oilseed rape

Ms8xRf3 (two years in Belgium) and maize 59122 (two years in Bulgaria). Field trials from more than two growing seasons were generally lacking, except the notification of oilseed rape Ms8xRf3, for which additional field trials in Belgium were conducted in the course of the notification update. The original notification from 1996 contained field trials from the years 1994-1995 and for the updated notification data of additional field trials from 2000 to 2002 were provided by the notifier.

For maize NK603 the Spanish CA requested a revision of the number of growing seasons as only a 2-year trial was conducted in Germany and only one season assessed in France. In its answer the notifier stated that all field trials together (2000-2002) would represent three growing seasons. Thus the notifer claimed to fulfil the requirement of considering several seasons and disregarded the fact that the trials were conducted in different countries. The notifier further argued that the results derived from the German and the French trials (representing the northern and southern regions of Europe, respectively) showed that maize had a similar behaviour in these locations and that thus the growing season would have no meaningful influence on the results.

Design of field trials and herbicide applications

The design of the field trials to assess agronomic parameters of the GM crop corresponded in most cases to a randomised complete block design with at least two treatments (GMO and non-GMO) and usually contained 2 to 4 replications. In some cases 1 of the 4 replications was dedicated to the assessment of other parameters such as expression values. Therefore the number of replications was reduced by one block (e.g. maize 59122 and maize 1507xNK603). Generally, in neither case the notifiers indicated whether the design of the field trials was appropriate to detect differences in the agronomic performance between the GM crop and the non-GM control and what effect size would be detectable by the design chosen for the individual parameters.

The inclusion of GMPs treated with the non-selective herbicide in the field trials in addition to an untreated GMP variant is relevant for GMPs with herbicide tolerance traits such as oilseed rape Ms8xRf3 and the maize lines Bt11, 1507, NK603, 59122, 1507xNK603 and NK603xMON810. Only untreated GM maize plants were used in the notifications of maize Bt11, maize 59122 as well as maize NK603. In the notification of maize 1507 glufosinate was used in the field trials in the year 2000 but not in 2002. In the notification of maize 1507xNK603 three different variants of herbicide treatments of both non-selective herbicides (glyphosate only, glufosinate only, glyphosate followed by glufosinate) were used but only the variant where glyphosate was applied followed by glufosinate was used for the evaluation of agronomic parameters. No untreated GMP variant was included in the trial. For the other stacked maize event no field trials were conducted in Europe. Both variants, i.e. GMPs treated and not treated with the relevant herbicide, were only used in the notifications of oilseed rape Ms8xRf3 (updated notification in 2004 presenting data from 2000-2002) and maize 1507 (2000 data only).

Plot sizes of field trials used to assess agronomic parameters differed among GMP notifications. Also the specification of the plot size varied among notifications. Frequently the length of the plots or rows and the number of rows were indicated. In other cases the number of seeds per row was indicated. Frequently, the length of the plots ranged from 5 to 8 metres and their width from approximately 2 to 3 meters or 2 rows. Plot size differed also within a specific notification between different field trials conducted in different years or at different locations. For example, in the oilseed

rape notification 6 rows were mentioned and a size of 6 x 1.8 m while in the updated notification a size of 5 x 2 m was indicated. In the case of potato EH92-527-1, oilseed rape Ms8xRf3 (1995 data), maize Bt11 and maize 1507 (2002 data) the exact size of the plots of the field trials was not indicated in the information provided by the notifier.

Non-GM comparators (controls)

Control plants used in agronomic assessments were either referred to as 'isogenic lines', 'non-GM hybrids with comparable or similar genetic background' or 'non-GM hybrids with a background representative of the GMO'. Non-GM control plants were not further specified in the case of oilseed rape Ms8xRf3 (1995 data), maize 1507 (2002 data) and maize 1507xNK603). In other cases the hybrid name or variety used as control was stated in the notification (Table 8). In the Annex of this report the specifications of the GMP lines and control lines used in the notifications reviewed for the evaluation of agronomic parameters are displayed (Table A5 in the Annex).

A breeding history of the respective GM crop was only included in the notifications of maize 1507 (2000 field trials) and maize 59122 (Table 8). In the case of maize 1507 the non-GM control seemed to be the parental line of the GM maize. The notifier stated that the GM hybrid had a genetic background equally close to the non-GM elite line and had been obtained through several rounds of backcrossing, selection and selfing. In the case of maize 59122 different maize lines derived from different inbred lines used during the breeding process of the GMO were used as control lines.

Table 8. Specification of non-GM comparators (controls) used in European field trials and indication of the breeding history.

"x"… yes; "-"… no data

GMP	Field trials Europe (year/country)	Specification of control	Indication of breeding history
Oilseed rape Ms8 x Rf3	1994/BE	x	-
	1995/BE, FR, SE, UK	-	-
	2000-2002/BE	x	-
Potato EH92-527-1	1993 (-1997)[1]/SE	x	-
Maize MON810	none		
Maize Bt11	1995/FR	x	-
Maize 1507	2000/FR, IT, BG	x	x
	2002/ES	-	-
Maize NK603	2000-2001/DE	x	-
	2002/FR	x	-
Maize 59122	2003/BG	x	x
	2004/ES, BG	x	x
Maize 1507 x NK603	2003/ES, BG	-	-
Maize NK603 x MON810	none		

[1] Field trials from 1993-1997 are cited but data from field trials are only given for the 1993 trials and one parameter from the 1996/97 trials

2.4.1.2 Agronomic parameters evaluated

In this report the agronomic parameters investigated by the notifiers were classified into four categories:

- plant growth and development
- plant morphology
- plant health
- yield characteristics

This classification is derived from the classification used in the majority of the GMP notifications. With a few exceptions the evaluated parameters are consistently assigned to these categories by the notifiers. An exceptions is, for example, the parameter ‘dropped ears’ which was in one case (maize 1507) classified under ‘plant growth and development’ while it was classified under ‘plant morphology’ in another case (maize NK603xMON810). Similarly, in some notifications the parameters ‘plant vigour’ (maize NK603) and ‘stay green’ (maize 1507xNK603 and maize 59122) were classified under ‘plant health’ and not, as in other notifications, under ‘plant growth and development’. Specification of parameters such as ‘plant vigour’ and ‘stay green’ was held general and the difference between these two parameters was generally not explained in the notifications. Only in one notification (maize 59122) these parameters were explained in more detail. As explained in this notification ‘seedling vigour’ ranged from short plants with small, thin leaves (category 1) to tall plants with large, robust leaves (category 9) while ‘stay green’ correlated from no visible green tissue (category 1) to approximately 90 % green tissue (category 9).

Table A6 in the Annex gives an overview of the agronomic parameters surveyed and their method of evaluation if indicated in the analysed notifications. In Table 9 the agronomic parameters evaluated in the notifications are comparatively listed.

With respect to the method of evaluation some parameters were frequently measured quantitatively, e.g. ‘germination’ or ‘early population’, ‘time to pollen shed or silking’, ‘final population’, ‘plant height’, ‘number of dropped ears’ and ‘yield’. Other parameters were mostly measured qualitatively, such as ‘susceptibility of the plants to herbicides’, ‘insect damage’, ‘disease incidence’ or ‘plant vigour’. A range of parameters was assessed by visual observation (e.g. ‘insect damage’, ‘disease incidence’, ‘stay green’, ‘lodging of plants’, ‘plant vigour’) and by rating on a scale from category 1 to category 9 (e. g. ‘disease incidence’). Certain parameters (e. g. ‘germination’) were in some notifications assessed quantitatively (e. g. in maize) but qualitatively in others (e. g. oilseed rape Ms8xRf3).

Various methods were used for the assessment of ‘insect damage’ and ‘disease incidence’. The qualitative methods ranged from assessing ‘slight or heavy stressors or symptoms’ or noting ‘heat, drought or dry weather’ as a stressor (see notification of maize NK603xMON810) or a visual estimation on a 1-9 scale (see above) to a simple yes/no classification. For example, ‘disease incidence’ was frequently visually estimated following a 1 to 9 scale where 1 corresponded to poor resistance and 9 to high resistance or no visible disease. Quantitative methods like the ‘percentage

of damaged ears', the 'number of larvae per stalk or ear' or the 'length of the tunnels' caused by a specific pest species were less often employed (e.g. maize 1507 and maize NK603).

The parameters 'frost sensitivity', 'foliage size', 'flower colour' and '% amylose in tubers' were specific for the potato EH92-527-1 notification. The parameters 'flower phenotype segregation' and 'seed quality' were evaluated only in the notification of oilseed rape Ms8xRf3. 'Maturity' was assessed in GM oilseed rape and GM potato but not in any of the GM maize notifications. Seed or tuber quality parameters such as oil content or the percentage of amylase were assessed in GM oilseed rape and GM potato only. Parameters specific for maize were 'ear height', 'dropped ears', 'leaf/ear deformities', 'leaf colour/shape' and 'pollen colour/shape'.

As shown in Table 9 agronomic data were collected in field trials at least to some extent in all of the notifications but parameters assessed were not consistent across the notifications. In maize frequently assessed parameters were 'plant growth' and developmental parameters such as 'germination' or 'early and final stand count', 'plant vigour' but also the 'time to pollen shed or silking' or 'lodging' of the maize plants. The parameter 'stay green' was inconsistently classified under 'plant growth and development' (maize 1507, maize NK603xMON810) or under 'plant health' (maize 59122, maize 1507xNK603). With respect to plant morphology, the parameters 'plant height/ear height' were assessed most frequently in maize notifications. Other morphological parameters were less consistently evaluated. 'Plant health', 'insect susceptibility' and 'disease incidence' were assessed in almost all maize notifications, but 'susceptibility to plant protection products' was only assessed in two notifications (maize NK603, maize NK603xMON810). 'Yield' was frequently evaluated in maize notifications and less consistently also the moisture content of the harvest.

Table 9. Type of data presented in the selected notifications for agronomic parameters.

"x" … parameter assessed,"(x)"… evaluation mentioned in the notification, but results are missing, "-"… parameter not assessed

		OSR Ms8 x Rf3	Potato EH92-527-1	Maize						
				NK603	1507	1507 x NK603	Bt11	MON 810	59122	NK603x MON810
plant growth and development	germination/early population/early stand count/emergence and establishment	x	x	x	x	x	-	x[5]	x	x
	plant vigour at various growth stages (e.g. seedling vigour)	x	-	x[1]	x	x	-	-	x	x

	time to pollen shed/GDU; 50% pollen shed; time to silking/GDU; 50% silking; % male/female flowering (maize) / flowering start (OSR)	x	-	x	x	x	-	-	x	x
	Stalk/root lodging/ lodging resistance	(x), x[2]	-	x[1]	x	x	-	-	x	x
	final population/final plant count	-	-	-	x	x	-	-	x	x
	stay green	-	-	-	x	x[1]	-	-	x[1]	x
	maturity (OSR, potato)	x	x	-	-	-	-	-	-	-
plant morphology	plant height, ear height	x	-	x	x	x	x	-	x	x
	dropped ears	-	-	-	x[3]	-	-	-	-	x
	leaf or ear deformities	-	-	x	-	-	-	-	-	-
	leaf colour, leaf shape	-	-	x	-	-	x	-	-	-
	pollen shape, pollen colour	-	-	-	-	x	-	-	x	-
	foliage size	-	x	-	-	-	-	-	-	-
	flower colour	-	x	-	-	-	x	-	-	-
	flower phenotype segregation	x	-	-	-	-	-	-	-	-
	anthocyanin coloration of different plant parts	-	-	-	-	-	x	-	-	-
	different morphological parameters of ear, tassels[4]	-	-	-	-	-	x	-	-	-
plant health	insect damage	x	-	x	x	x	-	-	x	x
	disease incidence	x	-	x	x	x	x	-	x	x
	susceptibility to insecticides, fungicides, herbicides	x	-	x	-	-	-	-	-	x
	frost sensitivity	-	x	-	-	-	-	-	-	-
yield character-istics	yield	x	x	x	x	-	-	-	x	x
	moisture	-	-	x	x	-	-	-	-	x
	grain density	-	-	-	x[5]	-	x	-	-	-
	seed quality (OSR); % amylose (potato)	x	x	-	-	-	-	-	-	-

[1] classified under 'plant health'/ evaluated as a 'general plant health' parameter, [2] assessed but results are only indicated in the additional information delivered in 2004, [3] classified under 'plant growth and development', [4] e.g. length of the main axis of the tassel, shape and length of ear, [5] only evaluated in non-EU field trials

Assessment of pest susceptibility and disease incidence

Insect damage, pest susceptibility or pest abundance were evaluated under European conditions in five notifications (maize lines 1507, NK603, 59122, 1507xNK603 and potato EH92-527-1; Table 10). For the potato EH92-527-1 no results on insect pest or disease evaluations were provided in the original notification except a statement that no evidence was found in the trials that the GM potato was more susceptible or resistant to several diseases or pest species. In the additional information delivered by the notifier in 2006, field studies on non-target organisms carried out in 2004 were submitted which contained also data on the abundance of several pest species. In the notifications of oilseed rape Ms8xRf3 and maize Bt11 the assessment of insect damage was indicated but no data were presented in the notification. In the notification of maize MON810 no EU field trials were conducted.

In the notifications in which infestation rates of pests were evaluated this was done mostly by visual observation and by rating on a 1-6 or 1-9 scale, where 1 stands for poor resistance and 6 or 9 for high resistance against a certain pest species (see Table 10)

Only in two notifications also quantitative methods were used (maize NK603, maize 1507). In the notification of maize NK603 at one location (France 2002) the number of borer larvae per stalk or ear, the length of the borer tunnelling in the stalk or ear and the percentage of damaged ears were evaluated. In one trial of the notification of maize 1507 (Spain 2002) the lengths of insect tunnels were measured.

For some field trials no exact information was given with respect to the pest species evaluated (e.g. maize 1507: 2000 field trials, 2002 field trials: infestation assessment). In two notifications the European corn borer (*Ostrinia nubilalis*) was evaluated (maize 1507, maize NK603). In these two notifications also the Mediterranean corn borer (*Sesamia* sp.) was evaluated at one location each. For the potato EH92-527-1 (2004 field trials) several pest species were covered in the assessment (see Table 10).

Table 10. Insect damage evaluated in European field trials.

n. r. … not relevant; "-"… no data provided

GMP	Field trial (year/ country)	Parameter assessed	Method	Insect pests assessed
Oilseed rape Ms8xRf3	1994/BE	-	-	-
	1995/BE, FR, SE, UK	-[1]	-	Aphids, flea beetles, pollen beetles
	2000-2002/BE	-	-	-
Potato EH92-527-1	1993/SE	-[1]	-	potato cyst nematodes, aphids, leafhoppers[2]
	2004/SE, DE, NL	abundance	Pitfall, beating, sticky traps	Aphids, Thrips, Colorado potato beetle, Auchenorrhyncha
Maize MON810	no EU trials	n.r.	n.r.	n.r.

Maize Bt11	1995/FR	-[1]	-	-
Maize 1507	2000/FR, IT, BG	infestation	1-9 visual estima-	-
	2002/ES	infestation insect tunnels	1-9 visual estimation tunnelling length in stalks in cm	- *O. nubilalis*, *Sesamia* spp. (2 of 3 sites) [3]
Maize NK603	2000/DE	infestation difference to other insects	1-6 visual estimation yes/no classification	O. nubilalis -
	2001/DE	Infestation Difference to other insects	1-6 visual estimation yes/no classification	O. nubilalis (3 sites) - (3 sites)
	2002/FR	abundance of larvae (for each pest) borer tunnels (for both pest species) % damaged ears (for both pest species)	no of larvae per stalk/ear length of borer tunnelling in stalk or ear	O. nubilalis (4 sites) *Sesamia* sp. (3 sites)
Maize 59122	2003/BG 2004/ES, BG	infestation	1-9 visual estimation	-
Maize 1507xNK603	2003/ES, BG	infestation	1-9 visual estimation	-
Maize NK603x MON810	no EU trials	n.r.	n.r.	n.r.

[1] indicated that it was evaluated but no further data provided, [2] only descriptive results are given, [3] at one site 50% of larvae were identified as *O.nubilalis* and 50% as *Sesamia* spp.; at the other site 5% were identified as *O.nubilalis* and 95% as *Sesamia*.spp.

The occurrence of diseases was evaluated in four GM maize notifications (maize lines 1507, NK603, 59122, 1507xNK603) using either a 1-9 scale for a visual estimation or a simple yes/no classification. Only in one notification at one location (maize NK603, France 2002) the percentage of plants infected was evaluated and the infestation severity of one specific disease assessed. In all other notifications the assessed diseases were not specified (Table 11).

Table 11. Disease incidence evaluated in European field trials.

n. r.…not relevant; "-"… no data provided

GMP	Field trial (year/ country)	Parameter assessed	Method	Diseases assessed
Oilseed rape Ms8xRf3	1994/BE	-		-
	1995/BE, FR, SE,	-[1]	-	-
	2000-2002/BE	-	-	-
Potato EH92-527-1	1993/SE	-	-	(late blight, early blight, Erwinia rots, other bacterial diseases) [2]
Maize MON810	no EU trials	n.r.	n.r.	n.r.
Maize Bt11	1995/FR	-[1]	-	-

Maize 1507	2000/FR, IT, BG	disease incidence	1-9 visual estimation	-
	2002/ES	disease incidence	1-9 visual estimation	-
Maize NK603	2000/DE	difference in susceptibility	yes/no classification	-
	2001/DE	difference in susceptibility	yes/no classification	- (3 sites)
	2002/FR	% plants infected (both diseases) % infestation severity (fusariosis only)	visual estimation	fusariosis Sphacelotheca reiliana (only 1 site)
Maize 59122	2003/BG, 2004/ES, BG	disease incidence	1-9 visual estimation	-
Maize 1507xNK603	2003/ES, BG	disease incidence	1-9 visual estimation	foliar diseases (not further specified)
Maize NK603x MON810	no EU trials	n.r.	n.r	n.r.

[1] indicated that it was evaluated but no further data provided, [2] only descriptive results are given

A statistical evaluation was not consistently carried out in the notifications in order to assess whether a GMP was less or more susceptible to a pest species or a particular disease than its non-GM counterpart. A statistical analysis was only carried out with the data from one field trial of the maize NK603 notification (France 2002), from one field trial of the maize 1507 notification (Spain 2002) and from the field trials of 2004 of the maize 59122 notification (Spain and Bulgaria 2004; see also next chapter).

2.4.1.3 Statistical analysis and presentation of results

In general, there was no consistent presentation of agronomic results in the technical dossiers of the different notifications. Depending on the number of locations and countries where field trials were conducted for a specific GMP different ways of calculating data and presenting results were chosen. Mean values of the agronomic parameters were calculated per location or per country or, more frequently, across all locations – independent whether these were located within one country or in several countries.

Also no consistency was evident for the statistical analysis of the agronomic data. Statistic evaluation of agronomic parameters differed between notifications but also between field trials within a specific notification. Often only few agronomic parameters were statistically analysed at all in order to test for any differences in the agronomic traits between the GMO and its non-GM counterpart. It was generally not clear why some parameters of a specific field trial were statistically analysed while others were excluded from the analysis. Statistical comparisons of agronomic parameters between different herbicide treatment variants of GMPs were generally not included. Also comparisons of agronomic parameters between stacked maize events and their parental lines were not carried out.

Presentation of results

In the technical dossiers of the notifications (corresponding to Part I according to the notification structure recommended by EFSA) the agronomic parameters were usually presented in tables indicating mean values of the evaluated parameters across all field trial sites. Data on individual field trial sites were often contained in the annexes of the notifications or selectively presented in the technical dossier. For example, in the technical dossier of maize 59122 the results of the agronomic evaluations were presented as means across locations (e.g. separately for Europe 2003 and Europe 2004). For the 2003 data means across locations but not results of the individual locations were presented in the technical dossier. The 2004 data were presented in the technical dossier also on an individual location basis but restricted to some agronomic parameters only (germination, plant and ear height and final population). Detailed results on a per location basis for the individual locations in 2003 and 2004 were presented in the annex. Data for the individual countries (e.g. mean per Bulgaria or Spain) were neither aggregated nor analysed.

Table 12 summarises the presentation and evaluation of the results of the agronomic parameters derived from field trials carried out at European sites. Means of the evaluated agronomic parameters were calculated and presented either on a per location basis, a per country basis or across all sites and countries. However, this was not consistently done across the notifications. Not in all notifications these means were compared between GM and non-GM plants using statistical tests (see also ‘statistical analysis’).

In those notifications where field trials were carried out at several locations **in several European countries** usually mean values across these countries (i.e. all European locations) were presented for a specific agronomic parameter (maize 1507: 2000; maize 59122: 2004; maize 1507xNK603: 2003 see Table 12). For the 1995 field trials of oilseed rape Ms8xRf3 mean values of 3-4 replications were indicated but it was unclear whether these results referred to the individual locations or across all locations.

In cases where means across several locations located in more than one European country were presented, the results were also presented on a per location basis but not on a per country basis (e.g. maize 1507: 2000 trial; maize 59122: 2004 trials and maize 1507xNK603).

In cases where field trials were carried out at several locations in **one single European country**, mean values across locations were calculated on a per country basis (oilseed rape Ms8xRf3: 2000-2002; maize 59122: 2003 and maize 1507: 2002). No means per country were calculated for oilseed rape Ms8xRf3 (1994 and 1995 data), maize Bt11, maize 1507 (2000 data), maize NK603, maize 59122 (2004 data) and maize 1507xNK603. In the case of potato EH-92-527-1 data were only provided for the 1993 field trials carried out in Sweden, but it is unclear if these were derived from 1 or 2 locations and if the values presented are means and refer to one or several locations.

Results from agronomic field trials **on a location basis** (i.e. for each location within each country) were mostly contained in the annex only.

Statistical analysis

It was not always explicitly indicated in the notifications whether the agronomic data were statistically analysed or not and which tests were applied (e.g. maize 1507: 2002 data). No statistical

evaluation in order to detect any differences in agronomic parameters between the GM and the non-GM plant was carried out in the case of oilseed rape (1994/1995 data), potato EH92-527-1, maize Bt11 and maize 1507 (2000 data). In order to justify the lack of statistical evaluation the notifiers mostly argued that only qualitative data were collected (e.g. maize Bt11).

Results of agronomic evaluations were statistically compared between the GMP and the respective control lines in the case of oilseed rape Ms8xRf3 (2000-2002 data), maize NK603, maize 59122, probably maize 1507 (2002 data) and maize 1507xNK603. If results were presented on a per location basis, the GMP and its relevant control line were compared. If results were presented as means across locations means for the GMPs of all locations were compared to means of the control lines of all locations. Generally not all agronomic parameters which were measured in the field trials were also subject to a statistical analysis but only parameters which were quantitatively assessed were also statistically analysed (e.g. plant and ear height, time to silking, time to pollen shed, early and final population). Notifiers did usually not state why only certain parameters were statistically analysed or not. Only in the case of maize NK603 the notifier stated that only replicated measurements were subject to a statistical test. In no case, it was indicated whether the data were suitable to carry out this kind of statistical test, e.g. whether the underlying data corresponded to a normal distribution.

In the original oilseed rape Ms8xRf3 notification (1994/1995 data) no statistics were applied to the data. In later field trials the data basis was improved and parameters such as plant growth and development, plant height and yield parameters were statistically analysed.

No statistical analysis was provided for the data for agronomic parameters derived from field trials with the Potato EH92-527-1 conducted in Sweden in 1993.

In the notification of maize MON810 the notifier argued that MON810 has been tested in Europe at 18 test sites since the years 1993/1994. The notifier stated that data collected from these trials included agronomic characteristics (vigour, disease, insect susceptibility). However, none of these data and analyses was included in the notification. Instead germination rates from US field trials of five sites were included without statistical evaluation. In addition the USDA petition for determination of non-regulated status of MON810 (Croon et al. 1995) was cited. This document was not included in the notification.

The agronomic parameters in the maize Bt11 notification were qualitatively assessed using a questionnaire. This way of assessment was based on descriptive observations. The data were not statistically analysed.

In the notification of maize 1507 no statistics were applied on the agronomic data in the year 2000 trials. For the trials carried out in 2002 in Spain it remains unclear whether statistics were used, as no statistical test was indicated in the notification but significantly differences (P-values) were marked in the tables presenting the results.

In the case of maize NK603 only replicated measurements carried out for the parameters ‘% male/female flowering’, ‘plant height’, ‘ear height or deformation’, ‘% lodging’, ‘insect and disease incidence’ and ‘yield’ were subject to a statistical analysis. Non-replicated measurements (e. g. yes/no classifications for susceptibility to plant protection products) were not statistically analysed.

The statistical evaluation of insect and disease parameters was restricted to the field trials carried out in France 2002.

In the technical dossier of maize 59122 not all agronomic data collected in 2003 and 2004 were statistically analysed in order to test for differences between the GM maize and the control lines. Several parameters ('stalk and root lodging', 'stay green', 'disease incidence', 'insect damage' and 'pollen viability') were not subject to a statistical analysis. The notifier justified this lack of statistical evaluation by the statement that no apparent differences between the GM maize and the comparator were identified.

In the notification of maize 1507xNK603 agronomic parameters were only collected from the herbicide treatment variant treated with glyphosate followed by glufosinate. Also in this case only some agronomic parameters such as 'plant and ear height', 'time to silking and pollen shed', 'early and final population' were statistically analysed. No statistical analysis was done for the parameters 'stalk lodging', 'root lodging', 'stay green', 'insect damage' and 'disease incidence'. The notifier justified this on account of no apparent differences in the visual measurements of these parameters.

Agronomic assessment of the stacked maize NK603xMON810 was only conducted in the USA and no European field trials were presented.

Comparisons of agronomic parameters of the two stacked maize events to their respective parental lines were not carried out (maize NK603xMON810, maize 1507xNK603).

Comparisons of herbicide treated and untreated variants of the GM crops were only considered in the case of oilseed rape Ms8xRf3. For the data of 2000 of maize 1507 both herbicide treatment variants were applied but no statistical comparisons carried out. In the other notifications of GMPs with herbicide tolerance traits (maize Bt11, maize 1507: 2002 data, maize NK603, maize 59122, both stacked maize lines) both herbicide treatment variants were not included thus making a statistical comparison impossible.

Table 12. Presentation of results of agronomic data derived from European field trials.

n.r. … not relevant (either because no European data are available or because they are only generated in one European country; "-"… no data; (?)…unclear/not indicated.

GMP	Years: no. of European countries/no. of locations	Mean across countries (all European locations)	Mean per country (all locations in a country)	Mean per location	Statistical analysis	Herbicide treatement differences considered
Oilseed rape Ms8xRf3	1994: 1/n.i.	n.r.	-	(?)[1]	-	- (glu+ only)
	1995: 4/1+1+1+2	(?)[1]	-	(?)[1]	-	- (glu+ only)
	2000-2001: 1/6 2001-2002: 1/6	n.r.	x	x	ANOVA[4]	x
Potato EH92-527-1	1993: 1/1-2[8]	n.r.	(?)[8]	(?)[8]	-	n.r.
Maize MON810	no EU trials[5]	n.r.	n.r.	n.r.	n.r.	n.r.
Maize Bt11	1995: 1/n.i.	n.r.	-	-	-[3]	- (glu- only)
Maize 1507	2000: 3/3+2+1	x	-	x	-	-[6]
	2002: 1/3	n.r.	x	x	unclear, P-	- (glu- only)

					value indicated	
Maize NK603	2000-2001: 1/4	n.r.	-	x	Student-Newman-Keuls test [2]	- (gly- only)
	2002: 1/4	n.r.	-	x		
Maize 59122	2003: 1/3	n.r.	x	x	ANOVA[4]	- (glu- only)
	2004: 2/3+3	x[4]	-	X[4]		
Maize 1507xNK603	2003: 2/2+3	x[4]	-	x[4]	ANOVA[4]	- (gly+ fb glu+ only)[7]
Maize NK603x MON810	no EU trials	n.r.	n.r.	n.r.	n.r.	n.r.

[1] unclear whether mean across countries or per location, [2] for replicated measurements only, [3] only qualitative data, [4] selected parameters only, [5] only one parameter from US trials was assessed ('germination'), but no details/separate study was provided; [6] no statistics applied; [7] glyphosate followed by glufosinate-ammonium. [8] Data only provided for the field trial in Sweden 1993 (unclear whether 1 or 2 locations; unclear whether values indicated are means).

2.4.1.4 Interpretation of agronomic data by the notifier

Frequently, the results of the agronomic evaluations were not specifically discussed in the notifications. Efficacy data demonstrating the product performance of the GMP were usually not presented. In the notification of maize 1507 the notifier concluded that the differences between the GMP and the control observed in terms of less insect damage, higher yield and less stalk lodging confirmed effective resistance of the GMP against attack from the target pest.

Agronomic results were used by the notifier for the statement that the GMP was 'comparable' to the non-GM control plant or that 'no unexpected changes between the GMP and the non-GMP' were detected. Independent of the parameters assessed or the results obtained the notifiers usually concluded that no phenotypic differences or unexpected agronomic differences between the GMP and the non-modified control were displayed or that the GMP had no altered survival, multiplication or dissemination characteristics (e.g. maize 1507). The GMP was consequently usually considered to be 'comparable' – or even 'equivalent' – to the non-GMP.

If statistically significant differences were observed between the GMP and the non-modified control in the analysis across locations (i.e. data pooled across locations), the notifier argued that these differences were not observed in the individual location analysis. Significant across location differences were thus dismissed by the notifiers as 'not occurring consistently at all locations' (e.g. maize NK603, maize 1507xNK603). On the other hand, if statistically significant differences were observed at individual locations (e.g. maize 59122: field trials 2004) such differences were also not considered to be meaningful by the notifiers as they were 'not consistently observed in the across all locations analysis'.

Any observed or statistically significant differences between the GMP and the control were commonly ascribed to small differences in the genetic background of the hybrids and not to the genetic modification of the GMP (e.g. maize NK603, maize 1507). Statistically significant difference were also considered to be numerically small and within the normal biological variability expected for maize or not to be of biological significance (e. g. maize NK603, maize 1507xNK603). In other notifications the notifiers stated that differences in the seed quality may account for the observed differences (maize NK603, maize 1507).

The agronomic results of the two stacked maize event notifications were not compared with those of their respective single event parental lines and thus no arguments for their similarity provided.

In the case of potato EH92-527-1, for which agronomic field trials were described but not exact data were provided, the notifier argued that the genetic modifications did not indicate any possible changes in sensitivity or resistance of the GMP (possibly meant against pests and diseases) and therefore no specific studies were conducted. Also the lack of any 'unexpected cases' of survival or persistence during the field trials was mentioned.

In the case of oilseed rape Ms8xRf3 the notifier argued that due to the absence of any change in the glucosinolate content of the GM oilseed rape no change in pest or disease resistance level would be expected. Although in this notification greenhouse and field studies to evaluate pest susceptibility and disease incidence were referred to (e.g. 1995 data), results of these studies were not contained in the notification.

In summary, if statistically significant differences were observed the following arguments were used by the notifiers to justify their evaluation that the observed differences were 'not biologically significant' because:

- they were due to small differences in the genetic background
- they were due to differences in seed quality
- they were not agronomical meaningful
- they were within normal biological variability
- differences in the across location analysis were not observed in the individual location analysis or only at a few of the respective locations
- differences which were not observed at every location were dismissed as not consistently observed across locations

2.5 Assessment of plant composition

2.5.1 Studies conducted for the evaluation of plant composition

The data for the compositional analysis were generally gained from field trials carried out by the notifiers at one or several locations. Often these field trials were also used to generate data on expression levels and agronomic performance of the GMP as well (see Table A3 in the Annex). In some cases separate field trials were conducted for the assessment of the plant composition of the GMP (e.g. potato EH92-527-1, maize Bt11) or field trials for the assessment of expression and composition combined (e.g. maize 1507: 1999 data, maize NK603, maize NK603xMON810, see also Table A3 in the Annex).

In the case of maize Bt11 the data for the compositional analysis were gained from numerous field trials for which only limited information was provided in the notification. Compositional data were derived from eight different sets of field trials, mostly located in the USA. In these trials a considerable variation is given with respect to the GMP lines and control lines used, the locations chosen

(greenhouse and field sites), the parameters assessed, the tissues analysed and the statistics applied.

2.5.1.1 Compositional parameters evaluated

Table 13 gives an overview on the compositional parameters assessed in the notifications. Details on fatty acids, amino acids and minerals are omitted here because they are not considered as primarily relevant for environmental interactions. These parameters were considered in detail already by Spök et al. (2003a, 2003b). Table 14 presents the information contained in the notifications with respect to the analysis of compositional data in grain (maize), tuber (potato) or seed (oilseed rape) focusing on secondary metabolites and anti-nutrients as these were considered to be of major ecological relevance.

Table 13. Overview of compositional parameters assessed in the GMP notifications.

Tissues Analysed	Parameter
Forage	Proximates: carbohydrates, crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, moisture
	Calcium, phosphorus
Grain/Seed	Proximates: carbohydrates, crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, moisture
	Oil content (oilseed rape only)
	Fatty acids
	Amino acids
	Minerals
	Vitamins (beta-carotene, B1, B2, B6, E, folic acid)
	Secondary metabolites (raffinose, inositol, 2-furaldehyde (furfural), ferulic acid, p-coumaric acid)
	Anti-nutrients (phytic acid, trypsin inhibitor for maize; glucosinolates, phytic and erucic acid for OSR)
Tubers (potato only)	Ash, protein, fat fibre (digestible fibre), starch (starch composition), carbohydrates, dry matter, sugars (fructose, glucose, saccharose), dry matter
	Chlorogenic acid
	Vitamin C
	Minerals
	Anti-nutrients: Nitrate
	Toxins: Glycoalkaloids (solanine, chakonine, total glycoalkaloids)

For GM maize the secondary metabolites analysed were inositol, raffinose, furfural, p-coumaric acid and ferulic acid (maize 1507, maize 59122, maize NK603xMON810). In the notification of maize 1507xNK603 the same parameters were analysed but raffinose was classified as an anti-nutrient. In the notifications of maize lines MON810, Bt11 and NK603 no secondary metabolites were analysed at all (see Table 14). Anti-nutrients analysed in GM maize notifications were phytic acid and trypsin inhibitors except from the notification of maize MON810. For maize Bt11 an analysis of these two anti-nutrients was mentioned in the update of 2003 but the data were not included. For the oilseed rape Ms8xRf3 the content of glucosinolates as well as erucic acid levels were analysed as anti-nutrients. For the potato EH-92-527-1 compositional data derived from the 1996 field

trials on glycalkaloids and nitrate were presented. In response to questions from the Scientific Committee on Plants the notifier attached additional data from field trials carried out in 1997 and 1998, however, without giving information on the design of the field trials (annex 29 of the update). This annex also included a statistical analysis of the compositional data from the field trials of all three years.

Table 14. Secondary metabolites and anti-nutrients presented in the compositional analyses of GMPs.

n.r. = not relevant; "-"= no data presented; PHY = phytic acid, TRY = trypsin inhibitor, RAF = raffinose, INO = inositol, FUR = furfural, P-Cou = p-coumaric acid, FER = ferulic acid; GLY = glyco-alcaloids, NIT = nitrate, GLU = glucosinolates, ERU = erucic acid; glu+ = glufosinate applied; glu- = glufosinate not applied; gly+ = glyphosate applied; gly- = glyphosate not applied; fb = followed by

GMP	European field trials	Secondary metabolites	Anti-nutrients	Herbicide treatment
Oilseed rape Ms8xRf3	1994/BE 1995/UK, BE, FR	n.r.	2 (GLU, ERU)	glu+
	2001-2002/BE	n.r.	3 (GLU, PHY, ERU)	glu+/glu-
Potato EH92-527-1	1996-1998/SE	1 (GLY)	1 (NIT)	n.r.
Maize MON810	1995/FR, IT	-	-	n.r.
Maize Bt11	1998/FR	-	2 (PHY, TRY)[1]	glu-
Maize 1507	1999/FR, IT	5 (INO, RAF, FUR, FER, P-Cou)	2 (PHY, TRY)	glu+(IT only)/glu-
	2000/FR, IT, BG	5 (as above)	2 (as above)	glu+/glu-
Maize NK603	1999/FR, IT	-	2 (PHY, TRY)	gly+
Maize 59122	2003/BG 2004/BG, ES	5 (INO, RAF, FUR, FER, P-Cou)	2 (PHY, TRY)	glu+/glu-
Maize 1507 x NK603	2003/BG, ES	4 (INO, FUR, FER, P-Cou)	3 (RAF, PHY, TRY)	gly+; glu+; gly+ fb glu+
Maize NK603xMON810	2000/FR	5 (INO, RAF, FUR, FER, P-Cou)	2 (PHY, TRY)	gly+

[1] data referred to in the update 2003 but not included

2.5.1.2 Design of field trials for the assessment of plant composition

Since compositional and agronomic evaluations were often combined in the same field trials, the designs of the field trials were often the same for both evaluations (e.g. maize 1507: 2000 data, maize 59122, maize 1507xNK603: 2003 data). The aspects of field design were already discussed in the assessment of agronomic parameters (see 2.4.1.1).

The field trials conducted for the generation of compositional data varied considerably between notifications or even between locations within a specific notification with respect to the herbicide-treatment variants (e.g. inclusion of a herbicide treated and an untreated GMP variant), the compositional parameters assessed, and the control plants grown at a specific location (e.g. non-GMPs or additionally other commercial non-GM hybrids). For example, in the case of maize 1507, in addition to the GMP and the non-GM control a GMP treated with the non-selective herbicide was included only at the Italian locations but not at the French locations in the 1999 field trials (see Table 14). As another example, in the oilseed rape notification phytic acid was included in the field trials from 2001-2002 but not in earlier field trials.

For the potato EH-92-527-1 information on the design of the field trials for the compositional data on glycalkaloids and nitrate of 1996 and field trials carried out in 1997 and 1998 were not presented although the data were referred to in Annex 29 of the ERA update (see also 2.5.1.1).

2.5.1.3 Statistic analysis and presentation of results

Comparators used

The results of the compositional assessment of the GMPs were compared to those of the control plants across locations in a first step. In cases where significant differences across locations of individual parameters were observed between the GMP and the control, compositional values were also compared between GMP and control on a per location basis.

In addition to the respective non-GMP grown at the same location as the GMP, compositional values derived from conventional varieties grown either at the same location or at other locations in other years were used to establish a 'range' for each compositional value. According to the notifiers this 'range' should represent the 'baseline for the consideration of natural variation' of a specific compositional parameter.

For example, in the case of maize NK603, the ranges for certain parameters obtained from commercial hybrids grown at the same location as the GMP/non-GMP or at other locations and/or in earlier trials by the notifier were used for comparison purposes. These ranges were defined as 'commercial range' (for EU field trials) or as 'historical/commercial/reported range' (for US field trials).

For maize NK603xMON810 'tolerance levels' were calculated which were composed of the values of other commercial non-GM hybrids grown at the same site combined with additional data from other commercial varieties grown elsewhere and in other years. Similarly, also in the notification of maize 59122 'tolerance intervals' were calculated. According to the notifier these intervals contained '99 % of the values expressed in commercial Pioneer maize hybrids'.

Frequently also compositional data from the published literature were used to establish a 'literature range'. This range comprised compositional values from the respective non-GMPs (maize, oilseed rape, potato) which were derived from different published studies. These ranges were - depending on the parameter - either based on one (e. g. maize 1507) or on several publications (e. g. maize 1507xNK603, maize 59122). In the notification of maize 1507 (data in Annex 19, Pavely 2002, n.st.) the literature range of certain compositional parameters was based on one literature citation, but for some parameters complemented by additional literature data (e.g. cystein, tyrosine). In the notification of maize 59122 compositional values were compared to 'normal ranges of variation for commercial maize'. This 'normal range' was represented by a combined range derived from diverse literature data (Watson 1982, Watson 1987, Codex Alimentarius 2001, ILSI 2006, OECD 2002a). In the notification of maize 1507xNK603 the following references to generate a 'combined range' were cited: OECD (2002a) and ILSI (2004) for secondary metabolites, Watson (1982), OECD (2002a), ILSI (2004) for anti-nutrients. For maize NK603 (EU field trials) the literature ranges were composed of Watson (1982) and Jugenheimer (1976) for some compounds and Watson (1987) for others.

Evaluation of differences due to herbicide application

Differences in the compositional values in the GMPs due to herbicide treatments were evaluated in only two (oilseed rape Ms8xRf3, maize 1507) out of seven GM crops with a herbicide tolerance trait (see Table 15).

In the oilseed rape Ms8xRf3 notification, total glucosinolates were assessed in the two herbicide treatment groups in the EU field trials 2001-2002. The notifier discussed the observed differences between GM (treated and untreated) and non-GM oilseed rape with respect to these anti-nutrients but not between the treated and the untreated GM variants.

In the maize 1507 notification, in addition to the non-treated GMP, a herbicide treated variant was included only at the Italian locations of the 1999 field trials. Potential effects of the herbicide treatment on the compositional values were evaluated. The notifier concluded that spraying with glufosinate-ammonium did not have an effect on the nutrient composition of maize forage or grain. Although in the field trials of 2000 a herbicide treated variant was also additionally inlcuded, statistics were only applied to test for differences between the GMP and the control, but not for differences between the herbicide treated and untreated variants.

In the other notifications of herbicide tolerant crops potential effects of the herbicide treatment on the levels of anti-nutrients or secondary metabolites were not assessed, either because not both herbicide treatment variants were included in the field trial designs or because no statistical analysis was made testing for differences between the treated and the non-treated variant (see Table 15).

Evaluation of compositional differences between stacked and single event GMPs

For the stacked maize NK603xMON810 the notifier stated that as additional control the respective single events (maize NK603 and MON810) were also grown and analyzed for their composition as part of the compositional study. However, the results were designated as ‘supplementary information’ and were not presented in the notification of the stacked maize. Instead, the notifier referred to the single event applications of maize lines NK603 and MON810. Similarly, the compositional values of the maize 1507xNK603 were also not compared to the respective single event maize lines.

Table 15. Significant differences between GMPs and non-GMPs in composition of secondary metabolites (or toxins in case of potatoes) and anti-nutrients.

n.r…..not relevant, i.e. parameters were not assessed; - ….no data provided,. x… significant difference found at one or more locations or in herbicide treatment variants (only maize 1507xNK603); o …. no significant differences, L = baseline composed from published literature; CH = baseline composed from non-GM commercial hybrids; glu+ = glufosinate applied; glu- = glufosinate not applied; gly+ = glyphosate applied; gly- = glyphosate not applied; fb = followed by

GMP	European field trials (years)	Secondary metabolites/toxins	Anti-nutrients	Baselines	Differences due to herbicide treatment
Oilseed rape Ms8xRf3	1994-1995	n.r.	x[1]	-	- (only glu+)
	2001-2002	n.r.	x	L	x
Potato EH92-527-1	1996-1998	x[2]	x[2]		n.r.
Maize MON810	1995	n. r.	n. r.		n.r.
Maize Bt11	n. r.[3]	n.r.	n.r.	-	- (only glu-)
Maize 1507	1999	o	o	L	x (IT)
	2000	x	o	L, CH[5]	-

Maize NK603	1999	x[4]	n. r.	L, CH	- (only gly+)
Maize 59122	2003	x	x	L, CH	-
	2004	x	x		
Maize 1507xNK603	2003	x	x	L	- (only glu+;gly+; gly+ fb glu+)
Maize NK603xMON810	2000	x	x	CH	- (only gly+)

[1] There is no indication if and what statistical analysis has been carried out; the significant difference is mentioned in the conclusions of meal quality; [2] not significantly different if yield as covariant is taken into account; [3] no relevant parameters were assessed in the field trials conducted for compositional assessment with Bt11 maize; assessment of 2 anti-nutrients mentioned in trials in France 1998, but no data provided; [4] 2 sites were not statistically evaluated; [5]only for the mineral calcium and the amino acid glutamic acid the literature range was complemented by a range calculated from 22 commercial Pioneer Brand Hybrids.

2.5.1.4 Interpretation of data on anti-nutrients and toxins by the notifiers

Significant differences between GMPs and non-GMPs in certain compositional parameters were found when analysed across locations or for individual locations (Table 15, Table A7 in the Annex). Observed statistically significant differences in anti-nutrients or secondary metabolites across locations were generally considered not relevant if they were not also consistently observed on a per location basis or if they were shown to fall within the values derived from a commercial or literature range or the calculated 'tolerance levels' (e. g. maize 59122, maize 1507xNK603, maize NK603xMON810).

In the case of maize NK603 levels of phytic acid were significantly higher at one site at French but not at Italian locations. These significant differences were considered as false positives and not considered relevant by the notifier as they were not consistently observed at all locations. For two other locations in France no statistical evaluation was carried out.

For maize 1507xNK603 a significant difference in inositol levels was observed across locations for two out of three herbicide treatment variants investigated (see Table 15). To follow up this result the notifier analysed the data for each location separately and still found significant differences in inositol levels at individual sites: for the application of glyphosate (gly+) at two of the five locations and for the application of glufosinate (glu+) at one out of the five locations. A similar picture was displayed for raffinose values. A significant difference between GM maize and the control was found across locations in the glyphosate treatment variant (gly+) and in the glyphosate followed by glufosinate (gly+ fb glu+) treatment variant. A statistically significant difference was observed at one individual location out of five locations for the glyphosate (gly+) treatment variant but no statistical difference was observed at any of the individual locations for the other herbicide treatment variant (gly+ fb glu+). Overall, the mean values for raffinose and inositol were within literature ranges and the notifier concluded that the stacked GM maize was comparable to non-GM maize.

The significant differences in glucosinolate values and apparent higher values in the oilseed rape Ms8xRf3 compared to the non-GM control were reported in the original notification of oilseed rape Ms8xRf3 although it was not indicated whether a statistical analysis had been carried out. In the updated data provided in 2004 again a higher glucosinolate level was observed in GM oilseed rape. The notifier cited glucosinolate values from the literature to conclude that observed values of GM oilseed rape are within the literature range. Additionally, the notifier calculated that the ob-

served increase in glucosinolates in GM oilseed rape corresponded to approximately 15 % and that this marginal increase would have no nutritional relevance as glucosinolates are destroyed during processing.

The levels of glycoalkaloids (chakonine, solanine) were significantly lower and nitrate was significantly higher in the GM potato EH92-527-1 than in the control. The notifier re-calculated these values taking yield as a covariant into account. This re-calculation of the data resulted in no significant differences between the GM and the non-GM potato. The notifier argued that higher nitrate contents in GM potato were to be expected as the amount of accessible nitrogen in the soil was correlated to the amount taken up by the plants. However, no information on nitrogen levels in soils where the GMPs were grown was indicated. The notifier concluded that the difference disappeared if all values over different years were compared thus assuming that the genetic modification did not influence the nitrate content of potato EH92-527-1. With respect to glycoalkaloids the notifer referred to the significantly lower glycoalkaloid levels in the GM potato if results from several years were taken into account. Despite these observed differences the notifier concluded that the genetic modification did not affect the level of this component in potato EH92-517-1. In conclusion the notifier related all the observed differences to the (intended) inhibition of the amylose synthesis without giving any further explanation.

2.6 Assessment of traits with relevance for dissemination and related processes

In the following the information provided in the analysed notifications relating to reproduction, dissemination, and survivability will be discussed separately from the assessment of the potential of the GMP to persist, invade or exhibit a potential selective advantage or disadvantage (including gene flow).

2.6.1 Assessment of reproduction, dissemination and survivability

Generally, specific studies for the assessment of differences of the GMP compared to the non-GMP with respect to dissemination, persistence or invasiveness were not conducted by any of the notifiers except in the oilseed rape Ms8xRf3 notification. The conclusions of the notifier on reproduction, dissemination, and survivability of the GMP were largely drawn on the basis of the agronomic assessment, the assessment of compositional equivalence or other assessments (Table 16). In the following the information and arguments submitted for the assessments of dissemination, persistence, and invasiveness will be discussed.

GM maize

In all notifications analysed the assessment of potential differences in reproduction, dissemination or survivability between the GM maize and the non-GM maize was based on the evaluation of agronomic traits of the GMPs in field trials (see Table 16). Generally, reference is made to specific agronomic parameters. The lack of observed differences between the GMP and the non-GMP in the following agronomic parameters was used in order to conclude that the GM maize is also not different to the non-GM control with respect to reproduction, dissemination, and survivability:

- **General agronomic characteristics**: reproductive morphology (Bt11, MON810), seedling vigour/plant stand count (NK603xMON810, 1507xNK603), plant maturity (MON810), vegetative vigour (MON810), stalk/root lodging (59122, NK603xMON810), plant/ear height (NK603xMON810, 59122), final population (59122), stay green (59122, NK603xMON810), disease incidence/insect damage (59122), stressor symptoms (NK603xMON810).
- **Flowering characteristics**: time to pollen shed/silking (NK603, 59122, NK603xMON810, 1507xNK603, 1507), pollen production (1507, 1507xNK603, 59122), pollen colour/shape (59122, 1507xNK603), tassel and silk features (MON810), synchronous pollen shed/silk emergence (MON810).
- **Seed characteristics**: seed production (Bt11, 59122, 1507xNK603), seed viability or germination (1507xNK603, 59122, 1507), ear deformities and barren plants (NK603), yield (MON810, NK603, NK603xMON810), germination (MON810), grain density (1507), seed maturity (MON810).

Notifiers of GM maize argued that, based on the agronomic assessment, the agronomic traits were generally not affected by the genetic modification. In all cases of GM maize the notifiers concluded that the agronomic characteristics, and therefore reproduction, dissemination and survivability of the GMP were not different compared to the non-GMP. In cases where the agronomic evaluation revealed differences between the GMP and the non-GM comparator, the notifier stated that these differences were not considered to be of biological significance as they were not consistently observed on a per location basis (e. g. maize 59122).

In some cases, however, a lack of difference between the GM and the non-GM maize in specific agronomic traits was cited but not substantiated by data, neither in the chapter discussing reproduction, dissemination and survivability, nor in the chapter presenting data on the agronomic assessment (e. g. maize MON810, maize NK603xMON810). In other cases it was unclear to which agronomic traits the notifier actually referred to, e. g. ‘reproductive morphology’ in the case of maize MON810 or ‘stressor symptoms’ in the case of maize NK603xMON810 as these were not mentioned in the agronomic assessments of the respective GMP.

As an example, the only data submitted in the notification of maize MON810 were data on germination of the GMP tested at five US field locations (see also chapter 2.4). The assessment of several other agronomic parameters cited (general agronomic, flowering or seed characteristics) was not substantiated by specific data. The notifier frequently cited the USDA petition for a non-regulated status of MON810 maize (Croon et al. 1995) in order to support some of these parameters cited (e.g. vegetative vigour, maturity). However, no data were provided which allowed verification of the statements. In addition, the notifier stated that no specific trials were conducted to assess pollen production or pollen dispersal of the GM maize. Instead the notifier referred to the ‘unchanged reproductive morphology’ of maize MON810, again without presenting data.

Two notifiers referred to the compositional equivalence of the GMP to the non-GMP (maize NK603xMON810, maize NK603) in order to demonstrate the lack of any differences of reproduction, dissemination or survivability of the GMP compared to the non-GMP.

Notifiers also argued that certain agronomic traits were not expected to be affected by the genetic modification (e. g. maize NK603xMON810, maize Bt11). As a consequence out-crossing frequency was considered unlikely to be different or no changes in seed dissemination would be expected. Some notifiers simply stated that the GMP did not show different ways of dissemination or differences in dissemination compared to the non-GMP (e. g. maize 1507, maize 1507xNK603) without referring to a specific assessment.

General statements were made such as ‘maize seeds cannot be disseminated without human intervention’ (assessment of dissemination) or ‘maize has a lack of dormancy and no traits for weediness’ (assessment of survivability). The notifications of maize 1507, maize 1507xNK603, maize 59122 and maize Bt11 referred to published studies in order to substantiate such statements (Table 16). Published studies on the following topics were cited to substantiate this argumentation:

- Dispersal/viability/deposition/settling rate of maize pollen (e. g. Raynor et al. 1972, Canadian Food Inspection Agency 1994)
- Seed dispersal of maize (e. g. Rissler & Mellon 1993)
- Lack of weediness traits of maize (e. g. Baker 1974)
- Sensibility of maize seed to temperature (e. g. Shaw 1988, Craig 1977)

GM potato

In the notification of potato EH92-527-1 observations of the following flower and tuber characteristics were mentioned in order to demonstrate that traits relevant for reproduction, dissemination or survivability of the GMP were unaffected by the genetic modification:

- **Flowering characteristics**: pollen production, flower frequencies, fruit setting
- **Tuber characteristics**: tuber formation, frost tolerance

The assessment of some of these traits was not substantiated by data. In the agronomic assessment only maturity, flower colour and frost sensitivity were assessed and data presented (see 2.4).

GM oilseed rape

In the original notification of oilseed rape Ms8xRf3 the following plant parameters were referred to in order to demonstrate that the GMP is not different from the non-GMP with respect to dissemination, reproduction and survivability.

- **General plant characteristics**: development of structures facilitating transport, vegetative growth
- **Flowering characteristics:** pollen dispersal
- **Seed characteristics**: seed dispersal, seed germination, seed shattering, shape and size of seeds, yield

Yield and germination were substantiated by data in the agronomic assessment (see 2.4). Data on the seed germination ability of oilseed rape Ms8xRf3 were provided although these tests included

different combinations of the GM oilseed rape lines Ms1, Rf1, Ms8 and Rf3 without containing specific information on the hybrid Ms8xRf3.

For the assessment of pollen dispersal the notifier referred to a gene flow model from the notification of oilseed rape Ms1xRf1. The notifier concluded that no differences in dispersal ability between GM oilseed rape Ms1xRf1 and non-GM oilseed rape were observed and thus dispersal of transgenic plants is identical to what is known for traditional oilseed rape varieties.

Reference to the absence of differences between GM and non-GM oilseed rape with respect to seed parameters such as seed shattering, shape and size of seeds, morphological or physiological parameters of the seeds and plant parameters such as vegetative growth were made, although no data were provided supporting this conclusion.

In the updated ERAs of 1999 and 2003 seed dispersal and pollen dispersal of the GM oilseed rape were again discussed. For **seed dispersal** the notifier referred to:

- Field trials of AgrEvo/PGS (later BCS) since 1990 (no data or references provided)
- Results from Dr. Sweet and collaborators since 1995 (no data or references provided). These results might correspond to annexes 8 and 9 provided by the notifier as a bulk of annexes in 1998. Annex 8 was a paper presented at a symposium on weeds but did not deal with Ms8xRf3 oilseed rape. Annex 9 was a publication with no indication of the journal and no indication which GM oilseed rape was dealt with. In the 2003 ERA update reference is made to 'NIAB' projects on seed dispersal citing two published studies (Norris et al. 1999, Astoin et al. 2000).
- Large-scale monitoring in Canada (no data or references provided). These results might correspond to Annex 12 of the additional information provided in 1998, consisting of surveys for GM canola and weedy relatives with the GM traits in Canada over two years.

Based on these references the notifier concluded that the distribution of seeds and spillage could occur up to several kilometres on roads and that post-harvest and seed loss from grain transport represented the main mechanism for long range dispersal of oilseed rape, regardless of its transgenic nature.

For **pollen dispersal** the notifier referred to:

- Published studies on cross pollination by wind and honey bees (Mesquida et al. 1982, Corbet et al. 1981, Jay 1986; updated by further references in 2003)
- Experiments conducted in the framework of the PROSAMO, BAP and BRIDGE projects (field trials in 1990 and 1994 in the UK, 1992-1993 in Belgium). Only summaries of the results of these field trials were included in the ERA but no details or references.
- Results of pollen dispersal studies by 'NIAB' and pollen flow studies from 1995-1997 by the University of Munich without providing a reference or specific data (only a summary).
- Further experiments studying pollen dispersal carried out either by different consortia and institutions (PROSAMO, BRIDGE, CETIOM, BBA, MAFF, University of Manitoba etc.) and referring to published studies (update 2003 only). No details on these studies were

given but outcrossing rates of oilseed rape for certain distances derived from these experiments were listed in a table.

Based on these references the notifier concluded that according to these studies cross pollination declined with distance and that long distance outcrossing due to bee pollination was likely to occur at traces only.

Survivability of the GM oilseed rape was specifically addressed in the updates of 1999 and 2003 in the assessment of persistence, invasiveness and selective advantage (see 2.6.2).

2.6.2 Assessment of persistence, invasiveness and potential selective disadvantages/advantages

GM maize

In the GM maize notifications specific assessments of persistence and invasiveness as well as of potential selective disadvantages or advantages of the GMP compared to the non-GMP were generally not carried out. Notifiers backed their argumentations either by referring to the agronomic assessments or by citing published studies (see Table 16).

Common arguments referred to the general biology of maize stating that:

- Maize is not known to be inherently persistent in the field or invasive into natural environments
- Maize cannot persist as a weed
- Maize has no traits for weediness
- Maize has poor dormancy
- Maize can occasionally persist from one growing season to the other only under favourable conditions

The following published studies were cited to back these argumentations:

- Maize as a volunteer/weed removal in agriculture (Shaw 1988, OECD 2003, Hicks & Thomison 2004, Finke et al. 1999)
- Lack of persistence of maize as a volunteer (OECD 2003)
- Occurrence of maize volunteers in set-aside fields (Bodet et al. 1994, Mamarot & Rodriguez 1994)
- Lack of weedy characteristics of maize (Baker 1974)
- Lack of invasiveness of maize (Canadian Food Inspection Agency 1994)

The expression of Cry-proteins (in Bt maize) or of proteins conferring herbicide tolerance to GM maize were considered not to change the above-mentioned inherent characteristics of the maize plant by the notifiers but this was generally not substantiated by specific data. This argumentation was in many cases also supported by the argumentation that phenotypic, agronomic or reproductive traits, survival and dissemination of the GMP was unaffected by the genetic modification (as

shown in other assessments; e. g. maize NK603, NK603xMON810) or that no biologically significant phenotypic differences were observed in the GM maize plant that could alter the biological fitness of the GM maize (e.g. maize NK603).

Further arguments included:

- Volunteers can easily be identified and controlled by herbicides and manual/mechanical removal (e. g. maize 59122, maize 1507xNK603).
- The fitness of the F2 generation of GM-maize (all hybrid seed) is generally poor because there is no 'breed true' of F1 cultivars (maize NK603, maize NK603xMON810).[2]
- The 'high unlikeliness' that GM-maize is more persistent in the field or more invasive in natural environments than traditional maize (e. g. maize Bt11, maize NK603xMON810).

Arguments particularly with respect to herbicide tolerant maize included:

- The selective advantage of the GM maize is limited and only effective under specific conditions in the field with negligible consequences to natural environments.
- Herbicide tolerant traits do not provide a selective advantage of the GM maize in areas not treated with the broad spectrum, non-selective herbicide.
- The advantage of the GM herbicide tolerant plant over weeds in the field is not ecologically meaningful when viewed in the context of today's baseline agronomic practices (e.g. maize NK603, maize NK603xMON810).
- The application of glufosinate-ammonium does not commonly occur outside the agricultural environment and does not confer a selective advantage outside the agricultural environment or managed habitats (e.g. maize 59122, maize 1507, maize 1507xNK603).

Arguments particularly with respect to insect resistant maize included:

- The introduced proteins do not confer any selective advantage to the plants in the natural environment, i.e. outside the agricultural environment.
- Insect attack is considered as only one of multiple biotic and abiotic factors that prevent growth of maize outside of agricultural environments. Therefore the expression of Cry-proteins conferring resistance to the target organism is not considered to be a selective advantage (e.g. maize 59122, maize 1507 and maize 1507xNK603).

2 The notifiers probably referred to the "heterosis effect", a higher performance of the F1 generation (the maize hybrid) than the parental (inbred) lines. As maize volunteers constitute the F2 generation they would exhibit a reduced fitness compared to the parental hybrid.

GM potato

The assessment of persistence and invasiveness as well as potential selective advantage of potato EH92-527-1 was argued by the comparability of the establishment and development of the GM potato to non-GM potatoes evaluated in field trials. Although the notifier referred to such field trials carried out from 1993 to 1995 and cited an Annex of the notification, this Annex did not contain any data on these field trials. Further arguments included that only the quality and not the quantity of starch had been modified and that the conferred kanamycin resistance did not constitute an advantage for the plant although the latter argument was also not substantiated by data.

GM oilseed rape

Persistence and invasiveness were considered to be unchanged for oilseed rape Ms8xRf3 by the notifier.

The following arguments were provided in order to support this conclusion relating to the **herbicide tolerance** trait of the GM oilseed rape Ms8xRf3:

- Transgenic oilseed rape will not create more volunteer problems than non-GM varieties in field margins or agricultural fields with unintended drift of glufosinate ammonium. These volunteers will be managed by good management practices.
- In oilseed rape fields GM oilseed rape will be managed by other herbicides (than the non-selective herbicide).
- In unmanaged or semi-managed environments non-GM oilseed rape will survive and continue to compete with the GM oilseed rape.
- The introduced herbicide tolerance trait does not confer any selective advantage in the absence of treatment with the non-selective herbicide, e.g. in wild habitats.
- There are no indications of an increased persistence or competitiveness of glufosinate tolerant oilseed rape in wild and semi-managed habitats were no selective pressure is present.

The following argument was provided in order to support this conclusion relating to the **male sterility/fertility restorer** trait:

- The constructs will not give any selective advantage to the GMP over the wild plant (although it is stated that male sterility alone will rather confer a disadvantage to the multiplication of a plant)

In the original notification of 1996 the notifier referred to the agronomic data (see 2.4) and an evaluation of competitiveness of GM oilseed rape. Competitive behaviour of oilseed rape Ms8xRf3 was assessed by a competitiveness evaluation comparing GM oilseed rape to certain weeds and a cereal volunteer. In this study, parameters such as the number of emerging plants, establishment and plant vigour were assessed. Parameters were observed by scoring (weediness score: 0-9) but not statistically analysed. Furthermore the notifier referred to the documentation package of Ms1 and Rf1 oilseed rape and stated that there were no reasons to assume that the incorporation of the

bar, *barnase* and *barstar* gene constructs would change the colonization capacity of transgenic oilseed rape or would give transgenic oilseed rape any selective advantage.

In later updates of the notification and the ERA (1999, 2003) reference to several published and unpublished studies was made.

With respect to **persistence and invasiveness** of GM oilseed rape the notifier referred to the following studies:

- Studies on seed dispersal by the notifier and several other institutions (see 2.6.1).
- Studies on colonization and establishment by citing:
- Studies conducted by the PROSAMO group in the UK without giving any specific data or a reference (summary of results only).
- PGS field trial monitoring (no data, no reference).
- Competition experiments performed in field trials in Belgium and Denmark (no data, no reference). This information may correspond to the Annex 2 of the 1998 additional information provided by the notifier. Annex 2 of this update contained information on seed dispersal and persistence of BAP post trial monitoring studies carried out in Belgium but did not provide any data and did not indicate with GM oilseed rape was used (Annex 2, Part2, Annex II.3). The other study included (Annex 2, Part II, Annex II.6) contained information on field trials on the competitiveness of transgenic oilseed rape in Denmark and Belgium. In these field trials either the GM oilseed rape used was Ms1/Rf1 or the GM line was not indicated.
- Monitoring results of NIAB of feral oilseed rape at roadsides in the UK (no reference, no data given).
- AgrEvo/PGS surveys in Canada of non-agricultural areas in 1996 and 1997 (no reference, only summary of results). This summary may be identical with the study provided as Annex 12 in the additional information provided in 1998 consisting of an interim report of AgrEvo presenting results of surveys for GM canola volunteers and weedy relatives with the GM trait.
- Field experiments of ADAS Terrington in the UK testing the competitive advantage of glufosinate tolerant oilseed rape (no data, no reference). This may be identical with Annex 2, Part 2, Annex II.4. of the 1998 additional information. However, this Annex provided only conclusions without giving any details on the experiments.
- Experiments conducted by NIAB on effects of the herbicide on oilseed rape plant populations in field margins (summary of data, no reference).
- Experiments conducted by NIAB in 1995 and 1996 on the incidence and persistence of oilseed rape volunteers across the UK (summary of data, referring to Norris et al. 1999).
- AgrEvo Canada monitoring of the occurrence and fate of glufosinate tolerant oilseed rape in 1996-1997 in cultivated fields (summary only, no data, no reference).

- Additional published studies provided in the 2003 update of the ERA.
- Additional results from project carried out in Europe by different institutions (BRIGHT, CETIOM, University of Halle etc.) summarizing the preliminary results (no data given) and partly supported by referring to published studies (update ERA 2003).

With respect to a potential **selective advantage/disadvantage** of GM oilseed rape the notifier referred neither to unpublished nor to published studies (in the ERA update of 1999 and 2003).

As neither a clear reference was made for most of the above mentioned studies nor original data or details of these studies were presented, it remains unclear which of these studies actually dealt with the GMO in question and would be of relevance for Ms8xRf3 oilseed rape.

Table 16. Data presented in GMP notifications for parameters relevant for spread and establishment of the GMP in the environment.

A…. notifier refers to agronomic assessment; I….notifier refers to assessment of invasiveness; Y …. Notifier study; L…reference to published literature; C…. reference to compositional equivalence, N…. reference to assessment in other notifications; " –"….no data presented.

GMP	Reproduction, Dissemination, Survivability	Persistence & Invasiveness / Selective Advantage / Disadvantage
Oilseed rape Ms8xRf3	A, I, N[1]	A, Y, L, N[1]
Potato EH92-527-1	A	-[4]
Maize MON810	A[3], L[2]	-
Maize Bt11	A, L	L
Maize 1507	A, L	L
Maize NK603	A, C	A, C, L
Maize 59122	A, L	L
Maize 1507xNK603	A, L	L
Maize NK603xMON810	A, C	A

[1] The notifier referred to notification C/UK/94/M1/1 (GM oilseed rape Ms1/Rf1); [2] reference to USDA Petition for MON810; [3] only 1 parameter was assessed ('germination'); [4] field trials to assess establishment and development of plants were cited but no data provided

Assessment of gene flow

Specific information or data generated for the particular GMP was generally not presented in GMP notifications in order to address the **co-existence issue** and gene transfer from the GMP to non-GMPs (see Table 17). In some maize notifications literature was cited in order to argue that pollen dispersal of maize was limited and depended on a range of environmental parameters (maize NK603, 1507xNK603, NK603xMON810). In other notifications gene flow to the same species was not discussed at all or no literature provided (see Table 17).

In the case of potato EH92-527-1 the notifier stated a lack of differences in flower morphology between the GMP and the non-GMP although it was not clear if this parameter was assessed in the agronomic evaluation of this GMP (see 2.4). Gene flow from GM potato to non-GM potato was not considered to be relevant by the notifier as cross-pollination and production of true seed was considered a very unlikely event.

In the notification of oilseed rape Ms8xRf3 in the updated ERA of 1999 and 2003 the following pollen dispersal studies were referred to:

- Published studies on cross pollination by wind and honey bees (Mesquida et al. 1982, Corbet et al. 1981, Jay 1986, updated by further references in 2003).
- Pollen dispersal studies in the framework of the PROSAMO, BAP and BRIDGE projects (field trials in 1990 and 1994 in the UK, 1992-1993 in Belgium). However, only a summary of the results of these field trials was included in the ERA but no details or references. Some of these experiments may correspond to information provided in 1998 as additional information. However, as these data were not cross-referenced it was not possible to deduce which studies were actually conducted and which results were relevant.
- Further experiments studying pollen dispersal carried out either by different consortia and institutions (PROSAMO, BRIDGE, CETIOM, BBA, MAFF, University of Manitoba etc.) and referring to published studies (update 2003 only). No details on these studies were given but outcrossing rates of oilseed rape for certain distances derived from these experiments were listed in a table.
- Results of pollen dispersal studies by 'NIAB' and pollen flow studies from 1995-1997 by the University of Munich without providing a reference or specific data (only summary). The latter report probably corresponded to Annex 10 of the additional info provided in 1998, a report of the results of field trials conducted in Germany on pollen flow and seed dispersal of GM oilseed rape.

On the basis of this information the notifier concluded that pollen spread between GM oilseed rape and its wild relatives or non-GM oilseed rape can only be controlled through isolation measures at small scale. At the same time the notifier stated that this would not be possible when oilseed rape was grown at large scale. The notifier further concluded that according to these studies cross pollination declined with distance and that long distance outcrossing due to bee pollination was likely to occur at traces only.

With respect to the issue of **outcrossing of the GMP to wild relatives** no specific assessments were made in notifications of GM maize. The notifier stated that no other cultivated or wild plant species were sexually compatible with maize within the European Union. Gene flow of GM potato to wild species of potato was not considered relevant in the respective notification, since potato was not sexually compatible with any other species of the Solanaceae in Europe supported by a range of published studies. The notifier concluded that hybrids of GM potato and non-GM potato should not have a competitive advantage. However, no specific evidence was provided to support this statement.

In the oilseed rape Ms8xRf3 notification of 1996 cross pollination of GM oilseed rape to wild relatives was discussed by referring to the documentation package of GM oilseed rape Ms1xRf1. The notifier stated that the male sterile line Ms8 did not produce any pollen while the fertility restorer line Rf3 and the fertile restored hybrid combinations (Ms8xRf3) did not differ in their pollination capacity. Without providing any data the notifier concluded that:

- Outcrossing to *B. napus* wild relatives is very unlikely under natural conditions.
- In case outcrossing to wild relatives occurs the environmental impact is considered as small as the *barnase* and *barstar* genes or the phosphinothricin resistance will not confer a selective advantage to recipient crucifers.
- In agricultural areas phosphinothricin resistant crucifers will be controlled d by suitable herbicides.

In conclusion the consequences of gene transfer of the introduced traits of GM oilseed rape to wild relatives were considered to be negligible by the notifier.

In the updated ERA of 1999 further published studies on successful outcrossing of oilseed rape to several wild relatives (Scheffler & Dale 1994) were mentioned. Additionally, the notifier stated that hybridization between *B. napus* and certain wild relatives (*B. rapa*, *B. juncea*, *H. incana*, *R. raphanistrum*) in the field had been observed and that intermediate hybrids between transgenic *B. napus* and these wild relatives revealed stable expression of the transgene and in some cases also silencing of expression. However, no further reference or data were provided which would make this statement more comprehensible.

In the updated ERA of 2003 further published study on the probability of successful pollination between oilseed rape and certain wild relatives were provided by the notifier. The notifier discussed in particular the possibility of interspecific hybrids of *B. napus* with *B. rapa*, *B. juncea*, *H. incana*, *R. raphanistrum*, *S. arvensis* and *B. nigra*.

In the 2003 ERA update also **multiple herbicide tolerant volunteers** were discussed by the notifier referring to monitoring studies in France by different institutions (the ‘inter-institutional trials’). Only a summary of the results was provided but no details or references. The possibility of establishment of multiple herbicide tolerant volunteers and the occurrence of multiple resistances as a major concern for farmers was addressed by the notifier. The notifier cited further published studies concluding that multiple tolerant oilseed rape could be treated with herbicides routinely used in cereal crops. The notifier further emphasized the need for a ‘good agricultural practice’ in order to minimize the occurrence of multi-tolerant volunteers.

The **consequences of outcrossing** and expression of the new traits in weedy relatives was further addressed by the notifier in the 1999 and 2003 ERA update:

- Assuming that no selective advantage will be conferred by the *barnase* or *barstar* gene to recipient plants in managed and unmanaged environments.
- Assuming that no selective advantage will be conferred by the herbicide tolerance trait in unmanaged habitats where the herbicide is not used.
- Assuming that a selective advantage will be conferred by the herbicide tolerance trait to recipient plants (wild relatives) in semi-managed and managed environments if glufosinate-ammonium is applied. The occurrences of transgenic wild relatives will be controllable with other available herbicides. Thereby no unmanageable herbicide tolerant relatives will develop. In order to support this assumption further studies were cited:

- Monitoring of spontaneous gene transfer from transgenic oilseed rape to other *Brassica* species by FORBIOSICH (no data, no reference, summary only).
- Monitoring of outcrossing in France by different institutions (e.g. CETIOM etc, no data, no reference, summary only),
- Monitoring of cultivated areas by AgrEvo (later: BCS) Canada in 1996 and 1997 for the occurrence of wild relatives (no data, no reference, summary only).
- New references of published studies generally showing no selective advantage or disadvantage of transgenes in a wild relative (*B. rapa*)

As a conclusion the notifier stated that even if the GM traits established in wild populations, this would not necessarily lead to a negative impact on the ecosystem. Furthermore the notifier referred to the AgrEvo/PGS stewardship program and a code for field management as well as an educational program to promote best practice among farmers.

Gene flow from plants to bacteria

Gene flow from plants to bacteria was generally not largely discussed in GM maize and the GM oilseed rape notifications and usually addressed only in short. The notifiers argued that:

- No elements were inserted in the maize known to be involved in DNA mobility/gene transfer and thus no changes are to be expected in the ability of the GMP to exchange genetic material with bacteria.
- There is no known mechanism for or demonstration of DNA transfer from plant to microbes under natural conditions.
- The introduced genes do not represent a risk to human or animal health or a plant pest risk.
- The GMP does not enable the transfer of genetic material to bacteria.

No data or published studies were submitted in the GM maize notifications in order to substantiate these arguments, except for Bt11 maize. In the case of Bt11 a published study was submitted assessing the potential of DNA uptake of a soil bacterium under natural conditions. Also in the GM oilseed rape notification update of 2003 reference was made to published studies which evaluated horizontal gene transfer of DNA to soil bacteria.

In the case of potato EH92-527-1 the notifier submitted an analysis assessing the presence of DNA fragments of the *nptII* genes in pulp, fruit juice and fruit water as well as a test of degradability of the APH(3')II protein and NPTII protein in ruminal fluid. Additionally, a study assessing kanamycin resistance in bacteria in soil was added. Published studies on horizontal gene transfer to bacteria in the digestive tract were also cited by the notifier.

Table 17. Information presented in GMP notifications for the assessmment of gene flow.

A…. notifier refers to agronomic assessment; Y…study conducted by the notifier or on behalf of the notifier ; L….reference to assessment in published literature; N…reference to assessment in other notifications; "-"…..no data/literature presented.

GMP	Gene flow to	Gene flow to wild	Consequences of gene	Plant to bacteria gene

	same species	relatives	flow	transfer
Oilseed rape Ms8xRf3	Y, L	N[1], Y, L	Y, L	L
Potato EH92-527-1	A	L	L	Y, L[2]
Maize MON810	-	-	-	-
Maize Bt11	-	L	-	L[3]
Maize 1507	-	-	-	-
Maize NK603	L	-	-	-
Maize 59122	-	-	-	-
Maize 1507 x NK603	L	-	-	-
Maize NK603xMON810	L	-	-	-

[1] The notifier refers to notification C/UK/94/M1/1 (GM oilseed rape Ms1xRf1); [2] Discussed by notifier in the toxicity assessment; [3] in Bt11 update 01(2003)

2.7 Assessment of effects mediated via target organisms

2.7.1 Studies conducted for the assessment of effects on target organisms

Generally, target organisms were only identified in GMPs with an insect resistant trait (*Bt* maize). In notifications of herbicide tolerant GMPs, no target organisms were identified. For GMPs with an insect resistance trait in addition to one or more herbicide tolerance trait(s), target organisms were only identified for the former trait (maize lines Bt11, 1507, 59122, 1507xNK603, NK603xMON810).

In the cases of oilseed rape Ms8xRf3 (traits: herbicide tolerance, male sterility, fertility restoration) and potato EH92-527-1 (trait: changed starch content) the notifiers did not identify or describe any target organism(s).

The target organisms of the maize lines MON810, Bt11, 1507, 1507xNK603 and NK603xMON810 expressing the Cry1Ab or Cry1F toxin were considered to be lepidopteran pests by the notifiers (see Table 18). For maize 59122, expressing the Cry34Ab1/Cry35Ab1 toxin, the larvae of rootworms (Coleoptera: *Diabrotica* sp.) were considered as the target organism by the notifier.

Table 18. Target organisms of the GMPs expressing a Bt toxin as specified in GMO notifications.

GMP	Cry protein expressed in GMP	Target organism specified by the notifier
Maize MON810	Cry1Ab	European corn borer (*Ostrinia nubilalis*) and certain lepidopteran pests such as pink borer (*Sesamia*).
Maize Bt11	Cry1Ab	European corn borer and maize-feeding Lepidoptera; lepidopteran pests*
Maize 1507	Cry1F	Certain lepidopteran pests such as ECB and *Sesamia* spp.
Maize 59122	Cry34Ab1/ Cry35Ab1	In general: Corn rootworm larvae (Coleoptera: Chrysomelidae; *Diabrotica* spp.) In EU: Western corn rootworm, *D. virgifera virgifera*.
Maize 1507xNK603	Cry1F	certain lepidopteran insect pests such as the European corn borer and *Sesamia* spp.
Maize NK603xMON810	Cry1Ab	European corn borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

* Notifier listed other lepidopteran maize pests occurring in the UK: Acronicta rumicis, Agrotis exclamationis, Agrotis segetum, Autrographa gamma, Agrotis ipsilon

Generally, in the notifications of GMPs conferring insect resistance the specificity of the respective *Bt* toxin was argued by providing a range of literature studies (Table 19). The notifiers of the stacked events additionally referred to the specificity assessments of the *Bt* toxins in the corresponding single event notifications. The notifications of maize Bt11, 1507, 1507xNK603 (for 1507) and maize 59122 contained specific studies conducted by or on behalf of the notifiers with an assessment of specificity to target organisms of the respective introduced proteins (Cry1Ab, Cry1F, Cry34Ab1/Cry35Ab1, Table 20).

Potential secondary pests, i.e. pest species replacing the target organism and being insensitive against the respective toxin, were not considered in any of the notifications. In the notification of maize Bt11 the notifier discussed secondary infestations of maize by pathogens such as *Fusarium* sp. due to damage by Corn borers and its relevance for food and feed safety. Literature on the beneficial effects of *Bt* maize on *Fusarium* infection was also provided in this notification.

Effects due to changes in the food or prey availability because of the loss of the target organism were not considered in any of the notifications, except maize Bt11. In this notification, literature was provided discussing the reduction of parasitoid populations of the European corn borer eggs due to the reduction of the target organism.

Resistance development of the target organisms was considered in all notifications of GMPs which contain an insect resistance trait (maize lines Bt11, 1507, 1507xNK603, 59122, MON810 and MON810xNK603) and an insect resistance management plan as a case-specific monitoring measure included (see 2.11).

Table 19. Overview of the studies conducted to assess the specificity of the introduced gene/trait and the consideration of potential secondary pests as well as effects such as resistance development and lack of food or prey availability.

T…study conducted by the notifier or on behalf of the notifier ; L…reference to assessment in published literature; N…reference to assessment in other notifications; -….no data presented/data missing; n.r. = not relevant (GMO does not contain the trait); HT = herbicide tolerance; IR = insect resistance; Y = yes

GMP	Assessment of effects on target organisms		Secondary pests evaluated	food/prey availability evaluated	Resistance development considered	
	HT trait	IR trait	IR trait	HT/IR trait	HT trait	IR trait
Oilseed rape Ms8xRf3	-	n. r.	-	-	-	n. r.
Potato EH92-527-1	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Maize MON810	n. r.	L	-	-	n. r.	Y
Maize Bt11	-	T, L	-	L	-	Y
Maize 1507	-	T, L	-	-	-	Y
Maize NK603	-	n. r.	n. r.	-	-	n.r.
Maize 59122	-	T, L	-	-	-	Y
Maize 1507xNK603	-	T, L, N	-	-	-	Y
Maize NK603xMON810	-	L, N	-	-	-	Y

Studies assessing effects of the *Bt* toxin on target organisms were submitted in the notifications of maize Bt11, 1507, 1507xNK603, and maize 59122 (see Table 19). Table 20 shows the species evaluated in these studies as well as the parameters assessed for the tested species.

In the case of the maize lines 1507 and 1507xNK603 studies assessing effects on target organisms were contained in the chapter dealing with the toxicological assessment of the GMP (Chapter 'Toxicology'). The aim of these studies was to demonstrate the equivalence of the plant-derived proteins and bacterially expressed proteins using different sensitive and insensitive pest species in insect bioassays. This was also the case for the studies submitted in the notifications of maize 59122 (Gao & Herman 2000, n.st.) and maize Bt11 (Meussen & Mettler 1994, n.st.). For maize 1507 in addition to the equivalence study (Evans 1998, n.st.), a field study assessing differences of target organisms in GM and non-GM maize (Vernier et al. 2001b, n.st.) and feeding assays with target organisms in a growth chamber (Castanera 2001, n.st.) were submitted. Both studies were attached as Annexes but not referred to in the ERA and the technical dossier. For maize 59122 two further studies were submitted, one showing an insect bioassay using different pest species (Herman 2000, n.st.) and one summarizing results of laboratory analyses using different pest and non-pest species (Zhuang & Dhidialla, undated, n.st.).

Table 20. Studies contained in GMP notifications using target organisms of the GMP (only GMPs containing Cry-proteins are listed).

"-" = no specific data provided by the notifier; SCR = Southern Corn Rootworm (*Diabrotica undecimpunctata howardi*); WCR = Western Corn Rootworm (*Diabrotica v. virgifera*); NCR = Northern Corn Rootworm (*Diabrotica barber*i); ECB = European corn borer (*Ostrinia nubilalis*); CEW = Corn Earworm (*Helicoverpa zea*); CLA = Corn leaf aphid (*Rhopalosiphum maidis*); MCB = Mediterranean Corn borer (*Sesamia nonagrioides*); TBW = Tobacco budworm (*Heliothis virescens*); BCW = black cutworm (*Agrotis ipsilon*); FAW = fall armyworm (*Spodoptera frugiperda*); CBW = cotton boll weevil (*Anthonomus grandis*).

GMP	Cry protein	Test substance	Pest species evaluated	Parameters evaluated	Reference
Maize MON810	Cry1Ab	-	-	-	-
Maize Bt11	Cry1Ab	plant and bacterial proteins	ECB , CEW	Mortality, weight gain	Meeusen & Mettler (1994)
Maize 1507	Cry1F	1507 maize plants	ECB, MCB	number of ears with feeding damage, number of larvae per plant, number of cavities in stalks, number and length of tunneling, (grain moisture and yield)	Vernier (2001b)
	Cry1F	plant and bacterial proteins	ECB, CEW, TBW, FAW, BCW	Mortality	Evans (1998)
	Cry1F, Cry1Ab	1507 maize plants	MCB	mortality	Castanera (2001)
Maize 59122	Cry34Ab1/ Cry35Ab1	Bacterial proteins	SCR	mortality, insect weight	Gao & Herman (2000)
	Cry34Ab1/ Cry35Ab1	Bacterial proteins	Lepidoptera: CEW, ECB, BCW Coleoptera: SCR,	mortality insect weight (except CLA, WCR	Herman (2000)

			WCR, NCR Homoptera: CLA	adults)	
	Cry34Ab 1/ Cry35Ab 1	Bacterial proteins	Lepidoptera: CEW, ECB Coleoptera: NCR, WCR, CBW Homoptera: CLA	mortality	Zhuang & Dhidialla (not dated)
Maize 1507xNK603	Cry1F	see maize 1507	see maize 1507	see maize 1507	Evans (1998)
Maize NK603x MON810	Cry1Ab	-	-	-	-

As a typical example of a study assessing the effects of *Bt* maize on targeted pests those provided in the notification of the maize 1507 are analysed in detail. Maize 1507 expressing the Cry1F protein aims to control not only the European corn borer (*Ostrinia nubilalis*) but also the Mediterranean Stalk borer (*Sesamia nonagrioides*). Two studies were submitted in the notification for demonstrating that this maize controls also the latter pest species: Castanera (2001, n.st.) and Vernier et al. (2001b, n.st.).

Castanera 2001

Two studies were carried out by the author: In one study, excised leaf disks were provided as food for neonate *S. nonagrioides* larvae and in the other study, the whole plant was used.

Leaf disk study: Minimal information was provided in the report regarding the basic elements of the experiment. The number of replications per treatment, the number of larvae per replication and treatment as well as the number of plants sampled remained unknown. Further, no statistical analyses were performed. Based on the information provided in the study, a total of 100 neonate larvae was used. The number of leaf discs as well as the number of plants from which these leaf discs were derived from was not indicated. However, the results table indicates that all 100 larvae were counted as one data point: This yielded one count for mortality, 98%, each on Cry1F and Cry1Ab maize. No isoline of Cry1Ab was used. The study consisted of a single run yielding exactly one mortality reading on each of the two *Bt* maize lines compared against one reading on one control line. Apparently, no replication of the study was carried out.

Whole plant study: As with the first study minimal information was provided. The analysis of data cannot be reconstructed from the information given in the study. However, from the result presented in Table 2 of the study, it appears that the approach was the same as for the leaf disk assay and consisted of a single run: all neonates were included in the analysis and one reading per treatment was made. Of the 100 neonates on the Cry1Ab and Cry1F plants survived one and three larvae after five days, respectively, while 84 survived on the control plants. No replication of the study over time or within the study was included.

Vernier et al. 2001b

An insect management study was carried out at one location in France. The study was carried out over one year only (2000) delivering 'preliminary results' as noted by the authors. The authors further concluded, that '*Despite the somewhat limited data obtained from this single location study, maize line TC1507 showed significant efficacy to control both European corn borer and Pink stalk borer PSB* (…) *This is the first study conducted against PSB and the results show excellent control of PSB.*' Treatments were Cry1F maize, unsprayed isoline and insecticide-treated isoline. Parameters measured included numbers of ears with feeding damage, total length of tunnels, total number of larvae present in stalks and yield. The data was, to the degree possible, differentiated for ECB and PSB. Significant differences were observed between the treatments for ECB but the number of PSB was too low to detect any significant differences, which was also noted by the authors. Also no significant difference for yield was observed between the the treatments which was attributed to the low number of locations and replication resulting in very low statistical power.

2.7.2 Argumentation of the notifiers

In notifications of GMPs expressing insecticidal Cry proteins, the specificity of the respective toxins was discussed either when describing the introduced traits (e. g. maize 59122), when assessing effects on target (e. g. maize 59122) or non-target organisms (e. g. maize NK603xMON810), when assessing the environmental impact of the product (e. g. maize MON810) or in the toxicology assessment (e. g. maize 59122). Often the specificity of the toxins was mentioned in several different chapters of the notification (e.g. toxicology, effects on target and non-target organisms). Effects of the GMP on target organisms were not consistently evaluated by specific studies conducted by the notifiers. If studies were provided to evaluate target organisms these were often not conclusive (see examples above).

With respect to the specificity of the introduced Cry-protein on the respective target organism, the notifiers generally concluded that besides resistance development no other immediate or delayed environmental impact resulting from direct or indirect interactions of the GMPs and the target organisms in the receiving environment were expected to arise (e.g. maize 1507xNK603, maize 59122). In other cases, notifiers concluded that a negligible risk for the environment through interactions with target organisms would be expected (e.g. maize NK603xMON810). Only in the case of maize 1507 the notifier concluded on the efficacy of the GMP to control the target organisms.

2.8 Assessment of interactions with non-target organisms and the biotic environment

2.8.1 Studies conducted for the assessment of interactions of GMPs with non-target organisms

All notifications contained or cited studies assessing effects of the respective GMP on non-target organisms. However, the number of studies attached or cited and the quality of these studies varied significantly among the notifications.

Initially, in the case of potato EH92-527-1 no studies on non-target organisms were provided in the notification. The environmental risk assessment in this notification referred to studies of the Swedish Agricultural University which conducted six field trials from 1996-1997. Except a letter from this University confirming the absence of effects of the GM potato on aphids, leafhoppers and several fungal and bacterial diseases, no data were presented. In response to Member State questions on effects of the GM potato on non-target organisms the notifier furthermore stated that amylopectin is generally present in the environment thus not having any adverse effects on the environment and that the safety of the APH(3')II protein had already been determined. The cited literature to underline these statements was not specific to the GM potato EH92-527-1. In addition the notifier referred to '*safety studies none of which have shown any indications of potential harm to humans, animals or the environment*' although no exact reference for these studies was made. Only in a later update, years after the original notification, field studies on non-target organisms were supplied by the notifier (see also below).

2.8.1.1 Assessment of exposure of non-target organisms to the GMP

A comprehensive and in-depth exposure asessment evaluating quality and quantity of exposure of different organisms in different environmental media (vegetation, soil, water) was not conducted in any of the GMP notifications reviewed. Only in the notifications of maize Bt11, maize 1507 and maize 59122 some discussion or evaluation of exposure of a few organisms or organism groups was presented.

In the notification of maize Bt11 general literature on the exposure of Monarch butterflies to *Bt* maize was cited, especially in the update from January 2003 and in Appendix 3 of the update from November 2003. Only in the update delivered in November 2003, the notifier presented a separate, 'formal' risk assessment of selected non-target organisms. Published and unpublished studies on the effects of the Cry1Ab toxin on Lepidoptera and non-lepidopteran non-target organisms were itemized. Lepidopteran species included in Annex IV of Directive 92/43/EEC (FFH Directive), their main food plant(s) and habitats were listed. The notifier argued that maize fields and their immediate surroundings were not important habitats for the 'Annex IV' Lepidoptera in Europe. In addition, no specific exposure values for individual species were calculated or assessed. The notifier argued that although the species for which effects of the Cry1Ab toxin had been tested so far may not be native to the EU, they would still be valid as representatives of other species of this group. These species were chosen either because laboratory methods were available or because they were representative of a particular route of exposure or of a taxonomic group.

In the notification of maize 1507 exposure was evaluated only for non-target Lepidoptera by estimating the predicted environmental concentration (PEC) based on pollen expression levels only (Wolt & Conlan 2001, n.st.). Pollen dispersal was estimated on the basis of published literature. Sensitivity data (LD_{50}) were calculated from toxicological studies of 15 lepidopteran species. Most of these lepidopteran species were pest species.

In the notification of maize 59122 the notifier followed the Environmental Risk Assessment Guidelines of the US EPA (1998), assessing exposure and effects, characterising the risks and analysing uncertainty and variability. The exposure estimation was carried out by estimating the 'high end exposure' (HEEE) or the 'environmental concentration' (EEC). The respective HEEE values for dif-

ferent tissues (pollen, leaves, etc.) were calculated based on the known expression values of the GMP and represented the 90% upper bound on the reported mean for each tissue. The expression data used as the basis for this calculation were based on expression values from field trials conducted in Chile but not from European field trials thus not representing European conditions. Exposure scenarios calculated for phytophages, pollinators and 'incidentals' (e. g. the Monarch butterfly) as well as aquatic habitats were based on these pollen expression values. Exposure scenarios for detritivores (Collembola) were based on senescent plant expression values (also from overseas) and calculated assuming ingestion of plant material in soil. Exposure of higher trophic levels was evaluated by calculating the transmission efficiencies of the Cry1Ab toxin in aphids and noctuid larvae feeding on GM maize (data from the published literature: Raps et al. 2001 and Head et al. 2001). Exposure scenarios for phytophages were also calculated based on overseas leaf expression values. The HEEE values were then compared to known toxicological endpoints (NOEC, NOEL) for the respective organisms.

Table 21. Exposure estimation studies for non-target organisms in GMP notifications.

Y = yes; "-" = no data presented/not considered; M = monarch butterfly, O = other; PEC = predicted environmental concentration; HEEE = high end exposure estimates.

GMP	Exposure estimation	Exposure value	Basis for exposure	Non-targets considered	Species of conservation concern	Reference
Oilseed rape Ms8xRf3	-	-	-	-	-	-
Potato EH92-527-1	-	-	-	-	-	-
Maize MON810	-	-	-	-	-	-
Maize Bt11	Y	-	Expression (pollen)	Lepidoptera, pollinators, foliar and ground dwellers, parasitoids	Lepidoptera (Annex IV, Dir. 92/43/EEC)	in notification and updates (no separate study)
Maize 1507	Y	PEC	Expression (pollen)	Lepidoptera (15 species)	Y (M)	Wolt & Conlan (2001)
Maize NK603	-	-	-	-	-	-
Maize 59122	Y	HEEE	Expression (different tissues), soil, aphids, noctuid larvae	phytophages, higher trophic levels, pollinators, detritivores, incidentals, aquatic habitats	Y (M, O)	Poletika (2003)
Maize 1507xNK603	-	-	-	-	-	-
Maize NK603 xMON810	-	-	-	-	-	-

2.8.1.2 Assessment of effects of the GMP on non-target organisms

Effects of a GMP on non-target organisms were usually assessed at different levels of containment (lab, greenhouse, and field) and with different test materials (the isolated synthetic GM protein, parts of the GM plant or the whole plant). Effects of the isolated and usually bacterial derived GM

protein or different plant parts (e.g. pollen or leaf discs) on non-target species were mostly evaluated by laboratory studies using a set of standard non-target species representing a typical toxicological testing regime, e. g. as used for pesticide testing. In only few cases, the whole GMP was assessed in the greenhouse exposing non-target organisms to the whole plant. In some notifications the GMP was additionally assessed in field studies for non-target effects. Table 22 presents an overview of the studies submitted in the notifications for the assessment of effects of the respective GMP on non-target organisms.

All notifications except potato EH92-527-1 contained information on laboratory studies (Table 22). Greenhouse studies were only provided in the notifications of oilseed rape Ms8xRf3 and maize NK603. Field studies were not provided in the notifications of the maize lines Bt11, NK603 and NK603xMON810. Tritrophic studies were provided in two notifications only. Specific studies using species of conservation concern or of aesthethical and cultural value, such as the Monarch butterfly, were submitted in two notifications (maize 1507, 59122, see also below).

The notifier of oilseed rape Ms8xRf3 referred to the notification of Ms1xRf1 oilseed rape of January 1994 for the assessment of non-target effects, stating that this GMP had a chimeric gene construct similar to Ms8xRf3 oilseed rape. Similarly, the notifiers of the two stacked event maize notifications referred to the safety assessments as submitted in the single event notifications without presenting specific tests of non-target organisms with the stacked GMP.

Table 22. Studies assessing adverse effects of GMPs on non-target organisms.

Lab studies = with isolated proteins/parts of the GMP; greenhouse studies and field studies = with whole plants; Y…study conducted by the notifier or by order of the notifier; (Y)…studies not conducted with the respective GMO, Y?...studies where the GMO used was not specified; N…reference to assessment in other notifications; "–"….no data presented or notifier referred only to the published literature; M = monarch butterfly, O = other.

GMP	Lab studies	Greenhouse studies	Field studies	Tritrophic studies	Species of conservation concern/ aesthetical/cultural value
Oilseed rape Ms8xRf3	(Y), N[1]	Y, (Y), N[1]	Y?	-	-
Potato EH92-527-1	-	-	Y	-	-
Maize MON810	Y	-	Y	-	-
Maize Bt11	Y	-	-	-	- (M, O)[7]
Maize 1507	Y[2]	-	Y	-	Y (M)
Maize NK603	Y	Y	-	Y	-
Maize 59122	Y	-	Y	Y[6]	Y (M)
Maize 1507xNK603	Y, N[3]	-	Y[4]	-	-
Maize NK603xMON810	Y, N[5]	-	-	-	- (M)[7]

[1] reference to notification of oilseed rape Ms1/Rf1; [2] 3 of the 9 studies cited are missing in the notification. The study with the Monarch butterfly is part of the additional information requested by MS CAs; [3] reference to notification of maize 1507 (C/ES/01/01); [4] part of the additional information delivered to the Spanish Competent Authority; [5] reference to single event notifications of maize MON810 and maize NK603; [6] study not attached to the notification; [7] reference to published studies, no toxicological studies submitted by the notifier

Laboratory studies using the isolated gene products or parts of the GMP

In all GM maize notifications laboratory studies were presented using either isolated proteins or parts of the GMP. The number of studies and the number of species tested in the laboratory varied only to some extent among the notifications. Often a similar set of species was used for testing. Rare, protected or endangered European species of non-target organisms were not specifically considered in any of the notifications.

The studies assessing effects of the isolated proteins or parts of the GMP on selected non-target organisms were generally studies generated by or on behalf of the applicant. In some notifications these studies were claimed to be 'Confidential Business Information' (maize NK603xMON810, maize MON810) but not in others. In some cases studies were cited but not attached to the notifications (e. g. three studies in the notification of maize 1507, one study for maize NK603, two studies for maize 59122). For the potato EH-92-527-1 no laboratory studies were submitted. In the oilseed rape Ms8xRf3 notification one study using isolated proteins was not attached (bee oral toxicity test).

Three notifications contained studies which did not deal with the respective GMP but used other GM events in their laboratory assessments (oilseed rape Ms8xRf3, maize Bt11, maize MON810; Table 23). In other cases the GMP used in these assessments could not clearly be identified from the information given in the notification. In the case of the Collembola study submitted for maize NK603 (Goldstein 2003, n.st.) it was unclear whether the Roundup Ready-corn tested was in fact NK603 maize or another event. Similarly, in the case of Ms8xRf3 oilseed rape, the feeding studies with mammals (rabbits) and birds were not conducted with the respective GMP but instead with another GM oilseed rape. As only the plasmids were indicated to identify the GMP it could only be inferred that not Ms8xRf3 oilseed rape was used. The bee oral toxicity test was not attached to the notification, therefore, the GMP used could not be determined. The notifier of oilseed rape Ms8xRf3 referred to the documentation package of oilseed rape Ms1/Rf1 and argued that no experiments specific for Ms8 and Rf3 lines with birds or mammals were conducted because the functions of the newly inserted genes and the expression patterns of the Ms1/Rf1 lines were identical to those of Ms8/Rf3.

For the stacked event of maize NK603xMON810 the notifier referred to the single event notifications submitted earlier and included the respective lab studies in the notification. In the case of maize 1507xNK603, it was also referred to the safety assessment of the single event notifications but without presenting the specific laboratory tests.

Table 23 gives an overview of the organisms tested in laboratory studies. While in some notifications no or only a few species were tested (e.g. maize MON810), in others a larger range of organisms was subject to laboratory testing (e.g. maize 59122).

The following organisms were tested in the laboratory studies of the analysed notifications:

- Adult or larval honeybees (*Apis mellifera*)
- Earthworms (Eisenia foetida)
- Green lacewing larvae (e.g. *Chrysoperla carnea)*,

- Parasitic hymenoptera (Nasonia vitripennis; Brachymeria intermedia),
- Lepidoptera (*Danaus plexippus*),
- Pest species (Lepidoptera: Spodoptera exigua; Helicoverpa zea; Heliothis virescens, Agrotis ipsilon; Ostrinia nubilalis; Coleoptera: Leptinotarsa decemlineata; aphids (Rhopalosiphum maidis)
- Coleoptera (Carabids: Poecilus cupreus; Coccinellids: Hippodamia convergens; Coleomegilla maculata),
- Collembola (Folsomia candida),
- Birds (Colinus virginianus; Serinus canaria domestica)
- Water organisms (Daphnia magna; Oncorhynchus mykiss)
- Mammals (Oryctolagus cuniculus).
- Other (Anthocorids: *Orius insidiosus*, soil micro-organisms)

Table 23. Test organisms used in laboratory studies using the isolated protein or parts of the GMP.

CBI = study attached as Confidential Business Information; x = study conducted; (x) = study not conducted with respective GMP, x? = GMP used in the study is unknown/not indicated; x (A) = adult; x (L) = larvae; x (A, L) = adult and larvae.

	Oil-seed rape	Potato	Maize						
Test organism			MON 810	Bt 11	1507	NK603	59122	NK603 x MON 810[3]	1507x NK603[5]
Earthworms				(x)[1]	x	x	x	x	
Honeybees	x?[6]		x (A, L)	x (A, L)	x (L)		x (L)	x (A, L)	
Collembola				(x)[1]	x	x?	x	x	
Green Lace-wings			X (L)	X (L)	X (L)	X (L)	X (L)	X (L)	
Ladybird beetles			x (A)	x (A)	x (A)		x (A, L)	x (A)	
Parasitic hymen-optera			x (A)	x (A)	x (A)		x (A)	x (A)	
Lepidoptera, Coleoptera, Aphids (pest species)						x	x		
Lepidoptera (other)					x		x[4]		
Carabids							x		
Bugs (Antho-corids)			(x)[2]						
Birds	(x)				x				
Fish							x		
Daphnids					x		x	x	
Micro-organisms						x			

Mammals	(x)		x

[1] study conducted with the lyophilized protein extracted from Bt176 maize leaves; [2] no data presented, only described in the Appendix; [3] all studies with single proteins; [4] study not attached to notification; [5]notifier refers to safety assessments of single event notifications – no studies attached.

In most studies the isolated, bacterially produced Cry-protein was fed to a test organism (see Table 24). In tests with Cladocera (daphnids), non-target Lepidoptera or Heteroptera pollen was used as a test substance. Pollen was also used for feeding ladybird beetles (e.g. maize 59122) or honey bees. Protein suspensions or solutions in water or honey were used for honey bees, adult ladybird beetles and adult parasitic hymenoptera and in some cases also daphnids. A protein solution mixed with moth eggs (i.e. coated eggs) was usually used to feed to green lacewing larvae used in the lab studies, except for the maize NK603 where green lacewing larvae were fed on aphids in a tritrophic study (see also below). Soil amended with proteins was generally applied in toxicological tests with earthworms and Collembola, although for the latter also mixtures with yeast or the whole GMP was used.

In stacked event GMO notifications, the notifiers usually referred to the single event notifications and the laboratory studies submitted therein. Lab studies with non-target organisms for stacked event notifications usually tested the isolated GM proteins individually and not a mixture of the respective transgenic products. Usually these studies corresponded to the studies submitted in the single event notifications. For example, in the notification of maize NK603xMON810 the studies with green lacewings (Hoxter & Lynn 1992a, n.st.), parasitic hymenoptera (Hoxter & Lynn 1992c, n.st.), ladybird beetles (Hoxter & Lynn 1992b, n.st.) and honey bee larvae and adults (Maggi & Sims 1994a, n.st.; 1994b, n.st.) were identical to those submitted for the single event maize MON810. The same studies were also submitted in the notification of maize Bt11. So, the same nontarget studies were recycled for different cases of *Bt* plants.

Table 24. Protein/GMP fed to the respective test organism in laboratory studies of GMP notifications.

Test organism	fed with	GMP notification
Earthworms	Proteins Mixed with soil (artificial soil substrate)	59122, 1507, NK603, Bt11, NK603xMON810
Honeybees	Pollen, protein suspension/solution in water or honey-water mixture	59122, 1507, MON810, Bt11, NK603xMON810
Collembola	Protein added to yeast or to artificial soil, GM maize	59122, 1507, Bt11, NK603, NK603xMON810
Green Lacewings	Protein (solution) mixed with moth eggs, aphids (tritrophic)	59122, 1507, MON810, Bt11, NK603xMON810, NK603
Ladybird beetles	Protein in mixture with sugar water or commercial honey, in honey-water solution, protein in artificial diet, Pollen mixture with corn earworm eggs, pollen alone	59122, 1507, MON810, Bt11, NK603xMON810
Parasitic hymenoptera	Protein in mixture with sugar water or commercial honey, in honey-water solution	59122, 1507, MON810, Bt11, NK603xMON810
Lepidoptera, Coleoptera, Aphids (pest species)	Protein in artificial diet	NK603, 59122
Lepidoptera (other)	pollen	59122, 1507
Coleoptera: Carabids	Protein injected in blowfly pupae	59122

Heteroptera: Anthocorids	Pollen	MON810
Birds	Grounded GM maize grain, oilseed rape leaves	1507, oilseed rape Ms8xRf3
Fish	Protein incorporated in fish diet	59122,
Daphnids	Microbial proteins added to water, pollen	59122, 1507, NK603xMON810
Micro-organisms	protein in soil	NK603
Mammals	Oilseed rape leaves, maize incorporated in artificial diet	Oilseed rape Ms8xRf3, NK603

The parameters evaluated were in most cases survival and mortality (e.g. NOEC, LC_{50}) or signs of toxicity. In relatively few studies sublethal parameters such as growth inhibition, body weight, effects on pupation or progeny and others were tested (see Table A8 in the Annex).

Tritrophic studies where a herbivore which fed on the GMP was fed to a predator were submitted only in two notifications (maize 59122 and maize NK603). In the maize 59122 study (Higgins 2000, n.st., cited in Annex 25; study not attached to notification) corn leaf aphids were reared on GM maize and then fed to ladybird beetles for a period of 10 days. Mortality and weight of the beetles were assessed without verifying their exposure to the toxin. Similarly, for maize NK603 aphids were allowed to feed on GM maize and then offered to green lacewing larvae for 11-12 days (also no verification of exposure; Chamornman et al. 2002, n.st.). Sublethal parameters such as consumption, development and reproduction of lacewings were assessed in this study.

Species of conservation concern or of aesthetical/cultural value were considered in four out of nine notifications: maize Bt11, maize 1507, maize 59122 and maize NK603xMON810. In all cases the Monarch butterfly *Danaus plexippus* was considered although this species does not occur in Europe and is not a protected species in the USA but rather represents a species of great cultural and aesthetical value[3].

In the notification of maize 1507 a laboratory study was conducted by the notifier using the isolated *Bt* protein fed to the monarch butterfly (Bystrak 2000, n.st.). This study was not contained in the original notification but submitted later on request. In the risk assessment conducted for maize 1507 (Wolt & Conlan 2001, n.st.) the notifier discussed effects of this GMP on the monarch butterfly by referring to literature risk assessments specific for the Cry1F protein. The notifier concluded that the assessment approaches and findings for the Monarch butterfly can also be applied to the consideration of other endangered lepidopteran species which may occur in the proximity of 1507 maize fields in Europe.

In the case of maize 59122 one laboratory study using the isolated protein fed to Monarch butterfly larvae was cited (Sears 2003, n.st.) but not attached to the notification. In a separate risk assessment study of this notification (Poletika 2003, n.st.), potential effects of this GMP on the Monarch

3 See for example: http://www.mbsf.org/

butterfly by using exposure/effects estimations were discussed. A low probability for risk was concluded as the exposure estimation value did not exceed the effects value for the Monarch butterfly. However, the notifier admitted that some uncertainty remained due to the fact that certain species which might be exposed to the GMP had not been tested for their susceptibility. For example, no assessment for endangered coleopteran species was presented for this GMP which expresses a Coleopteran-active Cry-protein.

In the notification of maize Bt11 (Appendix 3 of update November 2003) possible effects of pollen expressing the Cry1Ab protein to Monarch butterflies were discussed by referring to published studies. The notifier also cited Lepidoptera listed in Annex IV of Directive 92/43/EEC (FFH Directive) and stated that maize fields and their surroundings would not be important habitats for these lepidopteran species. Thus maize could be excluded as a food plant for most lepidopteran species. It was additionally argued by the notifier that, due to the low concentration of Cry1Ab in *Bt* maize pollen, the risk of adverse effects on these species would be negligible even if there were more sensitive species than monarchs among those listed in Annex IV, even if their food plants would trap maize pollen more efficiently than milkweed.

In the notification of maize NK603xMON810 the notifier concluded that MON810 pollen was unlikely to pose any significant risk to the sustainability of Monarch butterfly populations and backed this argument by citing published literature.

Greenhouse studies

Studies conducted in the greenhouse or under containment using the whole GMP were conducted only in the notifications of oilseed rape Ms8xRf3 and maize NK603 (Table 22).

In the original notification of oilseed rape Ms8xRf3 reference is made to a ‘honey bee foraging study’, referring to the documentation package of the oilseed rape notification Ms1/Rf1. As this study is not attached to the notification, it is not clear whether this study was actually conducted under greenhouse conditions and also other details of the study could not be evaluated. In the supplementary information supplied by the notifier in 1998 a ‘honey bee cage test’ study was submitted which was carried out by a toxicology laboratory (company LISEC) assessing the foraging activity and mortality of honey bees (see Table 25). Hence, it is assumed to be identical to the ‘honey bee foraging study’ study mentioned in the original notification. Similarly, the ‘bumble bee foraging study’ was referred to in the original notification as well as in several later updates (1999, 2003) but no data or studies were attached. In the update of 2003 this study was referred to as ‘Bayer internal studies’. The GMP used in the ‘honey bee foraging study’ was oilseed rape line Ms1xRf1 while for the ‘bumble bee foraging study’ no exact information on the GMP used was provided.

In the notification of maize NK603 a greenhouse study with honey bees was attached (Table 25).

Table 25. Evaluated parameters of greenhouse studies using the whole GMP.

Test organism	Parameters evaluated	Notification of GMP	Study provided
Honey bees	Foraging behaviour, egg laying rate, survival and development of eggs, larvae, pupae	maize NK603	Boonkrit et al. 2002
	Foraging behaviour/preference	oilseed rape Ms8xRf3	Reference to Notification of Ms1/Rf1 Oilseed rape/LISEC Ecotoxicology report
Bumble bees	Foraging behaviour/preference	oilseed rape Ms8xRf3	No study attached, no data provided

Field studies

Table A9 in the Annex gives an overview on the details of the field studies evaluating non-target organisms as submitted by notifiers using the respective GMP of the notification.

Seven out of nine notifications provided information on studies carried out by the notifier (or on behalf of the notifier) with the GMP under field conditions (Table A9 in the Annex). In two notifications notifier field studies were cited without including the respective studies (maize NK603 and maize NK603xMON810). In several cases field studies with the respective GMP were not submitted in the original notification but at a later stage of the notification procedure or upon request (e.g. potato EH92-527-1, maize Bt11, maize 1507xNK603, maize 59122, see also Table A1 in the Annex).

In the ERA of the original notification of oilseed rape Ms8xRf3 the notifier referred to 'PGS field trials' when discussing environmental interactions of the transgenic oilseed rape and its pollinators. In these trials the foraging behaviour of bumblebees, honeybees and wasps was assessed. However, no reference or details on these trials were given. Also in the updates of 1999 and 2003 reference was made to these trials without adding any data or supplementing information. In the 1999 update two additional studies were cited, one assessing epigean predatory arthropods, carried out by the Martin Luther University in Germany and 'field observations' by AgrEvo/PGS assessing pigeons, sparrows, hares and rabbits. Again no further details for the experiments were provided, although these studies are again referred to in the update of 2003 (see Table A9 in the Annex).

For the potato EH92-527-1 the original notification did not contain any information on effects of the GM potato on non-target organisms in the field except an attached letter of the University of Agricultural Sciences (Sweden) stating that six field trials were carried out between 1996 and 1997. The letter further stated that from these official trials there was no evidence that insects in GM potato fields were more or less abundant. In July 2006 the notifier submitted additional information containing field studies on non-target organisms conducted at four different locations in three different European countries.

Only two of the four field studies provided in the maize MON810 notification actually used MON810 maize, the other two studies used other GM maize lines.

In response to the questions of the Competent Authorities of the EU member states the notifier of Bt11 presented information on field studies in Appendix 4 of the information provided in November 2003. A summary report compiled by the Agricultural Biotechnology Stewardship Technical Com-

mittee (ABSTC 2002) was included in which various surveys of non-target invertebrate populations in *Bt* corn were summarised. Since this report was obviously not specifically compiled for Bt11 but for the US Authorities for the general extension of *Bt* corn registrations, studies conducted with different *Bt* maize events were included. In fact only five of the 16 studies were conducted in Bt11 maize fields (see Table A9 in the Annex). Of these five studies, two were published in scientific journals, for one an abstract was presented and for the other two unpublished reports were attached in the Appendix. Of the 16 field studies summarised in the ABSTC report five were conducted in Europe, none of these, however, with maize Bt11.

Only one study with data from field studies on non-target organisms was referred to in the original notification of maize 59122 (Higgins & Wright 2003, n.st., cited in Annex 25) but not attached. This field study used a GM maize line other than 59122 (Table A9 in the Annex). Another three field studies on non-target organisms were submitted after request of the Dutch Competent Authorities during the notification procedure.

In the case of maize 1507 three field studies were included in the notification.

For the notification of maize 1507xNK603 a summary report of a Spanish field study was submitted at a later stage on request of the Spanish Competent Authorities.

In the notifications of maize NK603 and maize NK603xMON810 no specific field studies with the aim to assess non-target organisms were conducted by or on behalf of the notifier.

Size of plots

For the assessment of non-target effects in field studies usually small plots or few plants per plot were sampled. Plot size in the different presented studies varied from 15-30 m^2 (oilseed rape, potato, maize Bt11, maize 59122) to around 100 m^2 (maize Bt11, maize 1507) or ranged from 400-900 m^2 (e. g. maize 1507xNK603, maize 59122, maize Bt11). In other cases plot sizes were not indicated (e. g. maize MON810).

Sampling methods

Sampling of non-target organisms was either done by different types of traps (pitfall, beating, and sticky traps) or by visual sampling or inspection of the plants (e.g. maize MON810, maize 1507, maize 59122). In most cases the abundance of the organisms was assessed or a simple inventory of species was made. In the case of oilseed rape Ms8xRf3 the foraging behaviour of pollinators and in the case of maize 1507 the leaf damage caused by leafhoppers was evaluated. Non-GM controls treated with insecticides were generally used in GM maize field trials (e.g. maize 1507, maize MON810, maize 59122, and maize 1507xNK603).

Citation of published studies

In all notifications studies published in peer-reviewed journals were cited by the notifier in order to demonstrate the safety of the respective GMP and/or the introduced protein on non-target organisms.

References were often provided in updates or additional information submitted by notifiers at a later stage during the notification procedure or in response to questions in the course of the scientific review by the national Competent Authorities or EFSA. In some cases the same literature as in

the original notification was cited in the updates. In some cases notifier studies already included in the original notification were again provided in the updates and not always complemented with new literature citations (e. g. maize 1507).

In the case of oilseed rape Ms8xRf3 no literature was provided in the original notification but in the updates of 1998 and 2003 (Table 26). Bt11 maize contained cited references in the original notification as well as in the updates of 2003. In the maize notification of 1507 new literature citations were provided in the answer to the questions posed by the EU member states. For NK603 maize literature was cited in the original notification as well as in 2006 and 2007 in response to the Spanish Competent Authority and EFSA, respectively. For the stacked 1507xNK603 maize literature on different GMOs was provided as an answer to Competent Authority questions only. For maize 59122 published studies were cited both in the original notification and in the ERA submitted as Annex (Poletika 2003, n.st.) as well as at a later stage where additional unpublished notifier studies were submitted (e.g. Scholte & Dicke 2005, n.st., provided as additional information). In the case of maize NK603xMON810, literature was provided in the original notification. During the review process the French Authorities requested further data with respect to potential effects of this stacked maize on non-target organisms. Since the review process is still ongoing for this GMO, futher data or published studies may be delivered by the notifier in the future.

In several cases a clear distinction was not made between published studies and unpublished studies (the latter mostly carried out by or on behalf of the notifier). This is for instance the case in the separate document submitted for maize 59122 (Poletika 2003, n.st.) in which exposure and effects data for selected non-target organisms were presented. For the effects data several references were provided, some of those referred to unpublished toxicological studies of the notifier while others referred to published studies. In the case of the update of 2003 of the maize Bt11 notification references were provided which were composed of a mixture of published studies and unpublished internal reports of the notifier.

The GMP used in the cited published studies was in several cases not identical to the GMP of the notification. For instance, in the case of maize NK603 seven published studies on non-target organisms evaluated under field conditions were cited in the notification. Of these six studies did not deal with NK603 maize but Roundup Ready soybean or Roundup Ready wheat. Similarly, citations of published studies in stacked event notifications mainly referred to studies carried out with the respective single event GMPs (e.g. maize NK603xMON810). In many cases the respective GMP used in the published study was not indicated when the notifier cited the study for the discussion of effects on non-target organisms.

Table 26. Studies published in peer-reviewed journals cited by the notifier in order to demonstrate the safety of the GMP or the introduced proteins to non-target organisms.

x…study cited; ”–“… no literature provided

GMP	Original notification	Update/additional info/response to Member state questions
Oilseed rape Ms8xRf3	-	x (1998, 2003)
Potato EH92-527-1	x	
Maize MON810	x	
Maize Bt11	x	x (2003)
Maize 1507	-	x
Maize NK603	x	x
Maize 59122	x	x
Maize 1507xNK603	-	x
Maize NK603xMON810	x	

2.8.2 Argumentation of the notifiers

The notifiers frequently argued the safety of the respective GMP to non-target organisms with the following arguments:

- The specificity of the biological and biochemical activities of the introduced proteins;
- The absence of toxic effects demonstrated in ecotoxicity studies on a range of non-target organisms;
- The safety of the introduced proteins and the GMPs was confirmed in field trials;
- The introduced proteins show a very limited persistence in the soil environment;
- The ecological interactions of the GMP and non-target organisms are not different from traditional maize;
- Compositional differences which might affect insects have not been observed

In the case of GMPs with herbicide tolerance the following argumentations for the safety of the EPSPS or PAT proteins to non-target organisms were provided by the notifiers:

- The introduced protein is not a novel protein in the environment / is common in the environment as it is derived from the genome of *Agrobacterium* sp., a common bacterium in the soil;
- The introduced protein does not target any organisms and does not have a toxic mode of action;
- The introduced protein is related to other EPSPS enzymes that are endogenous in plants and microbes;
- The introduced protein has a history of safety to non-target organisms as non-target organisms have historically been exposed to members of this safe class of proteins;

- There is no *a priori* reason to suspect that the introduced protein would show biological activity towards non-target organisms.

2.9 Assessment of effects on biogeochemical cycles and the abiotic environment

2.9.1 Studies conducted for the assessment of effects on biogeochemical cycles and the abiotic environment

Possible adverse effects of the GMP on biogeochemical processes were generally addressed by the notifiers of GMPs (Table 27). Only in one case, effects on biogeochemical processes were completely omitted from the ERA (maize MON810). Tables A10 to A12 in the Annex give an overview of the studies conducted by the notifiers (unpublished studies) as well as published studies cited for this assessment. For the specific assessment of potential effects of GMPs on the abiotic environment generally no separate data or studies were submitted (see Table 27).

Generally, the purpose of the studies presented by the notifiers in order to assess potential effects of the GMP on biogeochemical cycles differed considerably and was not consistent across the notifications.

In the notification of maize NK603 a specific study assessing the carbon or nitrogen transformation in soil was presented. In two other maize notifications (NK603, 1507) studies assessing non-target organisms of the soil compartment (Collembola, earthworms) were provided. These studies were also cited and discussed in the assessment of effects of the GMP on non-target organisms (see also chapter 2.8).

Soil persistence of the Cry-toxins was studied in three notifications of insect resistant maize lines (1507, Bt11, 59122). In the case of maize 59122, the soil persistence of the Cry34/35Ab1 proteins was evaluated in a study commissioned by the notifier (Herman et al. 2000, n.st.; later published as Herman et al. 2002b). In the original notification of maize Bt11 the notifier presented a study evaluating the fate of the *Btk* protein in transgenic plant material and soil (no author 1998, n.st.), estimating the half-life of the *Btk* protein in soil (Table A11 in the Annex). In the information additionally provided in November 2003 in response to the questions by the EU member states the notifier discussed also a study that assessed the potential for persistence and accumulation of Cry1Ab protein in soil (Dubelman 2003, n.st.). It is unclear whether this study was an internal study of the notifier. In the references this study was denoted as ‘report submitted to the US EPA’ but not attached to the notification. Also for maize 1507 the notifier presented a study on soil persistence of the Cry1F protein (Halliday 1998b, n.st.), estimating the half-life of the *Bt* toxin in soil.

In the original oilseed rape Ms8xRf3 notification the notifier referred to the documentation package of an earlier oilseed rape notification (Ms1xRf1 oilseed rape). In the ERA update of 1999 the notifier further referred to the results from post-trial monitoring in Belgian and Canadian field trials citing a study on bacterial rhizosphere populations and one study monitoring residual effects in previous transgenic oilseed rape fields. These studies were probably identical to two studies (Leyns 1994, n.st.; no author, no year) provided as additional information already in 1998. Both studies did not specifically deal with Ms8xRf3 oilseed rape but other GM oilseed rape lines.

The report presented in the potato EH92-527-1 notification assessed the presence of kanamycin-resistant bacteria in the soil.

In the case of maize NK603xMON810 the notifier referred to the safety assessment of the introduced proteins as provided in the single event notifications (NK603, MON810) and referred to several published studies generally dealing with the persistence and fate of *Bt* toxins in soil.

Table 27. Studies conducted by the notifier to assess potential effects of the GMP on biogeochemical cycles or on the abiotic environment.

Y…study conducted by the notifier or by order of the notifier ; L…reference to assessment in published studies; N…reference to assessment in other notifications, "-"…. no specific data/studies provided

GMP	Notifier studies conducted to assess… effects of the GMP on biogeochemical processes	effects of the GMP on the abiotic environment
Oilseed rape Ms8xRf3	Y, N[1]	-
Potato EH92-527-1	Y, L	-
Maize MON810	-	-
Maize Bt11	Y[2], L	-
Maize 1507	Y[3], L[6]	-
Maize NK603	Y	-
Maize 59122	Y, L	-
Maize 1507xNK603	N[4]	-
Maize NK603xMON810	N[5], L	-

[1] notifier refers to documentation package of GM oilseed rape Ms1/Rf1; [2] the notifier does not distinguish between biogeochemical/abiotic but refers to 'environmental fate of the *Btk* and PAT proteins'; [3] The two studies are part of the assessment of the GMP on non-target organisms; [4] reference to notification of 1507 maize C/ES/01/01; [5] reference to safety assessment of individual proteins as provided in the single event notifications (MON810, NK603); [6] reference to only 1 published document (OECD 1999)

2.9.2 Argumentation of the notifiers

The following arguments were provided by the notifiers in order to demonstrate that the GMP did not have any adverse effects on biogeochemical processes or the abiotic environment:

- The risk of an adverse effect of a GMP on biogeochemical processes in the soil is negligible (maize NK603, maize NK603xMON810).
- There is/are no evidence/indications that the GMP will alter the cycling of elements or organic nutrients in another way than a non-GMP (oilseed rape Ms8xRf3).
- The introduced proteins have no known negative interactions with the biotic or abiotic environment (maize NK603).
- The limited persistence and the natural ubiquity of the introduced proteins in the soil and the specific biochemical activity of the proteins confirm that these proteins will not cause any significant effects on biogeochemical processes (maize 59122, maize 1507, maize 1507xNK603).

- The expression of the introduced proteins does not alter the natural interaction of maize plants with the abiotic environment (maize 1507).
- No changes are anticipated given that the overall performance of the GMP is similar to the non-GMP (potato EH92-527-1).

2.10 Assessment of effects related to changes in land use or cultivation techniques

2.10.1 Arguments provided for the assessment of effects related to changes in land use or cultivation techniques

Generally, no description of the specific techniques with respect to cultivation or management of the GMPs was included in any of the notifications (see Table 28). The notifications contain the following arguments with respect to the description of potential differences between the cultivation and management techniques of the GMP and the non-GMP:

- The main change in cultivation is the possibility to use glufosinate ammonium as selective herbicide (oilseed rape Ms8xRf3).
- All agronomic practices currently used to grow maize remain applicable for GM maize (maize NK603, maize NK603xMON810).
- The specific cultivation, management and harvesting techniques used for the GMP are identical/comparable to those used for other commercially available (non-GM) plants , with the exception of the application of the IRM plan/the monitoring plan/the herbicide regime (maize 1507, maize 1507xNK603, maize 59122, potato EH92-527-1).
- The aim of the weed and pest control is neither new nor different for the GM maize compared to any other maize (maize NK603xMON810).
- The introduced herbicide tolerance trait/protein provides the farmer with an additional option or tool for weed removal (maize NK603xMON810) or gives the grower a wider choice for weed control measures (maize 1507).

In case of the herbicide tolerant oilseed rape Ms8xRf3, the notifier proposed the introduction of a ‘Good Agricultural Practice Guidance’ within the framework of a stewardship plan. The notifier stated that details of this guidance would be defined upon the launch of the commercial varieties. As cultivation was later excluded from the scope of this GM oilseed rape notification, this guidance was not submitted. In the update of the notification from 2003 the notifier referred to Directive 91/414/EEC for the safety assessment and the impact assessment of the herbicide use on the environment. Furthermore a range of interim reports of European projects were cited and project collaborations indicated (e.g. SCIMAC Interim reports, FACTT report, Inter-Institute trials in France, etc.). The aim of these projects was to evaluate the agronomic impact and the efficiency of glufosinate ammonium for weed control in herbicide tolerant oilseed rape. However, no data or results of these studies were presented. Additionally, some other references composed of published studies or industry booklets were cited with the statement that these also generated expertise for agro-

nomic practices with the new technology. Also in this case, no results or specific data for the oilseed rape Ms8xRf3 were provided in the notification. Thus it remains unclear whether the data contained in these citations actually refer to the GMP in questions.

For the potato EH92-527-1 the notifier stated that the cultivation management of the GM potato was identical to what was routinely applied for starch potatoes but referred to additional identity preservation procedures for the GM potato which would be implemented at commercialisation. No details of these procedures were indicated in the notification.

In the case of insect resistant GM maize reference was generally made to the Insect Resistance Management (IRM) plan in order to account for a potential development of resistance of the target insect (Table 28). In the notifications of maize MON810 the effects related to changes in land use or cultivation techniques were not separately discussed, although an IRM plan was added to this notification. For Bt11 maize the notifier stated that the use of Bt11 maize as an herbicide tolerant crop was outside the current scope of the application and the notifier would not promote the use of glufosinate ammonium herbicides in combination with the use of this maize. As for maize MON810, an IRM plan was submitted. Similarly, for maize 1507 and maize 59122 only an IRM plan was submitted but no effects due to herbicide use considered.

Upon request of the French Competent Authority asking for further information regarding the safety of the glufosinate-ammonium herbicide, the notifier of maize 1507 provided a study on residues of this herbicide carried out with 1507 maize (Robb 2002, n.st.). Beside information on the experimental design and tables summarising the analytical results the study mainly consisted of the analytical report by the laboratories that implemented the analysis. A separate discussion of the results was not included in this study. Residues of glufosinate-ammonium and its two major metabolites were analysed and discussed only with respect to analytical methodology. In the answers to the French Competent Authority the notifier stated that glufosinate-ammonium residues were not detectable in grain from 1507 maize plants treated with glufosinate-ammonium herbicide in most of the samples analysed. In cases where residues were detectable these were below 0.06 ppm which was below the tolerance level for glufosinate-ammonium residues in grain established by the US EPA (0.2 ppm). Regarding the safety of the glufosinate-ammonium herbicide the notifier referred to the Series on Harmonization of Regulatory Oversight in Biotechnology No. 25 of the OECD (OECD 2002c).

In three notifications of maize the potential effects of the broad-spectrum, non-selective herbicide on weeds were mentioned (maize 1507xNK603, maize NK603, maize NK603xMON810).

In the notification of maize 1507xNK603 reference was made to British Farm Scale Evaluations (FSE). In the notifier’s argumentation the GM maize was considered comparable to the GM maize used in the FSE with respect to the weed management and the possible positive effects on weed

biomass resulting thereof[4]. In the notifier's understanding the evaluation of herbicide use was not included within the legal scope of Regulation (EC) 1829/2003 or Directive 2001/18/EC, but should be covered by Directive 91/414/EEC. The Spanish Competent Authority required additional information on environmental impacts of the glufosinate ammonium tolerance trait. Additionally, an assessment of effects of glyphosate on weeds and the potential for resistance selection within species as well as weed shifts was required. In its answer to the questions of the Spanish CA the notifier:

- emphasized the labeling of 1507xNK603 maize seed with an indication that the use of glufosinate ammonium is not allowed,
- cited the EFSA GMO panel opinion for the notification of maize 1507 (C/ES/01/01),
- referred to questions on weeds asked in farmer questionnaires within the scope of the General Surveillance plan,
- referred again to the results of the FSE showing that herbicide tolerant maize had an increase in weed biomass,
- referred to the Directive 91/414/EEC, Annex III data (the biological dossier), for the evaluation of weed shifts and resistance development of glyphosate use as well as its environmental safety to non-target organisms without providing any specific data contained in this dossier.

In its evaluation of maize NK603 the Spanish Competent Authority requested information on the potential effects by the continued use of the non-selective herbicide glyphosate on **weeds** addressing also the potential **development of resistance** (September 2006). In its answer to this questions (December 2006) the notifier of maize NK603 emphasized that the regulation of herbicide use was not included within the legal scope of instruments regulating the placing of the market of GMOs (Directive 2001/18/EC or Regulation (EC) 1829/2003). The notifier also stated that glyphosate would not be the only weed control tool for maize NK603 and that a 'Technology Use Guide' for the EU market would be developed based on the existing guide for the US market.

The notifier of maize NK603 also referred to several field trials across EU member states and stated, without providing criteria, that among the trials conducted the ones with relevance for the safety assessment of NK603 maize were already included in the notification. However, field trials presented by the notifier considered the compositional analysis, expression analysis, agronomic and phenotypic characterization but not herbicide evaluations. The notifier stated that results of field trials assessing the herbicides were not included as their aim was to evaluate parameters that were not directly applicable to the present application, such as efficacy of the herbicide, selectivity of the herbicide, and residue quantification trials. According to the notifier, the results of the field

4 The maize used in the FSE was herbicide tolerant GM maize T25 (glufosinate ammonium tolerant).

trials relevant for herbicide application resulted in weed management recommendations for glyphosate-tolerant maize without presenting these results or giving any further information.

The notifier stated that glyphosate had been already included in the list of registered active ingredients (Annex I to Directive 91/414/EEC, Directive 2001/99/EC). According to the notifier, this assessment included the use of glyphosate in glyphosate-tolerant crops during the scientific review. However, no data of the assessment were presented.

The notifier also stated that data and risk assessments on the formulated product (i. e. data on the product Roundup Ready; Annex III data) were submitted to and assessed by individual EU member states according to local recommendations and use. This evaluation would take place during the next three years and would also include the assessment of the potential for weed resistance development. On this basis the notifier concluded that the formulated product would pose no unacceptable risks to man and the environment and that the risk for resistance development would be low. This conclusion was additionally supported by the argument that Germany had already approved the use of a Roundup Ready formulation in NK603 which would be effective from the moment NK603 is authorized for cultivation in the EU.

In this answer the notifier also mentioned that information was contained in Annex III (the 'biological dossier') of Directive 91/414/EEC on the Roundup Ready formulation with respect to efficacy and selectivity compared to reference products and controls but did not provide any specific data on the herbicide. With respect to **resistance development of weeds** to glyphosate the potential loss of efficacy was not considered an adverse ecological effect by the notifier but rather an agronomic problem. In this context the notifier also referred to the biological dossier, the consideration of resistance development and the resistance management plan contained in the biological dossier. Furthermore the notifier stated that label recommendations would be submitted. The notifier further referred to 'good agricultural practices' which should minimize the likelihood of weed resistance to develop. These and the 'customer complaints process' in case of poor performance were considered the basis of the notifier's resistance management plan.

The Spanish Competent Authority also addressed the potential **risks of weed shifts** associated with the herbicide crop management and requested further information on this issue. The notifier answered that weed shifts were not a problem specific to maize NK603 and did not consider weed shifts as an adverse ecological, environmental or agronomic effect as they were considered part of the accepted 'baseline' in agriculture. In this context the notifier also stated that weed management recommendations for NK603 had resulted from extensive field trials over several seasons in several EU member states and would be submitted as label recommendations for the evaluation of the herbicide in the framework of Directive 91/414/EEC. However, none of these results were presented in the respective notification. The notifier further referred to the notifier's collaboration with scientists during field studies on weed community change and attached several abstracts of ongoing and unpublished studies on this topic.

In a further question by the Spanish Authorities (February 2007), the Authorities again emphasized the need to assess indirect effects of herbicide treatments on the **farmland biodiversity** through the weed populations which was not covered by the safety assessment under Directive 91/414/EEC and requested information on such effects as well as a case-specific monitoring plan

under different European conditions to cover such effects. The notifier considered that no further data regarding indirect effects on biodiversity were needed to complete the risk assessment and attached Roundup Ready herbicide use recommendations from Spain (Cuaderno Tecnico no 6, in Spanish) and France (in French) as well as the Registration of a Roundup product for in-crop use in Spain (for non-GMO use, in Spanish) as well as a Safety Evaluation Summary for NK603 maize developed for Spain summarizing the safety characteristics and the good agricultural practices for its use over herbicide tolerant crops (in Spanish). The use recommendations of Roundup Ready applied in herbicide tolerant maize for Spain included 1-2 applications of Roundup Ready in different combinations with other selective, residual herbicides at a rate of 2,5-3 l/ha at the 4-6 and/or 8-10 leaf stage of maize (see Cuaderno Technico No 6 of NK603 maize). The recommendations also stated that best results would be obtained when the herbicide was applied post-emergence, before the weeds reach 10 cm in growth. Another application at 4 l/ha was recommended if annual herbs or sensitive perennial herbs occurred at a later stage. A similar recommendation for glyphosate tolerant maize was given in the French use recommendations, depending on the weed infestation.

The Spanish Authorities again requested information on potential effects of glyphosate and the monitoring of such effects under a general surveillance plan or a case-specific study. In its answer the notifier referred again to the safety assessment of the herbicide under Directive 91/414/EEC and did not consider a post-market monitoring of potential effects of glyphosate on the environment as necessary. The notifier referred also to other monitoring frameworks such as the Drinking Water Directive and the Water Framework Directive and the Thematic Strategy on the Sustainable Use of Pesticides which, according to the notifier's view, included the monitoring of pesticide residues.

As the Spanish CA again asked for the inclusion of potential effects due to changes in weed control management (weed shift, resistance development, effects on non-target organisms) in the risk assessment and in a specific monitoring plan (November 2007), the notifier finally included potential impacts of the weed control on weed population and biodiversity in its general surveillance plan (see also 2.11).

Table 28. Studies provided in GMP notifications assessing potential effects of the GMP through changes in agricultural practices.

Y…study conducted by the notifier or on behalf of the notifier; N…reference to assessment in other notifications; "-"….no data presented; 91/414/EEC = reference to Directive 91/414/EEC.

GMP	Description of specific cultivation techniques for GMP	Identification of differences to non-GMP	Effects of use of non-selective herbicide	Resistance development
Oilseed rape Ms8xRf3	-	-	91/414/EEC	-
Potato EH92-527-1	-	-	-	-
Maize MON810	-	-	-	IRM plan
Maize Bt11	-	-	-	IRM plan
Maize 1507	-	-	Y[1]	IRM plan
Maize NK603	-	-	91/414/EEC	91/414/EEC
Maize 59122	-	-	-	IRM plan
Maize 1507xNK603	-	-	91/414/EEC	91/414/EEC

Maize NK603xMON 810	-	-	91/414/EEC	91/414/EEC

[1] see text for explanation; reference to OECD (2002)

2.11 Proposed risk management and monitoring

2.11.1 Risk management measures and monitoring plans proposed

Generally, no adverse effects of a GMP to the environment were identified during the risk assessments carried out by the notifiers. The only exception was the development of insect resistance of the target organism for GMPs with insect tolerance traits. Some notifiers classified the proposed IRM plan both, as a risk management measure and as case-specific monitoring (maize lines Bt11, 1507, 1507xNK603, 59122) while others considered it only a case specific monitoring method (MON810, MON810xNK603, see Table 29). Specific risk management measures were only proposed in the notification of oilseed rape Ms8xRf3 as long as cultivation was included in the scope of the notification.

In the original notification of oilseed rape Ms8xRf3 from 1996 no monitoring plan was proposed. In the update of 1999 specific environmental risks of this GM oilseed rape were identified such as the occurrence of GM volunteers, outcrossing of the GMP to wild relatives and the environmental impact of the herbicide use. Therefore the notifier proposed a monitoring plan lasting for one year and a product stewardship programme for the monitoring of agricultural areas from seed production to large scale releases. Monitoring methods included the inventory of flora in the fields and their vicinity, the estimation of the amount of herbicide used in a large-scale demonstration field, a comparison of herbicide programmes based on Liberty® (glufosinate ammonium) and conventional herbicides (efficacy, number of applications, amount of active ingredient) and a biodiversity monitoring (impact of changed practices on farm land biodiversity) as well as interviews with farmers. A distinction between case specific monitoring and general surveillance was not made by the notifier. The notification update of 2003 contained the development of agricultural guidelines for growing the GM oilseed rape in order to address the occurrence of cross-pollination. It also contained weed and volunteer control recommendations. Monitoring provisions were proposed focusing on GM volunteers and out-crossing to wild relatives. Methods suggested included counting of GM oilseed rape volunteers in each pair of fields as well as counting of certain species of wild relatives in the field and field borders of 20 pairs of GM/non-GM fields in France, Germany and the UK. In view of the restriction of the scope of the application to import and processing, the notification update in 2004 identified no risks of the GMP. Hence, no specific strategies for risk management or provisions for case specific monitoring were proposed in the revised monitoring plan.

In the notification of the potato EH92-527-1 the notifier proposed a monitoring plan (2004 update) which related to molecular parameters (stable insertion of genes, lack of expression of ORF4), plant performance (amylase/amylopectin ratio, glycoalkaloid levels in tubers, several plant characteristics, susceptibility to diseases and pests, feed quality parameters) and ecological parameters (persistence, volunteer management inside and outside the managed field) although the notifier stated that no particular concern was identified in the ERA that required a specific monitoring effort.

However, by the proposed monitoring efforts the notifier aimed to verify the risk assessment assumptions over a prolonged period.

In the case of maize NK603 the Spanish Authorities repeatedly requested an assessment of effects of the herbicide use on farmland biodiversity due to changes in weed control management (weed shift, resistance development, effects on non-target organisms) under different European conditions to be included in a case specific monitoring plan. The notifier finally included the monitoring of potential impacts of the weed control on weed populations and biodiversity in its general surveillance plan (see also 2.10). In its evaluation the Spanish Authorities criticized the general surveillance plan as insufficient and requested information regarding the use of available information from monitoring networks established in different countries which should be taken into account. In its answer the notifier stated that many of the existing monitoring systems and networks collecting environmental data were unlikely to provide the relevant data for monitoring the impact of GMPs and that they would differ from country to country. In addition, they might not be feasible to modify these programs in order to make them suitable for general surveillance. The notifier stated also that information from these networks would only be considered on an *ad hoc* basis to assess whether an observed effect was associated with the GMP or not. Consequently, the Spanish Authorities requested further information on the availability of monitoring networks already established in different countries which resulted in an updated monitoring plan submitted by the notifier, mentioning the necessity of criteria for the selection of such networks but not listing them.

With respect to general surveillance all notifications contained provisions except maize MON810. Maize MON810 was approved under Directive 90/220/EEC not foreseeing the necessity of a monitoring plan (Table 29). The proposed monitoring plans generally differed little and contained similar suggestions such as:

- the use of pre-defined, adverse effects reporting format, distributed to sufficient number of users of GM maize / a subset of European maize growers cultivating more than 5 ha of GM maize in representative areas of the EU
- information provided via product briefings, technical literature, websites, official registers, government publications, telecommunications, media and the Internet
- information collection from selected existing networks: seed supply, distribution networks, key external networks such as organisations normally involved in agriculture, connected to agriculture, the environment, human and livestock health
- feedback from selected external networks; record keeping via the company network or toll-free telephone number

Details on selected networks in individual countries were not included in the notifications. Also details as to where and when the monitoring was going to take place were generally not included in the notifications. In some cases the notifier stated that the intensity of the general surveillance was unlikely to be the same in each of the different EU countries (maize NK603, maize NK603xMON810, maize Bt11) or that general surveillance activities would be proportionate to the extent of cultivation of the GMP (e.g. maize 1507xNK603) without further specification on how this could be done.

Table 29. Proposed risk management measures and monitoring provisions in GMP notifications.

Y = yes/suggested; IRM plan = insect resistance management plan; NTO = non-target organisms, ”-“ = none submitted/suggested

GMP	Risk management measures	Case-specific monitoring	General surveillance	Farmers questionnaires as main component of GS
Oilseed rape Ms8xRf3	Y?*[1]	Y[1]	Y	-
Potato EH92-527-1	-	Y	Y	-
Maize MON810	-	IRM plan	-	Not relevant
Maize Bt 11	IRM plan	IRM plan	Y	Y
Maize 1507	IRM plan	IRM plan	Y	Y
Maize NK603	-	-	Y	Y
Maize 59122	IRM plan	IRM plan	Y	Y
Maize 1507xNK603	IRM plan	IRM plan	Y	Y
Maize NK603xMON 810	-	IRM plan	Y	Y

*Agricultural guidelines to control cross-pollination, weeds and volunteers; [1] as long as cultivation was not excluded from the scope of the application

3 CRITICAL APPRAISAL OF THE ENVIRONMENTAL RISK ASSESSMENT IN GMO NOTIFICATIONS

3.1 General remarks

The analysis of the data presented in ERA of the notifications formed the basis of this report (see chapter 2). This chapter presents a critical appraisal of the data submitted by notifiers in view of the legislative and scientific requirements for the ERA. This includes the evaluation whether the conclusions drawn in the ERA were based on a robust data set and the identification of gaps in the data provided. The guiding question was whether the principles established for the ERA according to Directive 2001/18/EC were being followed and to what extent the requirements of Directive 2001/18/EC regarding the evaluation of potential adverse effects on the environment have been fulfilled. The conclusiveness and comprehensiveness of the data in view of the risk conclusions drawn in each notification are critically reviewed as well as any obvious inconsistency within or between notifications. Shortcomings in the ERA due to incomplete or incomprehensive data presentation are discussed.

This chapter is divided into two major subchapters. The first (cross sectional issues) deals with the identification of data gaps and shortcomings that are either relevant for several assessment categories or for the ERA approach in general, while the second (specific assessments) covers issues identified in each specific assessment category during the review of notifications.

Based on this critical appraisal the resulting needs for improvement of and further guidance for the ERA are outlined in chapter 4.

3.2 Cross sectional issues

In the notifications analysed in this report several shortcomings and data gaps were identified which are relevant for more than one or even all of the individual assessment categories considered. Such shortcomings refer to the generation of data for the evaluation of specific traits or of potential effects of the GMP on the environment. In other cases these shortcomings consider the way how these data are presented and referenced in the notifications. Additionally, shortcomings were identified with respect to how conclusions were made on a certain risk based on the evidence provided in the notification. Such difficulties with respect to the data generation, presentation, and argumentation are in many circumstances comparable across the different assessment categories and are discussed in this chapter.

3.2.1 The environmental risk assessment (ERA) model

Fundamental to the robustness of the delivered data in the ERA are the underlying assumptions of the applied risk assessment model which lead to a broad or narrow interpretation of the provisions of the ERA put forward in the relevant regulations. A narrow and exclusive approach to the ERA is more risk-prone and less precautionary as it has a higher chance to overlook potentially adverse effects, but may be more time and resource saving as it requires data from less complex testing systems. Such a narrow approach is applied when a strictly ‘trait-based’ ERA is carried out which focuses solely on toxicological endpoints. Consequently, the testing is thought to be faster and the

results are easier to handle for both applicants and regulators because restricted in scope and complexity. However, this approach has been critizised for its lack of ecological science and the lack of consideration of the receiving environment (Andow & Hilbeck 2004, Andow et al. 2006, Lang et al. 2007). In contrast, a broad and inclusive approach to the ERA is less risk-prone and more precautionary, but may require more ressources.

The current ERA model applied by the applicants of GMP notifications reviewed in this report is based on the assumption that a GM crop consists of two parts that function in a linear additive fashion:

Crop + novel GM transgene product = GM crop

The crop plant is declared 'substantially equivalent' and safe, consequently, the only novel aspect is the added transgene product, most likely a novel protein (e.g. *Bt* protein). So, the GMP is effectively reduced to the novel trait/protein and, if this novel protein is a known pesticidal substance, it constitutes the only stressor identified that requires testing. Consistent with this thinking, the novel transgene product is tested as a chemical (as purified microbially produced protein, not extracted from the GMP) following the guidelines established for pesticide testing. If this novel protein is not a known pesticidal substance, the GM crop is considered safe and no further testing for ecotoxicological purposes is required.

Under this model, a number of important, risk-relevant aspects are excluded: any secondary stressors such as the broad-spectrum herbicides required to realize the benefit of any herbicide-resistant crop (Hilbeck et al. 2008a); any unintended effects resulting from the transformation process (epigenetic, pleiotropic, etc.; Prescott et al. 2005); or any combination effects arising from interactions of the novel substance with existing natural plant defence (secondary) compounds. The scientific adequacy of this currently practised approach to the ERA is highly disputed (Andow & Hilbeck 2004, Birch et al. 2004, Andow et al. 2006, Lang et al. 2007, Andow & Zwahlen 2006), and argued to fail to comply with the provisions put forward by the EU Directive 2001/18/EC (Hilbeck et al. 2008a).

Improved ERA models have been developed that focus on the whole plant including any required chemical or agronomic measure. Such an improved ERA concept will be proposed and described in more detail in chapter 4.

Ecotoxicity testing: testing species – not from the receiving environment

Under the above discussed narrow approach to the ERA, the novel compound (e.g. *Bt* toxin) in isolation from the GMP is recognized as the sole stressor. This allows the notifiers to follow closely the familiar ecotoxicity testing methodologies developed for environmental chemicals like pesticides with universal applicability (Garcia-Alonso et al. 2006, Romeis et al. 2008). These methodologies are prescriptive with regard to testing organisms and detailed protocols. However, no standardized tests have been developed for GMOs so far. Most testing organisms are chosen from a list of universal standard test species that are not representative for or even present in a given receiving environment where the GMP is grown. Another aspect is that these tests rely heavily on ‘surrogate’ proteins (e.g. microbially produced) used as test substances. Very rarely the parts of the GMP or the GMP as a whole are used for testing. Although, sometimes pollen is used as test

substance, it remains debatable whether or not pollen qualifies as ‘organism’. The EU Directive requires the testing of the ‘genetically modified **organism**’ per se.

This additive risk concept, as currently applied in GMP notifications, does also support the recycling of 'old' data for other notifications of GMPs expressing the same transgene product (e.g. the Cry1Ab toxin). For instance, when hybrids of two single-gene GM maize varieties are conventionally crossed and the resulting 'stacked' hybrid maize now containing both transgenes is submitted for authorization, the notifiers simply added the relevant ecotoxicologial testing parts of two 'old' notifications of each individual single-gene parental events and submitted them for regulatory approval. No new testing with the actual stacked GM hybrid is carried out – as – in the understanding of the notifier - the stressor, the isolated protein, remains the same whether it is in a single-gene event or in a stacked gene event. This stands in contrast to the common knowledge about gene functioning varying in different genetic backgrounds. The same transgene can behave differently in different cultivars and lines. In contrast to the evaluation of the GMP for the ERA, this is fully understood in the development process of GMPs where every line containing the same transgene is tested individually for its performance before the most suitable is selected for commercial purposes.

Lack of clearly formulated risk research hypotheses for laboratory and field research for the ERA

In the ERA concepts and ecotoxicity testing programs of the notifications, neither formal risk research hypotheses are formulated nor followed through in a stringent, transparent fashion. This leads to the situation that an effect is measured but subsequently put at disposal whether or not the effect is actually meaningful. If no significant differences are detected the data are presumed to support safety claims while, in contrast, significant differences pointing towards an adverse effect do not mean that they may support the opposite. Consequently, they are always declared as 'biologically irrelevant' by the notifier. In other cases the effect of the GMP is compared to other, stronger effects of other treatments, such as effects due to the use of conventional plant protection products. Hence, the observed significant differences between the GMP and the untreated control are considered small, therefore meaningless and are used to support safety claims. This means that the experiments may not be suitable to assess the safety of the GMP and are of little rigor for risk assessment purposes (see also chapter 3.2.12).

Similar approaches are taken for experiments at the field level. As the information provided with the notifications show, field studies to measure 'non-target effects' are often conducted using broad 'community level’ biodiversity censuses but without proper risk research hypotheses. This approach stands in stark contrast to the tests carried out to measure target effects. Target effects – e.g. resistance against the European corn borer (ECB) - are always narrowly focussed, never compared to the entirety of similar target effects delivered by any or all other existing technologies (be these other cultivars also resistant to ECB or other control methods, like using *Trichogramma* spp. or cutting and ploughing of the maize remains). The only comparator here is the isogenic line (for criticism on the lack of efficacy evaluations in GMP notifications see also chapters 2.4 and 3.3.3). The above described differences in test designs lead to an unacceptable scientific double standard. The lack of a proper risk research hypothesis has also to be seen in conjunction with the

ignoring of the step-by-step principle and the lack of integrating results from Part B releases (field releases) for the notification of Part C (placing on the market) of a specific GMP (see also chapter 3.2.8).

The choice of comparators can pre-define the outcome, for example when 'normative' comparators are used that do not add scientific information on causes of effects but aim to 'norm' against another method or technology. For example, the choice of chemical pesticides as 'positive controls': their (known) effects on non-target organisms do not add any scientific explanation regarding an observed adverse effect of the GMP as it is a scientifically entirely unrelated treatment. Nor does it help predicting and modelling the future consequences for the agro-ecological systems of the adverse effect of the GMP but it does 'norm' the observed effect against an alternative technology. As can be seen in many notifications this is also the case when several unmodified cultivars in addition to the isogenic control are used as comparators. Trials with additional unmodified cultivars could only produce relevant scientific data if they included additional cultivars/lines that would also carry the same transgene construct. This would indeed deliver additional scientific data and knowledge about ‘genotype x transgene’ interactions. Therefore, choices of unmodified cultivars as comparators should be informed by clear research hypothesis and justified.

In conclusion, the currently applied approach of the ERA by the notifiers reveals a lack of acknowledgement of ecological sciences, weakens the role of the ERA in the decision making process and uses double standards in the assessment norms. It builds on the assumption that small effects compared to large variability are irrelevant and, thus, permits the conclusion of safety. However, this is not valid from scientific point of view. Ecological sciences hold many examples of ecological damage that began as a small adverse effect lasting possibly for fairly long periods of time (EEA 2001).

3.2.2 Lack of consideration of different exposure pathways

A thorough assessment of the potential exposure routes and potentially exposed non-target organisms was missing in most of the GMP notifications reviewed in this report. Either only non-target Lepidoptera exposed to GM pollen were addressed in the notifications (e.g. maize 1507, maize Bt11), or the expression values of the GMP and thus the data basis used for the evaluation of exposure of non-target organisms was not representative for European conditions (maize 59122).

Once grown commercially, GMPs and GMP transgene products can be introduced into the environment and affect target and non-target organisms via different pathways. Exposure of organisms in the agronomic context of the GMP cultivation e.g. by expression of *Bt* toxins in different plant tissues, is of particular relevance. Expression can vary significantly between and among plant tissues, depending on the age of the plant (Dutton et al. 2004a, 2004b), age of the leaves (Dutton et al. 2005) and the leaf section and may even depend on the position of the leaf on the plant (Abel & Adamczyk 2004). Exposure via pollen-flow is not only relevant for co-existence issues but also necessary to asess the exposition of non-target organisms outside the agricultural field.

After the cultivation period organisms may still be exposed to the GMP or its products when remaining parts of the crop are subject to decomposition processes. In the case of *Bt* maize plant residues such as leaves and roots remaining on the field constitute a considerable reservoir for

Cry-proteins after harvest (Baumgarte & Tebbe 2005). Cry-proteins from decomposing plant parts are available for organisms which degrade plant material above and below ground. Additionally, soil organisms such as isopods ingest and excrete *Bt* toxins thus making them available to other trophic levels (e.g. Wandeler et al. 2002). Ingestion and excretion of GMPs by livestock and consequently application of manure on agricultural fields may be a relevant exposure pathway. Apart from the presence of GMP transgene products in agricultural soils, their presence in headwater agricultural streams in corn cropping areas due to the presence of corn pollen or unharvested crop by-products were recently described by Rosi-Marshall et al. (2007). The relevance of exposure assessments has been further emphasized when it was observed that a range of herbivorous or predatory organisms present in or near agricultural systems contain the Cry-toxin and the indication of tri-trophic transfers of these toxins although the specific transfer mechanisms are still unclear (Harwood et al. 2005, Harwood et al. 2007). The requirement to consider different exposure pathways is also evident from other regulatory areas such as PPPs. The assessment of the 'fate and behaviour in the environment' of a PPP is a provision according to Directive 91/414/EEC. According to Annex III of this Directive the information provided should be sufficient to '…*predict the distribution, fate and behaviour of the PPP in the environment as well as the time courses involved; identify non-target species and populations for which hazards arise because of potential exposure and identify measures necessary to minimize contamination of the environment and impact on non-target species'* (Annex III, 9.). For this purpose predicted environmental concentrations (PEC) of water, soil and air, are to be provided.

Consequently, a well-founded assessment of the potential exposure routes and potentially exposed non-target organisms will constitute an important first step in the ERA, also relevant for a range of different assessments (Hilbeck et al. 2008b). If exposure via a particular pathway (e.g. phloem) can be excluded, this will consequently shape the following ERA process. However, in no case such an analysis was made in the reviewed notifications. Exposure pathways are generally not individually addressed thus resulting in a set of standard test organisms derived from pesticide or chemical testing without consideration of the relevant exposure in agro-ecological systems. This shortcoming again derives from the general lack of a broad ERA concept, as already addressed already elsewhere in this report (see also chapter 3.2.1).

3.2.3 Risk assessments often based on arguments rather than data

Current risk assessment practice shows that notifiers frequently base their conclusions regarding a certain risk on assumptions, on cross-referencing to other assessments or to the published literature rather than on data specifically generated for and with the respective GMP (see chapter 2). This is clearly not following the general principle of the ERA that the evaluation of potential adverse effects should be based on scientific and technical data and on common methodology for the identification, gathering and interpretation of the relevant data (Guidance Notes to Annex II of Directive 2001/18/EC).

This is in particular to be seen in conjunction with the rather general requirements for the ERA as outlined by the regulatory framework and guidance documents for GMOs, compared to other legislative areas such as chemical registration (Regulation (EC) 1907/2006) or pesticide regulation (Directive 91/414/EEC) which contain strict requirements on the tests and data to be submitted. Their

authorization procedure is generally guided by the principle 'no data no market' and requires generation of data for the substances to be authorized (see Regulation (EC) 1907/2006, Preamble 19 and Article 5). In general all information on that substance shall be contained in the dossier submitted or should provide a justification where data and information were not provided (Directive 91/414/EEC, Annex III). Such a rule is generally not followed by notifiers of GMP notifications. In particular relevant data from earlier experimental releases (Part B) are generally not contained in notifications of GMPs for placing on the market (Part C) thus not following the step-by-step principle (see chapter 3.2.8). The relevance of the generation of specific data for a GMP is also given in the context of the presentation of data in the ERA or the technical dossier. The evaluating authority must be able to judge whether the argumentations of the notifier and the risk conclusions are based on data specifically generated by the notifier or cited from published studies conducted by others, or on both. Thus, a clear distinction of results derived from data generated by the notifier for a specific GMP from results cited from the published literature is fundamental for the comprehensiveness of risk conclusions.

3.2.4 Shortcomings with respect to the compilation and presentation of information

When applying for market authorisation the evaluating authorities must be in the position to assess the information provided by the notifier within a certain time frame. The question of how information on the assessment of environmental risks in GMP notifications is presented is crucial, because it may significantly facilitate or impede the risk assessment procedure and thus the whole authorisation process. The ability to understand how the notifiers arrive at their conclusions on risks may not only alleviate the assessment by the relevant authorities but may also increase the confidence of the public in the authorization procedure of GMPs.

Although the proposed ERA structure as outlined by EFSA (2006) entailed some improvement with respect to the presentation of information in GMP notifications, the amount and complexity of information presented and the requests for additional information have constantly increased during the last years which still aggravate the need for a comprehensible structure in the notifications.

The notifier has to demonstrate the absence of potential environmental risks of the GMP in question in a comprehensible way. However, in risk assessment practice this is not always the case. As risk assessment of GMPs is generally less formalized than in other regulatory areas there is an urgent need for clear standards of how information is to be presented. The major shortcomings identified during this review with respect to the form of presentation of information are addressed in the following sections.

3.2.4.1 Lack of distinction of published and unpublished studies

Internal studies conducted by or on behalf of the notifier as well as unpublished studies conducted in co-operation with the notifier usually constitue the main information sources for the risk assessment. In order to support conclusions on a certain environmental risk notifiers frequently cite published literature. This is common practice in the ERA of GMPs and in principle appreciated by the Competent Authorities. However, it is not always apparent whether a particular study which is mentioned in the ERA is a notifier study or a published study as unpublished and published studies are generally not separately discussed. In some cases the question whether a specific study cited by

the notifier is a published or a notifier study can be clarified by looking in the reference list. However, in other cases the reference is not exactly specified (e.g. no journal or no study number indicated) or no reference is given at all. Then it is not possible to identify the source and – as may be necessary in some cases – evaluate the information and the data of the study in detail. Thus a clear separation of internal studies and unpublished reports of the notifier from published studies and a separate discussion of data generated by the notifier and of data derived from published studies would be desirable.

In this respect also the question of identity of notifier studies deserves particular attention. The references of internal notifier studies and unpublished reports provided need to be clearly identifiable by an author's name, a year and a study number. It must be clear whether the study was conducted by or on behalf of the notifier, by whom the study was carried out and when. Such studies also should be listed in the references separately from published studies.

The distinction of published and unpublished studies is not only relevant for the original notifications but in particular also for updates or additional information provided by the notifier later in the notification process (see also 'reiteration of information'). There is an urgent need for a clear separation of studies already provided in earlier versions of the ERA and the submission of completely new studies as it is for the separate discussion of published and unpublished (notifier) studies.

The case of oilseed rape Ms8xRf3 and the submission of information for the assessment of persistence and invasiveness by the notifier in the original notification and in several updates is particularly suited to demonstrate such shortcomings. For the notification of oilseed rape Ms8xRf3 the notifier submitted 12 annexes in 1998 consisting of a mixture of published and unpublished reports of field evaluations and safety evaluation programmes of GM oilseed rape. In later updates of the ERA results of these programmes and field trials were summarized by the notifer by citing as follows 'The results of the PROSAMO programme…' or 'as the studies by NIAB have shown…' without giving a further reference (see also chapter 2.6). The lack of specification of the reference made it impossible to identify whether the studies referred to were actually those contained in these annexes provided in 1998.

3.2.4.2 Identification of the specific GMO-event in submitted studies

The citation of studies by the notifier does generally not allow the conclusion whether the study cited was conducted with the GMP in question. Even the complete literature citation in the reference list often gives no indication which particular GMP was used in the study. As an example, in the case of maize NK603 a study was submitted for the assessment of non-target organisms, assessing Collembola (Goldstein 2003, n.st.). This study was a master's thesis from an US University using a Roundup Ready soybean and a Roundup Ready Corn. However, the exact event was not indicated in the whole study. Similarly, the reference to published studies using GM herbicide tolerant crops does usually not allow the conclusion whether the respective GM maize (NK603) was actually included in the study. Thus a clear indication of the GMP used when quoting a published study is a necessary step to be able to conclude on the relevance of the evidence provided by the notifier. The submission of data corresponding to the respective GMP of the notification is a prerequisite to fulfil the case-by-case principle of Directive 2001/18/EC (see also chapter 3.2.9).

3.2.4.3 Insufficient labeling of and cross referencing between different parts of the notification

The way data is presented in Annexes to the ERA and reference is made to these data differs considerably between notifications. The notification of oilseed rape Ms8xRf3 is a prominent example with several shortcomings in this respect. The original notification submitted in 1996 consisted of 3 main documents (A, B and C), each divided into several parts. Document C was designated as an Annex, however, consisted itself of several Annexes which were inconsistently numbered. Part I of Document C contained Annex VI.3.1., VI.3.2. etc., while Part II contained Annex II.1 and Annex II.2. Part 3 contained again an Annex II.1 and an Annex II.3.). Reference made by the notifier in the ERA to a particular Annex or Part of the Annex did generally not allow finding the respective data.

A similar problem arises when reference is made to tables in the Annexes. Table numbering is often not consistent throughout the technical dossier, the Annexes to the technical dossier and separate Annexes (e.g. of updates or additional information provided) thus adding confusion if data are claimed to be present in a specific table.

Some notifications consist of different updates that neither have a clear structure of their own nor are clearly connected to the original notification. If studies attached by the notifier are not labelled accordingly, it is hard to trace them in various parts of the notification. For instance in the case of oilseed rape Ms8xRf3, additional information consisting of twelve annexes was delivered by the notifier in 1998 containing several reports or studies. In many cases, neither an author is indicated nor a study number assigned to the study by the notifier. This is e.g. the case for a study monitoring residual effects in previous transgenic oilseed rape field trials (attached as Annex 2, Part 2, Annex III.2.) and a study comparing the rhizobacterial flora of transformed and non-transformed rapeseed plants (attached as Annex 2, Part 2, Annex III.1.). In later updates of the ERA in 1999 and 2003 the notifier referred to previously submitted studies for the assessment of effects of the GMP on biogeochemical processes, but only referring to ‘a study comparing bacterial rhizosphere populations in GM and non-GM oilseed rape fields and to a study monitoring residual effects by evaluating the growth of wild flora and agricultural crops in Belgian and Canadian field trials’ without giving any further details or references. Thus a clear identification of the specific studies was not possible and it can only be assumed that these studies were identical with the ones previously submitted.

3.2.4.4 Reiteration of information

Another shortcoming in GMP notifications is the reiteration of large parts of text. The repetition of large parts of text or whole paragraphs does not contribute to the understanding of the information provided but rather conceals the extent to which new information has been provided (e.g. in notification updates).

Reiteration of text is often the case when information is presented simultaneously according to Annex III B and Annex II (ERA) of Directive 2001/18/EC. In other words there is no clear separation between the information provided and their evaluation by the notifier in the context of the ERA. Similarly, in notifications according to Regulation (EC) 1829/2003 often a separate ERA document is provided, e.g. if such was already submitted within the framework of a previous notification ac-

cording to Directive 2001/18/EC, but without indication if the information provided in the notification and the ERA is identical or if any new information has been added.

Similarly, this is also relevant for the information provided in the monitoring plan of the GMP. Often several updates of the monitoring plan are submitted, e.g. on authority request. New information in such updated monitoring plans is never clearly indicated but interspersed somewhere in the text of the monitoring plan (e.g. maize 1507, maize NK603). In the case of maize NK603 the Spanish Authorities requested information on indirect effects of herbicide treatments on the farmland biodiversity due to changes in the weed control to be covered by a case-specific monitoring plan (see also chapter 1.10). After several rounds of commenting and answering between the notifier and the Spanish Authority, the notifier finally included potential impacts of the GMP of the weed control on weed communities and non-target organisms in the general surveillance plan. In the attached monitoring plan the respective commitment consisted of one or a few additional lines scattered in the text but not separately marked. Thus clear indication should be made what new information was provided in a specific update.

During the notification procedure Competent Authorities frequently ask notifiers for clarifications or further information if they consider the existing information insufficient. However, in many cases the notifiers provide information in these updates that was already previously submitted. For instance in the answers to the questions raised by EU member states the notifier of maize 1507 referred to studies that were already submitted in the original notification. With respect to effects on non-target organisms no new data were presented, except two new published studies. Also in other cases the same citations were provided in updates as in the original notification and only few new quotations were scattered in the updated ERA text (e.g. the assessment of dissemination, persistence and invasiveness of oilseed rape Ms8xRf3). In such cases the literature already cited in the original notification was redelivered without indicating which new citations were added.

3.2.4.5 Insufficient documentation and traceability of information

In order to enhance transparency and confidence in the notification procedure it is important that the conclusions of the notifier on a particular risk of a GMP are being substantiated by data and analyses. Since most of the information on the GMP at this state of the market release will have been collected by the notifier, field or laboratory studies need to be fully documented including the methodology used for data collection, raw data, and analysis, and need to be available to the authorities.

The evaluations of phenotypic characteristics or compositional parameters of a GMP are usually based on field trials conducted at various locations and in different growing seasons assessing different parameters. Details on these field trials was often only contained in one or several Annexes attached but no overview table with information concerning the design, methodology or conditions of the field trials presented in the ERA. Thus in order to get an overview of what was assessed where, when and how, was not possible without falling back on the annex(es) since the most important details on the field trials were provided in the (often divers and abundant) annexes only.

In the following notifications the notifier presented data without providing the background information of the corresponding studies.

Agronomic data of maize 1507 and compositional data and expression levels of maize Bt11 derived from various field trials were presented in the notifications, but no details, such as field trial design, methodology, location, replication etc. were provided. The presentation of results devoid of the background information of the study renders the comprehensiveness of the data and the conclusions of the notifier more difficult.

In the original notification of maize Bt11 compositional data were only provided for grain and not for forage and no anti-nutrients or secondary metabolites were included in the analyses. In the update of November 2003 the notifier referred to compositional data for forage gained in the US 1996 field trials and to the analysis of phytic acid and trypsin inhibitors in grain gained in field trials in France 1998. These data were claimed to be provided as 'replies to questions raised by the Danish Authorities', but neither this document nor the data were attached to the update. Thus the conclusions of the notifier were not comprehensible.

In the case of maize 1507 agronomic data were presented for two different set of field trials in Spain in the same year. One set of field trials consisted of three locations and the other set of two locations. Different agronomic parameters were assessed in these field trials. Details on the design of these field trials were incompletely indicated with respect to maize hybrids and controls, herbicide treatment etc. Hence, such an incomplete documentation of field trials and a lack of traceability of the data presented leads to a lack of understanding of the data provided by the notifier.

In many notifications the notifier commissions experimental studies, e.g. toxicological studies, to private or company internal environmental laboratories. As contracting work these studies are self-contained pieces of work and are as such usually attached to the notifications. In the case of maize 1507 the notifier referred to the results of such studies without making them available to the authorities. Three toxicological studies were not attached to the notification and the toxicological study with the monarch butterfly (Bystrak 2000, n.st.) was only provided by the notifier upon request of the Swedish Competent Authority. In any case, only from the notifier's statements ('no effects were observed') and without access to the details of the study it is hardly possible to follow the notifier's conclusions.

For the notification of oilseed rape Ms8xRf3 the notifier submitted the original notification in 1996. In 1998 12 annexes were provided by the notifier consisting of a mixture of published studies and various published and unpublished reports of field evaluations and safety evaluation programmes of GM oilseed rape. In the following updates of the ERA in 1999 and 2003 the notifier made reference to several field trials or results of monitoring programs or experimental field trials, only by indicating the institutional names (e.g. 'NIAB' or 'PROSAMO' etc.). As no clear cross-reference was made to which study the notifier actually referred (study number, author and year), it was not possible to establish a link between the summaries of the results provided in the ERAs in 1999 and 2003 and the studies provided in the Annexes in 1998. Again, in this case a clear traceability of the data underlying the conclusions in the ERA was not given.

3.2.5 Limitations of field trials for the phenotypic characterisation of the GMP

In the GMP notifications reviewed in this report field trials were the basis for the generation of morphological and phenotypical data of a specific GMP. Generally, notifiers carried out field trials for the evaluation of:

- the expression of the inserted transgenes in the respective tissues of the GMO (see chapter 2.3)
- any potential changes in the composition of the GMP as compared to the non-GM control and to establish substantial equivalence (see chapter 2.5)
- the agronomic performance of a GMP in the field (see chapter 2.4)

These assessments were either carried out in separate field trials in different years or at different locations or, in some cases, different assessments were combined in one particular field trial in the same year and at the same location (e.g. assessment of expression and plant composition). Field studies to assess potential effects of the GMP on non-target organisms were usually carried out separately from the morphological and phenotypical assessment of the GMP and thus generally displayed a different field trial design (see chapter 2.8).

Several shortcomings with respect to these field trials (e.g. locations, design, comparators etc.) and with respect to the interpretation of data derived from these field trials have been identified during this review which will be addressed and discussed in the following sub-chapters.

3.2.5.1 Shortcomings in the design of field trials

Several shortcomings and scientific flaws with respect to the data collection and the presentation of information in the field trials for the agronomic, the compositional and the expression assessment in the nine notifications have been identified.

The methodology of field trials differed considerably across but also within notifications especially with respect to the locations chosen and the parameters assessed. Sometimes details on the experimental set up of individual field trials were incomprehensively presented in the notifications (see chapter 3.2.4). With respect to the design of the field trials the following shortcomings have been identified.

Field trial locations and growing seasons

Field trials were generally conducted at locations overseas (USA, Canada and South America) and/or in Europe. In certain cases assessments were done using plants grown in the greenhouse only (e.g. expression). For the discussion of the relevance and representativeness of locations chosen by the notifiers see also chapter 3.2.10).

A major issue is the **characterisation of a location** where a field trial has been carried out. It is often unclear why a specific location was selected for a field trial. No reasoning with respect to the representativeness of locations chosen was usually given by the notifiers in any of the assessments. Neither an argumentation nor data were provided in order to show that the locations selected were representative for European environments with respect to the parameters assessed in the field trials.

The identification of a specific location or site varied significantly across notifications. For basic morphological or phenotypic assessments of the GMP usually the country (e. g. Bulgaria), regional or district names (e. g. Aragon) or the name of the town closest to the field trial (e.g. Aberdeen) was used to identify a specific field trial location. In some cases a figure (e.g. Aragon 1, Aragon 2) or a name (e. g. Zaragoza: Montanana, Zaragoza: Pastriz, Zaragoza: Cogullada) following a regional denomination was used to distinguish different field trial sites. In only few cases a rough map was provided with an overview of the field trial locations. Hence, it was difficult to assess whether the field trial locations represented different agronomic regions and conditions within a country or whether they were actually situated close to each exhibiting similar agronomic and environmental conditions. The locations were only characterised by the indication of average temperatures and rainfall data. Only in few cases additional information such as information on the soil type or wind speed etc. at the location was also provided. Detailed information, in particular on the agronomic and environmental particularities of the locations, was generally not included.

The difficulty of the exact delineation of representative locations for field trials is further complicated by the fact that data derived from field trials conducted in different locations were often pooled (also across EU countries) and discussed as 'EU data', e.g. as opposed to 'US data' (see also Table 12, e.g. maize 59122). Information on individual locations was generally provided in the Annex only and not in the technical dossier. This made it difficult to derive information on differences between European locations, e. g. due to different agronomic or environmental conditions.

The number of **growing seasons** over which field trials were conducted also varied considerably between the notifications. This is in particular relevant if considered in combination with a particular location. Often a specific assessment (e.g. expression analysis) was only carried out with plants grown over a single growing season at one particular location. Rarely additional assessments were made at a particular location over two or more than two consecutive years. Data from a specific location for more than one growing season in Europe was frequently missing in the agronomic assessments in GM crop notifications (six out of nine notifications). At maximum two consecutive years were assessed at a specific location (e.g. agronomic assessments of maize NK603, maize 59122 and oilseed rape Ms8xRf3). The agronomic assessments from one year at one location were often complemented by further assessments but carried out at other locations in different years.

Data from more than one growing season of a particular site are important, because several biotic and abiotic parameters such as pest pressure can considerably vary between years. Testing seed varieties usually covers two to three years depending on the crop plant. In the requirements for the authorisation of plant protection products efficacy data must be reported for at least two growing seasons (Directive 91/414/EEC; Annex III, EPPO PP 1/226(1)). EFSA states that the scale and number of experiments should be 'sufficient to reflect the experiences under field conditions in a range of geographic locations over more than one season' (EFSA 2006a). Additionally, more than one representative growing season and multiple geographical locations representative of the various environments in which the GMPs will be cultivated shall be chosen (EFSA 2006a). However, this was rarely achieved in any of the reviewed notifications.

Field trial design

EFSA requires the specification of the protocols of field trials, especially with respect to replicates (EFSA 2006a). This information is essential not only for the characterisation of the methodology but also for the statistical evaluation of the data derived from the field trials. Generally, the indication whether the field trial design was appropriate for the detection of differences between the GMP and the non-GM control was missing in the information provided on the field trials.

Usually, a randomized complete block design was used in the field trials with a varying number of replications across notifications and depending on the parameter assessed. In cases where all three assessments were combined in one field trial, one out of four replications was often used for the expression analysis leaving three replications for compositional and agronomic evaluations. Exact information on the number of replications in the field trials was frequently not provided by the notifiers (e.g. agronomic assessment). Hence, it remained unclear whether the replication used in a particular field trial was sufficient in order to detect any significant differences between the GMP and the control.

Also the plot size used in the field trials differed between notifications and among individual sites within notifications. The units used to indicate plot size also differed between notifications. Frequently the necessary information to infer the exact plot size (e.g. number of seeds planted; number of rows, row width, etc.) in the field trials was not or not fully provided by the notifiers (e.g. four notifications for the agronomic assessment). A sufficiently large plot size is a prerequisite for the collection of meaningful data, as plot size can significantly influence the occurrence and abundance of organisms, e. g. aboveground arthropods (Prasifka et al. 2005) which may have implications for the assessment of target or non-target pest species in agronomic assessments.

Comparators used

The establishment of a relevant baseline is essential for the comparison of the GMP when differences in phenotypic and morphological assessments are to be evaluated in experimental field trials (agronomic evaluation, expression analysis, compositional analysis). For the relevance of the comparators used for the outcome of the ERA see also chapter 3.2.1).

With respect to phenotypic and morphological assessments comparators used were generally non-GM control plants. The description of the **non-GM control** used as a comparator varied significantly across as well as within notifications, depending on the field trial. Specification of the comparators was not provided in all notifications. In the agronomic assessment in three notifications they were missing at all, in two notifications they were only provided for some of the field trials.

Characterisation of the non-GM control ranged from the indication of the use of an ‘isogenic line’ to the indication of a breeding history of the GMP and the plants used as comparators. In most cases the relatedness of the control line to the GM line remained unknown, since merely the names of the hybrids were indicated without further explanation. Often it remained unknown whether the control line was used during the breeding process of the GMP and thus whether the control line constituted an isogenic line with a genetic background comparable to the GMP. Only in selected cases the exact breeding history of the GMP was provided and the GM and control lines specified (e.g. maize 59122). In this respect also EFSA guidance (EFSA 2006a) is not specific enough.

EFSA (2006a) states that comparative assessments should use the 'most appropriate control'. However, further specification is necessary to define such an 'appropriate control'.

For assessments of **herbicide tolerant GMPs**, the question whether the GMP is treated with the non-selective herbicide in the course of the field trials is crucial in order to assess any potential effects of the herbicide treatment on the phenotype of the GMP. In risk assessment practice, however, the GMPs used were either not treated with the relevant, non-selective herbicide or only herbicide-treated plants were used (see Table 30). A herbicide treated variant in addition to the non-treated GMP was not consistently included in the field trials or not in all field trials of a specific GMP notification. In selected cases plants were used for the assessments which were untreated although a treated variant had also been included in the same field trial (e.g. agronomic evaluation, maize 59122).

For the assessment of plant composition the application of the relevant non-selective herbicide on the GMP was more frequently included than in the agronomic evaluations. In six out of seven notifications of herbicide tolerant GM plants at least at some locations the GMP was treated with the herbicide for the compositional evaluation while this was only the case for three notifications for the agronomic assessment. However, often only one treatment variant was included in the field trials of both assessments (e.g. maize Bt11, maize NK603 and maize 1507xNK603 and maize NK603xMON810). In these cases already the field trial design made a comparison between the treated and the non-treated variant impossible.

In the evaluation of transgene expression the herbicide regimes applied were often identical with those of the agronomic or the compositional assessments if the same plots were used (e.g. maize 59122). However, in three cases the herbicide application for the assessment of expression was not indicated.

As both variants, the treated and untreated GMPs were not constantly included and assessed across and within notifications a separate analysis and comparison of the treated GMP with the untreated GMP was not regularly included in the assessments of herbicide tolerant GMPs. For the compositional assessments in only three out of seven notifications both treatment variants (treated and untreated) were included (although not in all field trials), but differences in the compositional values in the GMPs due to herbicide treatments were evaluated in only two notifications (see Table 15, chapter 2.5.1.3). In other cases, even if the field trial design would have allowed for such an analysis (i.e. both herbicide treatment variants were included) only one treatment variant was used for the assessment (e.g. agronomic assessment, maize 59122). Consequently, potential differences in the agronomic performance, the expression of the inserted transgenes and the composition of the GMP due to the application of the non-selective herbicide were usually not addressed. The approach chosen by notifiers is also not consistent with the EFSA advice 'to include both blocks of GMPs exposed to the intended herbicide and blocks not exposed to the herbicide, which would facilitate the assessment of whether the expected agricultural condition might influence the expression of the studied parameters' (EFSA 2006a).

Table 30. GMP notifications considering different herbicide treatment variants in assessments of composition, expression and agronomic parameters.

Only notifications of GMPs expressing a herbicide tolerance trait are displayed (7 of 9 notifications reviewed). Empty fields indicate that no European field trials for the evaluation of this parameter were conducted. glu+…treatment with glufosinate; glu-… no treatment with glufosinate; gly+ …treatment with glyphosate; gly-…no treatment with glyphosate; fb…followed by.

GMP	European field trials (years)	Composition	Expression	Agronomic behaviour
Oilseed rape Ms8xRf3	1994-1995	glu+		glu+
	2001-2002	glu+/glu-		glu+/glu-
	2004/EU (greenhouse)		n.i.	
Maize Bt 11	1995			glu-
	1996/1998	glu-		
Maize 1507	1999	glu- (FR)		
		glu+/glu- (IT)		
	2000	glu+/glu-	glu- (FR)	glu+/glu-
			glu+/glu- (IT)	
	2002			glu-
Maize NK603	1999	gly+	n.i.	
	2000-2002			gly-
Maize 59122	2003	glu+/glu-	glu+/glu-	glu-[1]
	2004	glu+/glu-	glu+/glu-	glu-[1]
Maize 1507xNK603	2003	gly+; glu+; gly+ fb glu+	gly+ glu+ gly fb glu+ (2003, 2004)	gly+ fb glu+
	2004-2005		gly+/gly-glu+/glu-gly fb glu+/gly fb glu- (2005)	
Maize NK603xMON810	2000	gly+	n.i.	

[1] herbicide untreated variant was used although herbicide treated variants were also planted in the field trials

Stacked GMOs, represented by two maize notifications in this study (maize 1507xNK603, maize NK603xMON810), are derived from their parental GM lines by traditional crossing. In general the morphological and phenotypic characteristics of the stacked GMPs are considered to be equivalent with those of their parental GMPs by the notifiers. In order to evaluate this assumption, a comparison of the stacked GM maize with the respective parental GM lines was carried out only in one of the two notifications for expression values. No comparison between stacked and single event maize was performed for plant composition or agronomic characteristics in both cases. Thus the assumption that these maize lines are comparable to their respective parental lines was generally not backed by specific data. Also EFSA considers GM parental materials as well as appropriate non-transgenic genotype(s) as the most appropriate comparators for stacked event GMOs (EFSA 2007).

The baseline for comparison of an **insect resistant GMP** may differ as the current traditional cultivation practices in conventional crops differ between continents, countries and possibly regions. In many studies provided by the notifier it was not indicated whether an insecticide treatment has

been applied to the control. In field trials conducted for the phenotypic assessment of the GMPs a non-GM control treated with an alternative pest control, relevant for the respective agronomic region, was usually not included. This stands in contrast to the assessment of potential effects of insect-resistant GM maize on non-target organisms which frequently includes a control that is treated with a conventional insecticide (e.g. maize MON810, one study of maize 1507, maize 59122, maize 1507xNK603, two studies of maize Bt11). The choice of inclusion of an insecticide as well as the type of insecticide used can considerably influence the outcome (Wolfenbarger et al. 2008, see also 3.3.3).

A similar problem is evident for the assessment of potential changes in the cultivation or management techniques of GMPs as compared to conventional crops. No separate field studies were submitted by the notifiers in any of the notifications to assess the practical use of the GMP and its non selective herbicides. In no case potential differences of the GMHT crop to the conventional herbicide treatment practice were assessed (see also chapter 3.3.9).

3.2.5.2 Data evaluation and presentation of results generated from field trials

Generally, the data derived from field trials for the agronomic, compositional or expression assessment were analysed for differences between the GMP and the non-GM control. The statistics applied comprised predominantly descriptive statistics such as mean values, standard deviation and the range of values. Whether a statistical evaluation was carried out (beyond descriptive statistics) in order to compare the GM with the non-GM control or not varied between notifications but also within a specific assessment of a notification. In other cases, if statistical approaches were used for the comparison of these phenotypical or morphological parameters of the GMP and the non-GM control was not specified in the notifications.

Mean values of data generated in field trials were generally calculated across locations (e.g. expression; agronomics). Mean values were generated ‘across European locations’, also if the data derived from different EU countries. Means per country were often not calculated and presented in the notifications. Data on individual locations were frequently presented in the Annexes only and rarely separately discussed by the notifiers. Location-specific results are particularly of interest with respect to phenotpyic assessments such as expression, composition and agronomic behaviour of the GMP as they give an indication of the specific crop-environment interaction at a specific location. The practice of the notifiers to present results at first on an ‘across location’ basis may mask differences observed at individual locations. Pooling of data from a large geographical range does not allow deriving any information on regional or local effects. This may be in particular relevant for insect pest and disease pressure which may differ significantly between locations and years.

A comparison of parameters of a particular location between **growing seasons** was mostly not possible as data of consecutive years usually derived from different locations, countries or even continents in all phenotypic assessments (expression, composition, and agronomics). For example, compositional data for more than one growing season at the same location were missing in six out of seven GM maize notifications. An evaluation of plant parameters at a specific location over consecutive growing seasons was thus generally lacking in GMP notifications.

A **statistical analysis** for significant differences between the GMP and the non-GM control was generally carried out only for parameters which were measured quantitatively and if replicated measurements were made. No statistical evaluation of the assessed parameters was carried out if the parameters were qualitatively described or if no replicated measurements were made, as e. g. in the agronomic assessments of the GMPs. For example, for maize NK603 the number of the parameters assessed in the agronomic characterisation of the GMP subject to a statistical analysis varied not only between field trials of different years but even between locations of field trials of the same year. Whereas yield parameters of maize NK603 were statistically analysed in all three growing seasons, plant health parameters were only analysed in one growing season. The notifier stated that statistical analyses were applied 'where appropriate' without any further explanation. In addition, statistics were applied to certain parameters assessed in a specific field trial but not on the same parameters assessed in another field trial of the same GMP in another country and/or year.

If a statistical analysis was carried out to compare the GMP with the non-GM control, it was generally not indicated why a specific test was chosen. No indication was made whether the conditions for a specific statistical test (e.g. normality distribution of data) were fulfilled or which effect size could be detected by the chosen statistical test, e.g. by indication of the statistical power of the test. As statistical power is influenced by the experimental design and replications employed, often only large effects are detectable in field trials calling for increased replication to detect also moderate or even small effects (Lopez et al. 2005, Prasifka et al. 2008).

3.2.6 Insufficient specification of organisms, methods and parameters

In almost all assessment categories shortcomings with respect to the specification of the species chosen, the parameters assessed and the methods used were identified. This refers, for instance, to:

- Tissues analysed for expression
- Agronomic traits evaluated
- Anti-nutrients and secondary metabolites for the compositional analysis
- Organisms, parameters and methods to asses effects on target organisms
- Species, parameters and methods to asses effects on non-target organisms in the laboratory, greenhouse or in the field
- Organisms, parameters and methods to asses effects on biogeochemical processes

For instance, in the agronomic assessment the parameters 'stressor or symptom' or 'insect damage' were frequently evaluated (see Table 10). A detailed specification of these parameters was lacking in all notifications and it thus remained unclear what was exactly assessed with this parameter.

Taxa evaluated were frequently not taxonomically specified in non-target studies. For instance, the pooling of non-target organisms in groups such as 'predators' or 'bugs' in field studies did not allow

to conclude on a particular species, the response of which might be different from a different species evaluated in the same 'group'.

In assessments of potential effects of a GMP to non-target organisms the parameters assessed were not always clearly described. In toxicological studies the parameter 'signs of toxicity' was frequently evaluated but not explained which effects on or responses of the organisms tested were classified as a toxicity sign. With respect to assessment methods used for a specific parameter, a large variation across and within notifications was evident. Similar parameters were assessed either qualitatively (yes/no classification) or semi-quantitatively (e.g. agronomic assessment), depending on the notification. Assessment methods and modes of observation were frequently not specified in detail (e.g. non-target assessment). For instance, for GM oilseed rape the 'foraging behaviour' or 'foraging preference' of bees was assessed in greenhouse studies but it was not indicated how the parameter was actually measured (see chapter 3.3.7).

3.2.7 One species of conservation concern does not fit all

Species of conservation concern such as EU-wide, nationally or regionally protected species may be of particular concern if GMPs interfere with their life cycles, habitats, competitors or food resources. Directive 2001/18/EC and its guidelines for the ERA (EC 2002a) address endangered species in Step 2 of the ERA when evaluating the potential consequences of an adverse effect.

In risk assessment practice of GMP notifications, however, species of conservation concern were rarely addressed. In fact in no case a specific assessment for a protected European species was carried out. Species of conservation concern were addressed in *Bt* maize notifications only. Here, generally only Lepidoptera were addressed, apparantly because most *Bt* maize expresses lepidopteran-specific toxins. However, almost exclusively the Monarch butterfly (*Danaus plexippus*), a North-American species with no distribution in Europe, was taken into consideration. Potential effects on this species were mainly discussed by referring to published studies or supported by laboratory toxicological studies. European Lepidoptera were generally not considered in GMP notifications, except in one notification (maize Bt11). In this case the 'evaluation' was restricted to the presentation of the Lepidoptera listed in Annex IV of the FFH Directive. A separate risk assessment including an exposure assessment for European species which are of conservation concern was generally lacking in GMP notifications. Also other protected species, such as Coleoptera, which would be relevant in the case of *Bt* maize events expressing coleopteran-specific toxins (maize 59122) were not considered in the respective notification.

This apparent non-consideration of protected species, either EU-wide or nationally, has also led to considerable debate among EU member states on how national obligations to fulfil legal requirements of conservation of protected species shall be accomplished. If these are not addressed at the EU-wide, centralized authorization procedure of GMOs, ways must be found giving EU member states the necessary competence to address such questions before GMOs are placed commercially on the market (see also chapter 3.2.10).

3.2.8 Step-by-step-principle not realized

The step-by-step approach refers to the collection of data on GMOs from different steps, beginning with experiments in the contained use system through deliberate release up to the placing of the

market as outlined in the Guidance Notes supplementing Annex II of Directive 2001/18/EC (EC 2002a). In the context of GMP notifications for placing on the market (part C notifications of Directive 2001/18/EC) the step-by-step principle thus relates to the submission of 'relevant and available data of a GMO from deliberate releases from the types of environment where the GMO will be used' when carrying out an ERA (EC 2002a). According to the Directive, deliberate release refers to the release of GMOs for any other purpose than for placing on the market (part B).

Also the design of a monitoring plan should be established using this approach. Information derived from experimental releases and large-scale field trials should to be used as well as data gained through monitoring of experimental releases (Guidance Notes supplementing Annex VII of Directive 2001/18/EC, EC 2002b).

Table A13 in the Annex gives an overview of field trials of GMPs including Part B field trials carried out in the EU, as presented in the notifications reviewed in this report. The information on Part B field trials is generally requested according to Annex IIIB of Directive 2001/18/EC (information required in notifications, Point D. 13: information about previous releases of the GMP) for notifications submitted according to Directive 2001/18/EC (e.g. Bt11 maize, 1507 maize). In notifications according to Regulation (EC) 1829/2003 this information is either contained in the Summary (Part II) or/and as a separate document representing Annex IIIB according to Directive 2001/18/EC if such existed beforehand. In the case of maize MON810, submitted under Directive 90/220/EEC, the information on previous releases was provided in the general information on the GMP (part A).

The information and assessments derived from part B trials were generally not reflected in the ERA presented in GMO notifications. Additionally, a clear cross-referencing between the assessments carried out as deliberate release (part B) and their relevance for the placing on the market of the GMO (part C) was generally missing in GMP notifications.

Generally, the information provided on the part B trials is presented very roughly and does not contain any details on the methodology or results of these trials. In the case of maize MON810 the notification did not contain any information except the notification number of the part B trials. Hence, detailed information or results derived from part B trials were in no case available in the notifications for placing the GMO on the market.

In the technical dossiers and the ERA generally no reference was made by the notifiers to the part B trials as listed in Part II or Annex III. A comparison of locations and years used for the generation of data in the ERA (agronomics, composition, expression, non-target field study) and locations and years of part B trials in Europe shows that there is a large discrepancy between those field trials in several notifications (see Table A13 in the Annex). For example, in the notification of maize 1507 field trials were carried out in Bulgaria in 2000 in order to generate data on agronomic characteristics and plant composition of the GMP but no reference to part B field trials in this country was made. Also in the case of maize NK603 the field trials carried out in France 2002 and in Germany 2001 for the agronomic evaluations were not included in the part B trial listing. In the notification of maize Bt11 part B trials were carried out in Spain (1996-2003), Italy (1995-1998) and Portugal (1998) with the purpose to test this maize for tolerance to the target pest and other agronomic characteristics. However, in the technical dossier of the notification only data from the French trials (1994-2003) on agronomics and composition were presented but not from the other locations

Hence, a large discrepancy between the field trials conducted for the agronomic, compositional or expression assessment and Part B field trials carried out in the EU and indicated in the notifications is evident. Whether further part B field trials were conducted with the respective GMPs within the EU but not cited in the relevant notifications has not been specifically assessed in this review, but is considered likely.

Rarely were in any of the part B field trials effects of the GMO on non-target organisms assessed. For the notifications of potato EH92-527-1, maize Bt11, maize 59122 none of the field trials evaluating non-target organisms correspond to any of the part B field trials indicated in the notification. Two field studies on non-target organisms carried out in Europe for maize 1507 (Lefko 2002, n.st.; Vernier et al. 2001a, n.st.) corresponded to part B field trials. However, the indicated purpose of these part B field trials was the agronomic evaluation of the GMP rather than the evaluation of non-target organisms. For maize 1507xNK603 the European field study with non-targets was carried out in Spain in 2005 although it is unclear whether this study corresponds to one of the part B trials in Spain.

In the original notification of oilseed rape Ms8xRf3 of 1996 several field trials were mentioned in the Annex. Their purpose was to evaluate the restoration ability, phenotypic or agronomic parameters of the oilseed rape lines Ms8, Rf3 or Ms8xRf3. Part B authorization numbers and/or experiment numbers were indicated in most cases. Also experiments carried out under contained conditions were mentioned. In the ERA of this original notification the notifier referred to PGS field trials when discussing environmental interactions of the transgenic oilseed rape and its pollinators, stating that in these trials the foraging behaviour of bumblebees, honeybees and wasps was assessed. However, no reference to specific Part B trials was made. The update of the notification in 1998 contained 12 Annexes; some of these contained information or results on field trials. However, this information was not consistently summarized or denoted as ‘Part B field trial results’ but was spread across Annexes (e.g. in Annexes 2, 5, 10, and 11). In many cases the part B trials referred to did not deal with the GMP in question but with other events or GM lines (in particular Ms1xRf1, Rf2; e.g. Annexes 2, 3 and 5) or the GMO used in the experiments was not indicated. In the update of the ERA from 1999 the notifier again referred to results of field trials carried out by AgrEvo/PGS and other institutions since 1990 (e.g. PROSAMO and BRIDGE Programmes) but gave either no indication to which particular field trials it was referred to. Similarly, this was done in the ERA update of September 2003.

For maize NK603 the Spanish CA requested information from field trials in other EU member states than those carried out in France and Germany. The notifier answered that trials were carried out since 1999 in many EU regions (France, Spain, Germany, Italy, Belgium, Hungary, Czech Republic, and Sweden) but none would be indicative of any adverse effects of NK603. The notifier further stated that the purpose of these trials was not to generate data for the regulatory dossier of NK603. However, these trials were performed for the evaluation of parameters such as efficacy, selectivity, residue levels, trait integration, variety registration and demonstration trials. The argumentation of the notifier that those trials with relevance for the safety assessment had already been included in the notification for the composition, expression, agronomic and phenotypic characterisation, while results of the others were simply omitted, does not fulfil the requirement that all relevant data must be provided to perform the ERA (see also chapter 3.2.3). The purpose of the

field trials, not reported in this notification, makes clear that these data would have been highly relevant for several assessments of the GMP (e.g. efficacy assessment, assessment of herbicidal effects etc.).

3.2.9 Case-by-case principle ignored

The environmental safety of a particular GMP was often argued with data from a different GMP or a different GM event with similar traits (e.g. herbicide tolerance). Often the data provided even lacked the specification of the respective GMP used in a particular test or study. For the absence of adverse effects notifiers argued either with the safety of a GM trait in general (such as herbicide tolerance as such), with a particular GM trait (e.g. glufosinate-tolerance) even if expressed in a different crop (see e.g. effects on non-target organisms for NK603 maize), or referred to other notifications in which a similar GMP (e.g. oilseed rape) or a parental line (e.g. stacked event maize) was assessed. This is also to be seen in the context of the ERA approach applied by notifiers and the additive GM crop 'concept' (see also chapter 3.2.1).

Such a generalization of the particular GMP contradicts the case-by-case principle of Directive 2001/18/EC and ignores the particularities of the genetic transformation such as the importance of the insertion site and the number of insertions and their effects on the performance of the GMP (e.g. Purrington & Bergelson 1995).

The case-by-case principle established by Directive 2001/18/EC is defined as follows: 'The ERA should be carried out on a case by case basis meaning that the required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, inter alia, GMOs already in the environment' (Guidance notes supplementing Annex II to Directive 2001/18/EC; EC 2002a). The purpose of this principle is to recognize the broad range of individual characteristics of different organisms (GMO by GMO) and different environments (site by site and region by region; EC 2002a).

The GMO in this respect represents the 'case' and is per definition an organism ' in which genetic material has been altered in way that does not occur naturally by mating and/or natural recombination' (Directive 2001/18/EC). The GMO notified is usually a particular transgenic line derived through a specific technique of genetic modification, resulting in a specific 'event'. Thus the information of the genetic modification and the resulting modified organism are the starting point of the information required in the ERA according to Annex IIIB of Directive 2001/18/EC. In Regulation (EC) 1829/2003 this focus on a particular GMO has resulted in the need of information on the transformation event including an event-specific approach for detection, sampling and identification (Article 4). This implies that tests carried out in order to evaluate the human, animal or environmental safety of a GMO have to be conducted with the respective 'event' and cannot be replaced by tests used in other GMO notifications with other 'events'. Similarly, in the case of stacked events it is necessary that the field trials are conducted with the respective stacked GMO and data are not limited to those gained from the single event, parental lines only, as current practice shows. Stacked GMOs are understood, both legally and scientifically, as an individual transgenic plant which may have properties that are different from the individual single-event parental lines and which are subject to an individual ERA and a separate authorization.

3.2.10 Lack of specification and consideration of different environments

Directive 2001/18/EC and its guidance notes address the necessity to provide data from different environments where the GMO will be used (EC 2002a). Due to the range of characteristics of different GMOs and different environments the site-by-site or region-by-region principle is closely linked with the case-by-case principle of the ERA. The required information varies depending not only on the type of GMO and its use but also the potential receiving environment (see also discussion on the 'case' definition in the chapter 3.2.1).

A broad range of environmental characteristics, i.e. site- or regional-specific, may be taken into account in the ERA and it may be useful to classify regional data by habitat area, reflecting aspects of the receiving environment relevant to GMOs (EC 2002a). As an example, the occurrence of wild relatives of GMPs in different agricultural or natural habitats of Europe is cited.

The potential receiving environment is also addressed in the ERA methodology (EC 2002a). While information on recipient, donor, vector, genetic modification and the GMO is considered independent of the environment, information on the intended release, the receiving environment and the interaction between these relates to the particular environment into which the GMO will be released. This information then determines the extent of any potentially harmful characteristics of the GMO (Annex II, 4.1.).

Data derived from field trials are frequently submitted for the evaluation of a GMP with respect to basic morphological or phenotypic characterisations (expression, agronomic characteristics and compositional aspects) of the GMP in comparison to its non-GM control (see chapter 2). In some cases data were derived from assessments under contained conditions (e.g. expression analysis of oilseed rape Ms8xRf3, potato EH92-527-1, maize Bt11) while in most cases field trials took place overseas and/or in combination with European locations. For example, data for compositional analysis were derived from both, non-European (USA, Chile, Canada) and European countries.

The number of **European countries** where such field trials took place ranges from 1 to a maximum of 4. In the agronomic assessments in 2 notifications no field trials were carried out at European locations and in 4 notifications data from different (i.e. more than one) European countries were missing. Field trials in European countries for the compositional assessment were restricted to 1 (3 notifications), 2 (4 notifications) or at maximum 3 (2 notifications) European countries. Data from more than one European country were missing in 3 notifications. In some cases the number of locations used for the field trials was not indicated (e.g. in the agronomic assessment).

In several cases a specific assessment was carried out in a particular year in one European country only (e.g. expression of maize MON810, agronomic and composition of maize Bt11, expression and composition of maize NK603xMON810). Rarely a specific assessment was done in the same country for more than one or two consecutive years (e.g. expression of maize 1507xNK603 assessed in Spain from 2003-2005).

In conclusion, for the phenotypic characterisation (expression, composition, agronomics) of the GMPs 'different environments' are considered only to a very limited extent in GMP notifications. The choice of locations for field trials which are representative in terms of agronomic and environ-

mental conditions for the future commercial application of the GMP is crucial to be able to judge on the efficacy of the GMP towards several target and non-target pests, the quality of the product as well as for potential environmental effects such as resistance development, development of secondary pests or non-target effects under different conditions. Thus information on the performance of the GMP under conditions of regionally different target and non-target pests or different target pest generations, such as one or two generations of the ECB in Europe, is crucial. Different pest infestation and damaging levels in different agronomic sites and situations have been reported resulting in differential *Bt* crop performance (Archer et al. 2000, Archer et al. 2001, Horner et al. 2003). Toxin levels in the GMP and infestation rates of target pests are influenced by soil management histories and practices (Phelan et al. 1995, Bruns & Abel 2003, Bruns & Abel 2007) but also by other agricultural management practices such as planting time and irrigation (Horner et al. 2003, Pilcher & Rice 2001).

Field trials have further relevance for the assessment of GMPs with respect to potential adverse effects on non-target organisms and the biotic environment. Field trials under European conditions were carried out in a maximum of three countries in one notification (potato EH92-527-1; see Table A9 in the Annex). The majority of data were either derived from US locations (maize MON810, Bt11) or from one to two European countries (maize MON810, 1507, 59122, 1507xNK603) or even no country in Europe (maize NK603, maize NK603xMON810).

In almost all notifications limitations in the characterisation of field trials made it difficult to conclude whether the assessments were carried out in climatically or agronomically distinctive regions (see also chapter 3.2.5). Since the authorisation of the GMP according to Regulation (EC) 1829/2003 and Directive 2001/18/EC comprises several different European countries and regions, the GMP must be tested for its possible effects under various environmental conditions in Europe. This is also in line with the EFSA requirement that the comparison between the GMPs and the most appropriate comparator should cover multiple geographical locations representative of the various environments in which the GMPs will be cultivated (EFSA 2006a).

For the assessment of other potential environmental effects, i.e. effects on biogeochemical processes, the abiotic environment, effects on management and cultivation techniques, and the assessment of potential dissemination, persistence and invasiveness, data from different environments were neither provided nor discussed in any of the GMP notifications reviewed.

In addition, EU-wide or regionally protected species, areas or habitats have not been considered in any GMO notification so far (see also chapter 3.2.7). Environments which harbour a higher diversity than the ‘average’ agricultural landscape, such as areas protected under the Habitats Directive (Directive 92/43/EEC), ecologically sensitive areas or biodiversity hotspots (Traxler et al. 2005a), merit a specific and individual consideration if GMOs are to be placed on the market for cultivation. This is also justified by the fact that the responsibility of individual EU member states for the conservation of a particular endangered species may vary depending on distribution and population parameters of particular species (Petersen et al. 2003).

3.2.11 Lack of consideration of trait interactions

Notifications of GMPs derived from traditional crossing of two single event GMPs (stacked event GMPs) generally refer to the ERA and the assessments of the respective parental, single event GMPs in order to conclude on environmental risks. Potential interactions of transgene products, e.g. between different Cry-proteins when expressed in the same plant, were usually not addressed by the notifiers. This approach was justified by the argument that interactions of *Bt* proteins against target or non-target organisms were considered to be unlikely or based on the presumed narrow spectrum of insecticidal activity of the individual proteins. Although this approach is consistent with the concept of 'protein only' assessments of GMPs, currently applied by notifiers, it ignores the legal requirements of the 'case-by-case' approach (see also chapter 3.2.9) as well as scientific knowledge. The assessment of individual transgene products or proteins does not consider the potential of the introduced proteins to result in increased or decreased functions or novel mechanisms in the target or non-target organisms. Since the beginning of the research on *Bt* proteins it is known that the overall insecticidal activity of a *Bt* strain is considered due to the additive and/or synergistic interactions of the individual delta-endotoxins (Koziel et al. 1993b) and both, synergistic and antagonistic effects of insecticidal proteins including Cry-proteins have been described in the literature (for review see Spök et al. 2008). Toxin interactions towards invertebrates, especially of Cry and Cyt-toxins both when microbially expressed but also when expressed in GMPs and also interactions and synergistic effects of other plant proteins such as lectins and trypsin inhibitors are well known but constantly ignored in the current risk assessment practice of stacked GMPs.

3.2.12 Conclusions on environmental risks – role of uncertainties

In the GMP notifications reviewed the absence of any difference between the GMP and its non-GM control was concluded in the ERA. These conclusions were often not based on scientific data ('assumption-based conclusions') or were not comprehensible as test results did not unambiguously support these conclusions.

If conclusions were **not backed by specific data** the argumentations of the notifiers to demonstrate the lack of any environmental effects (lack of any difference between the GMP and the non-GM control) were as follows:

- Reference was made to other assessments (e.g. compositional analysis, assessment of reproduction-dissemination-survivability, persistence and invasiveness assessment, see also below)
- 'No unexpected changes/differences' were observed in certain assessments and thus no specific data (with relevance for a certain parameter) were generated (agronomics)
- The expression of the newly introduced sequences/proteins was not considered to 'change the inherent characteristics of the plant' (persistence-invasiveness)
- Certain traits were not expected to be affected by the genetic modification (e.g. assessment of agronomic characteristics, assessment of reproduction, dissemination and survivability)

- General statements on the crop were made (e.g. ‘maize is not known to be inherently persistent…’)
- General statements on the likelihood of an event were made (e.g. ‘gene flow is considered unlikely’)
- General statements on the general absence of any effect or difference between the GMP and the non-GM control were made:
- Generally no phenotypic differences are observed that could alter the biological fitness of the plant
- No effects/environmental impacts are expected to arise (e.g. target organisms)
- No *a priori* reason is given to suspect that the protein would show biological activity/no known negative interactions (e.g. effects of PAT, EPSPS proteins on non-target organisms; on biogeochemical cycles)
- No changes are anticipated as the overall performance of GMP is similar to non-GMP (effects on biogeochemical cycles and the abiotic environments)
- No effects are expected as the protein is not novel to the environment, does not target organisms as such, does not have a toxic mode, has a history of safety, organisms have been historically exposed to these proteins (HT crops); natural ubiquity and limited persistence (effects on biogeochemical cycles)
- Scientific data are not considered relevant to draw conclusions on particular environmental risks

Conclusions on environmental risks were often indirectly drawn by experiments designed to address different questions. In these cases conclusions on environmental effects were argued by **referring to other assessments**, such as:

- compositional assessment (e.g. for the assessment of reproduction, dissemination, survivability; effects on non-target organisms)
- agronomic assessment (e.g. for the assessment of reproduction, dissemination, survivability, assessment of persistence, invasiveness and potential selective advantage/disadvantages)
- assessments in other notifications (e.g. for the assessment of reproduction, dissemination and survivability, gene flow)
- competitiveness assessment (e.g. for the assessment of persistence and invasiveness)
- specificity assessment of the introduced protein only (for effects on non-target organisms in case of Cry-protein expressing GMOs)

In other cases the notifiers provided **specific data** to conclude on potential differences of the GMP from the non-GM control in a certain aspect. This is particularly relevant for the phenotypic and compositional characterisation of the GMP as conducted in order to assess agronomic characteris-

tics, plant composition and expression of the GMP. However, **differences were often dismissed** by the notifiers by arguing that the observed differences:

- were not observed consistently across locations or at all locations in an individual site analysis (e.g. agronomics, composition)
- were due to small differences in the genetic background or the seed quality of the GMP and the control (e.g. agronomics)
- were within normal biological variability expected or within ranges derived from literature or published studies (agronomics, composition)
- were numerically small (agronomics, composition), or small in comparison to the control receiving a pesticide treatment (non-target organisms)
- differences in the characteristics of the GMP were not considered 'ecologically meaningful/biologically relevant' (e.g. advantage of HT maize over weeds; compositional differences between GM and non-GMP; agronomic characteristics)

Since e.g. in non-target assessments the entire ecotoxicity testing program applied to the GMO ERA is copied from pesticide or chemical testing, which is not considered an adequate concept for testing of GMOs, its limits are revealed when it comes to the interpretation of the results – provided there are any. The discussion on the biological relevance of significant differences must be seen as the consequence of reseach hypotheses, which have not been clearly formulated (see also chapter 3.2.1). If proper risk research hypotheses are formulated, significant differences cannot be dismissed, questioned or relativized. The proposed improved concept as suggested in this report will provide guidance on how this could be done (see also chapter 4.2.1).

If the conclusions were based on data generated or provided by the notifier, several **shortcomings in the data basis** were often identified and data were not robust enough to support the conclusions. In this respect the following problems could be observed:

- Data were claimed that they have been generated but were not attached or presented (e.g. agronomics) or the parameters referred to were not assessed (e.g. maize MON810, agronomic characteristics).
- Data were presented for another GMO/event and not the GMO in question (e.g. Ms1Rf1 oilseed rape in the case of Ms8xRf3 oilseed rape notification)
- Data were not or not fully statistically analysed (agronomics, composition)
- Statistically significant differences were observed but dismissed without sufficient agrumentation (agronomics, composition; see below)
- Only few studies (published or notifier studies) were cited to support the conclusion (e.g. effects on biogeochemical cycles and the abiotic environment, effects non-target organisms)
- Data on non-European species (e.g. studies with the monarch butterfly in the US relying on the specific distribution of milkweed as host plant) were used to substitute data on

species of conservation concern in Europe (e.g. all butterfly species with different host plants in different geographical settings)

- Data were generated on a very general level (e.g. toxicological studies with a standard set of organisms or with only few functional groups) but conclusions were extrapolated to all non-target organisms

Specifically, the case of oilseed rape Ms8xRf3 and the assessments for persistence and invasiveness showed that conclusions on certain risks were often backed by data either not relevant for the specific GMP or for European conditions.

For oilseed rape Ms8xRf3 the notifier submitted the original notification in 1996. In 1998 12 annexes were provided by the notifier consisting of a mixture of published studies and various published and unpublished reports of field evaluations and safety evaluation programmes of GM oilseed rape. Of these 12 annexes, some considered persistence, survivability and competitiveness of transgenic oilseed rape. However, the majority of these reports either did not deal specifically with the oilseed rape Ms8xRf3 or the GM oilseed rape used was not exactly specified (e.g. data on seed yields from trials in Belgium, Annex 2, Part 2, Annex II.5; Annexes 8 and 9). In several annexes no data were presented but conclusions only (e.g. Monitoring report of transgenic experimental sites for Oilseed rape volunteers at ADAS, UK, Annex 2, part 2, annex II.4). Some reports were not carried out with the relevant GMO but another GM oilseed rape (e.g. Annex 3). In the presented monitoring studies carried out in Europe (Annex 9 to 11) the GM oilseed rape used in the trials was not indicated (Annex 9, 10, 11). Annex 10 consisted of unpublished results of field trials in Germany on pollen and seed dispersal. Annex 11 contained results from field trials in France but was the report was in French. Annex 12 consisted of surveys for GM canola and weedy relatives with the GM traits in Canada over two years.

This practice of drawing conclusions from data having limited relevance for the GMP in question was even complicated by the shortcomings in the presentation of the data (see chapter 3.2.4).

Scientific studies cited for conclusions on environmental risks did not reflect the standard of scientific knowledge

Published studies cited to back conclusions on the safety of a GMP did frequently not comprise the current standard of scientific knowledge. A balanced view as well as the integration of the latest scientific findings on a particular safety aspect of a GMP was frequently not reflected in GMP notifications. The safety of a particular GMP is often highly controversially discussed as studies with data exist that come to different – sometimes even opposing – conclusions. Both, the controversy and the contradicting studies, were typically not mentioned or discussed in GMP notifications.

For instance, the literature reporting adverse effects on lacewings when fed the *Bt* toxins and *Bt*-intoxicated prey over the entire larval period was entirely omitted from *Bt* maize notifications. While some have argued that these reported effects were not due to *Bt* (e.g. Romeis et al. 2006), others uphold that it is the *Bt* toxin in conjunction with other confounding factors and back this up with data (Hilbeck and Schmidt 2006, Hilbeck et al. 1998, Andow et al. 2006).

As another example, with respect to the mechanism of the *Bt* toxin in the insect, the citations presented in *Bt* notifications concluded that the toxin was highly specific to the respective target or-

ganism. Literature on influencing parameters of specificity and toxicity of these toxins – early but also latest findings – was generally not included or discussed (see e.g. Broderick et al. 2006).

Further, ecological and entomological science and literature with relevance to GMOs was largely ignored such as interaction effects of different secondary compounds that can affect crop-associated fauna (e.g. antagonistically, synergistically or additively).

Uncertainty is generally not addressed in risk conclusions

Risk assessment should also include an uncertainty analysis, in terms of variability of data and lack of information which is a critical component of ecological risk assessment (Henry 2006). The consideration of the precautionary principle is a basic requirement according to Directive 2001/18/EC (Art. 1, Art. 4) but is largely left unconsidered in current GMO decision making. Annex II of Directive 2001/18/EC specifies that the general principles of the ERA have to be framed by the precautionary principle. The guidance notes supplementing Annex II (EC 2002a) further specify this approach considering that 'the ERA may not always result in definitive answers to all the questions considered because of lack of data'. A particular focus should also be put on the consideration of the precautionary approach for long-term effects as the availability of data for such effects may be very low. In this case it is required to consider particular risk management (EC 2002a).

However, independent of the data basis provided to support a conclusion of a specific risk of a GMP, the conclusions drawn by notifiers generally resulted in no or negligible risks for the environment. Generally, no consideration was given to the evaluation of uncertainty when such conclusions on environmental risks were drawn. Only in one case a superficial uncertainty analysis was included in the ERA (notification of maize 59122) which was used to justify the flawed ERA methodology (see also chapter 3.3.7) rather than to outline specific uncertainties in the chosen approach. The uncertainty in the exposure data was justified by the use of the 90% upper bound on the mean expression levels, however, ignoring the fact that expression values chosen for the assessment derived from overseas and not European locations. Thus variations in expression levels of the respective toxins which might be different under European conditions were left unconsidered.

Based on the above mentioned shortcomings in current risk assessment practice of GMPs with respect to the inadequate ERA model, the inadequate data generation and interpretation the role of the precautionary principle has to be particularly emphasized. Chapter 4.2.12 outlines how the implementation of the precautionary principle could be achieved.

3.2.13 Long-term and cumulative effects

Long-term and cumulative effects refer to the 'accumulated effects of consents on human health and the environment, including inter alia flora and fauna, soil fertility, soil degradation of organic material, the feed/food chain, biological diversity, animal health and resistance problems in relation to antibiotics' (Directive 2001/18/EC, EC 2002a). A general principle of the ERA is that an analysis of the 'cumulative long-term effects' relevant to the release and the placing on the market of a GMO is to be carried out, as required in Directive 2001/18/EC (Annex II).

In considering the potential cumulative long-term effects, the ERA should take into account issues such as the long-term interactions of the GMO and the receiving environment, the characteristics

of a GMO which become important on a long-term basis, repeated deliberate releases or placings on the market over a long period and the GMOs deliberately released or placed on the market in the past (EC 2002a).

Although few comprehensive studies have so far been carried out addressing specifically long-term and cumulative effects, a range of potential long-term or cumulative effects have been identified (Henry 2006) although such a non-exhaustive list this does not preclude that the ERA also identifies other hazards, less obvious ones which may be just as well important:

- Effects of the use of herbicides with herbicide tolerant GMPs on weed populations and seed banks/usage of pesticide sprays
- Effects of *Bt* crops on non-target insect populations
- Development of resistance to *Bt*
- Effects of GM crops on soil decomposition
- Gene flow to wild relatives

The assessment of long-term effects has also to be seen in conjunction with the uncertainty analysis and the consideration of the precautionary principle in the ERA. Cumulative long-term effects of GMPs increase the uncertainty, thus an uncertainty analysis merits increased importance (Henry 2006) and if data on long-term effects are lacking then particular risk management has to be applied (see also above). However, in none of the notifications reviewed long-term interactions or cumulative effects have been specifically addressed or considered by suggesting specific risk management measures or in the monitoring plan.

From an environmental point of view long-term and cumulative effects gain increasing importance in the light of the increase in GMP applications and releases in the EU but have so far gained little attention, both in scientific literature and regulatory practice and decision making at EU level. Also at a political level, the need to take such effects into consideration when decisions on GMP authorisations are taken has been proclaimed by EU member states, but also by Commissioner Dimas who, during an EU conference on Co-existence, criticized the reliance of scientific opinions by EFSA on short-term effects only and demanded the consideration of long-term effects in the ERA of GM crops (Dimas 2006).

3.2.14 Guidance and assessment by EFSA

In 2002 the European Food Safety Authority (EFSA) was established by the European Commission as an independent agency with the aim to provide objective scientific advice on matters of food and feed safety. Since 2002 applications of GMPs for placing on the market have been increasingly submitted according to Regulation (EC) 1829/2003 shifting the responsibility for the risk assessment from individual EU member states to the EFSA scientific panel on GMOs (GMO panel). Hence, the GMO panel provided scientific opinions on GMOs to risk managers and published a range of guidance documents since then (EFSA 2006a, 2006b, 2007), with the aim to provide guidance for the preparation and presentation of notifications submitted within the framework of Regulation (EC) 1829/2003 (EFSA 2006a). In the case of notifications of GMOs or food contain-

ing or consisting of GMOs notified under the regulation, the notification must contain an ERA as required by Annexes III and IV to Directive 2001/18/EC as well as a Monitoring Plan conforming with Annex VII of Directive 2001/18/EC (Regulation (EC) 1829/2003; Article 5) which needed also consideration in the EFSA Guidance document (2006a). A comparative evaluation of the requirements for the ERA as specified in Directive 2001/18/EC and the EFSA Guidance Document on Risk Assessment (EFSA 2006a) has been made in the course of this report (see Table A2 in the Annex).

Although this guidance, in particular the Guidance Document on Risk Assessment (EFSA 2006a), has lead to an improvement of the structure and presentation of the GMO notifications provided by notifiers, its content as well as the scientific opinions provided by the GMO panel on GMOs have been controversially discussed and have not lead to an increased acceptance and majorities of votes for an authorisation of GMOs among EU member states.

From the GMP notifications reviewed in this report it became clear that the data basis of the ERA was in many cases incomplete or risk conclusions were not based on a robust data basis. However, in no case this was recognized by the EFSA GMO panel in its opinion, which so far exist for four of the nine notifications reviewed in this report (maize Bt11 and 1507, oilseed rape and potato). These opinions were all issued in 2005, at a time when guidance on risk assessment was already adopted by the panel (EFSA 2006a). Hence, the requirements as specified by this guidance were in many instances not fulfilled by the notifiers (see Table A14 in the Annex). In addition, for GMPs, originally notified under Directive 2001/18/EC (C-Dossiers) and later according to Regulation (EC) 1829/2003, the EFSA opinions on the C-Dossier considered also studies which were actually submitted only for the later notification according to the Regulation (see example subchronic toxicity study for potato, page 69). This and the fact that additionally published literature is cited in the EFSA opinions, which has not been cited in the respective notification does not increase the comprehensiveness and the credibility of the safety claims in view of the often incomplete data basis provided in the notification.

The requirements specified in the EFSA guidance document on risk assessment (EFSA 2006a) currently leave too much room for interpretation of the proposed standards by the notifiers (see Table A14 in the Annex). This leads also to substantial heterogeneity in the data basis provided in the different notifications on which conclusions are based. As an example, for the assessment of impacts of the specific cultivation, management and harvesting techniques EFSA (2006) requires the description of intended commercial management regimes including changes in applications of plant protection products, rotations, etc.) where these are different from the non-GMP. In herbicide tolerant GMP notifications notifiers generally argue that no differences in these specific techniques for the GMP are evident and do not provide specific data on e.g. time and amount of application of the complementary herbicide of the GMP as compared to conventional herbicides of non-GMPs. In such cases, additional guidance would be required with respect to the data requirements that need to be specifically submitted by the notifier in order to fulfil the respective provisions as outlined in the guidance document.

Both, the lack of compliance and the room for interpretability lead to the fact that the scientific opinions on GMP notifications issued by the GMO panel generally acknowledge the data presented by

the notifiers while several Member State authorities, not satisfied with the data submitted, reject their support for a common conclusion on the risk assessment, e.g. that the GMP in question poses no risk for the environment. This, in turn, supports the need for both, specification of requirements and development of further guidance in order to eliminate the existing room for interpretation as much as possible. In addition, a more stringent compliance by the notifiers to scientific standards and existing guidance will be a prerequisite for the improvement of risk assessment practice of GMPs and, consequently, of the confidence of EU member states in scientific opinions of risk assessors.

3.3 Shortcomings in specific assessment categories

In this chapter the shortcomings in the ERA identified in the individual assessment categories as defined for the analysis of the notifications (see chapter 2) are outlined and discussed. Major shortcomings are formulated in bold in each specific subchapter. A summary of the main shortcomings of each assessment category can be found at the beginning of each subchapter.

3.3.1 Molecular characterisation

The review of the molecular characterisation in the notifications revealed several shortcomings with respect to the experimental design and experimental methods, the comprehensiveness of the analyses and significance as well as the quality of the submitted data.

Submitted information on the genetic modifications of the GMPs is less sufficient than information on the genetic elements used for modification

The information submitted by the notifiers on the source of the transgenic elements, the genetic material that was potentially introduced as well as vectors and methods used for transformation was considered to be sufficient. Insufficient information was submitted to determine the number and nature of all genetic modifications, which were actually introduced into a specific GMP. Different transformation methods have different probabilities to introduce certain modifications, such as introduction of multiple transgenic insertions and induction of rearrangements at the insertion sites (Latham et al. 2006). Specifically transformation by biolistic methods frequently introduces several copies of the transgenic insert used for transformation fragments of the transgenic inserts and additional DNA sequences along with the transgenic insert, and lead to rearrangements at the genomic loci of insertions. Such additional insertions may be closely linked to functional transgenic insertion(s), which are characterised by the notifier during risk assessment, or located at non-linked loci (such as insertions present at different plant chromosomes). Unlinked insertions can be removed from the initial GM event by further breeding. During subsequent breed steps such additional insertions segregate differently than the insertion which shall be retained. Dependent on the nature of the modifications present in a certain event, as well as on breeding steps and the characterisation of the initial event by the notifier GMPs can be selected, which harbour only one functional transgenic insertion (Andow et al. 2004). None of the notifications analysed contained enough information to assess if and how this was achieved.

Furthermore, the quantity and quality of information for the characterisation of the genetic modifications as presented in the analysed notifications differed considerably with regard to comprehensiveness of the analysis, availability of experimental data to support conclusions, quality of experimental data, etc. (see also following chapters). These shortcomings are of significance for conclusions drawn by the notifier.

Lack of submission of comprehensive sets of data for the initial characterisation of the genetic modification

For most of the notifications initially only a core set of data from a limited number of individual experiments, e.g. Southern Blot experiments, for the determination of the number of integration sites and the number of insert copies present in the GMP, was submitted. Although the molecular characterisation was conducted by well established methods authorities frequently requested additional information for several of the notifications, because the initial characterisation was not considered adequate or sufficient.

Upon request additional information on the molecular characterisation was submitted by the notifiers, e.g. for oilseed rape Ms8xRf3, potato EH92-527-1, maize Bt11, maize 59122 and maize 1507. However, the later submission of additional information is time-consuming and impedes the fast assessment of the notifications by the authorities. The assessment is furthermore complicated because different sets of information usually contain overlapping information, which is presented and described in different ways by the notifiers. Another issue is that the results are not always comparable in case different test materials (e.g. samples taken from different GM lines with a different breeding history) were used for the experiments and/or if no details on the source of the test materials were indicated (e.g. pedigree of samples of GM material and non-transgenic controls).

Conclusions were rarely supported by complementing experimental results

For the molecular characterisation of the transgenic events usually only a limited set of data was submitted and no comprehensive assessment by complementing methods was conducted. This led in many cases to requests for additional information by the authorities due to concerns that the submitted data were insufficient evidence for the conclusions drawn by the notifiers.

Only for some notifications the results established by a certain method were corroborated with complementing results by using different methods. E.g. in case of oilseed rape Ms8xRf3 results from analyses by Southern Blot and PCR for the assessment of gross structural integrity of the insert and of flanking genomic sequences were submitted. Sequence data for the insert and flanking sequences provided a detailed analysis of insert structure and corroborated the results of Southern Blot and PCR analysis. Further PCR analyses were conducted to determine that flanking sequences were of native genomic origin. An additional bioinformatics analysis of sequence homologies to oilseed rape genomic sequences was submitted to identify the origin of the sequences at the locus of insertion. However, a comparable in depth analysis using different methods was not presented for all notifications and, in no case, in the initial submissions of the notifications.

Stacked event GMPs were not adequately characterised

For notifications of stacked event GM maize lines (maize 1507xNK603 and maize MON810xNK603) less data for the molecular characterisation were submitted by the notifiers in

comparison to notifications for single event GMPs. In these cases a limited molecular characterisation was used to support the notifier's conclusion that the genetic modifications had been inherited from the parental events and that no major rearrangements were detectable for the transgenic inserts present in the stacked events.

For the stacked events (but also for oilseed rape Ms8xRf3) only data from Southern Blot experiments were submitted for the stacked event itself. Based on these data the conclusion was drawn that the transgenic inserts from the parental events were inherited without any noticeable changes. For other requirements of the molecular characterisation (e. g. characterisation of the insert and flanking sequences, the stability of the inserts, etc.) the notifiers did not submit data established for the stacked event but referred to data established for the parental single event GMOs. Since potential changes of the genetic modifications present in the stacked events as compared to single events should be identified during the ERA, this practice is certainly not sufficient.

Insufficient design of experiments and data quality

For a number of notifications the data were regarded as insufficient and less than adequate to support in full the conclusions drawn by the notifiers. Inadequacies found during the analysis of notifications were twofold:

- Data which were not sufficiently conclusive due to inadequate design of specific experiments.
- Insufficient quality of presented data of specific experiments

In case only a limited set of data was available for the assessment of the number of integration sites and integrated copies by Southern Blot the data were frequently not fully decisive to support the conclusions by the notifiers. For maize NK603 the presence of a single insert was concluded from the detection of a high molecular weight fragment of 23 kb size in a Southern Blot experiment. However, since fragments of large size are very difficult to distinguish, such experimental designs are not fully conclusive and should be avoided. Furthermore the data for the molecular characterisation of maize NK603 by a different Southern Blot experiment were ambiguous as they were based on the assumption of the notifier that one of the recognition sites for the restriction endonuclease used in the experiment was not functional. However, experimental data should be interpretable without such assumptions.

Based on a reassessment by the notifier, the initial characterisation of the insert in potato EH92-527-1 had to be amended. The additional data requested by the authorities showed that indeed two copies of the insert were present in the GMP. Such deficiencies in the initial characterisation of insert locations can result in inadequate assessments of all transgenic inserts present in a specific GMP, specifically in case secondary inserts were overlooked and therefore disregarded at further assessment steps.

Similar shortcomings which led to the situation that initial conclusions by the notifier drawn with regard to the molecular structure of transgenic insertions were also identified for notifications of GMPs which were not analysed in this report but documented in scientific literature. One example for such a GMP is soybean GTS-40-3-2. For this GMO independent research (Windels et al. 2001)

identified additional transgenic sequences adjacent to the insert characterised by the notifier as well as additional insertion of a small segment independent from the main transgenic insert.

The quality of the prints to present data from Southern Blot experiments was frequently insufficient, specifically for notifications submitted according to Directive 2001/18/EC, which were distributed as xeroxed copies to the authorities (oilseed rape Ms8xRf3, potato EH92-527-1, maize lines 1507, MON810, and Bt11). However, the quality of data of Southern Blots from notifications according to Regulation (EC) 1829/2003, which were distributed as electronic documents, was also low and in many cases not satisfactory (e.g. for maize lines 59122 and 1507xNK603).

Sensitivities of methods were not adequately assessed

The determination of the sensitivities of methods for the molecular characterisation was insufficient in all the analysed notifications, specifically for the presented analyses by Southern Blot. No systematic determination of the sensitivity to detect smaller fragments of the inserted transgenic sequences was submitted by the notifiers. Only for potato EH92-527-1 an analysis of the sensitivity was submitted for the confirmation that vector backbone sequences were not present in the GMP. Generally, only positive control experiments to demonstrate that the used probes were able to detect control plasmids containing full length transgenic inserts were carried out. However, these controls merely demonstrated that single copy insertions of complete inserts can be detected in such experiments. No further data to assess the sensitivity of the methods for detecting partial inserts, like the determination of the minimum size of target sequence, which could be detected by the probes used in specific Southern Blot experiments at a given stringency were submitted.

The detailed characterisation of inserts was not sufficient

In all GMP notifications analysed the characterisation of the inserts was established by a combination of methods involving the determination of the insert sequence, except for both stacked events maize lines 1507xNK603 and MON810xNK603 (see also above).

The assessments were different with regard to the methods used and the amount of data presented. Southern Blot experiments with probes representative for the main genetic elements of the transgenic inserts were submitted for demonstration of gross identity with the transgenic constructs used for transformation. Sequence data for the inserts were generally included in the initial submissions for all GMPs, except maize MON810. Only for certain GMPs PCR analyses to provide additional evidence were submitted. However, without submission of sequence data a detailed assessment of insert structure is not possible. Detailed characterisation of the insert by Southern Blot and PCR complement these sequence data as well as corroborate the analysis of insert location and copy numbers. Furthermore these methods can be used to assess the unchanged inheritance of the transgenic inserts over multiple generations, since sequence data is not commonly available for more than a single generation.

For some GMPs (potato EH92-527-1, maize lines NK603 and 59122) base substitutions were recorded by sequencing of inserts. For these cases information is missing specifying the fidelity of the amplification reactions (indicated as number of introduced base changes per total amplified sequence length). Without this information it cannot be assessed whether these changes were due to actual modifications in the insert or due to the method used to determine the sequence.

The chromosomal locations of the transgenic insertions were not determined in all notifications and the quality of the assessment of flanking sequences was insufficient in many notifications

For some notifications analysed (e.g. maize lines MON810, 1507xNK603 and MON810xNK603) no sequence data of the flanking sequences was initially submitted to assess changes introduced into the genomic DNA at the locus of insertion by the transgenic constructs. For both stacked events the notifier only referred to data for the parental events.

The size of flanking sequences which was analysed by the notifiers differed considerably between notifications. Usually, the size of flanking sequences determined allowed a bioinformatics analysis in order to determine the potential for the generation of fusion proteins at the junctions between transgenic inserts and flanking sequences. However, it was not sufficient to conclude on the chromosomal locus of the insertion or on the detailed characterisation of the genomic sequence. This kind of information is considered necessary for an adequate characterisation of the modification and the assessment of potential pleiotropic effects. Since no other methods (like metabolic profiling techniques) were employed to assess the potential for pleiotropic effects on the molecular level, an adequate characterisation of the genomic flanking sequences, their origins and functions is necessary.

Sequence data for the flanking regions are an important tool to determine the chromosomal location of the transgenic insertions. For assessing the location of the inserts usually a segregation analysis of the insert (genetic segregation analysis by Southern Blot) and/or a segregation analysis of the inserted traits (phenotypic analysis by determination of expression or function of trait) were carried out. These data were used to conclude on the nuclear localisation of the inserts based on an observed Mendelian pattern of inheritance. However, these analyses do not point to a specific chromosomal location. For six out of eight notifications the chromosomal location could not be determined by sequence homology comparisons. For these no other complementing methods like Restriction Fragment Length Polymorphism (RFLP) mapping or chromosomal location by Fluorescent in situ Hybridisation (FISH) were used to pinpoint the location of inserts in the absence of sequence homologies indicating a specific locus. For maize Bt11 RFLP data were submitted to indicate a specific chromosomal location. For potato EH92-527-1 data of Southern hybridisation of insert sequences to undigested DNA were presented indicating the chromosomal location. The experiment of the latter notification did, however, not include adequate controls to validate the result. Such kind of analysis does not provide specific evidence with respect to the chromosomal location of the insert(s) in the GMP.

Data to determine the genetic stability of inserts were not sufficient in several notifications

The assessment of the stability of insertions present in the GMPs was usually based on a combination of direct genetic analysis of inherited inserts by Southern Blot and on a segregation analysis of the trait over several generations. However, the number of generations assessed and the number of analysed individual plants for each generation differed considerably between the notifications. With regard to the stacked events only for maize 1507xNK603 a segregation analysis of genetic data by Southern Blot was submitted.

In all notifications the analysis of genetic stability was done by a single Southern Blot experiment, which limits the conclusiveness of data. With such type of experiments minor modifications in the inserts cannot be assessed. No methods for analysis of stability of inheritance on a more detailed level, e.g. multiplex PCR analysis for simultaneously assessing specific segments of the insert and flanking sequences (Singh et al. 2007), were employed in any of the notifications.

The segregation analyses were not based on a hypothesis for the potential frequency of instability and thus did not indicate the statistical power of the presented assessment in order to confirm the stability of inserts.

3.3.2 Expression assessment

The current approach in GMP notifications not to deliver expression data of GMPs from environments representative for its future use, insufficient characterisation of expression in different parts of the GMP and over the course of the cultivation period (developmental expression) as well as expression in different genetic backgrounds and generational stability significantly hampers further assessments of potential exposures of non-target organisms but also the efficacy of the GMP in practice. Further shortcomings address the experimental method how transgene products were measured as well as the lack of assessment of the biological activity of the transgene in situ. Finally, the possibility of expression of potential fusion proteins was not sufficiently addressed.

Data from greenhouse trials do not reflect environmental conditions during the cultivation of GMPs

The expression of the transgenic components in properly designed field trials in relevant environments, selected with regard to cultivation of the respective crops in Europe, has not been sufficiently addressed in the notifications (see also chapter 2.3). For three out of nine notifications (oilseed rape Ms8xRf3, potato EH92-527-1, maize Bt11) the submitted data for the assessment of expression of transgenic components were established in greenhouse trials, which cannot properly reflect environmental conditions. Expression levels can considerably differ depending on the environmental conditions. Toxin expression of maize MON810 in close or open glasshouse and in the field differed significantly, with open glasshouse plants showing the lowest *Bt* toxin values (Dutton et al. 2004a, 2004b).

The sole assessment of expression under contained conditions implies that environmental conditions, which are encountered during the cultivation of a certain crop at different European locations, are not adequately considered. For GMPs, e.g. cotton expressing *Bt* toxins, it has been shown that environmental stresses like water deficit and elevated salinity caused decreased expression of the transgenes (Jiang et al. 2005, Martins et al. 2008). Such effects can only be assessed with the careful selection of field trial locations and the location-specific analysis of the transgene expression.

Lack of an appropriate field trial design for expression assessment

Shortcomings in the analysed notifications regarding the design of the field trials conducted by the notifiers for the phenotypic characterisation of the GMPs are presented in the chapter discussing cross-sectional issues (chapter 3.2.5).

Relevant for the assessment of expression are shortcomings regarding the use of GMPs grown under contained conditions (see also above), the selection of locations for field trials, the duration of field trials (commonly field trials were conducted for one growing season only at a specific location), the design of the field trials, as well as the agronomic treatment during the field trials (fertilizer, herbicide and insecticide treatment).

The current practice disregards that expression patterns for specific transgenic components, e.g. for *Bt* toxins, can vary considerably between individual plants from different locations and from subsequent growing seasons (Nguyen & Jehle 2007). It is also not taking into account that soil management practices, such as nitrogen fertilisation levels, can influence the expression levels of transgenes, like *Bt* toxins, as shown for GM maize and GM cotton varieties expressing such transgenes (Bruns & Abel 2003, Coviella et al. 2000).

For herbicide tolerant GM crops differences in the expression of transgenes due to a treatment of the respective GMP with the complementary non-selective herbicide were not systematically tested the field trials. Only for three out of seven notifications of GMHT crops (maize 59122, maize 1507, maize 1507xNK603) the design allowed for the comparison in expression of transgenes between treated and untreated GMPs, at least at some of the field trial locations. Such a comparison shall ensure that expression levels are unchanged under relevant and representative agronomic conditions.

Lack of consideration of different tissues and developmental stages of the GMP

Developmental expression data were not commonly submitted for all transgenic components of GMPs. This has to be seen in the context of several reports that *Bt* toxin levels of *Bt* crops can substantially vary between plant organs and plant stages, even between developmental stages of a particular plant organ (e.g. old leaf versus young leaf) and can also change during the vegetation cycle (Abel & Adamczyk 2004, Dutton et al. 2005, Kranthi et al. 2005, Nguyen & Jehle 2007). The variability of expression during the vegetation cycle can lead to problems controlling the target organism. This has been shown for *Bt* maize event 176, a *Bt* crop no longer on the market, and might be also relevant for other *Bt* crops such as *Bt* cotton (Kranthi et al. 2005). Such expression variability can have serious implications not only for the protection of the GMP from the target organism (e.g. second generation of ECB) but also for the exposure of non-target organisms.

Since currently no special guidance establishes a standard set of tissues or developmental time points, which should be used for the determination of transgene expression, the choice of tissues as well as developmental stages varied considerably between notifications. They even varied for notifications of the same crop species, e.g. GM maize notifications, and among different trials conducted for one specific notification. Whereas expression in certain tissues, like grain and leaves for GM maize varieties were commonly assessed, many other tissues were not constantly assessed in all notifications (for details see Table A4 in the Annex).

Basically the same observations were made for the assessments of developmental expression of transgenes presented in the analysed notifications. Considering different notifications of the same crop (e.g. maize), different growth stages were chosen for assessment of developmental expression. Specific tissues were not consistently used to assess developmental expression of transgenes. No relevant data for the assessment of developmental expression were submitted for five out of nine notifications (see also Table 4). In two notifications developmental expression data were established using samples from GMPs grown in the greenhouse. For one GM maize developmental expression data were only submitted for the Cry1Ab toxin but not for the PAT protein.

Thus, the design of the assessment of expression of transgenic components depended primarily on the choice of the notifier and was not consistently done throughout the analysed notifications in a systematic and consistent way.

Insufficient assessment of expression of transgenic components in different genetic backgrounds

The expression of transgenes in different genetic backgrounds was also not assessed in a systematic way. Submission of data for different GM varieties with different genetic backgrounds which were precisely identified by the notifier was not consistently done in GMP notifications. If expression of transgenes was assessed using varieties with different genetic backgrounds (for details see Table A5 in the Annex), no detailed information on the varieties (e.g. pedigree, breeding history) which were used to generate these test plants was submitted by the notifier thus impeding interpretation of results. This is even complicated as no information is generally given whether the tested GM line was an inbred or a hybrid. As hybrids generally exhibit a heterosis effect, different expression levels can also be expected in hybrid varieties compared to inbred lines.

Variability of expression of *Bt* toxins in different genotypes is usually assessed during the commercial development of a GMP. For example the selection of GM maize event 176 was favoured over event 171, also because the variability in expression of the toxin between different genotypes was less distinctive in event 176 (Koziel et al. 1993a). Also later expression of Cry toxins in GMPs has been shown to vary depending on the commercial hybrid and the parental background leading to different seasonal declines in the toxin levels between these hybrids (e.g. Kranthi et al. 2005).

Lack of standardized protocol for detection of transgene products

Another important issue that has been emerging recently –12 years after beginning of large scale commercial production of GM *Bt* crops in North America – is the lack of standardized protocols for quantification of *Bt* toxin concentration in *Bt* crops. Reliable quantification of *Bt* toxins is crucial for resistance management, quality control and risk assessment purposes (Crespo et al. 2008, Greenpeace 2007, Monsanto 2002).

Nguyen & Jehle (2007) conducted a multi-year study quantifying the *Bt* concentrations in various plant parts across different locations and over time (seasonal growth). The authors concluded that ‘the monitoring of Cry1Ab expression showed that the Cry1Ab concentrations varied strongly between different plant individuals’ (Nguyen & Jehle 2007). However, it is then often concluded that this is 'normal' in biology and, hence, requires no further investigation nor is it a matter of concern. Such a conclusion regarding the variability of a novel plant defence compound cannot be substan-

tiated with scientific evidence as the entomological and ecological literature is full with examples where variability in plant defensive compounds did cause concern. There is extensive research regarding the potential beneficial and adverse implications of this variability in agricultural and natural ecosystems (e.g. Lamb 1989, Swain 1977, and Hartmann 1996).

In fact a large source of variability stems from differences in the protocols used which includes everything just about every step of the applied ELISA protocols from materials to methods. This is documented by Crespo et al. (2008) and Ngyuen et al. (2008). Measured *Bt* concentrations of a given, pre-defined amount, can differ substantially. The reported concentrations by six laboratories quantifying the same given – but unknown to the researchers – *Bt* amount (50 µg ml-1) with their respective ELISA protocols ranged from 21 to 34 µg ml-1 (Nguyen et al. 2008).

Based on the differences of *Bt* concentrations reported by Nguyen & Jehle (2007) and those by Monsanto (2002) of the same event (MON810), Then & Lorch (2008) concluded in their review that 'it seems to be a matter of principle to understand more about the underlying mechanisms likely to influence the protein content in transgenic plants before any further commercialisation of *Bt* plants is authorised. It might also allow us to learn more about transgene functioning in other GMPs.'

While variability due to environment-by-transgene interactions is difficult to study let alone to control, variability due to lack of standardized protocols is a solvable problem (see chapter 4).

Lack of tests for biological activity of transgene products

The expression of transgenes was commonly assessed by ELISA, a method which allows quantification of the amount of a specific transgenic protein present in the analysed samples. However, none of the notifications contained a functional characterisation of the transgenes expressed under field conditions. Such a functional characterisation is most important for insect resistant GM crops with *Bt* toxins as transgenic component (Andow et al. 2004). Such a characterisation could be achieved e.g. by means of LD_{50} tests or leaf tissue bioassays. As also shown in the chapter agronomic assessment for *Bt* maize an establishment of efficacy is generally not conducted in situ (see also chapter 3.3.3).

No adequate assessment of the generational stability of expression of transgenes at the molecular level

The generational stability was commonly assessed by investigating the phenotypic stability of GM traits (specifically of HT traits) over a variable number of generations (for details see Table 2). The number of assessed generations as well as the number of individual GMPs investigated differed considerably between notifications.

The assessment of genetic stability was generally not accompanied by a determination of the level of expression of the transgenes in the respective GMPs. Only in two notifications (maize lines 59122 and 1507xNK603) the segregation of the transgenic trait(s) was assessed in parallel with the investigation of the molecular structure of the transgenic insertion by Southern Blot. Such segregation analysis by Southern Blot, however, was restricted to a single generation.

In the other notifications only a few individual plants of different generations were assessed to demonstrate comparability of the transgenic insertions over different generations. The number of

plants assessed should correspond to the probability of the occurrence of gene silencing effects in a given population of a crop species.

Lack of experimental data for the assessment of fusion proteins

Based on sequence data of the transgenic insertion and the flanking genomic sequences potential ORFs can be identified and analysed by means of bioinformatics analysis (e.g. by homology comparisons for homologies to known toxin and allergen sequences and by identification of homologies of neighbouring sequences to promoter sequences or other known regulatory elements). Although such an analysis was presented in all notifications based on the availability of the necessary sequencing data, in certain notifications the relevant sequence data were only submitted as additional information upon request by authorities.

In some notifications (oilseed rape Ms8xRf3, potato EH92-527-1, maize 1507) the bioinformatics analysis for sequence homologies to known allergens was not in line with existing guidance by FAO/WHO (2001). Specifically for the detection of homologies of small uninterrupted sequence elements to epitopes of known allergens the guidance was not followed. Instead, less stringent conditions were applied (scoring of identical matches to sequences of eight linear contiguous amino acids instead of analysing windows of six amino acids as specified in the FAO/WHO guidance).

Only in a few notifications additional tests were performed to experimentally assess also the potential expression of identified fusion proteins. Only for maize 1507 the expression of fusion proteins was systematically investigated by Northern Blot and RT-PCR methods. Expression of fusion proteins by Northern Blot was also assessed for oilseed rape Ms8xRf3. Although experiments to investigate specific fusion proteins were conducted in three other notifications, these were directed to assess either the expression of translational fusion proteins based on sequences of the inserted transgenes or the expression of single fusion protein ORFs identified by the bioinformatics analysis. This approach, however, does not constitute a systematic experimental assessment of the expression of potential fusion proteins.

3.3.3 Agronomic assessment

In the agronomic assessments performed in the GMP notifications, the characterization of the behaviour of the GMP in the intended agronomic and environmental setting indicating the performance of the GMP and checking whether the introduced traits are functional in situ, exhibited a range of shortcomings. The performance of the GMP was in no case evaluated in representative agronomic environments and the field trial designs employed lacked scientific strengths. The criticism also relates to the lack of the definition of target organisms/pests of the respective GMP and their distinction from non-target organisms/pests. As a consequence, a scientific assessment of the efficacy of the GMP towards the target organism(s) was not carried out. With such an elementary lack of proof, the performance of the GMP cannot be guaranteed which may have dramatic consequences not only for farmers but also for the environment.

Lack of specification of the field trial design applied for agronomic assessments

With respect to shortcomings identified in the design of the field trials in order to evaluate the agronomic performance see cross-sectional issues (chapter 3.2.5).

Lack of a consistent set of agronomic traits and of specification of the traits assessed

Generally, no explanation regarding the selection of the agronomic traits and the rationale behind them was provided in any of the notifications. It can thereby only be assumed that the selected parameters corresponded to common breeding parameters of the respective crop species (potato, maize, and oilseed rape). The parameters assessed in the agronomic evaluation varied even between notifications of the same crop (e.g. GM maize). Also the classification of the traits assessed into four categories – plant growth and development, plant morphology, plant health and yield was not consistent across notifications.

In certain cases agronomic assessments were not based on data but rather on statements. For instance, the lack of difference of the GMP as compared to the non-GM control with respect to the susceptibility to pests and diseases was based on simple statements but not supported by any scientific data in the case of GM potato, GM oilseed rape and GM maize Bt11.

Generally, a clear indication of what had been assessed at what developmental stage was frequently lacking for a specific agronomic trait or parameter. If there was any at all, the specification of certain plant parameters such as ‘stay green’ and ‘plant vigour’ (in case of GM maize) was often held very general. The definition of “stay green” as a “visual estimate of overall plant health” does not give any information on the exact parameter assessed. Similarly, the specification which pest species were assessed or caused the damage was frequently lacking. The indication of ‘susceptibility to pests’ or ‘difference to other insects’ does not give any information on the species assessed. Diseases assessed were generally not taxonomically specified.

Lack of distinction of target and non-target pest species

Generally, insect pest species assessed were not distinguished into target and non-target pests. As not all herbivorous species feeding on a particular GMP are considered target organisms these must be classified as non-target pest species (see also chapter 3.3.6). However, such a distinction was never made (see Table 18). In GM maize notifications expressing lepidopteran-specific *Bt* toxins, often, but not constantly, the European corn borer (*Ostrinia nubilalis*) was assessed as the target pest. In few cases, also the Mediterranean corn borer (*Sesamia nonagrioides*) was included. In certain notifications it was not indicated whether target or non-target pests were assessed as no information about the evaluated species was included. For instance, in the agronomic assessments only ‘differences to other insects’ was indicated, but lacked specification of which species were actually included (see also previous paragraph).

Lack of efficacy assessment of the GMP

An assessment of efficacy of the GMP was not included in any of the GMP notifications reviewed. This is in particular relevant for insect resistant and herbicide tolerant GMPs. As pest species were frequently not specified in the assessments of insect resistant GMPs an evaluation of the effectiveness of the respective GMP towards the defined target organism was not possible and most likely not done. Hence, it is not possible to judge on the efficacy of the GMP from the field trials conducted by the notifiers. In the case of herbicide tolerant GMPs, generally no target organisms were defined (see also chapter 3.3.9) and thus no efficacy of the GMP and its complementary herbicide

evaluated. This lack of efficacy assessment has also implications for the quality control of the GMP.

The assessment of differences of insect damage between the GMP and the non-GM control is an important element to demonstrate the efficacy of an insect resistant GMO. In addition, it was never indicated whether the background infestation levels of the target organisms in the control plants were sufficient. However, any efficacy assessment requires the demonstration of a relevant **level of infestation** in the control. For comparison, conventional plant protection products (PPPs) can only be placed on the market if their effectiveness is proven. PPPs have to be 'tested in circumstances where the target harmful organism has been shown to have been present at a level causing or known to cause adverse effects on an unprotect crop…or where the harmful organism is present at such a level that an evaluation of the plant protection product can be made' (Directive 91/414/EEC, Annex III, 6.2.). The new PPP must show the level of control of the species or harmful organism for which claims are made, including different stages of growth, different strains or races and different degrees of susceptibility, if relevant. The adequate control of the harmful organism must be proven in the range of circumstances likely to be encountered in practical use.

In the GMP notifications reviewed generally no reference was made to insect pressure of the respective year and locations of field trials. The only exemption to this was the notification of maize 1507, in which a rough indication of infestation levels is mentioned. The notifier states that a significant target pest pressure from the European and the Mediterranean corn borers was evident for the region of Aragon in the 2002 field trials but no quantitative measurement was provided.

The question of the efficacy of any PPP and thus also a GMP under relevant environmental conditions is also important for balancing the positive effect of the treatment (i.e. the control of the target organism) against any negative effects such as resistance development or effects on non-target organisms (EPPO Standard No. 1/223/(1)). For GMO risk assessment this means that the assessment of the successful control of the target organism is fundamental. It is in fact a matter of product quality control to protect farmers from fraud or loss due to insufficient product quality. If no sufficient control can be demonstrated under certain agronomic conditions then it is questionable whether a resistance risk or a risk for non-target organisms is considered acceptable. In this context, it is required that the dose of a certain protective agent used in GMOs (e.g. the *Bt* toxin) is as high as possible in order to avoid the development of resistances in the target organism while the dose used for conventional PPPs is generally considered the minimum dose to achieve the required effect – the control of the respective harmful organism.

Similarly, the assessment of differences of the agronomic behaviour of herbicide treated and untreated GM herbicide tolerant plants was generally lacking due to the common practice not to include both herbicide treatment variants of the GM herbicide tolerant plant in the field trials and the lack of evaluation of potential differences in the phenotypic characteristics of the GMP due to herbicide application (see also chapter 3.2.5)

In addition, no reference product (e.g. a conventional insecticide) was used in field trials to assess the agronomic performance or efficacy of an insect resistant GMO. This is in contrast to conventional PPPs where, besides the test product and the untreated control a reference product must be used in the effectiveness assessment. Although the relevance of a reference product in conven-

tional PPPs may be different from the GMO risk assessment (e.g. evaluation of application times etc.) it has to be considered that usually conventional pesticides are used as 'reference products' or a baseline in non-target studies while this is not the case for the evaluation of effects on the target organism. This – again – constitutes a double standard – if during the assessment of effects on non-target organisms (the 'environmental' performance of the GMP) the safety of the GMP is argued by the use of an presumed environmentally less favourable reference product (always insecticides as positive controls used) while the control of the target organism (the 'agronomic' performance) is not evaluated by a comparison with a conventional product.

Lack of information on the environmental behaviour of the GMP

Notifiers often draw on agronomic parameters in order to conclude on the behaviour of the GMP in the environment such as persistence, invasiveness, etc. (see chapter 3.3.5). It is questionable whether agronomic parameters of a GMP can be used for conclusions other than the agronomic behaviour of the GMP. It is currently not clear how e.g. the persistence or reproductive behaviour of the GMP could be scientifically assessed. In neither case a scientific reasoning was provided by the notifiers indicating why a certain agronomic parameter could be suitable for the assessment of processes such as e.g. persistence, outcrossing, invasiveness etc. If agronomic field trials are used for the assessment of dissemination parameters this has to be accounted for in the design of the field trials and the parameters assessed. Thus it is important not to limit the selection of parameters to merely evaluate the agronomic equivalence between the GMP and the non-GMP, but to incorporate the assessment of general biological features of the plant which are of ecological relevance. Parameters which are of environmental relevance such as frost tolerance, the occurrence of bolters, flowering time, duration of pollen viability etc. have generally not been assessed in the notifications reviewed.

Lack of consistent methods and analysis of agronomic characteristics

Generally, consistent methods for the assessment of agronomic traits of a GMP were not followed. For traits such as germination, disease or insect susceptibility the methods employed differed considerably between the notifications. Generally, no reasoning was given in any of the notifications why a certain parameter was assessed in a certain way, e.g. either quantitatively or qualitatively. This was in particular obvious in the case of the pest and disease assessments. In addition to the lack of specification of the pest species or diseases evaluated, the conclusions on the infestation levels or abundance of pests or diseases on the GMP as compared to the non-GM control were based on assessment methods that were suitable only to a limited extent to assess any difference. For example, the type of scale used in the assessments of abundance or infestation of certain pests (e.g. intervals - linear or not linear -; percentage) was frequently not indicated. Often, simple yes/no classifications were used for these assessments. Only rarely, quantitative assessments of pest damage were carried out, usually limited to one or a few field trials in selected notifications (maize NK603, 2002 field trials; maize 1507, 2002 field trials). Statistical evaluations of differences between the GMP and the non-GM control were frequently lacking. In no case it was indicated whether the selected method was actually suitable to assess any difference between the GMP and the non-GM control.

Therefore a more detailed assessment of potential differences in susceptibility of the GMP to important pests and diseases than currently provided in GMP notifications is needed. Methods do exist for the exact determination of infestation rates of certain pest species as shown in two notifications (maize NK603, maize 1507) where also quantitative assessment methods were employed (e.g. counting the number of larvae of a certain pest species, measuring the length of tunnels in stalk). Similar assessments are generally employed in the evaluation of plant varieties or for the authorisation of plant protection products (see also chapter 3.3.9).

Lack of follow-up of statistically significant differences between the GM and the non-GMP

General shortcomings with respect to the evaluation of differences during the agronomic characterisation of the GMP can be found in the cross sectional issues (chapter 3.2).

In several cases statistically significant differences were found between the GMP and the non-GM control in certain agronomic parameters either in the analysis across locations or at certain individual locations. The notifiers generally disregarded these differences using several argumentation lines (see chapter 3.2.12). In no case these observed differences were followed up or was their relevance evaluated. However, this is necessary if effects are observed only at a regional scale (e.g. at individual sites) as differences observed may be due to regional agronomic, environmental or climatic conditions (e.g. differences in pest pressure, e.g. Archer et al. 2000, Archer et al. 2001, Horner et al. 2003; see also chapter 3.2.10).

3.3.4 Assessment of plant composition of the GMP

The major shortcoming in the assessment of composition of a GMP was the lack of consideration of the environmental relevance of plant compounds. The focus of the assessment was generally put on food and feed safety but omitted potential effects for the environment. In particular novel metabolites produced in herbicide tolerant GMPs due to the application of the non-selective herbicides were generally not taken into consideration. Additionally, shortcomings with respect the interpretation of the results were evident. Differences between the GMP and the control at individual locations and their importance for the environment were left unconsidered.

No consistent approach in the assessment of plant compounds

The GM maize notifications which considered secondary metabolites or anti-nutrients generally assessed five secondary metabolites and two types of anti-nutrients. In addition to compounds suggested by the OECD (OECD 2002a), inositol, a secondary metabolite, and trypsin inhibitors, an anti-nutrient, were included in the GM maize notifications. In one GM maize notification neither secondary metabolites nor anti-nutrients were included and secondary metabolites were omitted in other two GM maize notifications. Assessed compositional compounds also differed between field trials conducted within a specific notification, e.g. in the case of GM oilseed rape where one anti-nutrient was not considered in earlier, but in later field trials.

Lack of consideration of environmentally relevant plant compounds

In the GMP risk assessments reviewed environmental aspects of plant composition were generally left unconsidered. The compositional analysis focused on food and feed safety only. Potential dif-

ferences between the composition of the GMP and the non-GM control of plant compounds were generally interpreted exclusively in view of the nutritional safety of the GM crops.

This focus on food and feed safety was also reflected in the choice of tissues subject to an analysis of anti-nutrients or toxins. They were chosen according to their relevance for food and feed purposes (i.e. grain in maize, seed in oilseed rape and tubers in potato). Secondary metabolites or toxins are frequently also present in other tissue types such as the green tissues which were generally not included in these assessments. The chosen parameters were not always comprehensive enough in order to corroborate substantial equivalence also in view of environmental safety. This criticism has already been expressed earlier with respect to nutritional safety assessments in GMO notifications (Spök et al. 2003b).

Lack of consideration of novel metabolites due to the application of the non-selective herbicide(s)

New metabolites in the GM crop due to the use of the non-selective herbicide have so far not been addressed or evaluated in any of the notifications of a herbicide tolerant GM crop. New metabolites produced due to the post-emergence application of glufosinate have been described both in herbicide tolerant oilseed rape and maize depending on the expression levels of the enzyme (OECD 1999a, OECD 1999b, OECD 2002c), in particular those being tolerant to glufosinate, with highest levels in leaves (Ruhland et al. 2002, Ruhland et al. 2004).

Artificial increase of variability for interpretation purposes

For general criticism on the comparators used in the field trials for the different assessments, including the compositional assessment, see also cross sectional issues (chapter 3.2).

The assessment of differences between the GMP and the controls with respect to a certain plant compound differed from the other phenotypic assessments.

For the evaluation whether a GMP differed in a certain compound from the non-GMP the notifiers usually established a ‘baseline’ in order to cover the natural variation of a certain compound, also named a ‘tolerance level’. This ‘tolerance level’ was either composed of conventional crops planted at the same location, at different locations and/or in different years than the GM crop or of an established literature range for different compounds. Different types of ‘ranges’ (e.g. literature range, commercial range) were calculated depending on the notification. Usually no explanation was provided in the notifications why a certain type of range was chosen for a certain compound while another was chosen for another compound. Additionally, no explanation was given why the literature chosen to establish such a range varied even among parameters (e.g. the number of literature sources considered).

The approach to pool compositional values of certain plant compounds from different locations and years may be acceptable for food and feed safety where commodities which are placed on the market derive from different sources but its relevance for environmental aspects must be challenged. For an insect species feeding on maize grown at a specific location a significant increase or decrease in a certain insecticidal plant compound may make a difference. Due to environmental variability this compound may, however, not be changed at other locations and thus not affect the same species at another location.

Plant composition is strongly influenced by growing conditions, especially soil composition (EFSA 2006a). By comparing compositional values derived from field trials at a certain location to a range of values from plants grown under different conditions, at other locations or in other years, any significant differences between the GM and the respective control plant grown under the same conditions are 'diluted'. This practice has apparently not changed since the first GMO notifications notified under Directive 90/220/EEC. An earlier analysis of GMO notifications already criticised this practice of comparing data derived from different locations as not suitable to detect potential changes in the composition or in the plant's metabolism (Spök et al. 2003b).

Lack of follow up of statistically significant differences

In all notifications reviewed statistically significant differences in anti-nutrients or secondary metabolites were found between the GMP and the non-GM control either in analyses across locations or at individual locations. In the interpretation of results of the compositional evaluation, notifiers generally followed a similar approach. They usually argued that any observed differences between the GM crop and the control did not have any biological significance. Statistically significant differences either across or within locations were considered not to be 'biologically relevant' if they fell within the 'tolerance level' or 'natural range' of values established by the notifier (see cross sectional issues (chapter 3.2).

The question whether a particular difference between the GM and the non-GMP is biologically 'relevant' or 'significant' can rarely be answered on an *ad hoc* basis. Notifiers argued that the values fell within the range presented and did not consider these 'biologically significant' with a view to food and feed safety of the GM crop. However, the biological relevance for an insect species at a particular location will have to be determined. EFSA clearly asks for statistically significant differences in composition between the modified crop and its non-genetically modified comparator grown and harvested under the same conditions to be followed up (EFSA 2006a) and states that 'modifications that fall outside normal ranges of variation will require further assessment to determine any biological significance'. Although in all notifications reviewed such statistically significant differences were observed, in no case they lead to further investigations by the notifier. This is consistent with results of Spök et al. (2003b) who reported that in several GMO notifications analysed observed significant differences lead in no case to a repetition of the test with further test parameters but were dismissed by referring to the natural range.

3.3.5 Assessment of survivability, selective advantage, dissemination, invasiveness and persistence

In general, the notifiers assumed that the introduced or modified GM traits did not change the biological characteristics of the GMP beside the ones intended to. This assumption was frequently not supported with relevant and conclusive data. In several cases no specific assessments were presented for the evaluation of dissemination, survivability, selective advantage, invasiveness or persistence based on the assumption that the novel traits were not considered to change the inherent characteristics of the plant. Data addressing the traits and processes in question were rarely specifically generated by the notifiers, most of the argumentations backed by 'surrogate' data derived from the agronomic assessments. These agronomic data, however, often did not aim specifically to evaluate the above mentioned processes or were based on data from a range of different sources.

In addition, many of the data were hardly comprehensible as information on their source (authors, institutions), status (published, preliminary) and relevance (GMO used) was frequently lacking.

Conclusions not based on specific data but on reference to other assessments

Evaluations of the persistence, invasiveness, survivability or potential selective advantage or disadvantage of the GMP was based on the assumptions of the notifiers that the crop plant was generally not considered to persist or to be invasive and did not have any weediness traits such as dormancy. However, this argumentation was rarely supported by specific assessments but largely based on the reference to other assessments, e.g. the assessment of agronomic traits, or by reference to the published literature.

Any differences in the reproduction, dissemination or survivability of GM maize were usually discussed by notifiers by referring to the agronomic evaluation or the assessment of plant composition (substantial equivalence). The notifiers referred to different traits evaluated in the agronomic assessments such as seed or flowering characteristics or general agronomic characteristics. Apparently, there is no general view which traits are useful for the assessment of the plant's reproductive biology or indicate the ability of the GMP to survive and disseminate, as the traits referred to differed considerably between GM maize notifications. Generally, no reasoning for the choice of the traits assessed was provided by the notifiers. This gives the impression that the parameters evaluated in the agronomic assessment, no matter which were assessed, were drawn on for the assessment of reproduction, dissemination and survivability without an evaluation which of the parameters are actually relevant for these processes. This is also reflected in the lack of consistency in the choice of parameters referred to in different notifications. Additionally, a lack of consistency between parameters referred to in the argumentation of unchanged reproduction, dissemination and survivability and the agronomic parameters was evident, i.e. agronomic parameters which were referred to were actually not assessed in the agronomic assessments or the reference made was held unspecific or general (e.g. 'agronomic traits'). In many cases the agronomic assessment itself to which the notifier referred was composed of insufficient or inadequate data (see also chapter 3.3.3).

Also for the GM potato agronomic traits were drawn on to argue that the GMP is unchanged to the non-GMP with respect to reproduction, survivability or dissemination although also in this case not all arguments were substantiated by data. A lack of differences in the flower morphology between GM and non-GM potato was referred to, but it remained unclear whether this parameter was actually assessed in the agronomic evaluation or not. The comparison of the potential for persistence and invasiveness as well as selective advantage or disadvantage between the GM potato and the non-GM potato was also based on argumentation rather than on specific data as relevant results from field trials, which were cited, were not presented in the notification.

Lack of specific assessment of gene flow in maize and potato

Gene flow was rarely addressed in GM maize or GM potato notifications. The notifiers concluded that pollen dispersal of the respective crop is limited. Consequently, also consequences of gene flow of GM maize were not addressed.

Reference to different information sources for the gene flow assessment in GM oilseed rape

For the assessment of survival, dissemination, gene flow as well as consequences of gene flow and the potential of the GM oilseed rape to have a selective advantage, to become persistent or invasive, agronomic traits were drawn on to argue that the GMP is not different from the non-GMP. Data on seed and pollen dispersal were discussed by the notifier referring to different sources such as other GM oilseed rape notifications, results from field trials by the notifier, preliminary, published and unpublished results from several research groups as well as results from monitoring studies of oilseed rape. In almost all cases, either the exact source of the citations was not clear or only summaries of the results were provided omitting details of the studies. A clear and concise overview which data were derived from the notifier's own research and which data were referred to from the published literature was lacking.

Unspecific referencing or lack of clear data presentation for GM oilseed rape

In the case of GM oilseed rape either no specific data were provided, results were presented on a very general level and/or the source of the data was unclear. Also the status of the results (preliminary – unpublished – published) and the GM event used in the studies were frequently not indicated. The main shortcoming of the assessment is thus the unstructured presentation of data and the lack of clear reference of the evidence provided by the notifier to conclude on the unchanged dispersal, outcrossing and survival ability of the GM oilseed rape (see also chapter 3.2.4). Similar shortcomings in the assessment of potential weediness of GM crops as provided by notifiers to the US Department of Agriculture (USDA APHIS) have been described as early as in 1995 (Purrington & Bergelson 1995).

Assumption-based assessment of selective advantage/disadvantage of the novel traits of GM oilseed rape

The possibility that the novel trait (for our example oilseed rape Ms8xRf3: male sterility or the herbicide tolerance) could confer any fitness benefit to feral GM oilseed rape was not addressed in depth or backed by specific data. Similarly, the assessment of any effect of the traits on wild relatives was also based on assumptions rather than on specific assessments. The basic assumption of the notifier behind these assessments was that the novel traits did not confer any fitness advantage either generally (male sterility) or outside the agricultural context (herbicide tolerance) where they could be controlled by other means. In unmanaged habitats the novel traits introduced in GM oilseed rape were assumed not to confer any selective advantage to the GM crop. The establishment of feral oilseed rape was considered not to lead to a negative impact on the environment. However, these assumptions were based on general information on gene transfer to and occurrence of wild relatives of oilseed rape without providing relevant data or studies. Although it was recognized by the notifier that in semi-managed habitats such as field margins where spray drift could occur a selective advantage would be conferred to the GM crop, in these habitats the herbicide tolerant crop was considered manageable through the use of other available selective herbicides.

3.3.6 Assessment of effects mediated via target organisms

In the notifications of herbicide tolerant GMPs generally no target organisms were defined and recognized. Consequently, resistance development of target organisms was not accounted for in

these notifications. For insect resistant GMPs such as *Bt* crops the definition of the target organism(s) was usually held very general. Notifiers generally did not specify the pest species which the GMP aimed to control. Hence, the mode of action of the *Bt* toxin and efficacy tests of the GMP against the pest species, including the establishment of minimum efficacy levels, were not provided for these crops. This also entailed that potential secondary pests or changes in food/prey availability due to loss of target organism were not considered as well as possible resistance development of non-target pest species.

Lack of definition of target organisms in herbicide tolerant GMPs

Target organisms were identified for GMPs with insect resistant trait(s) only. While it is acceptable that no target organisms were identified for the starch-altered GM potato, it is not acceptable that the issue of ‘target organisms’ was entirely omitted for herbicide resistant GMPs.

The lack of identification and evaluation of the target organisms for herbicide tolerant crops underlines a larger deficiency of the current regulations of herbicide tolerant crops: the exclusion of adverse effects caused by the application of the corresponding broad spectrum herbicides (such as glyphosate or glufosinate) in the ERA of these GMPs. The novel enzymes produced in herbicide tolerant crop plants, which enable them to survive the application of the corresponding herbicide, are considered the ‘novel’ trait, and in consequence, the sole 'stressor', but they are not expected to induce any adverse effects at all. Hence, the potential adverse effects of the herbicide tolerant GMPs and their associated secondary stressors, the broad-spectrum herbicides, are excluded from the assessment. This seems to be based on the conclusion, in analogy to insect resistant crops, that every plant other than the crop plant is considered a ‘target’ pest, and requires control or, even if not a ‘target’ pest, it is a pest as such (i.e. a weed). This is an outdated approach of pest or weed control. Modern sustainable agricultural systems acknowledge that in every cropping system only a fraction of the given non-crop flora (and fauna for that matter) actually reaches damaging densities and, therefore, qualifies to be a ‘weed’ (or a 'pest' if an arthropod) that might require control measures. Treatment of these weeds or pests is only initiated when their densities have reached a damaging threshold. Further, by no means, are these non-pest, non-crop plants in agricultural fields meaningless and have no function or service for the ecosystem they live in. In many areas in Europe, much if not all of the remaining and still declining biodiversity rests in what is called ‘cultural’ landscapes, an intertwined mosaic of agricultural and managed semi-natural habitats. These include plant species that grow in and alongside or nearby crop fields and constitute the most important food source for farmland arthropods and birds. Among these several species are protected either nationally or are even of EU-wide conservation concern (Traxler et al. 2005a). The Farm Scale Evaluations commissioned by the UK government and carried out from 2000 until 2002 documented significant additional impacts on the arthropod fauna associated with weeds growing in and nearby herbicide tolerant crop fields beyond and above those in conventionally treated fields (Heard et al. 2003a, b, c).

Lack of consideration of resistance development of target organisms in herbicide tolerant GMPs

No identification of target organisms for broad spectrum herbicides led to a lack of resistance management programs for weeds. Today, reports of weed resistance against the dominant herbicide

used in connection with herbicide tolerant crops are rising. Until the inefficacy of herbicide tolerant GMPs has not reached a certain economic threshold due to resistance development, significant environmental impacts will occur as a result of the steady increase in concentration and/or frequency of use of the 'doomed' herbicide and the additional use of alternative, supplementing herbicides compensating the declining control efficacy of the weeds.

This stands in stark contrast to insect-resistant GMPs where resistance of the target organisms has been recognized and acted upon as the most important risk associated with GM crops in North America. As for insect-resistant crops, the herbicide tolerant crops will become useless as resistance against its corresponding herbicide arises. However, while for *Bt* crops resistance only now begins to develop (Tabashnik et al. 2008), twelve years after their introduction, resistance in weeds against glyphosate began to rise very shortly after the large scale release of herbicide tolerant crops in North and South America and is now well on its way to render Glyphosate useless for a number of serious weeds worldwide (see for example: www.weedscience.org).

Lack of definition of target species and corresponding efficacy tests for *Bt* crops

Target organisms were frequently not specified in *Bt* plant notifications. Notifiers did not specifically and consistently provide lab and field assessments of the effects of the GMP on the target organism(s) in order to demonstrate the efficacy of the GMP. Tests using target organisms or pest species included in the notifications usually dealt with the establishment of equivalence of the plant-derived and microbially-derived proteins during the toxicological assessment of human health effects of the GMP but not for demonstrating efficacy of the GMP.

A considerable inconsistency between the specification of the target organism(s) of the GMP by the notifier and tests with the GMP and its target organism(s) was evident in some notifications. For instance, in the notification of maize Bt11 the notifier did not explicitly state which target organism the maize Bt11 intended to control. As a general statement the notifier considered all Lepidoptera occurring on maize as pests and listed a range of lepidopteran pests occurring in the UK. However, tests were only carried out with European corn borer and Corn earworm but not other lepidopteran pests.

In other notifications (maize MON810, maize NK603xMON810), no tests using the designated target organisms and testing the efficacy of the GMP were included at all (see also chapter agronomic assessment 7.3.3.) For GMPs expressing the *Bt* toxins Cry1Ab or Cry1F (five notifications) the lepidopteran pest species *Sesamia* sp. was included only in one notification (maize 1507). The fact that *Sesamia nonagrioides* represents a relevant target organism was ignored in the other notifications. No studies were provided assessing the effects on this pest species when exposed to the GMP. In the case of the maize 59122, expressing the Cry34Ab1/35Ab1 toxins, also no efficacy tests were presented either from the lab or the field. Only one out of six notifications with *Bt* crops contained a field study evaluating the actual *Bt* crops plant and its effects on the target organisms under field conditions (maize 1507, see also below).

Several shortcomings were identified in these typical studies carried out for demonstrating the effects of a *Bt* crop on targeted pests as shown for the studies submitted for maize 1507 (Castanera 2001, n.st.;Vernier et al. 2001b, n.st.; see also chapter 2.7). Although both studies were included in

the notification as attachments, the results of Castanera (2001, n.st.) were neither cited nor discussed in the ERA by the notifier.

Based on the data submitted in these two studies the demonstration of the capability of Cry1F maize to control target organisms rested on two mortality values from a single, probably non-replicated study, and the reading of four parameters from a total of 400 Cry1F maize plants at one location in one year. This is by any scientific standard a most rudimentary data basis in addition to at least one study not fulfilling even minimal scientific requirements in terms of sample size, replication, statistical analysis and their presentation. Studies showing reliable and sustainable control of *S. nonagrioides* have yet to be presented by the notifiers and robust data sets on the degree of mortality Cry1F maize at various growth stages and tissues inflicts on the relevant life stages of *S. nonagrioides* are still missing. Without such basic data, no reliable resistance management plan can be established nor are the necessary requirements given to assure the farmer minimal quality control standards of the commercial product. The claim of an excellent control of *Sesamia*, as stated by the study authors and consequently the notifier, was not supported by the data delivered.

In the notification of maize 1507, the notifier mentioned additional field trials carried out in Spain for agronomic purposes (not efficacy, resistance or biosafety purposes). Details of these studies were not included and, therefore, cannot be evaluated here. However, the notifier stated that the 'results confirmed that the two locations suffered significantly different insect pest pressure in terms of presence of insect pest species causing and the resulting damage.' This highlights another point that has not been convincingly demonstrated: to what degree *S. nonagrioides* is actually a problem for maize production in Spain or other EU countries. From the data delivered in the notification, *S. nonagrioides* densities appeared to strongly fluctuate locally and annually to the degree that it appeared to reach pest densities: locally and in certain years only. This goes along with the observation that the evidence delivered by the notifiers or in the supporting studies (e.g. Vernier et al. 2001b, n.st.) in support of the claims made regarding the severity of the pest problem *S. nonagrioides* poses in Spain are outdated with publication years ranging from 1940 to the most recent still being 20 years old (a proceedings publication from 1988) and regionally highly diverse from Turkey to Morocco (Vernier et al. 2001b, n.st.). If *S. nonagrioides* was such a broad and serious problem in Spain, more up-to-date and detailed data should be available and cited in the notification.

Most other studies using pest species, in particular those supplied for the characterisation of the novel protein in the context of the assessment of human health effects, were carried out with purified *Bt* toxins – derived from microbes, instead of GMPs. Such tests give only limited information of effects on target organisms, in particular if they are the only evidence for effects on target organisms provided. The *Bt* toxins were extracted and characterized based on a number of criteria that require purified solutions. The use of a purified protein, regardless whether derived from a microbial source or the plant, allows testing different doses of the toxin but excludes testing for plant x toxin interactions. Mortality and development of herbivores is known to vary considerably depending on food quality. Plants *per se* often constitute sub-optimal food, since they contain compounds that are either hard to digest (e.g. lignin, cellulose), toxic and/or anti-nutritive. If only purified proteins are used and mixed in an optimized artificial diet, these effects will be overlooked. Further overlooked will be the potential bioactivity of smaller fragments of *Bt* proteins either due to possibly

existing RNA fragments as were found for maize MON810 (Rosati et al. 2008) that might yield different unknown proteins or due to in-planta processing of the plant-produced *Bt* toxin.

In addition, none of the notifications accounted for the variability in toxin concentration levels as they were found at least in some regions in Germany (Nguyen & Jehle 2007).

In conclusion, baseline susceptibility values for the target organisms under field conditions and the proof that the target organism can be actually controlled by the GMP in the field as well as the establishment of a minimum efficacy level were lacking in all notifications of *Bt* plants.

Open questions in the mode of action (MoA) of the *Bt* toxin in target organisms unconsidered

The understanding of the mode of action of *Bt* toxins in target pests is crucial for the understanding of the functioning of a *Bt* crop and potential effects on other pests or non-target organisms. Generally, notifiers only presented published literature supporting a narrow specificity of the respective *Bt* toxin. Shortcomings and remaining questions on the mode of action and the specificity of *Bt* toxins were generally omitted and not discussed in the notifications. Here two examples are given:

Example1: Maize Bt11 – no inclusion of comprehensive and up-to-date literature

Although the long list of publications compiled by the notifier for the maize Bt11 seems extensive, it lacks an update. Of the 26 publications at least five were concerned with resistance development in target pests. All publications concerned with resistance were published during the 1990ies. Of the remaining 21, only four were younger than 1990. On other issues, publications until the year 2002 were listed. Hence, the majority of papers cited dealt with microbial *Bt* proteins and not with those produced in the GMP. However, even among the rather old literature, papers publishing data about new or unexpected effects and properties of *Bt* proteins were not included. For example, a series of papers that came out of the laboratory of Prof. David Ellar (Oxford University, UK) were omitted. For example, Haider et al. (1986) carried out a series of experiments where they activated microbial *Bt* proteins from a *B. thuringiensis* var. *colmeri* using gut extracts from susceptible insects like the mosquito *Aedes aegypti* and the moth *Pieris brassicae*. After activation with the mosquito larvae gut extracts, the *Bt* toxin affected all mosquito cell lines tested but only one caterpillar cell line (*Spodoptera frugiperda*), whereas an activated preparation produced by treatment with *P. brassicae* gut enzymes or trypsin was toxic only to the caterpillar cell lines not to the mosquito cell lines. Similarly, Knowles et al. (1986) reported about unpredictable cross-reaction of *Bt* delta-endotoxins from three different strains, *B.t. kurstaki*, *B.t. aizawai* and *B.t. thuringiensis*. So, the same *Bt* protein subjected to different gut extracts yielded different toxic fragments affecting different insect species contradicting the common notion that any given *Bt* protein only affects one taxonomic insect group.

Example 2: maize 59122 – no data provided on the presumed MoA for these new binary toxins

The two toxins expressed in maize 59122 (Cry34Ab1 and Cry35Ab1) are known to work best when delivered jointly but not even a crude explanation of the presumed mode of joint action was delivered in the notification. Only the MoA of single *Bt* toxins such as the Cry1Ab was summarized with

few supporting literature cited – one is a book (Glare & O'Callaghan 2000) published on *Bt* in general in 2000 which does not represent an expert document on binary *Bt* toxin mode of actions, and an unpublished, interim Canadian report (Mason & Schwartz 2000). For the latter it is entirely unclear whether that report contains original new data on the MoA of these particular binary toxins or is a literature study. Apparently at the time when this particular GM maize was notified – several years after the interim report – still no final report seemed to be available. In fact, in the report it is admitted that 'the mechanism of action (…) appears to be similar to that of other *Bt* toxins' and 'additional investigation is necessary to confirm this interpretation of the Cry34Ab1/35Ab1 mechanism of action.'

The Canadian study has been published in 2004 (Masson et al. 2004) providing insight into the capacity for pore formations of the individual toxins and the joint effect of both toxins. Another paper was published in the following year by a different researcher group (Schnepf et al. 2005). Both groups reiterate that both toxins exert the highest toxicity together with the 14 kDa (Cry34Ab1) – a small *Bt* toxin by comparison – obviously having the most toxic effect when provided as singular toxin but its effect is significantly enhanced by the presence of the 44 kDa (Cry35Ab1). The 44 kDa toxin by itself seldom formed pores but added to the destabilization of the membrane. However, even this literature was ignored when the assessment of non-target effects was performed. For the evaluation of effects and exposure data of certain non-target species only the small protein (Cry34Ab1) was considered in the relevant study (Poletika 2003, see also chapter 2.8), hence ignoring the combined MoA and the higher toxicity of both toxins.

Resistance management programs restricted to ECB

Currently, the only commercially available insect resistance traits are all based on toxins from *Bacillus thuringiensis*. Identified target organisms always include only those that are aimed to be controlled. For GMPs containing Cry1 these include *Ostrinia nubilalis* (European corn borer, ECB), *Helicoverpa zea* (Corn Earworm, occurring in the US) and occasionally *Sesamia* spp. (occurring in Spain). Occasionally, other lepidopteran pests which feed on maize were listed as target organisms in GMP notifications but were generally not further specified or considered.

However, insect resistance management as proposed in the reviewed notifications concerned exclusively certain target organisms defined by the notifier, i.e. European corn borer for Cry1Ab and Cry1F containing GMPs and *Diabrotica* for the Cry34/35Ab1 containing GMP. Insect resistance management of other lepidopteran species, even although often listed as 'target organisms' in Cry1Ab or Cry1F expressing GMPs, was usually left unconsidered.

In many agricultural systems, a number of lepidopteran pest species exist that are affected to lesser degree or sublethally by the Cry1 class of *Bt* toxins. These include species of the genus *Spodoptera* (various armyworms), *Agrotis* spp. and others (DeMaagd et al. 2003, Binning & Rice 2002). These are ideal candidates for developing resistance fairly quickly against Cry1 *Bt* toxins. Many of these pest species are polyphagous and also feed on other crops, including vegetables. Lower susceptibility acquired in *Bt* crops may interfere with control measures using *Bt* sprays in other cropping systems. This negligence of resistance development in other lepidopteran pests other than the target organism stands in contrast to the fact that 'Lepidoptera' as such occurring in

maize cropping systems were considered generally as pests by the notifiers and were as such also often listed as target organisms (e. g. maize Bt11).

Lack of assessment of potential secondary pests of the GMP

For maize Bt11 a fungal disease was identified (*Fusarium* spp.) and tested as the only potential secondary pest. For all other *Bt* maize events the notifiers did not consider secondary pests at all. This must be scientifically challenged since there are a number of plant sucking pest species on maize (e.g. spider mites, bugs, aphids) that are expected to be unaffected by the *Bt* toxins. Released from competition by the target insects and from pesticide pressure due to reduction of application frequency, they might well be at a serious advantage and cause problems as secondary pests. Predictions (Hilbeck 2001, Hilbeck 2002) and indeed, reports about sucking insect pests increasing in *Bt* crop production have surfaced at least from *Bt* cotton production regions in the southeastern US and China (Wang et al. 2008). Laboratory studies by Rovenska et al. (2005) have shown that while predatory mites tended to feed less on *Bt* containing prey spider mites, the spider mites (a serious pest) in contrast preferred *Bt* maize. Also Faria et al. (2007) reported higher densities of aphids on *Bt* maize compared to the isogenic maize.

Lack of assessment of food/prey availability due to the loss of the target organism(s)

The loss of organisms associated with the target organism and affected by a change of the target organism's abundance due to the *Bt* crop has been observed. Larval parasitoids of the European corn borer *Ostrinia nubilalis* were reduced in *Bt* maize fields due to the lack of *O. nubilalis* larvae in *Bt* corn plots (Bruck et al. 2006). Similarly, beetles of the family Nitidulidae were reduced, probably due to a lack of *O. nubilalis* tunnelling (Bruck et al. 2006). None of such effects has been evaluated or even discussed in any of the notifications of *Bt* maize.

3.3.7 Assessment of interactions of the GMP with non-target organisms and the biotic environment

A number of key issues emerged from the detailed analyses and evaluations of the notifications reviewed regarding non-target assessments. The main shortcomings derived from the fact that no research hypotheses resulting from a science-based ERA approach were formulated and tested. Consequently, a realistic exposure assessment, the selection of relevant test species from representative environments and the alignment of laboratory and field studies on a particular question were not included. Consequently, a rudimentary problem formulation, the lack of consideration of important aspects of toxin function and activity in non-target organisms, the use of prescribed test organisms for ecotoxicity testing that have limited value for the ERA as well as a lack of ecologically more realistic studies were evident. In addition, several methodological shortcomings in lab and field studies have been identified, including the omission of relevant information on methodology or test organisms. Another major issue is the fact that for the assessment of non-target organisms published field studies were increasingly cited, which were often irrelevant to the GMP or the environment in question, rather than providing specific non-target assessment studies conducted

by the notifier. Last but not least, the need to consider also relevant plant compounds, such as toxins or secondary metabolites and their interaction with non-target organisms has been identified.

Lack of characterisation of the GMP and natural compounds

The ERA for non-target organisms should be science-based and requires a solid characterisation of the GMP, in particular if it expresses a novel pesticidal compound(s), such as *Bt* plants do. This includes not only the quantification of a novel compound(s) (pesticides and others; see Expression analysis in chapter 3.3.2) but also potential changes in naturally produced primary and secondary plant compounds. Such compounds have an important role for feeding and oviposition stimulation, deterrence/attractants of pests and pathogens or their parasitoids. In the ecological and entomological scientific literature, evidence for the mutual influence of plant compounds and herbivores on the evolution of both, the plants and their herbivores has been reported (e.g. Berenbaum 1995, Scheirs et al. 2003). For instance, Berenbaum (1995) postulated that 'Variation in primary metabolism is likely to be particularly effective as a defense against highly oligophagous herbivores with limited mobility, especially those confined to structures containing allelochemicals that could neutralize the benefits associated with compensatory feeding*'*. She argued that the 'degree to which variation in plant primary metabolism results from the selective impact of herbivory may be greatly underestimated […]' (Berenbaum 1995).

Thus, unintended changes in these compounds due to the genetic modification may change the chemical cues for insect herbivory and related processes. Unintended and unknown changes in the metabolism of GMPs caused by the genetic engineering process can be subtle and possibly chronic (i.e. long-term) and easily escape the attention when testing for a limited number of certain primary compounds and agronomic performance only. Scientific evidence for such effects is accumulating and several reports exist to date on unexpected changes in the metabolism of GMOs due to the transformation process (e.g. Birch et al. 2002, Saxena and Stotzky 2001, Prescott et al. 2005). Such changes can affect associated arthropod communities and, for instance, change the pest status of certain herbivores for better or worse. Both is important to be informed about prior to the commercial use by farmers. The challenge in the ERA is to identify those changes in the plant's composition that can be linked to potential environmental adverse effects. Therefore, it is important to know the differences between the GM and the non-GM crop in relevant toxins, anti-nutrients or secondary metabolites as they can provide important indications for changes in certain ecological responses in the crop plant to the associated fauna – be it beneficial or pest organisms.

However, the crude data from compositional analyses submitted for establishing substantial equivalence is by far not sufficient for this (see chapter 3.3.4). Little if any data is currently provided by the notifiers on naturally occurring secondary plant compounds.

Further, no research data is submitted aiming at identifying new metabolites in the GM crop due to the use of the non-selective herbicides in any of the notifications of a herbicide tolerant GM crops. New metabolites produced due to the post-emergence application of glufosinate have been described both in herbicide tolerant oilseed rape and maize depending on the expression levels of the enzyme (OECD 1999a, OECD 1999b, OECD 2002c), in particular those being tolerant to glufosinate, with highest levels in leaves (Ruhland et al. 2002, Ruhland et al. 2004).

Ecotoxicity testing – little and crude testing

In the following the shortcomings of ecotoxicity data provided in the notification of maize 59122 – representative for ecotoxicity testing in all evaluated notifications – will be discussed in detail. For this purpose a scientific critique of the non-target risk assessment study provided for this GM maize notification (Poletika 2003, n.st.) is provided.

Poletika (2003) documents the typical approach taken essentially by all notifiers regarding ecotoxicity testing of non-target organisms. These tests are being criticized for their lack of scientific rigor and ecological relevance for at least eight years (Hilbeck et al. 2000). Here, the most serious shortcomings are summarized. In essence, the ecotoxicity testing provided by the applicants is of only limited value for the ERA.

Problem formulation not adequate

Although at least an attempt was made to formally follow the EPA Ecological Risk Assessment guidelines and include a 'problem formulation' section, the outcome of that assessment is lacking rigor and ecological depth. One problem is that the entire ERA hinges on the conceptual model that a *Bt* – or any GM plant – is the linear addition of the conventional, non-modified plant + an added protein. Hence, only the protein is considered the only stressor tested in isolation of the context of the GMP. This conceptual model is based on an outdated understanding of molecular genetics where a gene codes for exclusively one protein and that this protein has only one singular effect. It neglects any interaction and/or combination effect at both the genetic and the metabolic level – let alone any epigenetic or pleiotropic effect due to the transformation process itself (see also chapter 3.2.1). Further, the problem formulation only draws on scientific literature that supports the pre-conceived understanding omitting any uncertainties or alternative scientific concepts. Many claims were not even supported with scientific data altogether but simply postulated.

Bt mode of action (MoA) in adversely affected non-target organisms unknown

All of our current knowledge and understanding of the MoA of *Bt* toxins stems from research with target insect pests, i.e. herbivores. In fact, much information became available through extensive research conducted to learn about mechanisms of resistance in these target insect pests (e.g. Heckel et al. 2007). Except for one study (Rodrigo-Simón et al. 2006) no study was carried out investigating the potential MoA in documented cases of adversely affected non-target organisms outside of the typcial range of target pest herbivores.

Throughout the document, it was taken as a given that the expressed *Bt* toxins only and exclusively affect the target insects of a particular order. This conclusion cannot be uphold in face of many published studies reporting unexplained adverse effects of *Bt* proteins or *Bt* plants in bi- and tritrophic trials on a broad range of arthropods also outside of the usual taxonomic orders of target organisms (for reviews and supportive scientific literature see Lövei & Arpaia 2005, Hilbeck & Schmidt 2006). For none of these, the MoA have been solved. Some attempts were reported to disprove a direct *Bt* toxin effect (see the Green Lacewing example evaluated in detail in Hilbeck &

Schmidt 2006). However, a closer evaluation of the applied methods and materials revealed that these studies yielded complementary but not contradictory data. Cited literature on reported non-target effects in the evaluated notifications was meager to non-existent, and focussed on the hypothesis that there are no adverse effects of *Bt* crop plants on organisms outside of the target taxonomic orders.

In addition, the ERA provided by Poletika (2003, n.st.) only considered one of the two binary toxins – the Cry34Ab1 toxin (14 kDa molecular weight) - although the necessity of both toxins for insecticidal action is known and acknowledged in the introduction of Poletika (2003) as well as in the chapter on the mode of action of this toxin in the notification (see also chapter 3.3.6).

Selection of test species – few organisms from the receiving environments of Europe

Although a field census – 'field monitoring study'- carried out by the notifier at two locations in the central US corn belt in two years yielded long lists of taxa found to inhabit or exist in maize fields – only two species in addition to the typical universal standard species were chosen for testing: the 12-spotted ladybird beetle and the monarch butterfly. From all the species tested the only species relevant also to European ecosystems are the honey bee, the convergent ladybird beetle and the green lacewing. All other testing organisms are the typical species list that can be found in all notifications of *Bt* crop plants and were not selected with respect to the receiving environment. No organisms from the receiving environments in Europe were included in the tests.

Also, no attempt was made to conider species of conservation concern from Europe. The only substitute species for endangered Lepidoptera is the Monarch butterfly. That species does not occur in Europe. However, in many regions of Europe, larvae of the peacock butterfly (*Inachis io*) live on weeds growing in and around maize fields. In Hungary, *Inachis io* is a listed endangered species and therefore would be a more adequate test-species. None of these issues were considered in the notifications. Also in other European countries several butterfly species occurring in agricultural regions have a protection status (for example see Traxler et al. 2005a).

Exposure assessment

Expression values used for the calculation of the exposure estimates for different taxa were derived from the field trials conducted in Chile. Expression values derived from overseas locations cannot be considered representative for Europe and do not provide the information on the variabiity of expression under European conditions. One can only speculate why these expression values were chosen, considering that in the same notification expression levels of several European locations were reported.

These expression values, but of the Cry34Ab1 toxin only, were then used as the basis for the exposure calculation for non-target organisms. The consideration of only one Cry-toxin was justified by the author stating that 'no single ratio of the both toxins would represent the expression pattern in any given tissue, but rather variability results in a wide array of ratios' (Poletika 2003, p 13).

In addition, calculating the expected exposure concentrations was highly speculative and often oriented towards the lowest possible exposure scenario. Typically, in ecotoxicity tests multiples of at least 10 are used, more typically ranging from 100 to up to 1'000 or 10'000 fold depending on the toxicity of the compound. In Poletika (2003, n.st.), however, a maximum safety factor of 21 was

applied for one honey bee test and the minimum safety factor was as low as 1 for the Collembola test and 2 for most other tests.

Serious shortcomings of laboratory trials

Most applied protocols had serious scientific shortcomings. In the following the most serious flaws are detailed:

General critique applying to all ecotoxicity tests and protocols:

In the introduction of the notification and studies attached, it is stated that 'both proteins are required for significant insecticidal activity' (e.g. Herman et al. 2002). However, the dominating protein in terms of concentrations was reported to be the small – 14 kDa – Cry34Ab1 fragment. Cry35Ab1 is larger and 44kDa in size.

From the summarizing description of the effects data provided in Poletika (2003, p 22-23), only the toxin Cry34Ab1 was used for comparison of effects and exposure data although the laboratory studies cited for the effects data used both proteins. This is in particular problematic as it was made clear earlier that for achieving maximum insecticidal activity even against the target pests the joint action of the two binary toxins is required. Hence, it is a serious shortcoming that in the ERA only one of the two toxins was used.

General critique applying to specific trials:

Convergent ladybird beetle – *Hippodamia convergens*:

- Direct feeding trial with purified single Cry34Ab1 toxin with estimated safety factor based on pollen concentration (low) of approx. 2
- Result: no effects
- Scientific shortcoming: Adult beetles (page 21, 22) were used for testing – most known insensitive life stage used. *Bt* toxins are larvicides.

Twelve-spotted ladybird beetle – *Coleomegilla maculata*:

- Direct feeding trial with purified single Cry34Ab1 toxin with estimated safety factor based on pollen concentration (low) of ca. 12
- Noteworthy inconsistency: Here larvae were used for testing. Not adults like with *H. convergens*. No explanation provided why.
- Result: Significant weight reduction
- Scientific shortcoming: Sublethal effect counted as irrelevant.

Tritrophic feeding trial with aphids:

- Noteworthy inconsistency: here both larvae and adults were used. Since plants were used here both toxins were present in food for the prey.
- Result: no effects

- Scientific shortcoming: Likely no to minimal exposure to toxins because aphids do not ingest and mediate toxins as no toxin was documented to be present in phloem sap – the predominant food of the used aphids. Therefore only accidental exposure or minute amounts can be expected.

Parasitic hymenoptera adults – *Nasonia vitripennis*:

- Results: no effects
- Scientific shortcomings: *N. vitripennis* is of low ecological relevance. It is the parasitoid of the house fly; an ecological more meaningful organism should have been selected; adults, as the most insensitive live stage - were tested.

Green lacewings – *Chrysoperla carnea*:

- Result: no effects
- Scientific shortcomings: No exposure to the *Bt* toxin via ingestion. Lacewing larvae have piercing-sucking mouthparts. They penetrate skins of prey and shells of eggs, inject enzymes that liquefy the content and suck out the content without consuming the shells or skins. In this test, purified *Bt* toxin is mixed with meal moth eggs resulting in externally applied *Bt* toxin outside of the egg shell.

Compost worm – *Eisenia foetida*:

- Typically, a single dose test evaluating toxicity is carried out.
- Exposure time: very (too) short: Worms are long-lived (years) but are only tested for several days (typically 14)
- Exposure route: unproven. It needs to be demonstrated whether *E. foetida* ingests any *Bt* protein in this test. *E. foetida*, a typical edaphic species, does not feed through soil but ingests concentrated organic debris. Typically corn leaf protein powder or microbial protein is mixed into a test soil substrate (consisting of peat, clay and industrial sand, OECD guideline no. 207), the study tests primarily for contact toxicity but it needs to be proven that it is an adequate system to test for adverse effects due to ingestion of corn plant residues.
- Result: no effects
- Scientific shortcomings: limited ecological relevance. Although *Eisenia foetida* is a standard test organism for ecotoxicity testing of industrial pollutants and synthetic pesticides it prefers habitats that contain high amounts of decomposing organic matter (compost worm). *E. foetida* therefore does usually not occur in agricultural ecosystems. Therefore, *E. foetida* as a species is of minor ecological relevance in corn fields.

Water fleas - *Daphnia magna*:

- This is typically a 48-hour static renewal toxicity of pollen from *Bt* maize to water fleas (*Daphnia magna*).

- An exposure time of 48 hours is too short for toxicity to manifest. Even in susceptible insects, adverse effects of *Bt* toxins are reliably measurable only after time spans of 24 to 48 hours.
- Result: no effects
- Scientific shortcomings: Ingestion and exposure questionable. Toxicity tests with *D. magna* are designed for substances soluble in water. In order to exert any activity, *Bt* proteins, in this case whole pollen grains, have to be ingested. *Daphnia'*s natural food sources are various groups of bacteria, yeast, microalgae, detritus, and dissolved organic matter. All these food groups range from 1 to 5 µm in diameter. Corn pollen on the other hand has a size of around 70 µm in diameter. From daphnids of the family Cladocera it is known that by filtering their food, particles of inadequate size are excreted unprocessed via the abdomen (which can cause a yellow colouring of the animals). Therefore, it needs to be proven first that *D. magna* can actually ingest – not just pass through - pollen or pollen fragments before conclusions based on this testing procedure can be drawn.

Honey bees – *Apis mellifera*:

- In the example of maize 59122 it is unclear how the test was carried out in detail. On page 24 it is stated that Cry34Ab1 toxin was mixed in sugar water and probably simply added to the cells that contain the larvae.
- Result: no effects.
- Scientific shortcomings: Unclear exposure via consumption; the degree to which the larvae consumed the added *Bt* sugar solution or not is unclear. Typically bee larvae are fed by worker bees who pre-digest the food for them; unclear exposure duration.

Springtails – Folsomia candida:

- In the example of maize 59122 the testing method is unclear. Typically, a 28-day survival and reproduction study should be carried out. From the documentation provided it appears that the purified *Bt* toxin was added to soil.
- Result: no effects.
- Scientific shortcomings: Unclear exposure via ingestion. Typically also yeast is added as this the actual food the springtails would eat. Proper foods for *F. candida* are saprophytic fungi found on decaying plant matter. Consequently, a laboratory experiment adding purified *Bt* toxin to soil with yeast is a poor simulation of the field environments especially since the *Bt* toxin must be ingested to unfold its effect.

Serious shortcomings of field studies

The field studies were general 'biodiversity' studies using methods that all have strengths and weaknesses. None of them was suitable to investigate the dynamics of particular populations or natural enemy - prey interactions. The field trials were designed as stand-alone trials with no connecting research hypotheses linked to laboratory studies or an overall risk research hypothesis.

Example 1: Field trials reported by Poletika (2003, n.st.)

The most revealing result was the 'low level' or often 'no level' effect, despite the fact that the chosen comparators were chemical insecticides – tefluthrin or bifenthrin. This indicates that the sample sizes and statistical rigors of these trials were minimal and that the statistical trial designs were insufficiently able to detect the immediate lethal effects of the two pesticides. The approach therefore is not suited for detection of effects that require days to realize even with the most sensitive target organisms. The results show that the statistical design was too weak and lacked the necessary power to detect even the strong effects of the two pesticides. Hence, the data is of limited value for ERA of this particular *Bt* crop.

Example 2: Higgins and Hong – Pioneer field trials in Spain and Hungary

The field trials carried out by the notifier with a number of different single and stacked *Bt* maize events in Hungary and Spain (2005 and 2006) used three methods – sticky traps and pitfall traps which both catch active, mobile species and life stadia as well as visual observations. These were carried out only at four sample dates in a period of at least six to seven weeks in each country. Only crude community analyses (in essence estimating abundances and comparing their means) were carried out that, again, hardly detected any effect even of the conventional pesticide treatments. Only effects of glyphosate could be shown to have some effects on certain ground beetles in Hungary. No subtle, chronic, sub-lethal or long-term effect or any more specific effect could be detected with the used experimental design. Therefore, the data from these trials are of little value for ERA. In addition, all observed effects were dismissed as irrelevant and minor by the authors of the respective studies.

'Pooling' of taxa and lack of specification of methods in field assessments

When evaluating abundance of insects in certain studies no differentiation was made between different insect taxa. Usually, terms such as 'predator abundance' or abundance of 'spiders' were indicated without further specification or differentiation. In the field trials of maize MON810 carried out in France and the USA pooled numbers of 'beneficial arthropods' or 'predators' were presented without further discrimination of taxa. Similarly, in the study of Higgins (1999, maize 1507), assessments were not made on a species level but taxa were assessed as groups (e.g. 'spiders', 'predatory beetles' etc.) although the notifier stated that different genera or species were found. Taxa differentiation in the other studies mostly referred to the family level (e.g. Lefko 2002, n.st.) or differed depending on the taxa assessed. Heteropterans of the genus *Orius* spp. were mostly identified as such while other taxons (e.g. leafhoppers) were not assessed at the genus level.

In addition, important information on the methods used in field trials – a standard scientific requirement – was frequently missing. Details on the experimental design, e. g. plot size or replication (maize MON810), the exact GM lines or control lines used (e.g. oilseed rape Ms8xRf3, maize MON810), the time of sampling (e.g. maize MON810) or herbicide/pesticide treatments were frequently omitted. Small plot sizes can affect abundance of non-target arthropods and it has been recommended that small plots with a width of less than 9 m should be avoided for non-target stud-

ies (Prasifka et al. 2005). In other cases it was not indicated which statistics were used (e.g. oilseed rape Ms8xRf3, maize MON810, maize 1507).

3.3.8 Assessment of effects of the GMP on biogeochemical processes and the abiotic environment

No consistent methods and tests are currently used to assess GM crops in relation to their effects on biogeochemical processes. Although some minor requirements for the assessment of effects of GMPs on biogeochemical processes have been specified by EFSA (2006), focusing on CO_2 evolution, organic matter turnover or nitrogen fixation, soil microbial communities and earthworms as well as unspecified 'deleterious organisms', these requirements are currently not followed by notifiers. As already outlined in other assessment categories (see e.g. effects on non-target organisms, chapter 3.3.7) the 'protein-only' approach was generally followed also in this assessment category. This approach ignores in particular the importance of the plant composition, which is known to be important for degradation and decomposition processes of plants. In addition, the relevance and explanatory power of protein-based laboratory experiments for degradation studies is severely limited due to several methodological shortcomings as well as the lack of consideration of in-situ effects which can only be tested in field experiments. As for non-target organisms, this approach also reflects the lack of a scientifically based, comprehensive ERA model incorporating the step-by-step principle and gaining knowledge from different steps of release of the GMP (lab – greenhouse - field). In addition, trait interactions were generally not considered. Last but not least, effects of the GMP on the abiotic environment lacked a specific assessment.

Proteins instead of whole GMPs

The conclusions of the notifiers for potential effects of the GMP on biogeochemical processes in the notifications reviewed were frequently based on assumptions than on specific data, in particular if no novel pesticide was produced by the GMP, such as herbicide tolerant GM crops. In the case of insect resistant crops such as *Bt* crops the conceptual separation of the newly expressed protein from the whole plant (see also chapter 3.2.1) leads to a simplified assessment of effects of the isolated, purified protein, i.e. the *Bt* protein, either on soil organisms or its degradation. This 'protein-focussed' ERA approach also entails the complete omission of an assessment of effects of herbicide tolerant GMPs on biogeochemical processes as the novel proteins expressed in GMHT plants (i.e. the PAT or EPSPS proteins) were generally assumed to be ubiquitous in the soil compartment and, hence, of no risk for soil organisms or processes. The presumed irrelevance of these proteins for biogeochemical processes is further argued by the specificity of these proteins. This approach limiting the assessments of potential effects of the GMP on biogeochemical processes on the newly expressed (*Bt*) protein only stands in contrast to arguments frequently provided in order to conclude on the safety of the protein/toxin, which conclude the absence of any risk from the 'overall performance of the GMP'. This, again, represents a double standard applied in the safety conclusion of the GMP.

In addition, the absence of any risk was frequently argued by the absence of evidence of any known effects of the GMP on biogeochemical processes in the soil (e.g. 'There are no indications that the GMP will alter the cycling of elements' or 'The proteins have no known negative interac-

tions', see also chapter 2.9). Certainly, in a science-based ERA approach, the absence of evidence of a risk cannot be drawn on to provide an evidence of absence of a risk.

Lack of consideration of protein/toxin metabolites

In GMPs expressing a transgene product, such as *Bt* crops, the biochemical properties of the toxin may change depending on the GM event as well as depending on the degradation processes in the plant, in different organisms (consumers) feeding on the plant and in different environmental compartments. Different fragment sizes of the *Bt* toxin have been detected in different GMPs expressing the same toxin, such as in maize MON810 and maize Bt176 (Andow & Hilbeck 2004). *Bt* proteins have been shown to degrade within the GMP (Andow & Hilbeck 2004), when consumed by a herbivore and excreted (e.g. Lutz et al. 2005) or when not bound to clay or humic substances in soil but available as free protein (see review in Isocz & Stotzky 2008).

Currently, the knowledge of differential toxicities of *Bt* protein fragments is very limited. Although it is known that, depending on the *Bt* toxin, different toxin fragments can exert different toxicities (Haider et al. 1986, Chilcott & Ellar 1988), the influence of sequential proteolytic degradation and the subsequent change in the biological activity of the toxin has not been systematically studied so far and was generally not addressed in GMP notifications.

Lack of consideration of plant composition for degradation processes

Effects of the whole plant are relevant for degradation processes of the GMP as the chemical composition of the GMP is an intrinsic factor influencing decomposition and persistence (Isocz & Stotzky 2008). In no case the degradation studies were complemented by studies on the composition or lignifcation of the *Bt* plants although there is evidence that changes in the lignifcation of *Bt* plants can mediate changes in the decomposition rate of the GMP (Flores et al. 2005).

Lack of consideration of interactions between transgene products and/or plant compounds

Stacked maize events containing a *Bt* trait usually contained no studies carried out with the respective stacked GMP but referred only to studies using single proteins. Interactions of proteins were not taken into consideration in the assessment of effects on biogeochemical processes, as already in other assessments categories. While interactions of Cry-toxins are relevant for their mode of action towards non-target organisms (e.g. Cry34Ab1/Cry35Ab1; see non-target chapter 3.3.7), their potential synergistic or antagonistic effect on biogeochemical processes is still to be determined. As Cry-toxins derived from *Bt* plants have been shown to bind to clay minerals and humic acids or clay-humic acid complexes reducing their availability to soil microbes and enhancing persistence (see review in Stotzky 2004) interactions in the soil compartment should not be dismissed *per se*.

In addition, combined effects of *Bt* toxins and herbicide applications merit further investigation but have so far never been considered in the ERA of GMP notifications although there is evidence that Cry-toxins are able to enhance the persistence of herbicides such as glyphosate and glufosinate in soil (Accinelli et al. 2004). Both herbicides were significantly longer present in soils when the *Bt* toxin was present.

Methodological shortcomings of laboratory experiments assessing protein degradation and persistence

For protein degradation studies submitted in GMP notifications a range of methodological shortcomings were evident. The studies submitted assessing the degradation of the introduced protein in soil usually comprised laboratory studies in which the purified proteins were added to soil and incubated in the laboratory at room temperature for a short time period (maximum 28 days). In certain intervals samples were taken and the insecticidal activity determined by bioassay with susceptible insect larvae. As a result the DT_{50} (time until 50 % of the concentration of the toxin is not detectable) or any other endpoint related to the insect larvae's growth inhibition (EC_{50}, GI_{50}) was presented as a measure for the bioactivity of the protein. This relevance of such short-term, protein based degradation studies must be challenged as the effectiveness of toxin recovery is known to vary with soil and toxin concentration (Palm et al. 1994). In addition, the toxin decay does not always fit an exponential decay curve as shown by several authors (Palm et al. 1996, Baumgarte & Tebbe 2005, Dubelmann et al. 2005) and the half-life concept for this kind of proteins has in general been fundamentally challenged (Stotzky 2004). In addition, the decay rates of these toxins differ depending on the experimental conditions applied, especially with respect to soil type, composition and clay content (Tapp et al. 1994, Tapp & Stotzky 1998, Saxena & Stotzky 2002), protein source used (purified toxin versus plant material) and type of plant material used (Zwahlen et al. 2003a, Saxena & Stotzky 2001, Baumgarte & Tebbe 2005), environmental conditions, in particular temperature (Zwahlen et al. 2003b), initial toxin concentrations (Clark et al. 2005) and insects and methods used for bioassays when determining the larvicidal activity of Cry-toxins.

The reliance in the ERA on a single laboratory test with several methodological limitations in order to predict 'real-life' degradation processes of the whole GMP under field conditions must therefore be challenged. It is very likely that cry-toxins persist and retain their insecticidal activity when bound on surface active soil particles thus being resistant to microbial degradation for periods significantly longer than 28 days (Saxena & Stotzky 2002, Tapp & Stotzky 1998, Zwahlen et al. 2003a, Baumgarte & Tebbe 2005).

No consideration of the presence of GMPs and GMP products in other environmental compartments than agricultural soils

The fact that litter of GMPs is not only limited to agricultural soils but enters different environmental compartments including water and water sediments (Rosi-Marshall et al. 2007, Douville et al. 2005, Douville et al. 2006) has been completely omitted in the current ERA approach of GMPs.

'Recycling' of studies

Apparently there is no common understanding, neither among notifiers nor among authorities, how potential effects of GMPs on biogeochemical processes should be assessed. The lack of a well founded ERA approach and a research hypothesis further fuels these weaknesses in the assessment of potential effects on biogeochemical processes. As a consequence notifiers submitted studies for the assessment of potential effects of GMPs on biogeochemical processes which dealt with soil organisms, mostly earthworms and Collembola, which had originally been carried out for the purpose of assessments of non-target organisms. This 'recycling' of studies derived from other

assessments, which themselves are limited in relevance due to several conceptual and methodological shortcomings (see chapter 3.3.7), is not only evident for assessments within a specific notification but also between notifications if identical *Bt* toxins are concerned (e.g. Cry1Ab toxin in Bt11 and MON810).

Lack of assessment of potential effects under field conditions

Studies assessing degradation or potential effects of the GMP under field conditions were generally not submitted except in one *Bt* maize notification (maize Bt11) in which a reference was made to published studies dealing with *Bt* toxins in soils under field conditions.

In recent years a range of studies were conducted and published evaluating persistence of Cry-toxins and their potential effects on soil organisms and processes either in the laboratory, under glasshouse or field conditions. Although some sort of effects of GMPs on soil communities and organisms have been detected in several of these studies these were often transient or comparatively small. These effects have thus been classified as relatively minor compared to differences due to cultivars, soil management and environmental variables (e.g. Baumgarte and Tebbe 2005, Zwahlen et al. 2007, Griffiths et al. 2006, Griffiths et al. 2007). However, indications of adverse effects of *Bt* maize were shown for some soil organisms, e.g. soil microorganisms, or some parameters although the underlying cause is still matter of debate (for review see Bruinsma et al. 2003, Dolezel et al. 2005, Icoz & Stotzky 2008). Additionally, a range of soil organisms potentially exposed to *Bt* toxins in fields have not been subject to experimental testing so far (Zwahlen et al. 2007) or pattern, duration and extent of exposure in experimental testing have not matched those experienced by organisms in the field (Marvier 2002 cited in Icoz & Stotzky 2008).

An assessment of the degradation of Cry toxins under field conditions gives an indication of the fate and behaviour of these toxins under natural conditions. Persistence of Cry-toxins in fields using whole GMPs may result in prolonged persistence estimations compared to laboratory studies due to different temperature regimes (Zwahlen et al. 2003b) and differences in the bioavailability of the transgene product which, *in situ*, is protected in the plant matrix and thus not as quickly available to micro-organisms as under laboratory conditions. Additionally, degradation of *Bt* toxins differs depending on the part of the plant assessed (Baumgarte & Tebbe 2005). Thus a holistic approach has been called for as results from laboratory testing are often not corroborated by glasshouse or field studies and *vice versa* (Birch et al. 2007).

Incomplete information on studies conducted or cited by notifiers

In this report many of the studies discussed by the notifiers could not be evaluated in depth because:

- the study was cited but not attached to the notification (e.g. maize Bt11, maize 1507)
- methodological details (e.g. oilseed rape Ms8xRf3) or information on the GMP used (e.g. maize NK603) were not included

Lack of assessment of effects of the GMP on the abiotic environment

In current risk assessment practice potential interactions between the GMP and its abiotic environment were generally not assessed. Although there is no common understanding of what such

effects could be, notifiers generally referred to the absence of evidence for negative interactions of the GMP with the abiotic environment. In no case these statements were supported by specific data. Interactions between GMPs and the abiotic environment may be mediated by elevated atmospheric CO_2 levels which may increase the need for higher nitrogen resources in order to keep the necessary *Bt* toxin expression at the relevant levels (Coviella et al. 2000).

3.3.9 Assessment of effects related to land use and cultivation techniques

In the reviewed GMP notifications weeds were generally not acknowledged as the target organisms in GMHT plants (see also chapter 3.3.6). Consequently, an assessment of any direct or indirect effects of the complementary, non-selective herbicides was generally omitted from notifications of GMPs with a herbicide tolerance trait. This includes the lack of an evaluation whether such GMPs will require different herbicide regimes at different times and the consequences thereof for flora and fauna in agro-ecosystems. The general approach of the notifiers of GMHT plants was to shift the responsibility for the assessment to the authorization of the pesticide according to Directive 91/414/EEC where environmental assessments of the herbicide must be provided. Even although this information is frequently with the notifier, it is generally not included in notifications of GMHT plants. However, these assessments do not cover all environmental aspects specifically relevant for GMPs.

No evaluation of effects due to altered herbicide/pesticide regimes in GMPs

In the GM notifications neither a description of current cultivation, management or harvest techniques nor a description of potential differences due to the use of a herbicide tolerant or insect resistant GMP was provided. The argumentation of the notifiers that none of the above mentioned methods would be changed by the use of the GMP was in no case substantiated by specific data but based on argumentation and assumptions only. In the notifier's view the use of the herbicide tolerant plant includes only an additional option for weed removal and gives the farmer an additional choice for weed control measures. For insect resistant plants notifiers only considered insect resistance development and referred to the IRM plans attached. In GMPs with both traits, herbicide tolerance and insect resistance, generally only the insect resistance was addressed (e.g. maize lines Bt11, 1507, 59122 as well as both stacked maize events).

The lack of an evaluation of the environmental effects of the herbicide application in conjunction with the GMP has also to be seen in the context of the notifier's argumentation that herbicide tolerant crops do not have any target organisms (see chapter 3.3.6). In no case, notifiers considered weeds as the target organisms of the GMHT plant. This was based on the argumentation that weeds are not directly targeted by the genetic modification of the GMHT plant. However, as the herbicide tolerant GM crop is part of a specific crop and weed management package, it is not reasonable for the farmer to use the crop without the complementary herbicide in order to realize the intended effect and the economic benefit. Thus the target organism is indirectly linked with the GMP via the plant protection product.

This lack of an assessment of the herbicide tolerant GMP in conjunction with the relevant non-selective herbicide is in clear contradiction to the requirements of Directive 2001/18/EC claiming an

assessment also of indirect effects which in particular should take information on changes in the use or management of the GMP (EC 2002a, objectives) or, more specifically, on multiple herbicide resistances into account (EC 2002a; general principles).

Lack of inclusion of data from assessments carried out in the framework of Directive 91/414/EEC

Although the need for assessing potential effects of changes in herbicide use due to the GMHT plant on the environment has also been acknowledged by Competent Authorities (e.g. see member states comments of the Spanish CA carrying out the ERA for maize NK603), it remains controversial to what extent this has to be considered in the scope of GMO notifications. Thus notifiers generally push the full responsibility for the evaluation of environmental effects of the non-selective herbicide applied to the GMHT plant to the obligations under Directive 91/414/EEC.

Recital 26 of Directive 2001/18/EC requires working in close liaison with the implementation of other instruments such as the Council Directive 91/414/EEC. The overlap of these two Directives fuels an ongoing controversy with respect to which data have to be submitted by the notifier for herbicide tolerant GM crops in view of potential direct effects on weeds or indirect effects on biodiversity.

According to Directive 91/414/EEC the harmonised procedure of the regulation of plant protection products in the EU follows a two-step process. First, the evaluation of an active substance is carried out by the European Commission in cooperation with the EU member states. The active ingredient is evaluated by a rapporteur member state and authorization is granted by inclusion of the active ingredient into Annex I of Directive 91/414/EEC. Second, the commercial products and formulations are subject to an authorization procedure at national level by the application of harmonised evaluation criteria. A main component of the national evaluation is the evaluation of the efficacy of a plant protection product which is carried out at national level.

Notifiers generally argued in the notifications of the GMHT plants that the respective herbicide had been tested in field trials in several EU member states but the results were not applicable or relevant for the GMP notification. As can be seen from notification reports of Part B trials according to Directive 2001/18/EC, notifiers do carry out specific assessments of the respective GM crop on weed communities but do not report their outcomes in the Part C notification of the GM crop. In the following the purposes of such Part B trials of maize NK603 are listed:

- Efficacy and selectivity assessment of NK603 using Roundup herbicide and other herbicide formulations (B/DE/03/148, B/DK/07/04, B/DK/08/01)
- Agronomic performance…with and without glyphosate (B/DE/06/181, B/DE/06/185)
- Establishing guidelines on the appropriate use of glyphosate containing herbicides (B/DE/06/185)
- Effects on target organisms (weeds) (B/CZ/07/02)
- Assessment of weed treatment strategies in maize (B/SK/08/03)
- Herbicide registration (B/RO/08/09)

- Influence of Roundup Ready maize on weed population regarding the maintenance of biodiversity, examination of field flora (B/PL/07/02-01)

Although the notifiers refer to and dispose of data necessary for these evaluations of efficacy and selectivity of the herbicide, residue quantification, effects on the environment etc., these were generally not presented in the notifications (see e.g. maize NK603). Neither direct effects on weeds nor the potential for weed shifts were covered in any of the notifications of GMHT plants.

No assessments of the use of the non-selective herbicide

In certain notifications the notifiers referred to 'a Guidance for good agricultural practice' (e.g. oilseed rape Ms8xRf3) or a 'Technology Use Guide' (e.g. maize NK603) which were proposed to be submitted at a time when the GMOs would be commercially launched. For NK603 maize use recommendations for Roundup Ready products as suggested for Spain and France were attached by the notifier. These descriptions (submitted in French and Spanish) were only referred to but not discussed by the notifier with respect to any differences in timing, amount and frequency of the non-selective herbicide to the current baseline of herbicide application in conventional maize or with respect to its applicability for other EU member states.

Lack of assessment of biodiversity effects

Assessment of effects other than direct effects of the PPP on weeds, e.g. indirect effects on biodiversity resulting from changes in the weed community, were generally omitted from the ERA of herbicide tolerant GMPs. The Farm Scale Evaluations have demonstrated that herbivores, detritivores and many of their predators and parasitoids in arable systems are sensitive to the changes in weed communities that result from the introduction of new herbicide regimes (Brooks et al. 2003, Haughton et al. 2003, Hawes et al. 2003, Roy et al. 2003). Thus biodiversity effects of the GMP-herbicide complex need to be an integral part of the ERA.

3.3.10 Proposed risk management and monitoring

Generally, no risks were identified in the ERA as carried out by the notifiers (see also chapter 3.2.1). Hence, CSM was generally not considered relevant in GMP notifications. The lack of identification of any risk(s) was mainly based on flaws in the risk characterization and the problem formulation in the ERA. Factors which influence the decision whether a risk might be subject to CSM or not even if the risk is considered negligible were generally not taken into consideration by the notifiers. As the only risk identified, the development of insect resistance in target organisms in *Bt* crops was considered by the notifiers although there is no consistency whether this risk and the Insect Resistance Manangement plan (IRM plan) proposed constitutes a risk management strategy, CSM, or both. Apart from IRM plans, risk management measures as such were generally not proposed in the notifications. GS plans were generally mainly composed of farmer questionnaires not specifically addressing environmental aspects in a scientifically sound manner and are thus considered limited for the detection of environmental effects. The proposals of the notifiers to include 'environmental networks and/or programs' in their GS efforts pose several yet unsolved

questions with respect to the availability of these programs in different member states as well as their suitability for GMO monitoring.

Flaws in the ERA lead to a general conclusion of no risks

In the ERA the notifier generally outlines the characteristics of the GMP which may cause an adverse effect, the consequences of the adverse effect, if it occurs, and the likelihood of occurrence of the potential adverse effect in order to estimate the risk posed by each characteristic of the GMO. With this procedure the notifiers generally follow the formalized steps of the ERA as outlined in Annex II of Directive 2001/18/EC (EC 2002a). According to this approach a risk is defined as a combination of the consequences of a harmful characteristic (the hazard) and the likelihood that the consequence occurs.

Hazard identification is usually the first step in the ERA process which relates to the identification of any adverse effect of the GMP on the environment. This first step is crucial as it scopes out the problem and determines what to assess at which level of detail (Hill 2005). The second step, the evaluation of the likelihood of occurrence of a particular potential adverse effect, is based on an assessment of the potential exposure. Consequences of a specific hazard should be evaluated if exposure occurs (Hill 2005). In the current risk assessment practice of GMO notifications notifiers generally do not specify hazards but define them on a general level, such as ‘the expression of the transgene’ or ‘the presence of GM trait’. The fundamental flaw is thus the delineation of the transgene or the introduced trait from the GMP thus ignoring the whole GMP as a stressor. Consequently, this leads to the omission of inclusion of effects of the whole plant or the management method as well as indirect effects (see also chapter on the ERA model; chapter 3.2.1). Thus already the first step in the ERA, the hazard identification, does not allow deciding which data must be gained in the following assessments. Similarly, when exploring on the likelihood of occurrence of potential adverse effects and their consequences, only general and qualitative descriptions, not quantitative assessments are provided. In several cases, assessments are assumption-based (e.g. ‘no adverse effect is expected’) rather than based on scientifically sound data. On the basis of the ‘protein-focussed’ approach, the likelihood of occurrence is generally assumed to be negligible, without discrimination of specific effects or mechanisms. However, risk characterisation as such needs some kind of evaluation of exposure in addition to the evaluation of the potential adverse consequences (National Research Council 1983, EPA 1998). A risk characterisation assuming negligible likelihoods for the occurrence of any potential adverse effect implicitly assume that no exposure will occur in any case, a view that could in no case be substantiated by facts and data.

Risk management or monitoring?

According to Directive 2001/18/EC the decision on an appropriate risk management strategy will be taken during the ERA before the overall risk of the GMO is determined. Hence, risk management is part of the ERA procedure and the conclusion on the overall risk of a specific GMO already takes into account any risk management strategy. Consequently, risk management measures should not be mixed with monitoring efforts.

The only risk management measure proposed in GMP notifications was the establishment of an IRM plan for target organisms of *Bt* crops. However, there is disagreement among the notifiers

whether the IRM plan for *Bt* maize constitutes a risk management measure or represent case-specific monitoring. Also in the original oilseed rape (Ms8xRf3) notification, environmental risks were identified and specific agricultural guidelines proposed to control or monitor these risks but also CSM efforts were proposed. While the identification of no risk may mean that no risk management measures are necessary, this may not be the case for CSM (see next paragraph). However, a particular activity may also be both, a risk management measure and CSM. For instance, volunteers of GM oilseed rape may be monitored for their occurrence, persistence or dissemination (CSM) while they may also be classified as risk management measures, e.g. if such volunteers are removed by chemical or physical means. However, in any case, a clear distinction between these efforts shall be made by the notifier.

No case-specific monitoring (CSM) if no risks are identified

According to the result of the ERA carried out by the notifiers in the reviewed GMP notifications, all identified risks were generally considered to be negligible and were thus not subjected to CSM. As a consequence, generally no case-specific monitoring plans were proposed by the notifiers, except IRM plans for *Bt* maize (see also above).

According to the view of the notifiers, as CSM is directly linked to the ERA, a risk is only monitored in CSM when the ERA defines it to be a risk. If the ERA defines no or a negligible risk, CSM is not considered necessary by the notifiers (see also chapter 3.2.1). This approach is inconsistently followed among notifiers of GMPs. In the case of *Bt* maize the risk for resistance development was also considered negligible or limited in the ERA of most *Bt* maize crops, although an IRM plan was generally proposed in most cases as CSM. In the case of the GM potato specific monitoring actions were suggested although no particular risks were identified. The notifier aimed at verifying the assumptions of the ERA over a prolonged period, a view that correctly reflects the aim of CSM according to Directive 2001/18/EC and its guidance notes to Annex VII (EC 2002b). For herbicide tolerant maize NK603 a CSM plan covering the effects of the changed herbicide management regime was requested by the Spanish authorities assessing the ERA but ignored by the notifier. Thus, there is disagreement among the notifiers how the ERA is linked to case specific monitoring although notifiers generally seem to avoid taking responsibilities for monitoring actions under CSM.

General surveillance (GS) – only based on questionnaires

In GMP notifications GS is seen as a ‘routine observation’ without specific hypotheses of environmental effects of the GMO. Generally, no explicit monitoring actions were specificed or clear monitoring objectives defined, except the use of questionnaires given to a selected number of farmers growing the respective GMP. According to the Guidance Notes to Annex VII (EC 2002b) monitoring does require an appropriate methodology prior to the commencement of monitoring programmes and is considered a ‘means to evaluate or verify results and assumptions arising from previous research and evaluation of potential risks and research’ (EC 2002b). Thus results and assumptions from previous research establish the starting point for every monitoring action which results in the definition of a specific hypothesis to be verified or falsified by a specific monitoring action. This, again, calls for the compliance with the step-by-step principle, and the inclusion of results from part B trials in GMP notifications for placing on the market. These may give important

indications on potential environmental effects of GMPs to be used for specific monitoring actions in GS.

Questionnaires not suitable to detect environmental changes

In the sense of the legal provisions of Directive 2001/18/EC – to identify non-anticipated effects on the environment and to confirm assumptions from the ERA - monitoring needs to consider in particular environmental aspects. Hence, the use of farmer questionnaires as a key tool for environmental monitoring has to be fundamentally challenged. While the use of agronomic questionnaires may be useful for assessments of agronomic information collected by farmers or according to the records they keep, a thorough scientific approach and established methods from vegetation analysis, entomology, pedobiology or other scientific areas is necessary to collect ecologocial information and detect potential environmental effects in and around GMO fields. As currently foreseen, general questions on wildlife and weed infestations in the fields in the farmers' questionnaires are unsuitable to allow an assessment of environmental effects of the GM crop on individual species, taxa or ecological processes. Also agronomically relevant parameters such as weeds or pests need to be taxonomically identified to the highest taxonomic level possible and should not be generalized within a 'weeds' or 'pests' category. Such a generalization does not allow any conclusions on the particular abundance of a certain species and, hence, a potential environmental effect of the GMP. This is also valid for the assessment of other species such as non-target organisms or incidentials which are currently subsumed under the category 'wildlife' in the questionnaire without further specification. Methodologically these questionnaires focus on visual or descriptive assessments rather than quantitative measurements. Such quantitative measurements, however, require the involvement of scientists with expertise in their scientific fields in order to generate meaningful data based on a robust monitoring design and statistical evaluation.

Shortcomings in the methodology applied by questionnaires

The following methodological shortcomings in the proposed farmer questionnaires have been identified:

<u>Lack of a representative choice of farmers for questionnaire use</u>

The proposals of the notifiers of GMPs to submit the farmer questionnaires to a 'subset' of farmers growing the GMO do generally not contain information on how this subset of farmers will be selected. The farmer selection should be based on a representative subsample of all farmers of an individual member state growing the GMO. No indications are made with respect to the selection of farmers and the criteria applied for selection to ensure that a representative sample of farmers is actually drawn.

<u>Lack of a quality control of questionnaires</u>

There is no check in the proposed farmer questionnaires whether the questions posed in the respective questionnaires will actually measure a specific parameter. This should generally be assessed in a pre-test, evaluating whether the respondent correctly understands the questions posed.

<u>Information in questionnaires is based on memory rather than data</u>

Respondents of farmer questionnaires are not sensitized to the parameters assessed in questionnaires beforehand. Assessment of certain parameters after the growing season will rely on the respondent's memory if there is no need for the farmer to record information during the growing season. In this context also the variation in the farmer's observation sensitivity, education and attention towards changes must be considered. For instance, a change in a certain pest species' abundance may be noticed by one farmer but not by another. Thus a standardization of observation is not guaranteed.

Lack of validation of farmers questionnaires

The measurements made by the use of the questionnaire are not subject to a validation procedure. A scientific validation of the observations (e.g. increase in weed infestation) would ensure that this kind of method actually correctly assesses the parameter(s) of interest.

Lack of specification and evaluation of existing environmental monitoring networks/programmes for general surveillance (GS)

Although the use of existing networks for GS monitoring was suggested in most GS plans, no specific networks or programs in individual EU member states were indicated in the submitted monitoring plans. The availability and suitability of such programs and networks for the GMO monitoring with respect to sampling design, indicators, frequency and location (sampling points) has so far never been assessed by notifiers. It is unlikely that the existing monitoring programs in different EU member states, in case they exist, will fulfil the requirements of the different GMO monitoring plans. For example, so far in only few EU countries specific biodiversity monitoring programs have been developed or even implemented on a national level which could potentially be useful for GS. In Switzerland an investigation of potential synergies between existing monitoring programs and GMO programs has concluded that current programs would have to be adapted to satisfy the specific requirements for a GMO monitoring (Hintermann & Weber 2003). In Germany a similar evaluation based on the 'ökologische Flächenstichprobe' has been carried out (Middelhoff et al. 2006). Although potentials for synergies and the usefulness of certain parameters for a GMO monitoring were evident, an extension of the networks was considered necessary. To the authors' knowledge these have been the only efforts to evaluate the suitability of existing monitoring programs in Europe so far. With respect to the monitoring of the distribution of GMPs outside of agricultural areas Hintermann & Weber (2003) concluded that a separate set up of a monitoring program would be more feasible and cost-effective than the adaptation of existing programs, such as the biodiversity monitoring program. A prerequisite that has to be met in order to integrate GS into existing monitoring programs but which is rarely met is the possibility of application of existing data to any stratification, e.g. to the agricultural landscape (Bühler 2006). The question of the use of an existing monitoring program is strongly associated with the question which indicators and parameters need to be covered by GS of GMOs. Only if these are identified the usefulness and suitability of existing programs or networks can be evaluated. However, such a thorough analysis has so far never been presented in any of the GMO notifications.

Apart from the contents and scopes of existing networks and programs that would have to be analysed with respect to their applicability, organisational aspects would also have to be clarified by the notifiers. This refers mainly to agreements with relevant monitoring programs and networks,

access to data, collection and analysis of data and evaluation of results in view of the specific question.

Information efforts as part of GS insufficient as early warning system

The information efforts mentioned in the GS plans mainly refer to ‘general’ information activities of the companies in the framework of their general product marketing measures (e.g. product briefings, internet etc.). The information collection from the internal company network or from externals (e.g. via a toll-free telephone number) mentioned by notifiers in their GS plans, does not constitute an active and suitable monitoring method for the detection of adverse effects. If GS aims at functioning as an early warning system for unexpected effects on the environment (as specified in Directive 2001/18/EC), then appropriate and active monitoring methods need to be employed rather than passively collecting information which will more than likely not provide any indications of adverse effects of the respective GMO.

3.3.11 Conclusions: Presended data do not support the conclusion of the ERA

The review of GMP notifications analysed for this report shows that the conclusions of the risk assessment and the evaluation of potential environmental risks of GMPs are mostly based on few scientifically robust and relevant data. The conclusions on specific environmental risks of a particular GMP are drawn from few assessments for which specific data are generated.

The major assumption is that the introduced novel GM trait(s) do not change the inherent characteristics of the GMP in question and thus no adverse effects are expected. Hence, the focus of the risk assessment is almost exclusively on the molecular characterisation, the expression analysis, the compositional assessment and to some extent the agronomic characterization of the GMP. For most other assessments only cross-reference is made to the above assessments, to authorities’ opinions, to other GMPs or the published literature. In many cases the assessments are based on major assumptions or general statements rather than on data specifically generated for the GMP in question by the notifier. The evaluation of risks of the GMP to the environment, such as to non-target species or the assessment of potential effects due to the use of non-selective herbicides is based on few scientifically robust data.

Generally, no assessment of long-term and cumulative effects is presented. A separate evaluation of potential effects of the GMP on species of conservation concern occurring in Europe is not contained in GMP notifications.

Based on the weak data basis notifiers generally conclude on ‘no’ or ‘negligible’ risks of the GMP to the environment and, consequently, do not consider risk management measures or case-specific monitoring as relevant. Instead of following a scientific approach leading to concise results the argumentations in the notifications give the impression that the ERA is used to demonstrate the absence of any risks of the GMP to the environment.

In addition, many assessments are often not concisely presented and the data cited arehardly traceable and not presented in a comprehensive way. This leads to uncertainty on which data basis the conclusions were actually based on. Major flaws were identified for some specific data generated and provided by the notifier. Common flaws include the choice of models and general approach of the ERA, methodology of data generation and analysis, results presentation, and conclu-

sion finding. In general, the approach of the notifiers is to focus the risk assessment on the introduced trait of the GMP, ignoring the whole plant as being the stressor in combination with its management techniques. Hence, the current risk assessment practice is largely not following the legislative requirements of Directive 2001/18/EC and its Annexes, considering the whole GMO, the modified trait(s) and the receiving environment.

Despite the above mentioned shortcomings in the ERA, the notifiers' assessments and argumentations are frequently considered sufficient in EFSA GMO panel opinions, even although in many cases the requirements outlined in the guidance document on risk assessment (EFSA 2006a) were not or not fully followed.

Shortcomings have also led to considerable dispute among EU member states with respect to the claimed 'environmental safety' of GMOs. While for some the evidence presented in the GMO notifications is sufficient to proof the environmental safety of the GMO, for others there are fundamental flaws in the way this safety is evaluated. The basic requirement of Directive 2001/18/EC that the ERA should be based on scientific and technical data (EC 2002a) is considered as largely not fulfilled. Therefore it is essential to arrive at a common understanding on which scientific and technical data the ERA should be based on. In the next chapter, suggestions are made on how such improvements and, possibly standardization, of the ERA could be achieved.

4 RECOMMENDATIONS FOR IMPROVEMENTS AND STANDARDIZATION OF THE ENVIRONMENTAL RISK ASSESSMENT OF GMOS

4.1 General Remarks

This chapter makes suggestions for the improvement of the current risk assessment practice of GMP notifications with the aim of implementing the ERA as specified in Directive 2001/18/EC and its Annexes. The suggestions address both, the improvement of the scientific data basis for the ERA, its comprehensiveness and explanatory power as well as the format for presentation of data and results in order to achieve common minimum standards in the ERA and to arrive at comprehensive risk conclusions. Such common standards still consider the case-by-case aspects as outlined in Directive 2001/18/EC.

Recommendations for improvements of the ERA as proposed in this chapter are neither exhaustive nor final, but form a first basis for discussion which may be extended depending on further needs and issues identified. The recommendations focus on the GMP notifications reviewed in this report. Conclusions on and recommendations for the ERA as outlined in this report may differ if other GMP notifications are reviewed, as the data basis provided in other GMP notifications, the format and the conclusions of the ERA may be different.

In the following chapter general and specific recommendations for improvements of the ERA of GMP notifications are delineated. The general recommendations cover aspects which are relevant for more than one specific, in several cases, all assessment categories. These recommendations are to be seen in analogy to the cross sectional issues in the critical appraisal of the ERA (chapter 3.2) where a similar approach was chosen. The specific recommendations refer specifically to individual assessment categories of the ERA. For reasons of clarity most recommendations are drafted as requests in bold with additional short explanatory statements underneath or are itemized with bulletpoints.

Different levels of improvements are addressed in this chapter. The need for improvements in ERA approach and methodology, interpretation of data or presentation of results is identified. For these improvements suggestions result in specific recommendations such as how to select non-target organisms for testing or how to present results derived from field trials. For some of these suggestions the development of further guidance will have to be elaborated. In case of other suggestions for improvements the need for the development of standardized methods and protocols is outlined. Certain aspects in the ERA need standardization which should apply for all GMPs of a certain type, such as standardized protocols for the detection and quantification of *Bt* toxins expressed in *Bt* crops. Also knowledge gaps are addressed in this chapter for which further research will be necessary before certain improvements of the ERA of GMPs can be scientifically addressed.

In several assessment categories reference is made to the risk assessment requirements of plant protection products regulated under Directive 91/414/EEC or the guidance documents issued by the European and Mediterranean Plant Protection Organisation on specific issues, e.g. on the design of field trials (EPPO Guidelines; www.eppo.org). These risk assessment requirements for

plant protection products are much more formalized and the guidance documents much more elaborated than the ERA requirements outlined for GMPs. In this chapter the reference to the ERA requirements of plant protection products is to be understood in the way that similar guidance is needed for GMP risk assessment rather than a simple copying of specific data or formal requirements. If further guidance for GMP risk assessment is to be developed in the future then it needs to be assessed in detail whether and to what extent the ERA requirements for plant protection products may also be suitable for GMP risk assessment.

The suggestions and recommendations for improvements outlined in this chapter address several stakeholders. Mainly risk assessors such as notifiers and EU member states authorities or the EFSA are addressed. However, the suggestions and recommendations are also of significance for decision makers and risk managers as the outcome of the ERA and a common understanding of the risk conclusions derived from the ERA shall provide the basis for decision making and risk management.

4.2 General Recommendations

4.2.1 The Environmental Risk Assessment (ERA) Model

An urgent need for improvement of the currently applied ERA model was identified during this review of GMO notifications. In its first steps problem formulation and hazard assessment, the current ERA model narrowly defines potentially adverse effects (see also Romeis et al. 2008, Garcia-Alonso et al. 2006). This leads in many instances to an exlusion of for the ERA relevant issues. It is therefore stongly suggested to broaden the scope of the assessment to be compliant with the provisions of Directive 2001/18/EC and the guidance notes for risk assessment (EC 2002a).

The proposed approach towards the ERA of GMPs aims at being as inclusive as possible at the beginning and aims at eliminating potentially adverse effects only after rigorous evaluation and testing. The proposed ecotoxicological testing strategy is prescriptive with regard to a procedure developed for selection of test organisms ecologically relevant for the receiving environments and the proper testing protocols. This is in contrast to the ecotoxicological testing strategy under the current narrow, i.e. exclusive, approach to ERA focusing on the use of universal standard testing species and testing protocols.

A broadened, more inclusive ERA model focusses on testing the actual whole GMP. Testing according to a broad ERA model includes adverse effects arising from direct, indirect, and/or long-term exposure to the whole GMP. Also potential adverse effects deriving from secondary stressors that are required to realize the benefit and intended effect(s) of the GMP, such as the application of broad spectrum herbicides are included in the assessment. Ideally, all organisms of an agro-ecosystem and all potential adverse effects/risks arising from direct and indirect, short- and long-term exposure to the GMP should be tested. This would be the most risk-averse approach but is neither feasible nor fundable. Hence, an intermediate approach focusing on the greatest potential adverse effects is called for that is broader than the current reductionistic approach but nevertheless feasible. Such a broadened ERA concept has been developed by a large international group of public sector scientists of the IOBC Global Working Group on 'Transgenic Organisms in IPM and Biocontrol' and proven to work well in a number of test runs, the outcomes of which were published

in a newly established CABI book series 'Environmental Risk Assessment of Genetically Modified Organisms' (Hilbeck and Andow 2004, Hilbeck et al. 2006, Andow et al. 2008). In 2008, a fourth test run is planned in Germany.

In the following, the different components of such a broadened ERA concept will be shortly presented. Details can be found in the above mentioned book series.

Identify the hazard and the scope of ERA (Component I)

In this first component, EU legislation requires the 'identification of characteristics which may cause adverse effects.' This is the most critical step as it is here where the scope of the ERA is determined. Under the current narrow approach to ERA important potentially adverse effects are eliminated. A fully comprehensive ERA would have to include all adverse effects caused by the novel protein(s), the application of any chemical required for realizing the benefit of a GM crop (e.g. broad spectrum herbicides for HT plants) and all potential adverse effects of the GMP caused by any intended and unintended changes in its phenotype. As testing all potential adverse effects is impossible, those potential adverse effects should be identified that most likely pose a high risk. Such effects should be tested in a step-wise fashion prior to field release.

Define the 'case'

The ERA should initially start as comprehensively and inclusively as possible based on a broad and inclusive scoping exercise called 'Problem Formulation and Options Assessment (PFOA)'. This broad stakeholder process was developed for the use in ERA of GMOs (Nelson et al. 2004, Capalbo et al. 2006, Hilbeck et al. 2004) and recently transformed into a guiding handbook (see also Nelson & Banker 2007). An inclusive scoping exercise at the beginning of the assessment will ensure that at least initially as many potential adverse effects as possible are carefully considered. Potential adverse effects will only be excluded after a broad scientific evaluation and the taking into account also of societal, ethical, cultural, and political aspects involving those who will be the users or otherwise affected by the introduction of the GMO. Although this inclusive approach is broader than the current EU regulations (e.g. including societal, ethical aspects) it is reasonable to begin a similar process with the defitnition of the 'case' as a starting point. This constitutes the basis for building the process in a systematic and transparent manner.

Based on the provisions put forward by the Directive 2001/18/EC and, similarly by the Cartagena Protocol on Biosafety, for each GMO (event) a case is described by the 3 elements: 1) the crop plant, 2) the novel trait relating to its intended effect and phenotypic characteristics of the GMP and 3) the receiving environment relating to the intended use of the GMP. For each element, information must be compiled and synthesized.

For the crop plant, any information on its biology, ecology and current spatio-temporal agronomic use and limitations of use is compiled. For the novel trait, this includes comprehensive information on the molecular characterization of the GMP, its introduced genetic material and tissue-specific expression of the novel proteins. Information on the intended effect(s) includes for example all available data on the problem to be solved with the proposed GMP, efficacy data of the GMP demonstrating the ability to solve that problem, the severity of the problem, how widespread the problem is and who is mostly affected by the problem (Nelson et al. 2004, Nelson & Banker 2007, Hil-

beck et al. 2004). This will allow anticipating the main users of the GMP, and estimate the adoption rate and spread of the GMO after release. From this in turn, the potential receiving environments can be delineated (see also below) which helps to focus the analysis on those where the adoption of the GMO is expected to be highest, based on the assumption that potential adverse environmental effects will likely manifest firstly and foremost where the GM crop is grown most frequently and most widespread. Finally, the identification of the potential receiving environments is essential to characterize the existing biodiversity and ecological processes that might be affected and from which the candidate testing species will be selected (see description below). The outcome of this first critical step is the scope and context of the ERA and the testing strategy tailored to the particular GMP case. For more details on the case definition see also 4.2.9.

Choose what species to test

Two main issues emerge that form the core of the persisting controversy regarding ecotoxicity testing of GMOs for environmental risk assessment: a) What organisms should be tested and b) how should they be tested? For the proposed methodology for ecotoxicological testing of non-target organisms see also specific recommendations for non-target organism testing (chapter 4.3.7; Hilbeck et al. 2008b).

Assess the exposure (component II)

For guidance and recommendations on how to conduct an exposure assessment and how to arrive at testable adverse effect scenarios and hypotheses see chapter 4.2.2).

Determine the effect (component III)

The main activity in this component of the ERA framework is the implementation of the testing plan developed in the two previous components. It corresponds in such directly to the provision for ‘evaluation of the potential consequences of each adverse effect, if it occurs’ of the Directive 2001/18/EC. The aim is to measure whether the GMP, its intended or unintended use, or the transgene product can affect structural (i.e. related to individual species) or functional (i.e. related to services provided by the whole community of species) endpoints. Testing follows a systematic hierarchical scheme. Hierarchical – or step-wise - testing is expected by law (Directive 2001/18/EC, EC 2002a). The underlying idea is to gain increasing realism at the expense of safety control only if testing at less complex but more controlled (i.e. confined) and therefore ‘safe’ levels do not give reason for concern. However, controversy exists over a number of related issues. Firstly, whether the evidence for ‘reason for concern’ should be experimental (i.e. original data produced) or could be extrapolated from theory and experience in related fields of science (e.g. Lang et al. 2007, Andow et al. 2006, Romeis et al. 2008, Garcia-Alonso et al. 2006). Secondly, whether or not an absence of a ‘reason for concern’ (i.e. evidence) constitutes evidence for safety, i.e. no more testing required at higher levels if lower level testing does not yield results of concern (Lang et al. 2007, Romeis et al. 2007). Just as the target effect(s) of a GMP cannot be fully predicted based on laboratory studies only, also non-target effects cannot. Both – evidence of risk and safety - needs to be confirmed at every hierarchical level for the same reasons – interactions with the environment which can induce significant differences in evolutionary and ecological parameters for

better or worse but certainly unpredictably. For other aspects on the step-by-step principle see also chapter 4.2.8.

Characterise the risk (component IV)

In this component of the ERA framework, the risk is characterized by combining and comparing the obtained data and information of the previous three components. While the emphasis is placed on quantitative data, all gathered qualitative information is also integrated here. This concurs with the provision of the 'estimation of the risk posed by each identified characteristic of the GMO(s)' put forward in the Directive 2001/18/EC. The outcome of activities in this component is a list of confirmed risks with an estimation of their strength (high, moderate or low). Likewise important, the delimitation of the ERA and transparent documentation of remaining uncertainties is identified here. From this, guidance for possible risk management strategies and monitoring plans can be derived.

4.2.2 Exposure assessment as the starting point in the ERA

A solid understanding and assessment of the various possible exposure routes of transgenic material will inform best the development process of adverse effect scenarios from which the proper testing protocols will be derived.

Define the relevant exposure pathways and assess the exposure of species

For the species ranked highest in the previous step, the scoping of the ERA, an exposure analysis is conducted to determine whether or not and to what degree the species come into contact with the primary stressor, i.e. the GMP including the transgene product (e.g. a *Bt* toxin) or the altered composition of primary metabolic compounds (e.g. starch), or any secondary stressor required for realizing the transgenic function of the GMP, e.g. the broad spectrum herbicide for herbicide tolerant GMPs. Exposure can be bitrophic via the GMP including any metabolites in residues, fluids (e.g. phloem) or secretions (e.g. nectar, root exudates). Exposure of higher order consumers can also occur through multi-trophic exposure routes (moving through food chains), or after movement and expression of the transgenes into other genetic contexts (e.g. wild relatives), or after spread of the transgene products including any metabolite separately and away from the GMP e.g. via wind dispersed GM pollen and GMP residue input into aquifers, or leaching of transgene product into the soil.

Determination of the possible exposure pathways requires a solid characterization of the GMP and the expressed novel traits and applied secondary stressors. Hence, this step builds on and is only as good as the information collated in the previous component.

Because GMPs can multiply and spread via pollen and seed flow, this exercise will differ significantly from an exposure analysis of chemicals. To facilitate this exercise and allow it to be done in a systematic and transparent fashion, the use of ranking matrices as a tool is recommended (for details see Birch et al. 2004, Hilbeck et al. 2006, 2008a).

From the information compiled on spread and input routes of GMPs and their transgene products/metabolites, the potentially receiving environments can be identified. Some important gaps of

knowledge regarding spread and exposure of testing organisms to GMPs and their transgene products/metabolites may have to be investigated and verified experimentally. The information compiled in this component will allow refining and further reducing the number of testing species from the previous component to those that are most critically exposed to GMPs and their transgene products or metabolites under the assumption that these will be the ones most likely experiencing adverse effects. Modelling exposure scenarios may assist in this effort.

Develop adverse effect scenarios and define testable hypotheses

Understanding exposure routes and pathways of introduction of GMOs and their transgene products into the environment is critically important to develop adverse effect scenarios and research hypotheses for the testing of the selected candidate species. This is illustrated by using the case examples of GM *Bt* and herbicide tolerant crops. For *Bt* plants, the stressor potentially triggering adverse effects, the *Bt* protein, is expressed in almost all plant parts and therefore must be expected to be ingested by all herbivores feeding on these crops and moving through the associated food chain. During this process, the novel protein can take on new properties as it is biochemically altered during the passage through the various gut milieus and exert effects at higher trophic levels in an entirely unexpected way. Such effects cannot be predicted for example from the known mode of action stemming almost exclusively from target pest herbivores (Hilbeck & Schmidt 2006).

For GM herbicide tolerant crops, the stressor is the GMO that triggers a secondary stressor, the application of a broad spectrum herbicide like glyphosate or glufosinate. The use of such herbicides can differ significantly in conjunction with herbicide tolerant crops from its conventional use and give rise to much different adverse effect scenarios than under its conventional use. This was largely confirmed by the Farm Scale Evaluations (FSE), the largest commercial size field trials ever conducted with GM herbicide tolerant crop plants. Certainly, for oilseed rape and sugar beet an additional loss of farmland biodiversity beyond and above current conventional practices was documented (Hawes et al. 2003). Therefore, for herbicide tolerant maize alone (without the insect resistance trait), results were mixed yielding higher numbers of certain plant residue-feeding organisms and no differences for others.

Developing adverse effect scenarios builds on the confirmed exposure routes and the information compiled on the ecological function(s) of the candidate species. Only those candidate species remain until this step, that have an important function and hence, any adverse effect would be significant. It may well be possible to eliminate a number of adverse effect scenarios already at this early stage if a critical exposure pathway can be proven to be non-existent or highly unlikely. For instance, if it can be determined that *Bt* toxins are not present in phloem and xylem sap of *Bt* GMPs at this stage, a whole range of adverse effect scenarios arising from exposure of aphids, that feed exclusively on plant sap, and their associated food chain(s), including many important natural enemies, can be eliminated. The outcome of this step can be a map of all identified exposure pathways and routes of spread of the GM crop plant, its transgenes and transgene products or the secondary stressors required for the realization of the benefit of the GM crop. To do this in a

transparent fashion the use of well-known risk analysis tools called ‘Event-Tree Analysis’ and ‘Fault-Tree Analysis’ is recommended (National Research Council 2002). Fault- and Event Tree Analyses are complementary tools used in risk assessment that were originally developed by engineers identifying critical steps in complex engineering processes, e.g. aviation or large scale industrial production facilities. In a modified form, they have been used for environmental purposes and different ecological systems (Hayes 1998, Hayes 2003, Hilbeck et al. 2008a). Fault-trees are ‘top-down’ risk analysis tools where the analyst specifies a failure event (i.e. ‘top-event’) and, by combining logical functions such as ‘and’ and ‘or’, identifies all events that can or must contribute to the specified failure. An Event Tree is the complementary ‘bottom-up’ approach where an analyst specifies an ‘initiating event’ and lays out the logical chain of events that can occur and lead to a number of possible consequences. Both tools yield more or less complex tree-like charts where each event chain forms one branch of the tree. They do graphically model all of the parallel and sequential combinations of events that can lead to a particular ‘top event’ or arise from a particular ‘initiating event’. This structured, logical approach is based on scientific data and expert knowledge and identifies what data and information is necessary to determine reliably the outcome and the gaps of knowledge associated with the possible events in a transparent manner. Both tools provide a fairly good understanding of the reliability of the analysis and the involved uncertainties and identify research priorities for closing the most critical data gaps.

4.2.3 Data requirements for the ERA

The question of which data must be provided for a specific assessment of the ERA in order to be able to conclude on a specific environmental risk is highly controversal among risk assessors. The conclusiveness and comprehensiveness of the data basis provided in the ERA decides whether a risk conclusion will be unanimously accepted or whether scientific controversies arise among decision makers. This disaccord about what data should form the basis of the ERA is thus a central question and is tightly linked with the ERA’s underlying model, the questions posed at the start of the ERA, the hypotheses defined, the methodologies used and the conclusions drawn (see also chapter 4.2.1).

Carry out assessments of individual environmental risks on a stand-alone basis

The ERA is generally comprised of several individual assessments of environmental risks (e.g. assessment of risks for non-target organisms), each of which should represent a separate and stand-alone assessment. This implies that an assessment cannot be carried out exclusively by referring to other assessments or to the published literature. However, as shown in this report, this practice was frequently applied for certain ERA assessments where risk conclusions for one specific assessment were mainly or even exclusively based on risk conclusions of one or several other assessments (see Review of Notifications; chapter 2). If cross-referencing to other assessments or the literature is made then details of these assessments must be provided and their relevance for the assessment in question justified. This includes also the presentation of the data derived from these assessments in a concise and comprehensive manner (see also chapter 4.2.4).

Present a basic data set for each assessment

Notifiers frequently do not present data specifically generated for the GMP in question. Thus conclusions on potential differences between the GMP in question and the conventional plant or on specific environmental risks are not based on the notifier's own data basis but rely exclusively on reference to other assessment categories, opinions of regulatory authorities' or the published literature. This approach stands in strong contrast to other regulatory areas such as those for pesticides or chemicals where all available data for a specific product must be provided in the notification or dossier. In principle, a basic notifier data set is required for each individual assessment and conclusions should be firstly drawn from these data. If data are drawn on which were generated for other assessments (e.g. evaluation of agronomic characteristics) these should be presented and discussed in the context of the environmental risks addressed in the specific assessment but not only cross-referenced. If no notifier data are provided a thorough justification must be provided. Similarly, data derived from the published literature might be presented in the assessment. If relevant (i.e. relevant for the GMP in question) published studies are included and the aims, results and conclusions of these studies clearly presented (see chapter 4.2.4), they may be used to support a specific risk conclusion of the notifier. If differences between conclusions of the notifier's data and published literature are evident these should also be stated and possible reasons discussed. Raw data and analyses (e.g. statistics) should generally be made fully available to the risk assessor, e.g. by inclusion in the annex and not only on request.

4.2.4 Compilation and presentation of information

The clear and concise presentation of the data and information provided in the ERA in order to conclude on a potential environmental risk of the GMP is fundamental for risk assessors and decision makers who need to decide on the comprehensiveness of these conclusions and on the relevance of the data submitted. In the following recommendations are drafted with respect to structural and formal requirements of the data presentation in GMP notifications.

- Clearly delineate unpublished studies (e.g. notifier internal studies) from published literature
- Clearly identify the GMO, i.e. the event used, in published and unpublished studies
- If data from field trials or toxicological tests are cited in the ERA (e.g. the technical dossier) provide relevant background information also in the technical dossier as well as a clear reference if full details are contained in a separate report (e.g. results of field trials for the agronomic assessment presented in a table in the technical dossier)
- Exactly specify citations (Author, year) and provide the full reference in the reference list
- Clearly identify unpublished, in particular notifier internal studies, indicating the author's names, year and study number (in the reference list)
- Present data and results derived from unpublished, in particular notifier internal studies, separately from published literature
- Discuss data and results generated by the notifier specifically for the respective GMP notification separately from results of other notifications or cited from published literature

- For citation of published literature: indicate the type of study (literature review, original research), the aim of the study, the GM event, the GM material used (plant part etc.), results, conclusions of authors and relevance for ERA of respective GMP
- Provide a separate reference list of unpublished/notifier internal studies and published literature
- Clearly delineate information provided in the ERA of the original notification from additional information provided in updates (e.g. later versions of the ERA) or provided at a later stage of the notification procedure
- Clearly identify new citations (published literature) in updates, if they were previously not cited in the ERA
- Do not re-submit information in the updates which was already submitted in earlier ERA versions (e.g. literature citations, studies, text) or clearly identify information that was already submitted in previous documents
- Explicitly identify and clearly structure and label updates (including Annexes)
- Provide a complete and comprehensive table of contents for the ERA and each update
- Avoid reiteration of information and text (e.g. in Annex II, the ERA, and Annex IIIB of the same notification or between updates of ERAs)
- Clearly cross-reference between ERA text (e.g. technical dossier) and annexes
- Clearly cross-reference between updates of the ERA and studies previously submitted
- Clearly cross-reference between text and information contained in tables (either in the ERA or in annexes)
- Number tables and annexes continuously and consistently throughout a notifications and its updates
- Attach all reports of notifier internal and/or unpublished studies (e.g. field trials conducted for compositional assessment, non-target toxicological studies etc.)

4.2.5 Field trials for the phenotypic characterization of the GMO

Field trials provide the basis for the generation of data for the expression analysis (see chapter 2.3.1), for the agronomic assessment (see chapter 2.4) and for the compositional analysis (see chapter 2.5). The recommendations outlined in this chapter with respect to the methods of such trials refer to their design, the presentation of data derived from these trials as well as the interpretation of results.

Develop further guidance for field trial design, data evaluation and data presentation

The large variation in locations chosen and field trial designs applied in the GMP notifications reviewed in this report make a clear guidance for notifiers necessary. This guidance should cover the following aspects:

- The aim of field trail(s) or trial series
- The number of locations to be chosen
- Criteria to be considered for the selection of locations
- Guidance for the set up of experimental units (plot sizes, number of plants, sampling plan etc.)
- Guidance on the statistical evaluation and interpretation of results derived from field trials

In this respect it has to be pointed out that a range of guidance documents exists for the evaluation and assessment of PPPs (EPPO Standards), including detailed guidance on the design and the analysis of efficacy trials of PPPs or on the evaluation of specific pests in different pest/crop combinations. For instance, for the efficacy evaluation of PPPs guidelines are available regarding the number of efficacy trials and their design (EPPO Guidelines PP1/226 and PP1/152). Similarly, EPPO lists the information to be included in trial reports with respect to experimental conditions, applications of treatments, mode of assessment, recording and measurements and results (EPPO Standard PP1/181, Appendix I).

Comparable guidance is required for GM crops in order to enhance consistency between notifications and enable comparability and interpretability of results derived from such field trials.

4.2.5.1 Design of field trials

The field trials presented in the GMP notifications reviewed in this report did not follow a common methodology. Field trials varied considerably with respect to their locations, their plot sizes, the number of replications or the herbicide treatment variants and controls included. Most of these aspects are independent of the type of GMO but are rather linked to the objectives of the trials and the underlying statistical approach to detect differences between the GM crop and the non-GM control. Thus specification and guidance is needed for the aspects listed below.

Specify field trial scope, objectives, design and conditions during field trials

Field trial designs should be comprehensible and should correspond to the aim of the field trial. In order to achieve this, the scope and the objectives of the field trial should be outlined. The scope should define the context in which the experimental observations are made and the objectives should outline the questions to be answered by a particular field trial. In general an overview of all field trials conducted with a specific GMP for a particular assessment should be provided in the ERA. For reasons of clarity the layout of the field trials should additionally be presented in a figure.

Further information should include:

- List of all Europen trials carried out
- Field trial environmental conditions (temperature, rainfall, soil conditions etc.) and, if relevant, any stress conditions during the trial (e.g. heat, drought, wind)

- GM crop (variety/breeding history) and controls used (see also below)
- Details of the field trials design applied (e.g. randomized complete block design, split plot design etc.) including the number of replications for each treatment and the power of the trial (i.e. the probability of detecting a given difference between treatments if such exists)
- Details of experimental units, e.g. plot size, plot length and width in meters, numbers of plant rows, spacing of plants, plant density, spacing between plant rows etc.
- Sowing and planting date, tillage and irrigation regimes if applied
- Treatments of plants (GM crop treated/untreated, non-GM control, other controls): timing, numbers and amount of fertilizer, pesticide and herbicide applications
- Variables assessed including sample size and the mode of observation for each variable assessed (e.g. plant height)
-

Select representative field trial locations

The locations chosen for conducting field trials need to be representative for the different environments in Europe (see chapter 4.2.10). Locations selected for field trials should thus be representative for the various agronomic and environmental conditions within the EU where the GMP is intended to be commercially grown in order to take different agronomic structures and environmental variables into consideration. For a detailed discussion on representative environments in the ERA see also chapter 4.2.10.

Fully characterise field trial locations

Locations of field trials are generally not characterised in detail in GMP notifications. In order to be able to judge whether field trials were conducted in representative environments basic geographical and climatic information on the trial site is needed. Such information should include:

- Full address of the trial location
- Name and distance to closest town(s)
- Name of the region, if available (e.g. Aragon)
- Geographic specification of the trial location (geographic co-ordinates)
- Soil characteristics at each location (e.g. predominant soil types)
- Climatic characterisation of each location (e.g. long-term meterological data including average precipitation, temperature)
- Typical agronomic conditions applied in the relevant (non-GM) crops at each location (e.g. insecticide application, irrigation etc.)
- A map of the field trial location(s)

- Justification for the selection of the locations chosen (agronomic description of the area, agronomic peculiarities; i.e. whether the location is located in a typical maize growing region of the country or not)

Include more than one growing season at a specific location for a specific phenotypic assessment

It is recommended to conduct field trials over more than one growing seasons at the same location in order to account for across-season variations in climate, pest and disease pressure or other factors which are subject to seasonal variations. Depending on the conditions in a specific growing season (e.g. extreme weather conditions) more than two seasons at a specific location may be necessary.

Specify the non-GM control and demonstrate the use of an isogenic line

Generally, the purpose of field trials is to evaluate whether there are any significant differences between the GMP and its conventional counterpart. In order to assess differences that are due to the genetic modification of the plant appropriate controls need to be used. The notifier has to make clear that the genetic background of a GMP and the non-GM control are as similar as possible. This can be done, e.g. by indication of the breeding history of the respective GMP and the hybrids or lines used for the field trials. This allows concluding on the genetic relatedness of the GMP and the control plants and increases the comprehensiveness regarding the often stated ‘similar genetic background’ of control plants as claimed by notifiers. If the genetic background of the control is shown to be similar to the GMP this has also consequences for the interpretation of results, as the argumentation that differences observed in any parameter may be due to differences in the genetic background, as frequently stated by notifiers (see chapter 3.2.12), will no longer be valid if proper non-GM controls are used.

Include the respective parental, single event GMPs as comparators for stacked events

When carrying out field trials with stacked event GMPs, the inclusion of the respective parental single event GMPs grown under the same conditions in addition to a relevant non-GM control will give an additional indication of any unintended effects in the stacked event.

Include a variant treated with the respective, non-selective herbicide and a variant not treated with the respective herbicide in case of GMHT plants

In order to evaluate possible effects of the intended agricultural practice (e.g. the herbicide treatment) on plant composition, agronomic traits or expression of the novel trait, the inclusion of both treatment variants is needed. This is also consistent with the provisions in the authorization of PPPs which require showing that the product does not exhibit any unacceptable effect on yield or on the quality of the plant (Directive 91/414/EEC, Annex III). The possibility that any other quality aspect or agronomic behaviour of the treated plant is affected by the product must be investigated. Hence, this information should also be provided for GMHT crops.

Include relevant comparators (insecticide treated, untreated) for IR GMPs

Depending on the currently prevailing or traditional agronomic practice of pest control in the representative regions where the GMP is intended to be grown, an insecticide treated variant and/or an untreated variant should be included in the field trials (see also chapter 4.3.3).

4.2.5.2 Data evaluation and presentation of results generated from field trials

Data derived from field trials should be presented in a concise and comprehensible way suitable to support the conclusions of the notifier with respect to any potential differences between the GMP and the non-GM control. This refers to the way how data are analysed but also to the formal presentation of data and results derived from these field trials.

a) Recommendations concerning the evaluation of data

- Indicate which parameters were statistically analysed (separately for each location).
- Indicate which parameters were not statistically analysed (separately for each location) and the reasons therefore.
- Provide the statistical method of the comparative assessments for each individual location and each parameter assessed (e.g. GM versus non-GM at site x, site y, site z etc.).
- Indicate which statistical power was achieved by the field trial design chosen at a specific field trial location and whether the data fulfilled the requirements for the statistical method chosen.
- Include a comparison of treatments for each location separately (and not only across all locations) in order to be able to assess crop-environment interactions at a specific location. Include relevant environmental information for each location which might be relevant to explain observed differences.
- Include a comparison of treatments between growing seasons for each location separately if two or more consecutive growing seasons at a specific field trial location were included.
- Include a comparison of the GMHT with the GM non-HT variant in order to evaluate potential effects of the herbicide treatment on the assessed parameter (for each location/each parameter separately) in the case of GMHT plants.
- If additional controls (e.g. conventional hybrids) are included at specific field trial locations (e.g. in the case of the compositional assessment) include a separate comparison of the GMP and the additional controls for each location separately.
-

b) Recommendations for the presentation of results derived from field trials

There is an urgent need to present the available information of the ERA in a concise and comprehensible way. The problem, as evident from many GMP notifications, that the relevant information is generally scattered throughout the notification can only be solved if a concise format for present-

ing the basic information on the field trials conducted for a specific GMP is followed. This can substantially contribute to a faster evaluation of data and results by the authorities.

The European and Mediterranean Plant Protection Organisation (EPPO) has elaborated standards to facilitate the conduct and evaluation of efficacy trials for plant protection products. Standard number PP1/181 on the conduct and reporting of efficacy evaluation trials provides guidance on how information collected in trial series ought to be presented in the Biological Dossier according to Directive 91/414/EEC. In this EPPO standard detailed suggestions with respect to the presentation of the relevant information in a harmonized form are made. Examples can be found for the presentation of test material parameters, site details, trial reports and for a multi-trial summary. In a similar way it will be also necessary for GMP notifications to develop guidance on how to present overviews of all field trials conducted and of each individual trial at a specific location as well as details for individual trial sites. The recommendations thus comprise:

- Include an overview table of all field trials conducted for a specific GMP notification with the relevant information in the ERA (technical dossier).
- Present results in a clear and concise manner for each parameter assessed on a per location basis and on a per season basis.
- Give an overview which statistical comparisons were made (between locations, between seasons, etc.) for each parameter assessed, including the statistical test and the outcome (i.e. any significant differences).
- If relevant, provide tables summarizing the results across locations separating European and locations overseas. Across locations analysis may be relevant if several locations are similar with respect to their agronomic or environmental representativeness for a specific parameter (e.g. pest occurrence).
- Provide raw data in the annexes only.

4.2.6 Specification of organisms, methods, parameters

The parameters or variables assessed in a specific test or field trial influence which statistical method will be chosen for the analysis and hence the interpretation of the results. The choice of the assessed parameters needs to be relevant and comprehensible for a specific assessment. It must be clear, which parameter or trait was assessed when and where and what the relevance of this parameter is for a specific assessement and for the ERA. The organisms chosen and the parameter assessed must fit in the overall scientific ERA approach and the hypothesis formulated at the start of the ERA process (chapter 4.2.1; see also the separate chapters on individual assessment categories, chapter 4.3). Here, only general improvements are outlined:

- Specify as detailed as possible what was exactly assessed (e.g. which pest species, what trait of the GMP).
- Indicate the category of variables (binary, nominal, ordinal, quantitative) used.

- Indicate the mode of observation of a particular variable (measurement method: measurement, visual estimation, ranking, scoring).
- Indicate the timing and, if relevant, the area/location of observation/assessment (which plant stage, which leaf…).
- Indicate the sample size for a specific variable/measurement (e.g. plant height).

4.2.7 Consideration of Species of Conservation Concern

Species of conservation concern will have to be considered during the scoping of the ERA (see also chapter 4.2.1). In the hazard identification step, the compiled list of species for non-target testing should be checked for key species which allow evaluating possible consequences for species of conservation concern. Such species may include species ecologically or taxonomically close to rare or endanged species. Due to their vulnerability highly protected species should not undergo toxicity testing. Thus emphasis has to be put in particular on an in-depth and thorough exposure analysis. In addition the lowest no-observed-effect-concentration (NOEC) for sublethal effects for species ecologically or taxonomically close to rare or endanged species should be applied instead data from acute toxicity testing. The application of safety margins on the NOEC will then give an indication of the risk for a particular protected species.

For EU-wide GMP notifications it is important to identify regional differences in the endangered status of a particular species. A species may be listed as endangered in one country/region but not in others. Such species must receive specific scrutiny and consideration. For example, *Inachis io* - the peacock - is one case in point. While this species is included on the list of endangered species in Hungary, it is not so, for instance, in Germany. Whether the consideration of such species can be achieved during the individual risk assessment procedure of a GMP or whether individual EU member states will have to be given the possibility to address such questions after EU-wide commercialization, e.g. also in conjunction with the consideration of protected habitats, is currently still under discussion (see also chapter 4.2.10).

4.2.8 Consideration of the Step-by-Step Principle

Define the ‘red thread’ connecting different steps in the ERA

Since it must be evident that GMPs do not cause an adverse effect on the environment, one or several testing steps with the GMP in question may be required at different levels of confinement: laboratory, greenhouse, and field. Especially, if significant uncertainties remain at one level, it is necessary to proceed to the next level of (lesser) confinement with caution. Precaution is operationalized by lifting the level of confinement successively and not moving in one step from the laboratory straight to the field. Given that GMPs can self-reproduce and spread, it may be difficult or impossible to recall them once released into nature. As GMPs and their biochemical products can exhibit different properties in different environments and at different ecological organisational levels (e.g. when moving up the food chain), data documenting the lack of evidence of adverse effects

must be produced at every testing level. In contrast, if at a lower hierarchical level, i.e. laboratory or greenhouse, a high, difficult to manage adverse effect is determined, no further testing may be necessary if the GMP will not pass the minimum safety requirements. However, failure criteria for environmental safety assessments of GMPs have yet to be determined and examined in practice.

If data obtained at higher hierarchical levels do not support or confirm findings at lower hierarchical level, additional laboratory testing with modified experimental protocols may be necessary to complete the scientific understanding of the functioning of the GMP before moving to experiments at yet higher hierarchical levels with less or no confinement. Hence, the developed testing strategy as outlined in this report (see chapter 4.2.1) is iterative and grounded in generated scientific data. The primary function of lower hierarchical level testing is to provide data that allow focusing and inform the designing of experiments to be conducted at higher hierarchical levels. The testing strategy has to be driven by coherent research hypothesis and strategy from the lowest to the highest tier of testing – the 'red thread' connecting the tiered testing program must exist. Therefore, the quality and reliability of higher hierarchical level testing is intimately tied to the testing carried out at lower hierarchical levels.

Integrate information gained from part B trials

The information and data gained from field trials carried out according to Part B of Directive 2001/18/EC are largely not presented in part C notifications. The inclusion of this information, however, would facilitate the evaluation of specific risks and the conclusion on the environmental safety of a GMP, in the sense of the step-by-step principle, i.e. that the introduction of the GMP into the environment is carried out in a step-wise approach after the absence of risks in a particular step or testing hierarchy has been confirmed. In addition, the inclusion of this information could probably avoid the request by authorities for additional information. It may also ensure that data need not be specifically generated for part C notifications if they had already been gained from part B field trials.

The following approach is suggested:

- Upgrade the information presented according to Annex III B of Directive 2001/18/EC by addition of information on previous (part B) releases.
- Upgrade the database on European Field trials (https://snif.jrc.it/GMP_snif_search.asp) by expanding the searchability of data, enabling the CAs to check for respective field experiments with certain events, traits or genes in all EU countries.
- Include detailed information on the purpose, the methods and results of each single part B field trial or trial series.
- Provide reference to information gained from part B trials in the respective section of the ERA and present results where appropriate and relevant.
- Provide full data gained from part B field trials for a respective GMP in the Annex of the part C notification.
- If field trials from overseas (non-EU) are included, make a clear reference and include details of the data (aim, methods, results) in the annex of the part C notification.

Environmental effects are not always addressed in part B field trials (see also chapter 3.2.8). The interplay of the data collection for the deliberate release of GMPs (part B) and the data basis to be submitted for the market authorization of GMPs (part C) needs further guidance. A recently commissioned study aims at the elaboration of a modular concept for ERA data gained in deliberate release (BfN 2007).

4.2.9 Consideration of the Case-by-Case principle

In the context of a comprehensive and inclusive ERA and the broad scoping exercise of the 'Problem Formulation and Options Assessment (PFOA)' shall be performed (see also chapter 4.2.1). For such an inclusive approach of ERA to be compliant with the EU regulations from the start, it is reasonable to begin this process by defining and describing the 'case' to be assessed. As already outlined in the chapter 4.2.1, the definition of the case comprises for each GMO the 3 elements (Hilbeck et al. 2008b):

1) The crop plant,

2) The novel trait relating to its intended effect and phenotypic characteristics of the GMP and

3) The receiving environment relating to the intended use of the GMP.

For the crop plant, for the novel trait and the potential receiving environments relevant data and information need to be compiled. The outcome of this first critical step is the scope and context of the ERA and the testing strategy tailored to the particular GMP case (see also chapter 4.2.1).

This approach broadens the context in which the ERA is to be performed from the novel transgene product (current practice) to the whole GMO and its specific environment. It is considered that such a broader approach fulfils the legal provisions as outlined in Directive 2001/18/EC and the Cartagena Protocol on Biosafety, in contrast to the currently applied 'protein-focussed' approach. The broader approach considers specific features of the transgenic construct and the transformation event, and integrates differences in morphological or phenotypic plant characteristics due to different expression patterns of the transgene leading to different exposure scenarios for plant-associated organisms. This has implications for the ERA applied in GMP notifications, in particular with respect to the GMP used in laboratory tests and field assessments or published literature cited for the conclusion of a specific risk. Consequently, the following approach is suggested:

- Specify the GMP used in each assessment for lab tests, greenhouse and field trials conducted by the notifier as well as in published studies cited (see also chapter 4.2.4)
- In addition to the novel protein use the respective whole GMP for testing
- Use the GMP in question, but not other GM events with same traits – in that respect be consistent with what is done during the GMP development process – risk assessments require the same level of scrutiny as the same biological principles/processes and variation apply.

4.2.10 Consideration of Different Environments

Test the GMPs in representative environments

Since GMPs are intended for authorisation throughout the European Union it is essential to consider the importance of regional aspects for the evaluation of specific characteristics and the environmental behaviour of the GMO as well as of interactions of the GMP with the environment. Directive 2001/18/EC clearly outlines the requirement of an assessment of potential adverse effects of the GMP on the '**potential receiving environments**'. In the Guidance Notes on Risk Assessment supplementing Annex II of the Directive the case-by-case principle is clearly recommended for the ERA because 'of the broad range of individual characteristics of different organisms (GMO-by-GMO) and different environments (region-by-region)' (EC 2002a). The review of GMP notifications in this report (see chapter 2) demonstrated that this requirement has not been fulfilled so far and that guidance for its implementation is urgently needed.

Hence, regionally differing factors that may influence the characteristics and the behaviour of the GMP as well as the interactions of the GMP with the environment must be taken into account during the risk assessment procedure. Regions and locations selected to collect data or conduct field trials should thus represent the range of agricultural, plant health and environmental conditions the GMP is expected to encounter when commercially cultivated.

Different environments may be defined e.g. by the differences in occurrence or in the number of generations of target organisms (e.g. European corn borer), different agricultural practices and agronomic structures (e.g. nitrogen input), different cultivation systems (e.g. low-tillage farming), different crop rotation practices, different climatic conditions, different occurrence of non-target organisms as well as other abiotic and biotic conditions.

Such relevant factors of a specific region or location should be determined at the start of the ERA which calls, again, for a broad and integrative ERA concept. This is important as these factors may lead to differences in potential adverse environmental effects which only become evident if assessed on a regional level. For instance, increased available nitrogen can increase the *Bt* delta-endotoxin concentration in *Bt* plants (Bruns & Abel 2003). As organisms differ in their sensitivity towards *Bt* toxins, changes in the *Bt* toxin concentrations may influence the exposure of both, target and non-target organisms in regions where high nitrogen input is expected.

A comparable situation exists if PPPs are assessed under the requirements of Directive 91/414/EEC. The authorisation of the active ingredient is regulated on an EU wide basis while the specific formulations of PPPs are evaluated on a national level. The relevance of the data submitted for the efficacy evaluation of PPPs under local conditions must be established. The performance of a PPP, however, may vary with sites and seasons. To provide guidance the European and Mediterranean plant protection organization (EPPO) has developed standards for the harmonization of efficacy evaluations. Directive 91/414/EEC explicitly refers to these EPPO guidelines (e.g. Gudelines152 and 181) for testing effectiveness (Annex III, 6.2). The 'Guidance on Comparable Climates' (EPPO Guidance PP1/241) defines four comparable agro-climatic zones for Europe: the Mediterranean zone, the Maritime zone, the North-East zone and the South-East zone. However, climate is only one factor to be considered and the guidance clearly states that also other condi-

tions may be taken into consideration. Focus is to be put on those factors relevant for the product that may affect performance or crop safety and on the biology and pathogenicity of the target organisms.

As for GMPs a 'two-step authorization procedure' is not in place, regional aspects need to be considered in the EU-wide authorization procedures according to Directive 2001/18/EC or Regulation (EC) 1829/2003. Considerations need to be given to criteria for the selection of representative environments taking into account existing concepts, such as the agro-climatic zones (EPPO; PP1/241) or the European Biogeographical Regions mentioned in Art.1c (iii) of the Habitats Directive (Directive 92/43/EEC). An indicative Map of European Biogeographical Regions was developed for the purpose of environmental reporting and for assistance to the Natura 2000 process (ETC/BD 2006).

Consider habitats and areas of particular ecological sensitivity or protection status

In all EU member states certain areas and habitats are subject to environmental protection efforts of different strengths in order to halt the loss of biodiversity or for other conservation reasons. Species and biotope types are frequently subject to a regional protection status and/or are nationally listed in 'Red Lists' (Traxler et al. 2005b, Essl et al. 2008). The areas and species concerned are under the protection of either national or EU law, such as the Fauna-Flora-Habitat and the Birds Directives (Directive 92/43/EEC; Directive 79/409/EEC). In any case the implementation of legislation regarding nature conservation is in the responsibility of the EU member states.

This national responsibility to protect rare and endangered species and habitats stands in contrast to the EU-wide, centralised regulation of GMPs for which the ERA is conducted on a supra-national level. The legal relationship between the GMP regulation and the requirements for nature protection at EU level and its implications for the authorisation of GMPs are a matter of continuous debate (Kerschner & Wagner 2003, Winter 2007a, Winter 2007b).

Apart from legal questions the lack of feasibility and practicability of taking into account aspects of nature protection in an EU-wide authorisation process has led to discontent of EU member states which have repeatedly requested to be given the necessary leeway for such issues (see e.g. GMO orientation debate at the Environmental Council of June, 5th 2008). Although the Guidance Notes on Annex II of Directive 2001/18/EC (EC 2002a) specifically demand to consider the various receiving environments in which the GMO is to be released ('region-by-region' principle), in practice it is hardly feasible to consider the diversity of species and habitats in its entirety at the EU level. Similarly, nature protection aims and obligations of individual member states cannot be considered at this level as protected areas represent individual phenomena - unique by nature - and their potential impairment by the cultivation of GMPs cannot be evaluated satisfyingly at the EU-level. Hence, areas of particular ecological sensitivity or areas and habitats of conservation concern are not being considered in GMP notifications. Legally, on these grounds, the validity of GMP authorisations has to be challenged (Winter 2007a, Winter 2007b).

The basic question remains how such protection entities can be considered in the risk assessment of GMPs. The approach suggested by Winter (2007a, 2007b) is to complement the ERA by adding weight to potentially affected habitat types and species during the evaluation of the overall risk of

the GMP. Authorisations issued should be subject to conditions imposed by the EU member states, such as further assessments according to Article 6 of Directive 92/43/EEC. In this respect it will be necessary to elaborate guidance for the distinction between assessments in the course of the centralised authorisation procedure and specific post-authorisation assessments on a national level in order to avoid the re- or double assessment of certain aspects.

Beside specific nature protection aims also aspects of biodiversity need to be taken into account in GMP risk assessment. The EU has defined the goal of halting the loss of biodiversity until 2010 (COM/2006/0216) and has codified this aim in the 6th Environment Programme (Decision 1600/2002/EC). As a result, in the common agricultural policy (GAP) efforts are made to classify the agricultural landscape in Europe in view of biodiversity aspects. The aim is to evaluate the integration of environmental concerns into the Community's agricultural policy. For this purpose the European Commission has presented a set of agri-environmental indicators (AEI) and started the so-called IRENA-process (Indicator Reporting on the Integration of Environmental Concerns into Agricultural Policy). One of the indicators presented is the indicator 'High Nature Value Farmland' (HNVF). The core piece of this concept is the combination of agricultural habitats and biological diversity. 'HNVF comprises those areas in Europe where agriculture is a major (usually the dominant) land use and where that agriculture supports or is associated with either a high species and habitat diversity or the presence of species of European conservation concern or both' (Andersen et al. 2004 in Bartel et al. 2008). Although this concept is still on its way to implementation it will sooner or later enable the identification of agricultural areas with special concern for biodiversity in each Member State. Depending on the GMP and its possible interactions with the environment in the near future this concept might facilitate the identification of environments with special relevance for the risk assessment process of particular GMPs.

An identification of 'hotspots' of biodiversity within the agricultural landscape based on data on the distribution, endangerment and ecology of plants, biotopes and butterflies has already been conducted at national level in Austria (Traxler et al. 2005a). Areas which are important for the conservation of plants and agro-associated butterflies but which are not part of protected areas but integrated in the agricultural landscape are of high significance for nature conservation and can be considered as areas of high risk with respect to GMP cultivation. Such areas need specific consideration when a GMP is intended to be placed on the market.

4.2.11 Consideration of trait interactions

Use stacked GM hybrids for testing

Instead of referring to single event GMPs or their introduced traits individually, stacked GM hybrids need to undergo testing. As for single event GMOs, this testing should be guided by formulated research hypotheses and aimed to verify and confirm the assumptions that they indeed behave as expected (see also chapter 4.2.1). If there are deviations pointing towards potential adverse effects, these must be followed up by more research at the laboratory and field level. This is the only way that the multiple possible interactions can be taken into account. It is impossible to test all of the above separately because they actually will act in concert in reality. This has been shown by

several examples, such as synergistic or antagonistic interactions of plant-produced proteins or other substances as well as herbicides and *Bt* proteins in soil (see also the relevant subchapters in the chapter 3.3.8).

4.2.12 Interpretation of results and consideration of uncertainty

Base conclusions in the ERA on relevant and scientific data

As a general principle any environmental risk assessment of a GMO should be carried out in a scientifically sound and transparent manner based on available scientific and technical data (Guidance Notes supplementing Annex II of Directive 2001/18/EC, point 3). For authorities to be able to evaluate a specific ERA the conclusions drawn by the notifier on a certain environmental risk must be based on scientific data and must be comprehensible. Thus the notifier should present the way how conclusions were drawn, on which scientific data they were based on, which uncertainties were addressed and which uncertainties remain when concluding on a particular risk.

Thus conclusions should be also based on relevant data, i.e. data generated for and using the respective GMP (the GM event) in the context of a sound research hypothesis (see also chapter 4.2.1). Hence, it should be clearly indicated which conclusions were not based on specific data generated by the notifier. The conclusions drawn should not or not exclusively base on:

- Presumptions on the likeliness of an incident ('…not likely to be…') or anticipations ('…not expected to be...').
- Argumentations derived from other assessments of the same GMP (such as no differences observed in other assessments).
- Assessments of other GMPs or other GM events with same GM traits (e.g. herbicide tolerance).
- Published literature only (see also chapter 4.2.3)
-

Do not dismiss significant differences as 'irrelevant' per se but follow them up

Differences observed during testing in the ERA must not be dismissed on the ground that they are either 'numerically small', 'within biological variability', 'not observed consistently' or assumed to be due to 'other (unconfirmed) factors'. If relevant scientific questions are posed and the ERA approach framed by meaningful research hypotheses, the outcome will clearly indicate whether the hypothesis is confirmed or rejected and will guide further through the whole ERA either leading to higher tiered tests or refining the research question at that level (see also chapter 4.2.1). Confounding factors or parameters influencing the research outcome but not included in the respective research question, either due to methodological or conceptual difficulties may be addressed in an uncertainty analysis (see below).

General recommendations for the interpretation of results gained during the ERA include:

- Follow up statistically significant differences at individual locations.

- If statistically significant differences are observed, further analyses are needed to determine whether these are biologically relevant.
- Consider the potential influence of environmental variables, such as abiotic and biotic stresses (e.g. soil conditions)
- Consider which of these parameters may or are known to be influenced when crossed into different varieties (varietal effects).

Include a characterization of scientific uncertainties for conclusions in the ERA

When risk assessment is the basis for decision making, scientific uncertainty should be fully characterized and communicated in order to inform decision makers as much as possible (Hill et al. 2004). The documentation of uncertainty in the ERA is a fundamental requirement addressed in a range of risk assessment guidelines worldwide, some of which even address methodologies or techniques to be applied to estimate uncertainty (see overview in Henry 2006). Uncertainty analysis promotes transparency and credibility and leads to improved decision-making (Hayes 2003). Such an analysis should not only contain an assessment of the main uncertainties in the models applied, data generated or assumptions made in the ERA but also evaluate whether decision making is possible and outline possibilities to reduce uncertainties.

Different types and sources of uncertainties have been identified by several authors. In particular epistemic uncertainty reflects our limited knowledge of ecological systems and occurs as measurement errors, bias, natural variation, model error, subjective judgement and ignorance (Henry 2006). In addition there are inherent uncertainties such as the nature of the scientific approach to data analysis (Hill et al. 2004). While some of these can be reduced with empirical effort others can only be described (Henry 2006). Uncertainties depend on how environment is valued, how scientific questions are posed about cause-effect pathways and how experimental methods are designed to answer those questions (Levidow 2003). Considering the narrow ERA approach chosen in current risk assessment practice of GMPs ignoring influencing factors of the whole plant and the receiving environment, the uncertainties are much higher than if a broader, integrative approach was applied (see also chapter 4.2.1).

Consider the Precautionary Principle

The Precautionary Principle is an important element in various international, European and national biosafety regulations.

At the international level the Precautionary Principle is one of the guiding principles for the Cartagena Protocol on Biosafety (CPB), the most important international agreement concerning GMOs. Like in other pieces of international agreements the application of the Precautionary Principle in the Cartagena Protocol on Biosafety is based upon Principle 15 of the Rio Declaration on Environment and Development (1992). Principle 15 states that *'…where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation'*.

At the European level the Precautionary Principle is of importance for regulations concerning deliberate release and placing on the market of GMOs as well as for regulations in other related

fields, e.g. safety of chemicals. Regarding GMOs the Precautionary Principle is not only one of the guiding principles of the European Directive 2001/18/EC on the deliberate release into the environment of GMOs but also of the Regulation (EC) No 1946/2003 on transboundary movements of GMOs, which implements the Cartagena Protocol on Biosafety into the EU legal framework. The European Commission guided the implementation of the Precautionary Principle into European law with the 'Communication from the Commission on the precautionary principle' in the year 2000. In addition, the Precautionary Principle is also incorporated into many different pieces of national environmental regulations in Europe.

However, based on current experience the practical implementation of the Precautionary Principle in GMO regulation is not a straightforward matter. A number of open questions and deficiencies when implementing the Precautionary Principle are apparent at the EU and international level. The deficiencies in the current decision making of GMPs and relevant guidance documents for the practical implementation of the Precautionary Principle show the need for further specific guidance and development of procedures on how this principle can be practically addressed in risk assessment practice and decision making of GMPs. Thus the development of a common understanding among stakeholders of the Precautionary Principle within the ERA framework is recommended, in particular addressing the following questions:

- Clarify the role of Precaution in risk assessment and decision making (see also Hill et al. 2004).
- Is current guidance on the ERA of GMOs reflecting the Precautionary Principle?
- How could insufficiencies of scientific data and uncertainty during the ERA be addressed with a view to implementation of the Precautionary Principle?
- What are the different approaches in dealing with precautionary issues at EU level, national level and notifier level?
- How is the diversity of scientific opinions being addressed in the ERA?

4.2.13 Long-term and cumulative effects

Assess long-term and cumulative effects

As outlined in the critical appraisal of the ERA in GMP notifications (chapter 3) long-term and/or cumulative effects arising from the cultivation of a particular GMO are generally not addressed. Although effects that arise only after a substantial time of cultivation of a GM crop alone or in combination with – possibly at the moment not specifiable - other GMPs may currently not be obvious, some effects have been described as possible even if their likeliness may currently not be predictable. As in particular long-term effects may be less obvious and predictable than short term effects or may differ in the degree or level of uncertainty from short term effects, the conceptual requirements for their assessment will need a much broader approach in order to identify other hazards, less obvious ones which may be just as well important.

Suggestions for the consideration of these effects goes beyond the scope of this review, however, others have already proposed a framework for the risk assessment of long-term and cumulative effects (Henry 2006) which might be used as the basis for further guidance and specifications for appropriate risk assessment procedures.

4.3 Specific recommendations for individual assessment categories

In consistency with the critical appraisal, separating general issues from specific shortcomings in the notifications, specific recommendations for improvements and the need for standardization of individual assessment categories in the ERA are outlined and discussed in this chapter. The most important claims and needs are formulated in bold in each specific subchapter.

4.3.1 Molecular Characterisation

The molecular characterisation of GMPs shall provide an understanding which genetic material is introduced into and expressed and inherited by GMPs. Its function is to frame the overall environmental risk assessment of GMPs as it may indicate which adverse effects of the respective GMP need to be considered in the course of the ERA. Like the whole risk assessment the molecular characterisation of GMPs shall reflect the state of the art with regard to the methods applied and the interpretation of results.

The molecular characterisation is necessary to assess which genetic elements have been introduced into a particular GMO and if and which other genetic modifications are present in the GMO. This is relevant for the ERA because knowledge of the inserted genes, their regulation, and the sites of integration within the host genome can provide indications on intended or unintended effects due to the genetic modification(s). The molecular characterisation can specifically support the assessment of unintended effects which may be due to transformation-induced genomic deletions and rearrangements or pleiotropic effects caused by the introduced trait(s). However, the molecular characterisation itself may not be sufficient for predicting any possible unintended effects.

Deficiencies in the initial molecular characterisation of the GMO can result in an insufficient assessment of the properties of a GMO, e. g. in case specific genetic modifications remain undetected and their effects on the phenotype are therefore not properly assessed. Since the risk assessment of GMPs is based on the case by case principle the other assessments (e.g. selection of issues to be addressed in the risk assessment) are to some extent influenced by the specific results of the molecular characterisation of the GMO.

Submit a comprehensive and conclusive set of data for molecular characterisation

Together with information describing the origin and nature of the sequences which are used for the process of genetic modification, a comprehensive set of data on molecular characterisation of the GMP should be available. The notifier should specifically consider the following points:

- Provide adequate quality of data for molecular characterization to draw unambiguous conclusions

High quality data should be submitted with regard to two issues: the chosen test designs and the presentation of results. The submitted data should unambiguously support the conclusions drawn by the notifier.

- Provide information on the initial characterisation of transformants in relation to the chosen method of transformation used to construct a specific GMP.

This information is relevant to assess whether the GMP contains only the minimum transgenic modification required to confer the intended transgenic phenotype, as recommended e.g. by Andow et al. (2004). Specifically, information on further breeding steps with the selected initial transformants should be submitted by the notifier to assess the probability that in addition to a functional transgenic insert further unlinked modifications could be present in the GMP. Since the commonly used transformation methods (biolistic transformation and *Agrobacterium*-mediated transformation) have a substantial potential for creating transformants harbouring multiple insertions with multiple copies of whole and rearranged transgenic inserts any steps taken to remove additional (non-functional) insertions should be documented.

- All transgenic insertions present in a GMP as well as the copy number of (functional and non-functional) inserts should be assessed by complementary methods to achieve robust evidence.

An initial characterisation evaluating the number of transgenic insertions should be presented which allows the detection of all full-length and partial inserts in the genome of the GMP, e.g. by means of a carefully designed analysis by Southern Blot. Data from several different Southern Blot experiments should be submitted (using different restriction enzyme digests of the genomic DNA of the GMP as well as appropriate controls with probes that cover the whole length of the transgenic DNA used for transformation) to increase the conclusiveness of the results. The interpretation of these data should take into account the results from an assessment of the sensitivity of the respective experiments (see also below).

Information on the breeding history of the GMP should be submitted and results of single tests should be corroborated with data from differently designed tests.

The genetic modifications present in stacked event GMPs should be characterised in the stacked event hybrid itself with methods that demonstrate the molecular similarity to the modifications present in the parental single event GMPs.

Assess and indicate the sensitivity of methods used for molecular characterisation

An important issue for the evaluation of the data submitted by the notifier for molecular characterisation is the sensitivity of the methods used for the assessment. As shown in this study the sensitivity of methods is rarely assessed with adequate experiments. Specifically for the detection of transgenic insertions and for the demonstration of absence of vector (backbone) sequences, the respective Southern Blot results were not accompanied by an analysis of the sensitivity of the experiments. Thus no determination of the minimum size of transgenic sequences was possible, which could be detected by the probes used in specific Southern Blot experiments at a given stringency. However, in the absence of a conclusive demonstration that all additional transgenic inserts present in the initial transformant have been removed via selection at further breeding steps, an

analysis of the sensitivity of the methods is needed for a complete assessment of all transgenic insertions in a specific GMP.

The assessment of sensitivity is also an issue for other types of experiments, e. g. the analysis of expression by Northern Blot and RT-PCR and the analysis of expression of transgenic proteins by ELISA. However, only for the latter notifiers commonly present an evaluation of the sensitivity.

It is recommended that all experiments which are crucial for assessing the presence of transgenic insertions in a specific GMP are accompanied by an adequate analysis of the sensitivity of detection.

Characterise all transgenic insertions present in a GMP by detailed PCR or Southern Blot and sequence analysis

It is recommended to use PCR, Southern Blot and sequence data to achieve a detailed characterisation of the insertions. Only a description of the structure and nuclear acid sequences of the transgenic elements used for transformation cannot substitute a detailed experimental characterisation. Together the above information can identify issues which are important for the risk assessment, e. g. an assessment of the effects of potential sequence rearrangements.

The design of experiments should enable a detailed assessment of the structure of the transgenic insertions and indicate all genetic elements present in these insertions as well as their organisation. Results of this characterisation should guide the nucleic acid sequence determination of transgenic and flanking genomic sequences present at the loci of insertions. Sequence data in turn can then corroborate the results of the characterisation by Southern Blot analysis and PCR analysis at a fine level of resolution. Carefully designed methods for the assessment of the structure of transgenic insertions can aid the subsequent characterisation of GMP lines developed from the characterised GMP by further breeding steps. Southern Blot and PCR experiments are valuable tools for the assessment of the genetic stability of a certain GMP at an appropriate level of resolution (see below).

With regard to stacked events similar experiments should be used for the assessment of similarity of the transgenic insertions present in stacked events compared to the respective modifications present in the parental single event GMPs.

Comprehensively characterise genomic sequences at the loci of transgenic insertions

In addition to a detailed analysis of the internal structure of transgenic insertions present in a GMP the notification should contain a detailed characterisation of the genomic sequences flanking the transgenic insertions (Andow et al. 2004). This analysis should comprise:

- Analysis of the presence of additional sequence elements at the border regions of transgenic inserts, which were inserted during transformation together with transgenic sequences (Latham et al. 2006).
- Assessment of sequences of the junction regions of genomic and transgenic sequences (e.g. to determine the potential for the expression of fusion proteins).

- Analysis of functional genetic elements of the recipient plant (e.g. genes and regulatory elements) present at the loci of transgenic insertions.
- Analysis of potential rearrangements of and modifications to native genomic sequences of the recipient plant (e.g. deletions, etc.) at the loci of transgenic insertions.
- Determination of the chromosomal origin and localisation of the flanking sequences in the genome of the recipient plant, dependent on the availability of sequence data for certain plant species (see also following recommendation).

The analysis of notifications presented in this report showed that in some notifications information on flanking sequences was only submitted in additional submissions on request by the authorities (for details see Table 3). Information on nature and origin of flanking sequences, as outlined above, should be obligatory already in initial notifications.

The analysis of flanking sequences should not only be restricted to the analysis of the potential for expression of fusion proteins and the identification of additional sequences inserted during transformation. An important issue connected to a comprehensive evaluation of flanking sequences is the amount of sequence data necessary for this assessment. To address the following issues more sequence data may be necessary than is presently included in the GMP notifications:

- Identification of functional genetic elements of the recipient plant present at the loci of transgenic insertions and potential changes to these sequences.
- Determination of the chromosomal origin and localisation of the flanking sequences in the genome of the recipient plant.

For the comparison of native genomic sequences present at the 5´and 3´ junctions to the transgenic insertions, an analysis of the respective loci in the genome of the unmodified recipient plant is required. A comparison of native genomic sequences in the GMP and the unmodified recipient plant, e.g. by PCR analysis should generally be presented.

Assess cellular and chromosomal location of inserts with complementary methods

It is recommended that the chromosomal location of inserts is assessed with available techniques. An adequate characterisation of the nucleic acid sequence of native genomic sequences flanking transgenic insertions can indicate a specific chromosomal location in case sufficient sequence data is available for a certain plant species in public sequence databases or proprietary sequence databases accessible to the notifier. For a number of important crop species (maize, rice, etc.), which are currently used as recipient plants for GMPs the sites of transgenic insertions could be localized in the respective genomes because a substantial amount of sequence data is already available for these species.

Additionally, a number of complementary methods to directly assess the localisation are available. The use of such methods, like Restriction Fragment Length Polymorphism (RFLP) mapping or chromosomal localisation by Fluorescent in situ Hybridisation (FISH), is recommended to address the chromosomal location of transgenic insertions.

Assess genetic and phenotypic stability of inserts with a combination of methods that are designed to yield significant results

For certain GM events (e. g. maize lines MON810 and Bt11 also analysed in this study; as well as soybean GTS 40-3-2, and maize lines T25 and Bt176) the results of the molecular characterisation conducted after placing on the market of these GMPs differed from the information submitted by the notifier upon initial notification. This indicates that rearrangements due to genetic instability have occurred (Collonier et al. 2003). It is therefore recommended that genetic methods (e.g. Southern Blot or PCR analysis) which allow the assessment of the integrity of the transgenic insertions are used to assess genetic stability over a number of generations. The development of such methods should be based on test designs and results of the analysis of the detailed structure of transgenic insertions (see above).

The notifications analysed in this report contained data regarding stability of the GMPs which were established for different numbers of individuals from a variety of different generations. Additionally, the individual GM plants had different genetic backgrounds depending on the different breeding histories of the test lines. Therefore guidance for the assessment of genetic and phenotypic stability of inserts should specify:

- The documentation of the breeding scheme of the GMP and test lines used for the analysis
- The number of generations which should be analysed
- The specific generations from a breeding tree to be included in the analysis (e.g. BC3 or BC5)
- The number of individual plants per generation which should be analysed with regard to a defined level of stability

For the generations specified as above it is recommended that both genetic as well as phenotypic data are submitted.

4.3.2 Expression assessment

The assessment of expression of the inserted transgene(s) or changes in expression resulting from the suppression of certain gene sequences is considered fundamental for the ERA of GMPs. The assessment of expression of transgenic components should be established in a way to facilitate the risk assessment of other issues, like the analysis of effects of the GMP on non-target and target organisms (Andow et al. 2004). The expression of the introduced transgenic sequences not only determines the performance of the GMP in the agricultural setting and the efficacy of the GM trait but also mediates the environmental behaviour of the GMP and potential adverse consequences to the environment.

For the ERA the analysis of expression of the introduced genetic elements in different parts of the plant and throughout the life cycle of a plant is of particular concern. The determination of exposure of any target or non-target organism either directly or indirectly via the food chain is important to assess the occurrence of potential adverse environmental effects. For the assessment of risks for

non-target organisms any tissues which may be relevant for non-target organisms should be considered also in the expression assessment. With respect to effects of insecticidal transgenes on target organisms all tissues which are potentially consumed by these organisms should be considered. In particular, the assessment of different plant tissues and developmental stages are specifically relevant to determine the exposition of the environment and any target and non-target organisms when releasing the GMP into the environment (see also chapter 3.2.2). Expression analysis in the context of the specific environment where a GMO will be commercially grown is a prerequisite for the assessment of exposure of target and non-target organisms.

In addition, the expression of a GM trait and thus the exposure of organisms to the novel traits of a GMO may differ depending on the genetic background of the plant. Thus an analysis of the expression of specific traits in different varieties of the crop is a prerequisite for the assessment of the environmental effects of GM traits in different genetic backgrounds. This is also to be seen in conjunction with the analysis of the stability of expression of the targeted traits which is important in order to judge whether the traits are stable when GM traits are transferred into different commercial hybrids.

Also the expression of potential fusion proteins in the GMP may have consequences for the evaluation of several types of risks including potential environmental effects.

Assess expression in representative environments

The assessment of the expression of transgenic components in relevant European environments is a prerequisite to specifically evaluate potential effects of the respective GMPs on the environment and organisms living in these environments. The determination of expression needs to be established in field trials reflecting representative conditions of the different environments where the GMP is intended to be commercially grown.

Hence, data on the expression of transgenic components should be based on field trials in representative European locations. Expression of transgenes (including assessment of developmental expression and tissue specific expression as discussed below) should be investigated for more than one consecutive growing season at relevant European locations in order to be able evaluate changes which may be due to different climatic or agro-ecological conditions.

Expression of transgenic components in GMPs should be evaluated in parallel to the assessment of other phenotypic properties. Important for the assessment of the expression of transgenic elements are the conditions of cultivation of a certain GM crop. For instance, with regard to herbicide tolerant crops one of the specific issues for the expression analysis is whether the trial plots were treated with the respective non-selective herbicides or not. The assessment should thus indicate whether non-selective herbicides were used and provide a comparison of the expression levels in GM plants treated and untreated with non-selective herbicides. Therefore the recommendations for the design of field trials given in chapter 4.2.5.1 of this report apply similarly.

Standardize experimental protocols for the detection of transgene products in various environmental media

Comparing expression levels is important during the risk assessment as well as in the context of enforcement, traceability, resistance management, research and for quality control. Hence, detection methods for novel transgene products should be standardised. However, a standardisation of methods does not exist to date. Differences due to variable protocols for the detection of transgene products should be avoided by standardization of experimental procedures.

Guidance should therefore consider the following points:

- Use of standardised sampling procedures
- Use of standardised methods for all tests of expression of transgene products conducted in a specific notification
- Validation of detection methods for certain transgene products, like specific *Bt* toxins, which are expressed in a number of different GMPs (including stacked events constructed from these GMPs) to enhance comparability of results of expression between notifications.

For transgene products like *Bt* toxins, which may have environmental effects, in addition to quantification of the amounts expressed in the GMPs an assessment of the biological activity of these transgene products would aid the evaluation of environmental effects. Since an assessment of the biological activity of transgene products is not standard in the assessment of GMPs further guidance should address this issue and suggest when and how the biological activity of a specific transgene product should be assessed.

Standardize tissue-specific expression analysis of transgene products for each crop species

As tissue specific expression data are relevant for the assessment of environmental effects of GMPs, guidance is necessary specifying in a crop-specific manner which tissues should be sampled and analysed for expression evaluation of the transgenic products by standardised methods. Additionally to tissues which are commonly assessed (e. g. grain and leaves for GM maize lines) other tissues of environmental relevance should also be included (root, stalk, pollen, etc.). For GM maize expressing *Bt* toxins the following tissues were suggested for an assessment of expression: Leaves, pith, phloem, pollen, male flowers, roots below ground, adventitious roots above ground, tillers, ears leaves, silk, kernels and cob (Andow et al. 2004).

Guidance is further needed with respect to the time of sampling of individual tissues for expression analysis and for the part of individual plant organs which are sampled (especially leaves). The same set of tissues should be assessed at all locations of field trials for a certain GMP and during all test seasons to ensure comparability of results.

Standardize the analysis of developmental expression of transgene products

For the assessment of expression of the GM trait(s) during the growing season crop-specific standardization is necessary with respect to:

- The types of tissues to be sampled throughout the growing season

- The time points for sampling during the growing season (e. g. at which growth stages sampling should be done)
- The maximum timeframe between measurements

Standardize the assessment of expression in different genetic backgrounds

If no clear information on the variability of the expression in different varieties and their specification is provided, then the interpretability of effects, e.g. to target or non-target organisms will be hampered because the influence of the genetic background on the expression will also determine effects down the food chain. This is in particular relevant for the control of the target organisms by the respective *Bt* crop. If different varieties of the same event exhibit different protection levels of the toxin this has severe consequences for the efficacy and, hence, the usefulness of the *Bt* crop in Europe (as different varieties are usually grown in different regions), resistance management and quality control of the product (see also chapters 3.3.3 and 3.3.6). As *Bt* toxin levels are currently not assessed during variety testing, this must be considered in the ERA before approval for placing on the market can be given.

However, as shown in this report the expression of transgenes in different genetic backgrounds of the GMP was not assessed in a systematic way in GMP notifications. Where different varieties were used for the assessment of transgene expression the information submitted to identify the genetic background of the test varieties was found to be insufficient.

Guidance should therefore include:

- Exact information on the GMP varieties used for assessing the expression of transgenes
- Indications of the lines from a breeding program which should be used for assessing the expression of transgenes (inbred lines, backcrosses, F1 generation, etc.)
- Specification of how many different hybrids should be assessed

Standardize the assessment of generational stability of expression

The assessment of stability of the genetic modifications present in GMPs and of the traits conferred by these modifications is a crucial issue for the release of GMPs into the environment. In addition to the potential instability by genetic rearrangements of the modified insertions, the possibility that the integrated transgenes are subject to gene silencing effects needs to be taken into account (Andow et al. 2004).

Since expression of the transgenes inserted into GMPs may change over generations in unpredictable ways, the stability of expression over a certain number of generations should be assessed to conclude on phenotypic stability. Guidance for the assessment of generational stability of expression should specify:

- The numbers of generations to be assessed
- The number of individual plants per generation which should be sampled
- The methods which should be applied to assess generational stability of expression

Experimentally assess expression of identified fusion proteins

In addition to the evaluation of the expression of transgenic components, which are intentionally present in the GMPs, the expression of potential fusion proteins possibly created by the genetic modification needs to be assessed. Such fusion proteins may be created at the junctions of genomic border sequences present at the loci of insertion and the integrated transgenic sequences. Additionally, at complex insertion loci (e.g. loci containing additional integrated fragments other than single copies of the inserts or multiple insertions of full-length or partial inserts) open reading frames (ORFs) for potential fusion proteins can exist, which need to be evaluated.

It is recommended that the expression of identified fusion proteins is examined with analytical methods to substantiate conclusions from sequence analysis and homology comparisons of identified ORFs that could be expressed as fusion proteins. As a first step the transcription of the ORFs into specific mRNAs should be analysed by Northern Blot or RT-PCR. In case specific transcripts are observed, the possible translation into proteins needs to be examined as a second step.

The experimental analysis of the expression of fusion proteins should be accompanied by an assessment of the sensitivity of the conducted experiments.

To achieve a consistent assessment of the expression of fusion proteins it is further recommended that standardization of the bioinformatics analyses to detect and evaluate potential fusion proteins is included in the guidance for risk assessment of GMPs. The following issues should be subject to standardization:

- Minimal length of ORFs that should be further analysed. Currently the minimal length of ORFs, which are further assessed, is not standardised and thus ORFs are differently assessed in different notifications.
- Criteria for the detection of homologies of flanking sequences to ORFs for potential fusion proteins to regulatory sequences, like promoters for transcription and regulatory sequences for translation.
- Criteria for assessment of homologies of potential fusion proteins to previously identified toxins and allergens. Currently the criteria by FAO/WHO (2001) are not constantly applied in the assessments. Furthermore the toxin and allergen databases which are used for the analyses should be state of the art.

4.3.3 Agronomic assessment

The aim of the assessment of agronomic traits of a GMP compared to the non-GM control is to characterise the behaviour of the GMP in the intended agronomic and environmental setting. In addition, it provides an indication on the performance of the GMP compared to the non-GM control and whether the introduced traits are functional in situ. This is in particular relevant for the assessment of the efficacy of a GMP e.g. with respect to control a specific pest or disease. It is not only important as a quality check of the product but also indicates possible environmental changes mediated by the introduced trait(s). The evaluation of a positive effect of a treatment (in this case the insect resistant or herbicide tolerant GMP) which is measured by the control of the target organism

is essential for any GMP. An evaluation of efficacy is also fundamental in the assessment of any conventional plant protection product according to Directive 91/414/EEC. For the efficacy assessment the specification of the pest and disease spectrum relevant for a particular GMP is a basic requirement which also allows the determination and distinction of target and non-target organisms of a specific GMP as well as the assessment of potential exposure pathways for non-target organisms (chapters 2.7 and 2.8). Additionally, an assessment of potential food/prey availability effects and potential secondary pests also requires that pests and diseases relevant for a specific location are known.

A thorough agronomic characterisation of a GMO can thus provide important information relevant for the ecological characteristics of a GMO. This concerns for example information on how the GMO differs from the recipient plant in its mode and/or rate of reproduction, dissemination and survivability. This information in turn contributes to the information of the likelihood for a GMP to become more persistent in agricultural habitats or more invasive in natural habitats. Also according to Directive 2001/18/EC there is the need to assess the possible selective advantage or disadvantage of a GMO and the potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMP and target and non-target organisms (Annex II, D.2). For the evaluation of these aspects the assessment of agronomic parameters may form an important basis if relevant parameters are assessed.

In addition, an assessment of the agronomic behaviour of a GMP can detect an unintended phenotype. Thus the assessment should comprise not only agronomic traits but also plant traits in general. Such unintended phenotypes may be caused by position effects, epistatic interactions, pleiotropic effects, mutations and unidentified causes. In breeding programmes phenotypically abnormal individuals are usually identified during extensive screening among locations and years and can be eliminated. However, ‘small, unintended effects may remain undetected, because they may depend on cumulative action, specific environmental conditions or introgression into different genetic backgrounds’ (Snow et al. 2003). Such unintended effects may manifest themselves for example ‘through changes in susceptibility to important pests and diseases, through morphological or developmental changes or through modified responses to agronomic and crop management regimes’ (EFSA 2006a). An altered phenotype may also have consequences for the plant’s interactions with other organisms, such as pollinators or pest species. Thus phenotypic changes that may mediate potential environmental effects need to be identified in the agronomic assessment.

Define a set of agronomic parameters and use standardized assessment methods for each GM crop

A minimum set of standard parameters to be assessed for each crop plant in field trials needs to be defined. Similar sets for the assessment of agronomic performance already exist for the value analysis of varieties and variety testing (AGES 2002, BSA 2000). The International Union for the Protection of New Varieties of Plants (UPOV) has elaborated guidelines for testing (UPOV 2002). Despite the fact that in the value analysis of plant varieties the focus is on the evaluation of the susceptibility of the plant to diseases and on yield parameters, to a certain extent, these protocols may be suitable or may serve as a starting point for the evaluation of agronomic parameters of GM crops.

Further guidance is needed to what extent the currently assessed parameters in GM crop notifications need to be supplemented by parameters of environmental relevance. This is in particular necessary if other assessments of the ERA are not separately conducted but draw on results of the agronomic assessment for the argumentation of safety of the GMP. It will thus depend on the respective assessment (e.g. assessment of differences in the dissemination ability of the GMP) which crop-specific parameters should be additionally assessed in field trials and whether this can be done in the framework of the assessment of the agronomic performance of the GMP.

Define agronomic parameters as clearly as possible

A specification on what is exactly assessed (e.g. parameter 'plant health') should be given for all agronomic parameters assessed. This refers in particular to the commonly assessed parameters 'insect damage' and 'disease incidence' which should be specified with respect to the pest assessed at the species level, the timing of assessment in the growing season, the variable assessed (e.g. abundance) and the mode of observation (see also next point).

Assess pest damage/incidence and disease incidence for each location

The evaluation which pests and diseases affect the GM crop in the relevant environment where it is intended to be released is particularly important in view of secondary pests or prey-mediated effects on non-target organisms. Pest species that are not the target of the GMP may also be affected by the GMP thus being at risk for resistance development. If non-target pests are unaffected by the GMP they may develop into a major pest if the target organism is sufficiently controlled by the inserted GM trait. Thus, all pests and diseases occurring at a particular location on the GMP should be reported.

In the value analysis of plant varieties major pathogens are usually assessed for each crop plant in order to evaluate the susceptibility of a certain new plant variety to pests and diseases (BSA 2000, AGES 2002). Although the national specifications vary according to regional differences in the emergence of pest and diseases they may give some indications which pests and diseases are regionally relevant. These specifications may also be helpful for reporting and evaluating relevant pests and diseases of GMPs.

Standardize methodology for the assessment of pests and diseases

Standardized methods for the evaluation of pests and diseases are necessary in order to get a reliable estimate on the pest and disease incidence at a particular location. Standardized methods are also of value if target organisms are assessed during the quality control of the GMP and when baseline susceptibilities relevant for resistance management plans are established.

Methods to assess pests and diseases may be derived from plant variety testing (e.g. BSA 2000, AGES 2002) or the evaluation of plant protection products (e.g. EPPO guidelines), or they must be specifically developed for GMPs. For instance, methods are available for the assessment of European corn borer (ECB) infestation which are by far more precise than the methods currently used in GMP notifications. In plant variety testing in Germany plants infested by the ECB are enumerated as close as possible to the harvest and results presented relating to the total number of plants in the plot (BSA 2000). In field trials for the assessment of plant protections products, as conducted by companies, ECB infestation is assessed by opening 20-30 plants per plot and counting the

number of living larvae (Püntener 1981). Beside general standards for the assessment of effects of plant protection products, EPPO has also elaborated specific standards for the assessment of certain pest and diseases, e.g. Standard Nr. PP1/13(3) for *Ostrinia nubilalis*.

In any case the type of variable assessed should be indicated (binary, nominal, ordinal or quantitative) as it also influences the statistical method chosen for the interpretation of results. Variables should be measured as accurately as feasible and the mode of observation should be indicated (e.g. measurement, visual estimation, ranking, scoring).

Hence, clear guidance is needed how notifiers should proceed when assessing and evaluating pests and diseases. Specific assessment procedures may be necessary for target pests and diseases. The focus of the observations should not only be on the plant damage but also on the effect of the GMP on the target organism in the field (see also chapter 4.3.6). The consideration that pest and diseases differ between regions should play a role in the selection of the field trial locations (see also chapter 4.2.10). Comparable guidance can be found, for instance, in the EPPO Guidance on the Design and Analysis of Efficacy Evaluation Trials (EPPO Guidance PP 1/152(3)).

Record baseline infestation rates of pests and diseases

In the assessments of disease incidence and insect damage in GMP notifications it was generally not indicated whether the level of infestation corresponded to an average infestation in the respective region or whether it was significant enough to demonstrate the performance of the product. The latter aspect is not only crucial for the performance of the product and quality control but also for the evaluation of exposure pathways for non-target organisms. The question if and to what extent target organisms are affected by the plant-incorporated toxin also makes a difference for the evaluation of effects to non-target organisms (see chapter 4.3.7). The requirement to report baseline infestation rates is also to be seen in the context of the need to define the efficacy of the GMP (see also below) and is consistent with the requirement for testing plant protection products for which ‘the product must be tested in circumstances where the target harmful organism has been shown to have been present at a level causing or known to cause adverse effects on an unprotected crop (…) or where the harmful organism is present at such a level that an evaluation of the plant protection product can be made’ (Directive 91/414/EEC, Annex III).

Establish the efficacy of the product

The efficacy and performance of a GMP is not only an issue of product quality for the farmer but also has environmental consequences. If e.g. *Bt* toxins do not work properly under specific environmental conditions, then resistance risks in target but also non-target pest species may increase. As has been shown in this report, performance and efficacy of *Bt* crops have generally not been assessed in GMP notifications. Together with expression values, data on the performance of the crop in the field and efficacy towards the target organism give an indication of possible dose responses to *Bt* toxins. Such dose responses are relevant for resistance risk evaluations. Efficacy evaluation is a basic requirement in the registration procedure of plant protection products and can be defined as ‘the balancing of positive effects of the treatment against the negative effects such as direct damage to the crop, effects on pollinators and natural enemies or development of resis-

tance' (EPPO PP1/223(1)). The information needed to establish efficacy according to EPPO guidelines includes that the **level, consistency and duration of control** was shown to give a defined benefit under the range of conditions (including agricultural, climatic, plant health and environmental) likely to be encountered in practical use. If performance is not shown for all conditions, then the proposed label could specify that the product is intended for use in certain specified circumstances, e.g. under particular growing conditions (see EPPO guidance PP1/223(1)). At least for GMPs producing a plant-incorporated pesticide, such as *Bt* crops, similar requirements are urgently needed. The need for a reference product with proven and sufficient performance in practice, in addition to an untreated control, as generally required for plant protection product efficacy trials, will give an indication on the level of control given by the *Bt* crop under different environmental conditions. Several EPPO guidelines for the efficacy evaluation are available (e.g. EPPO PP 1/223(1), 1/214(1), 1/152(3)) which could give some indication of how to draft such a guidance.

4.3.4 Assessment of plant composition of the GMP

The assessment of the composition of a GMP compared to the non-GMP is important in GMO risk assessment as differences in the plant's composition may lead to potential adverse effects on human and animal health. However, certain compositional parameters have also relevance for potential environmental effects of GMOs. Changes in key macro-elements such as nitrogen may modulate the specific environmental response of the GMP to its target or non-target organisms. Plant compounds also play a role for feeding and oviposition stimulation, deterrence of pests and pathogens or parasitoids. Thus unintended changes in these compounds due to the genetic modification may change the chemical cues for insect herbivory and related processes. Consequently, the challenge in the ERA is to identify changes in the plant's composition which may indicate potential environmental effects. Differences between the GM and the non-GM crop in relevant toxins, antinutrients or secondary metabolites may give an indication for changes in certain ecological responses in the crop plant to environmental stresses.

During the ERA compositional differences that might have arisen due to the genetic modification a GMP are evaluated in comparison with an appropriate comparator. This concept of substantial equivalence is considered to be a starting point in the risk assessment process of novel foods and is generally applied in the current risk assessment practice of GMOs (see e.g. Spök et al. 2003b). The concept was originally attributed to the safety assessment of food and food components and has been adopted in several European and international recommendations as guidance in the framework of the assessment of novel foods. Although several different interpretations of substantial or compositional equivalence were suggested (see Spök et al. 2003), analytical studies focusing on the composition of plant specific ingredients (micro-, macroelements), toxins and antinutritives are of major importance for the risk assessment of GMPs.

Chemical composition of plants can influence a range of phenotypic traits which relate to the ERA, in particular plant decomposition (see chapter 4.3.8) and potential effects on non-target organisms (see chapter 4.3.7). For the compositional analysis as carried out in the ERA the following recommendations for improvements are given:

- For each crop define a minimum set of plant compositional parameters plant relevant for environmental aspects taking into account latest findings in chemical ecology and phytochemistry.
- Assess and discuss anti-nutrients/secondary metabolites for individual crops with a view to environmental safety and not only food/feed safety.

The OECD has published a range of consensus documents on compositional considerations for new varieties of several crops addressing composition of major crops by identifying the key food and feed nutrients, anti-nutrients and secondary plant metabolites (e.g. OECD 2001, OECD 2002a, OECD 2002b). However, these documents consider predominantly nutritional aspects. For the ERA of GMPs additional compositional aspects should be taken into consideration:

For maize secondary metabolites (inositol, raffinose, furfural, feroulic acid, P-coumaric acid) and anti-nutrients (phytic acid, trypsin inhibitor) as suggested by OECD (2002a) are relevant for food or feed use of maize as they either influence bioavailability (e.g. phytic acid) or digestibility in the gastro-intestinal tract (e.g. raffinose, phenolic acids) or may be toxic to man at higher levels (e.g. furfural; OECD 2002a). In contrast, other components such as trypsin inhibitors are not considered to be nutritionally significant (OECD 2002a) but are highly relevant as storage or reserve proteins, as regulators of endogenous enzymes and as defensive agents against attacks by predators and insect pests (Blanco-Labra et al. 1995, Chen et al. 1992) as well as plant-pathogenic fungi (Chen et al. 1999). Also the assessment of DIMBOA levels in maize are not recommended by the OECD, mainly because of the high level of variability of this compound in maize tissues and its fragmentary knowledge on its toxicity for man. However, its relevance and toxicity for insect pests has been described (Klun et al. 1970). Similarly, the role of phenolics such as ferulic acid and p-coumaric acid in pest resistance is known (OECD 2002a, Bily et al. 2003, Santiago et al. 2005). Also interactions among plant-produced allelochemicals and *Bt* toxins have been described (Navon et al. 1993, Olsen & Daly 2000, Santos et al. 1997) and may affect fitness costs associated with *Bt* resistance (Carrière et al. 2004).

In oilseed rape glucosinolates are known to influence herbivory, parasitoid behaviour and oviposition in crucifers with an influence of the side-chain structure on herbivore response (Raybould & Moyes 2001). Also effects of glucosinolates on disease resistance have been described (Giamoustaris & Mithen 1997).

In potato glycoalkaloids and sesquiterpenes exert protection against predation and disease in potato and have been shown to be influenced in tuber tissues by genetic modification (Matthews et al. 2005). Proteinase inhibitors and lectins, present in potatoes, are mentioned but their specific assessment not recommended by OECD (2002b) because they are largely inactivated by thermal processing. Both, trypsin inhibitors as well as lectins have potent ecotoxic properties, interfere with digestive processes in insects and are increasingly used in insect-resistant transgenic plants (Ussuf et al. 2001).

- For herbicide tolerant crops: consider new metabolites and their ecotoxicological relevance due to the post-emergence application of the non-selective herbicide.

- Define more accurate ranges when establishing the ‘natural variation’ of each compositional parameter. This could be achieved by using data from modern commercial varieties currently used in the EU. If additional controls, e.g. modern varieties, are used to establish a ‘natural range’ of compositional values, then these commercial hybrids should be included in the respective field trials conducted with the GMP and not at other locations or in different years.
- If such a ‘natural range’ is established, it should be calculated per location for a particular crop. This is particularly relevant for assessments of traits with environmental relevance which may vary considerable between locations.
- Develop guidance for the assessment of the relevance of statistically significant differences in plant composition between the GMP and the non-GM control.

4.3.5 Assessment of dissemination and related processes

The assessment of biological features of the GMP that affect dissemination and, potentially, persistence in the environment is a key component for GMPs which, due to their biology, dispose of the ability to disseminate, persist and survive in or particularly outside agricultural fields. Although oilseed rape is a prominent example for such processes, other crops may also be able to disseminate seeds or pollen and build up stable populations. The difficulty of an assessment of these processes before a GMP is placed on the market is due to the fact that the ecological behaviour of an organism is not solely determined by its inherent characteristics but also by the environmental conditions and habitat characteristics where the GMP will be grown. Although these dispersal processes as such may not be considered as negative environmental effects *per se* they may pave the way for adverse environmental effects occurring at a later stage even if a selective advantage or disadvantage can currently not be envisaged. Thus an evaluation of the characteristics of a GMP with respect to potential changes in its ability to reproduce, disseminate, establish and survive as compared to the non-GM comparator is a crucial factor of the environmental risk assessment of GMPs. This includes also the evaluation whether a GM crop-wild hybrid exhibits a selective advantage, survives better, persists more or invades better a particular habitat compared to the non-GMP. In this context the perceived risks of GM crops comprise the following (Conner 2003, Andow & Zwahlen 2006):

- GM crops or GM crop-wild hybrids may become agricultural weeds thus compromising current weed management systems
- GM crops or GM crop-wild hybrids may invade natural habitats changing their biodiversity value
- Gene flow from GM crops may replace wild genes (genetic assimilation) and reduce genetic diversity of a recipient population
- Lower fitness of GM crop-hybrids may drive wild populations to extinction (demographic swamping)

- Higher fitness of hybrids may lead to increased invasiveness replacing wild populations and other species
- Gene flow from GM crops may contaminate seed pools and reduce seed quality

Many agricultural crops are restricted in their distribution to the agricultural context and the cultivated fields, being largely dependent on human intervention with respect to their ability to survive, establish and reproduce. For maize the above mentioned processes are currently not considered to be relevant as maize does not establish feral populations and has no wild relatives in Europe (Craig et al. 2008). However, almost all crops have the capacity to appear as volunteers within fields due to seed loss or incomplete destruction depending on the crop management and the local environmental conditions. This is also relevant for maize volunteers which frequently occur in crop rotations although no self-maintenance of such volunteer populations is assumed until now (Connor et al. 2003). However, certain GM traits may favour the ability of volunteer GM maize to thrive under particular conditions thus compromising the farmer's weed management options. In addition, gene flow is relevant for maize in the context of the coexistence issue (Messean et al. 2006) and possible seed quality or seed purity issues (Andow & Zwahlen 2006).

Other crop species such as oilseed rape are not highly domesticated, have the ability to escape from the agricultural context and are able to survive as a wild plant in a range of different habitats and under different environmental conditions. Oilseed rape is known to occur as a volunteer in crop rotations and GM oilseed rape has frequently been shown to occur in regions with extensive GM oilseed rape cultivations beginning to constitute major agronomic problems to farmers with the occurrence of multiple herbicide traits derived from different spontaneous hybridisation events (Hall et al. 2000). Additionally, persistence of oilseed rape volunteers, including GM oilseed rape in agricultural environments over several years has been observed even without selection pressure (D`Hertefeld et al. 2008). Feral oilseed rape is also known to build up stable and self-dispersing populations outside cultivated fields which persist for at least several years or even longer (Pessel et al. 2001, Crawley & Brown 2004, Claessen et al. 2005a, Claessen et al. 2005b).

When sexually compatible wild relatives are present and grow next to the crop, hybridization may lead to the creation of crop-wild hybrids. While the hybridization between oilseed rape and its wild relatives as well as the fertility of the resulting hybrids and their occurrence in the wild is relatively well known (Raybould & Gray 1993, Pascher & Gollmann 1999, Wilkinson et al. 2000, Chevre et al. 2000), the behaviour of such crop-wild hybrids is currently largely unpredictable, especially as it depends not only on the plant but also on the habitat where the recipient plant is likely to survive. As crop-wild hybrids are not restricted to a controlled area (i.e. the cultivated field) the ecological consequences of such a scenario is currently difficult to predict. Theoretically assessments of the impacts of gene introgression on fitness are uncertain due to the lack of knowledge of the ecological context into which a transgenic hybrid might spread (Johnson 2002). Previous experience with invasive plant species, the fear of the creation of novel 'superweeds' and general the inability to keep plants under human control or fetch these plants back as well as the unknown and unpredictable ecological and evolutionary consequences justify a thorough assessment in the ERA before a release of the GMP into the environment is envisaged. Several species of wild relatives of oilseed rape occur in agricultural regions in Europe and overlap in flowering with oilseed rape populations

(e.g. Pascher & Gollmann 1999). This includes potential weed species such as *Brassica rapa*. It has been shown that introgression of GM traits of GM oilseed rape into wild relatives and their persistence over time can occur even in the absence of selection pressure (Yoshimura et al. 2006, Warwick et al. 2007). For herbicide tolerant crops which can produce interspecific hybrids a particular ecological consequence of cross pollination and introgression has to be considered as the novel herbicide tolerance trait confers an additional adaptive trait compared to 'traditional' interspecific hybrids which usually inherited no new advantage to resist against herbicides since all species within a botanical family share the same resistances (Darmency 2002). In addition, evidence for fitness effects comes from other GM crop plants where the weedy GM crop plant experienced reduced herbivory and increased seed production (Snow et al. 2003).

Provide clear and concise data for the respective GMP

In general, any assessment of traits or processes related to the reproductive ability, survivability, selective advantage/disadvantage or dissemination, invasiveness and persistence of the GMP should be substantiated by specific data of the respective GMP (see also chapter 4.2.3). The data provided for the assessment must be clear and concise with respect to the evaluated trait (e.g. reproductive potential) or process (e.g. dissemination).

4.3.5.1 GM crops without wild relatives in Europe

The assessment of reproduction, selective advantage, survival, dissemination, persistence or related processes will depend on the crop species and the introduced trait. Crops with no wild relatives under current European conditions such as maize or potato will thus not be subject to an assessment of outcrossing of the GMP to wild relatives.

Standardize crop-specific parameters to be evaluated in the agronomic assessment

The focus for GM crops with no wild relatives should be on intra-species gene flow and the assessment of the general dissemination and reproduction ability and survivability of the GMP under current agricultural conditions. This should include the assessment of phenotypic characteristics of the GMP in relation to the above-mentioned processes, such as seed set, seed loss, volunteer formation, pollen production and morphological and physiological parameters of flowers, pollen and seed. Addressing certain phenotypical traits of the GM crop may give a first indication of any potential differences between the GM crop and its non-GM isogenic line in the capacity for gene transfer (Conner et al. 2003). For instance, any difference in a reproductive trait such as flower colour or flower period might indicate the potential of the GMP to change pollination frequencies. Similarly, fertility or sterility traits of GMPs may remove pollen competition. These parameters can be assessed in combination with the evaluation of agronomic characteristics of the GMP in representative regions where the release of the GMP is intended.

As current risk assessment practice shows there is an urgent need to define clear parameters and corresponding measurement methods for specific crop species complementing the current agronomic assessment of a GM crop (see chapter 4.3.3), in particular for flowering and seed parameters, volunteer formation as well as vegetative reproduction (e.g. tuber formation in GM potato).

Address the composition of the plant, the expression and segregation of the transgene

The assessment of crop specific phenotypic parameters indicates the potential of the GMP to survive under agronomic conditions. In particular, such an assessment should be conducted in combination with an assessment of the GMP's composition as well as information on transgene expression and segregation of the transgene in the GM crop (see chapter 4.3.2). Expression of the GM trait in the crop and in volunteers means that a novel protein or substance is produced in the plant. Hence, the behaviour of these transgene products and any new metabolites must be addressed during the ERA.

Assess the competitiveness of the GMP

The evaluation of the occurrence and survival of the GMP (e.g. GM volunteers) should be carried out as a first step and is especially important for plants known to occur also as weeds (e.g. potato or oilseed rape). The focus of the assessment should be on the behaviour of the transgene and its impact when expressed in volunteer plants on agriculture and the environment.

Such an assessment should comprise an evaluation whether the novel trait in the GM crop is linked to a fitness parameter that is relevant outside the agricultural context. Such a fitness parameter may be, for instance, the resistance to a specific lepidopteran pest feeding on the crop or the herbicide tolerance in habitats where the herbicide is applied (Meier 2007).

In addition the competitiveness of the GM crop under pest pressure and/or herbicide application should be evaluated. This should include an assessment of basic reproduction and dissemination parameters of the GM crop. For this purpose, comparative experimental assessments may be needed considering key factors influencing reproductive success (Johnson 2002) such as:

- plant survival from germination to seed production
- number of flower heads, seeds per head, seed viability, seed size
- predation of seeds
- seed survival over winter/seed dormancy

Results from such controlled experimental assessments may also be derived from glasshouse experiments or during Part B experimental releases (see also chapter 4.2.8).

Particular attention should be paid to crops in which the novel genes might improve the competitiveness in agricultural and/or natural habitats. The novel trait may not necessarily translate into a higher fitness or selective advantage of the GMP. This will depend on whether the specific trait confers any fitness advantage and if the function of the GM trait(s) (e.g. the resistance to certain pest species) is a limiting factor of the wild plant population. A fitness effect of a *Bt*-trait mediated resistance to insect herbivory has been shown by Snow et al. (2003).

4.3.5.2 GM crops with wild relatives in Europe

Evaluate the occurrence of wild relatives in relevant environments where the GMP is intended to be released (on a regional scale)

For crops with wild relatives such as oilseed rape the identification of relevant wild relatives, the assessment of the potential for hybrid formation and fertility of hybrids is needed for the ERA. The ERA should in this respect also consider centres of origin as well as contact zones of major bio-

geographic regions which harbour a high potential for hybridisation (Pascher & Gollmann 1999). Endangered or rare species and species known as weeds (e.g. *Brassica rapa*) should also be taken into consideration. The assessment will have to take into account any differences in the occurrence and probability of hybridisation events of the GM crop with wild relatives under worst case (lab) and natural conditions.

Take receiving populations and receiving environments into consideration

There is a wealth of literature discussing the probability of spontaneous hybrid formations of crops such as oilseed rape with several wild relatives and their hybridization rates. In contrast, there is much less information on the consequences if such an event occurs. There is substantial uncertainty if and what selective advantage or disadvantage a novel GM trait might confer to a newly formed hybrid. The sole assessment of the hybridization rate or the probability of hybridization does not give any insight into these processes.

The relevance of the novel trait for a plant's fitness will not only be determined by the function of the transgene and the relevance of this function for the recipient population but also by factors such as the recipient population size and density as well as genetic drift. Consequently, the receiving populations and the receiving environment must also be considered. Regions where feral crops or wild relatives are sympatric with GM crops have to be particularly addressed. This can be done, for instance, by considering the habitat into which the plant is likely to spread or by relating experimental results to population dynamics using life tables for specific habitats (Johnson 2002). Also the assessment of the progeny of crop/wild hybrids is particularly valuable since their reproductive output may be restored compared to the parental plants (Warwick et al. 2007).

Strengthen monitoring and develop risk assessment methodologies for addressing the consequences of gene flow and the behaviour of the GMP and GM crop/wild hybrids

Independent of the ongoing debate on whether the escape of transgenes from GMPs constitutes a risk or enhances the possibility of risks or not, a range of environmental, social or agricultural implications have to be faced if GM crops outcross to wild relatives (Marvier & van Acker 2005). As knowledge on the establishment and behaviour of GM crops and GM crop/wild hybrids in the environment is only emerging, adequate risk assessment methodologies are still to be developed. To address the invasiveness of a GM plant, field experiments comparing ecological fitness between GMPs and conventional counterparts over a range of ecological conditions and several years have been considered inevitable (Hails 2002). As long as no adequate risk assessment methodologies are available, intensive environmental monitoring of relevant habitats where hybrids or ferals are expected to occur will be necessary. If this cannot be achieved in a satisfactory way or in regions of high environmental sensitivity, such as centres of origin of a crop or hotspots of biological diversity, exemptions from GMP cultivation will have to be considered.

4.3.5.3 Improve data presentation and referencing

Last but not least, structural improvements with respect to the presentation and cross-reference of data and studies submitted by notifiers for the assessment of survivability, selective advantage, dissemination, invasiveness and persistence are necessary (see also chapter 4.2.4). In this respect notifiers should:

- Make a clear reference to parameters/traits assessed in the agronomic assessment, if these are used for the evaluation of the relevant processes such as dissemination, reproduction, survivability, gene flow, persistence, invasiveness, selective advantages or disadvantages and present details of the results.
- Indicate why a specific agronomic trait assessed is useful for the assessment of any of the above mentioned traits/processes.
- Make clear reference to published studies and cited literature including a clear indication of the GMO used in the studies, the methods, the results and the conclusions of the authors (see also chapter 4.2.3).

4.3.6 Assessment of effects mediated via target organisms

Defining the 'target' and 'non-target' pests which a particular insect-resistant GMO aims to control is important but by no means trivial. A target pest of one application may be a non-target pest of a related application somewhere else. For example, Colorado potato beetles (*Leptinotarsa decemlineata*) and potato tuber moths (*Phthorimaea operculella*) are both pest species on potato and tomato in southern Italy. On tomatoes that contain the coleopteran-active *Bt* toxin of the Cry3 class, the 'target' pest is the Colorado potato beetle, while the lepidopteran potato tuber moth is a 'non-target' pest. Vice versa, potatoes that contain the lepidopteran active *Bt* toxin of the Cry1 class 'target' the potato tuber moth while the Colorado potato beetle is the 'non-target'. Even more complicated is the situation where lepidopteran active *Bt* toxins work well only against certain lepidopteran pest species out of several that co-occur on the same crop. For instance, in the US, the GM maize varieties expressing Cry1Ab *Bt* toxins work best against the European corn borer but much less or only sublethally against the various species of the *Spodoptera* genus, commonly called armyworms. Hence, for herbivores, a careful scientific analysis of which is the target species and which is the non-target species has to be carried out. For pest control purposes, for a *Bt*-crop expressing lepidopteran-active Cry1Ab toxins, *Spodoptera* species are not necessarily 'targets' because it does not reliably control them below a damaging threshold. Hence, they are considered 'non-targets'. However, from the perspective of resistance managers, *Spodoptera* species are also 'targets' because they are affected – sublethally or even lethally after some time - and pests on both cotton and maize that contain the same or very similar *Bt* toxins. Hence, the risk of resistance is fairly significant in these species if they are only intermediately affected. For insect-resistant GMOs, a careful statement and justification of target and non-target pests is therefore important.

The evaluation will thus not only include the specific target organism itself but also potential environmental effects mediated by the loss of the target organism, such as the loss of food or prey for other organisms. Such effects have been described in literature for organisms closely associated with the target organism of *Bt* maize (Bruck et al. 2006).

Clearly define and list target organisms that the GMP or the related management technique aims to control

Organisms targeted by the GM trait or by the related management techniques have to be clearly specified (for GMHT plants see also issues related to land use and cultivation techniques in chapter 4.3.9). For insect resistant GM crops this should not only include a statement that the relevant insect resistance trait is effective against 'lepidopteran pests' but clearly distinguish which lepidopteran pests are targeted in combination with the proven efficacy of the GMP for the specific pests. Information should include a delineation of pest species which are not the primary target of the GMP but may or are known also to be affected by the GM trait. The potential of development of new major pests and resistance development also in these species should be included in the evaluation. This is necessary for reasons of quality control, product liability and resistance management (see also below).

Evaluate efficacy of the GMP on target and non-target pests in representative environments

For all listed target organisms, data on efficacy of the GMP and data on basic biological effects (i.e. mortality, development time, fecundity, etc.) of the expressed transgene product and the relevant GM plant tissues should be delivered. Standardized procedures should be developed that specify which tissues and growth stages of the GM plant for each relevant target pest organism are to be tested. Guidance should also include from how many locations and biogeographic regions the data should be derived. This serves multiple purposes. For one, it provides the necessary data basis for the development of effective resistance management plans (e.g. the 'high dose' towards the target pest). Moreover, efficacy data ensure quality control standards which benefit the farmers. Necessary experiments may be included in the course of the assessment of agronomic traits and characteristics of the GMP in relevant and representative environments (see also agronomic characterisation of the GMP, chapter 4.3.3).

Laboratory and field data of each target pest should allow judging whether or not the product will work in different environments and under different conditions and should allow delineating the likely main production regions. The main production regions will be those regions where the listed target pests are causing most frequently problems. Hence, target species data and expression data should derive from a variety of environments into which the GMP will be released in the EU (see also chapter 4.2.10).

Information for *Bt* crops provided should include:

- Estimation of mean *Bt* concentration and variability thereof in different plant tissues (see also chapter 4.3.2)
- Tests including plant material – not only microbial *Bt* toxins
- Identification and testing of potential secondary pest species using GM plant material
- Resistance management plans or surveys in related but less affected pest species
- Separate tests for stacked event GMPs – at least to the degree that they can confirm that they function as their single event parental lines

All other organisms that are not targeted or aimed to be controlled are 'non-target' organisms. They will be included in the selection procedure outlined in the chapter on effects on non-target organisms (chapter 4.3.7). During the ERA their ecological relevance will be established and it will be

determined whether or not they shall be included in a testing program. It will be decided if they are relevant pest species that may be at risk for developing resistance and become secondary pests either in the same crop or other crops. As an example, *Spodoptera* species are a pest species that is not or only sublethally affected by most *Bt* crops. In regions where *Bt* crops are grown, *Spodoptera* species are likely to become a serious secondary pest in lieu of the eliminated target pest.

Consider resistance development not only for the target but also non-target pests and/or weeds

For reasons outlined in chapter 3.3.6 insect resistance development should not be evaluated for the target organism only, but should also comprise non-target pests and/or weeds. This implies that the baseline susceptibility of relevant non-target pests and/or weeds needs to be established. For weeds information derived from the evaluation of the complementary, non-selective herbicide according to Directive 91/414/EEC may be used (see also chapter 4.3.9).

Provide and discuss all relevant studies on the mode of action of the *Bt* toxin in the target organism(s)

The mode of action of a specific *Bt* toxin as expressed in the GMP should be discussed considering all relevant published studies available at the time of the notification. Published literature cited should reflect the current state of scientific knowledge on the mode of action and include also the reporting of uncertainties in the mode of action or known interactions of *Bt* proteins with other substances. The literature cited should be consistent with the approach chosen in the ERA, i.e. if both toxins are known to be relevant for the toxicity, then both toxins must be considered in non-target studies.

Develop standardized protocols for the establishment of minimum efficacy levels and for toxin measurements

Minimum efficacy concentrations are important for resistance management control and quality control of the GMP (i.e. the *Bt* crop, see also above). To reliably establish minimum efficacy and to do this in a comparable fashion with other studies, standardized procedures for the establishment of efficacy of the GMP in the lab as well as under field conditions are necessary (see also agronomic assessment, chapter 4.3.3). Similarly, standardized procedures are needed for the measurement of *Bt* toxins in GMPs (see also expression assessment, chapter 4.3.2).

4.3.7 Assessment of interactions of the GMP with non-target organisms and the biotic environment

Similar to conventional crops, also transgenic crops can have an impact on other organisms in the environment (Craig et al. 2008). All transgenic crops have some non-target species, i.e. organisms that are not the target of a transgenic plant (Andow & Zwahlen 2006).

The assessment of effects of a GM plant on other organisms than the target organism(s) is a key process within the environmental risk assessment of GMOs. The discussion on potential environmental risks of GMOs to non-target organisms and methods how to assess these risks fuels ongoing discussions not only among scientists but also between notifiers, risk assessors and EU member states (see e.g. reasons outlined by several EU member states for a national ban for placing

on the market of GM maize MON810). Hence, a thorough and science-based risk assessment for non-target effects of a particular GMP is fundamental in each GMP notification, in particular if the GMP is intended for cultivation (see also chapters 3.2.1. and 3.3.7).

Several functional groups of non-target organisms have been defined, at least for insect-resistant GMPs, although all GMPs are associated with non-target organisms (see Craig et al. 2008 and references therein):

- pollinators and natural enemies of pests/beneficial species
- non-target herbivores
- soil organisms
- species of conservation concern/protected species
- species contributing to local biodiversity

Risks to non-target organisms are of particular concern when the affected organisms are beneficial species such as pollinators, species of conservation concern or flagship species that are perceived as indicators of ecosystem health (Snow et al. 2004 cited in Hill et al. 2004). In other cases non-target herbivores may be considered as highly relevant because they may increase the risk of secondary pest problems (Andow & Zwahlen 2006). With the improvement of concepts for non-target risk assessment, a trend has been observed towards assessing risks using non-target species that occur locally where transgenic crops will be planted. As this approach corresponds to a case-specific risk assessment, its expansion in the future is foreseeable (Andow & Zwahlen 2006).

Consequently, as a first step in the ERA it is important to identify and describe non-target organisms and related ecological processes which are actually exposed to a certain GMP in a certain environment (Exposure Assessment). This process will lead, in turn, to the decision about which organisms to select and use in toxicological tests with the gene product or assess in greenhouse of field trials using the whole plant. By this approach the assessment of non-target organisms also takes into consideration species of cultural or aesthetical value or species of conservation concern.

Comply with minimum data requirements for non-target testing: bioactivity spectrum of GMP-produced toxin(s) and metabolites including modes of action

Minimum data on the mode of action of novel insecticidal proteins like the *Bt* toxins should include:

- pH – dependent activity spectrum
- conditions for solubilization and activation
- respective molecular weights of expressed toxins and its metabolites in the GMP
- steps of activation required in the target insect
- pore formation
- possible in-planta present *Bt* toxin metabolites and their spectrum of activity
- potential interactions with naturally occurring secondary compounds of the plant

Minimum data requirements should include data that were obtained with the purified microbially produced transgene product as well as from the GMP, as it is only possible to understand potentially (synergistic, additive, antagonistic or neutral) interaction effects if the actual GMP is used. If the plant is known to express potent and highly bioactive secondary compounds such as glucosinolates in Brassiceae or alkaloids like Solanin in Solanaceae like potato, tomato and egg plant, basic experiments with these substances and the novel protein must be conducted to explore the potential of such interaction effects (see also chapter 4.2.11).

In addition to the knowledge of the mode of action of the *Bt* toxins in non-target organisms, the two main issues forming the core of the persisting controversy regarding ecotoxicity testing of GMOs for environmental risk assessment are: What organisms should be tested and how should they be tested?

Select organisms in a step-wise procedure

The proposed methodology for ecotoxicological testing of non-target organisms is prescriptive with regard to the use of a procedure for selection of testing species and the development of testing protocols tailored to each case and the receiving environment. The methodology builds on a procedure that was developed and tested with three case examples by the GMO ERA Project (Andow et al. 2008, Hilbeck et al. 2006, Hilbeck et al. 2004). For a detailed description of the selection procedure and the outcomes of the test runs, see the series of publications by Birch et al. (2004) and Hilbeck et al. (2006 and 2008a, including the relevant chapters in each volume). Here, only a brief summary is provided. The selection procedure was further expanded by proposing additional criteria related to aspects of practicality.

The selection procedure is a step-wise process that begins with identifying the most important ecological functions relevant to the sustainable production of the GMP. Based on the information obtained from the characterization of the existing biodiversity in the identified receiving environments, a list of the most relevant functional groups for the given cropping system is compiled and the identified species are classified according to their known ecological functions (Step 1). Next, a defined set of ecological criteria is used to select the most important species of each functional category. Each species is ranked according to its geographic distribution, habitat specialization, abundance, phenology, linkage and association with the crop (Step 2). This step is largely independent of the genetically engineered novel trait of the crop plant. The goal is to select those species that rank highest in ecological criteria and, therefore, have an important functional role in that cropping system. The rationale is that if the selected species are adversely affected by a GMP, it would indeed result in an adverse environmental effect. To facilitate the ranking process, matrices were developed as tools (Hilbeck et al. 2008a). The selection steps greatly reduce the number of potential testing species existing in a given cropping system and surrounding habitats. Only those candidate species that were ranked highest in the two preceding steps are taken further along in the procedure. The goal is that neither all nor too little is required for testing but a reasonable set of species

with greatest relevance to the receiving environment and an important ecological function in the given cropping system (Hilbeck et al. 2008b).

Improve current ecotoxicology testing protocols

The development of testing protocols will be informed by all previous steps required for selection of the most important testing species of the receiving environment but in particular by the exposure analysis during the ERA (see also chapter 4.2.1). A higher trophic level organism should be tested in a tritrophic set-up while for a herbivore a bi-trophic set-up will be sufficient. In every testing program, the whole plant and its relevant tissues must be included as test material. Using microbially produced proteins can provide complementary data to establish dose-effect relationships. Test with microbial toxins cannot suffice by itself as the protein in the GMP typically differs from the microbially produced product. Additionally, unintended effects can arise from the transformation process that can only be tested if the whole GM plant is used as testing material.

Identify key species and processes for each GMP in soil ecosystems

The assessment of potential effects of the GMP on soil organisms and functions must always focus on the individual GMP, its introduced trait and the receiving environments. Plant modifications and the specific GM trait introduced may target specific processes or taxa in soil ecosystems via impacting functional groups utilizing the transgene product (Oger et al. 2000). The choice of target groups and processes will have to be adapted depending on the type of plant and transgene product as well as its origin, function and persistence in soil. GMPs expressing transgenes aiming at regulating fungal infections (e.g. chitinases) may require more intense assessments of soil fungal communities than herbicide tolerant GMPs, whose major effects on soil ecosystems may rather derive indirectly from changes in the cultivation or management techniques than from direct effects of the transgene product. With the increase of stacked event GMPs, particular attention should be paid on combined effects of transgene products.

The question of appropriate criteria for soil assessments in the ERA of GMPs is controversial, not only because of scientific controversies over the explanatory power of individual soil assessment methodologies but also because of the high temporal and spatial variability of soils. Controversies exist on which organisms should be used for the assessment of GMPs on soil ecosystems and which test methodologies and protocols are to be applied. However, recent suggestions for the improvement of the risk assessment of GM crops on soil ecosystems comprise the following:

- Assessment of both functional and structural components (functional soil properties and biodiversity; Mendoca-Hagler et al. 2006).
- Broad and specific assessments integrating soil ecology (organisms and processes) as well as soil quality (capacity to provide and sustain defined functions; Lilley et al. 2006).
- Assessment of specific microbial groups and processes most likely to be susceptible and general analyses for effects outside the scope of predictions (Bruinsma et al. 2003).
- Assessment of potential bio-indicators such as mycorrhizal fungi, symbiotic N_2-fixing bacteria, nitrifying bacteria, wood-decaying fungi and antagonists (Bruinsma et al. 2003).

- Assessment of general keystone indicators which may indicate the loss of a particular soil function (mycorrhizal fungi, plant growth promoting rhizobacteria, wood lignin decomposing fungi, N-fixing and nitrifying bacteria) as well as more specific indicators depending on type of GMP and likely to be affected by the introduced trait (Lilley et al. 2006).
- Assessment of larger soil invertebrates (e.g. earthworms, millipedes, isopods) as they are known to process large amounts of dead plant material (Zwahlen et al. 2007).
- Evaluation of general community parameters as well as specific, potentially vulnerable indicator groups or processes for soil borne microbial communities (Kowalchuk et al. 2003).
- Testing of effects on different soil food web components and ecosystem-level processes (ecosystem approach, e.g. Bogomolov et al. 1996), in order to differentiate effects and get insight into ecological mechanisms.
- The use of an holistic approach with feedback loops and increasing complexity comprising laboratory testing, glasshouse or mesocom studies as well as field studies (Birch et al. 2007).
- Testing species from organism groups which are ecologically relevant for the receiving environment and which cover different exposure routes, taxonomic and physiologic groups (Hilbeck et al. 2008b).

As outlined above, the determination of key functional groups, ranking of species and functions and determination of exposure pathways should frame the selection of non-target organisms also in soil ecosystems (Hilbeck et al. 2008a, b). Once relevant non-target organisms were selected the practicability of species tests can be assessed and test methods chosen. Existing standardized test protocols, as applied in ecotoxicity testing of chemicals or PPPs may be useful depending on the exposure scenario and the species selected although some need a modification step before they may be applied for GMPs (Hilbeck et al. 2008b). Currently, earthworms, collembolans and isopods have been identified as the most likely candidates. However, the need for adaptation of the species range to be tested to account for the receiving environment, exposure pathways and behavioural types has been emphasized (Hilbeck et al. 2008b).

4.3.8 Assessment of effects on biogeochemical processes and the abiotic environment

GMPs and GMP products and their in-planta metabolites will be introduced into soil and other environmental compartments in several different ways – as/in fresh and decaying plant material, as free protein leaching from plant material or bound to soil particles. Depending on the environmental conditions the transgene product (e.g. the *Bt*-toxin) may persist in fields over prolonged periods and may undergo further degradation yielding other or additional metabolites with unknown bioactivity. The knowledge of persistence of such toxins and their metabolites is therefore a prerequisite for the assessment of effects of novel GMP proteins in different environmental compartments. Hence, the evaluation of persistence of transgene products is a first and crucial step in order to evaluate potential exposure of non-target soil organisms and is relevant for the assessment of the

potential exposure of the fauna and flora of soil communities. Adverse effects due to GMP cultivation on soil organisms may result in changes or losses of particular soil processes.

Soils contain a large range of different species of bacteria, fungi, micro-, meso- and macrofauna, of which the bacteria and fungi are the numerically dominant group and perform important soil functions including nutrient cycling. Soil and soil functions are affected by plant-derived material, such as plant and root residues or root exudates. Soil-plant root interactions can affect above-ground ecological relationships of crops such as plant defence (Guerrieri et al. 2004). As currently notified GMPs express transgenes in all tissues, plant-incorporated proteins or toxins will be released into the soil compartment via degradation of plant material present in the field after harvest, via pollen dispersal or via root debris and root exudates during plant growth. Especially root exudates have been identified as a relevant factor influencing the bacterial rhizosphere community associated (Brusetti et al. 2004). In soil, transgene products are subject to ingestion, degradation, adsorption or leaching. Impacts on soil ecosystem functions are generally mediated via effects on soil biota responsible for key soil processes such as soil microbial communities but also higher trophic level organisms. Physical and chemical conditions in soils vary considerably both spatially and temporally depending on soil texture and type, crop management techniques, climate and plant variety used. Consequently, species diversity and functional processes in soil exhibit a wide variability.

The recommendations outlined in this chapter do not comprise potential effects of the GMP on non-target soil organisms which are dealt with in the previous chapter discussing recommendations for the assessment of interactions of the GMP with non-target organisms and the biotic environment (see previous chapter 4.3.7). Although this distinction may be arbitrary at first sight, it enables a clear assignment of recommendations and fits with the suggested overall approach of the ERA.

Assess the fate of the transgenic product and its degradation products/metabolites in different environmental compartments as a starting point of the ERA

GMP-derived *Bt* toxins have been shown to be present in soil and water compartments (see chapter 3.3.8). Verification of the presence and the fate (e.g. persistence) of the plant-expressed substance, protein or toxin in different environmental compartments needs to be one of the first steps in the ERA of a GMP in order to determine if and which organisms are exposed or which processes may be affected. In this context it is important to consider relevant environmental conditions (e.g. different soil types) reflecting the conditions under which the GMP will be cultivated (see also below).

Risk assessment requirements in other regulatory frameworks such as PPPs according to Directive 91/414/EEC require an assessment of the fate and behaviour of the active ingredient in the environment. Environmental compartments to be considered are generally soil, water, and, if relevant, air. The ERA requirements of Directive 91/414/EEC may also demand an assessment of the mobility and the spread of any degradation products in relevant environmental compartments. This includes a persistence estimation for any secondary metabolites/toxins and their environmental relevance.

Develop standardized protocols for the assessment of degradation and persistence

The current approach in GMP risk assessment using the chemical approach to measure a DT_{50} or DT_{90} value to describe persistence of *Bt* toxins is not sufficient as degradation does usually not follow first order degradation kinetics (see also chapter 3.3.8).

No general standards are currently available in order to assess e.g. persistence or degradation of these toxins under laboratory or field conditions. Due to the range of influencing variables standardised protocols for the assessment of *Bt* toxin persistence and degradation are needed in order to avoid experimental shortcomings which are evident in the currently applied *Bt* protein degradation studies. As plant material used and the experimental conditions (temperature, soil type) are major factors influencing the outcome (i.e. the estimated persistence of the protein) it is useful to assess persistence under a range of different conditions. This enables reflecting the relevant degradation processes and the potential persistence of transgene products to be expected under cultivation conditions of the GMP. Common test protocols available from testing of plant protection products, such as the litter bag test for the decomposition of organic matter or the test of microbial respiration in soils, may be useful as a starting point but most likely need to be adapted to meet the specific demands of GMP testing (Hilbeck et al. 2008b).

Consider the whole plant for degradation studies

Decomposition of the GMP in comparison with the non-GMP should be evaluated considering the specific composition of the GMP. Differences in composition between the GMP and the non-GMP may significantly influence decomposition rates of the plants (see also chapter 3.3.8).

Address metabolites of transgene products

As the environmental consequences of the presence of protein fragments or metabolites of e.g. *Bt* proteins derived from GMP cultivation are currently unknown, a minimum requirement for GMP notifications should be to address the most relevant metabolites of the transgenic product. This is in accordance with other regulatory frameworks, such as PPPs, for which an assessment of metabolites including products resulting from degradation or reaction of the active substance, as well as relevant metabolites of toxicological and /or ecotoxicological concern must be identified (Directive 91/414/EEC). The ecotoxicological relevance of such metabolites will then have to be established on a case-by-case basis according to identified exposure routes.

Include assessments under field conditions

Laboratory assessments using isolated proteins can give some indications of the persistence or of potential toxic effects of the GMP on non-target soil organisms. However, field studies have several advantages compared to laboratory assessments:

- Consideration of the whole plant in field studies as opposed to laboratory studies using the isolated protein enables taking confounding effects due to plant material into consideration. For instance, stimulatory effects of microbial activity due to plant material will not be mirrored in laboratory tests using the microbial produced protein (Donegan et al. 1995, Palm et al. 1996).
- Possibility of inclusion of different crop management (e.g. soil tillage) and pesticide regimes (Birch et al. 2007).

- Possibility of inclusion of relevant controls such as different non-GM varieties (Griffiths et al. 2007) and locally adapted GM and non-GM cultivars (Mendoca-Hagler et al. 2006).
- Multiple sampling to cover natural variations.
- Prolonged studies integrating continuous release of *Bt* toxins into the soil ecosystem.

The above advantages clearly demonstrate the urgent need for the inclusion of field studies in the assessment of potential effects of the GMP on biogeochemical processes. The inclusion of field studies also complies with the step-by–step principle as outlined in Directive 2001/18/EC and a generally broad and holistic ERA approach integrating results from laboratory, glasshouse and field studies (Birch et al. 2007).

Take spatial heterogeneity (different soil types) into consideration

The inclusion of representative soil types may constitute a major challenge to risk assessors. However, similar requirements exist in other regulatory frameworks such as microbial PPPs (Directive 91/414/EEC). According to Directive 91/414/EEC information on several cultivated and uncultivated soils representative of soils typical of the various Community regions should be provided. Soil types should cover a range of organic carbon contents, particle size distributions and several pH ranges in case degradation is expected to be pH dependent. With respect to field studies soil conditions should be as close to normal agricultural practice as possible covering a range of soil types and climatic conditions representative of the area of use.

The importance of different soil conditions for the risk assessment is also evident for the ERA of GMPs. With respect to soils that will be exposed to GMPs in the EU relevant agricultural soil types should be chosen. EU-wide soil classification systems such as the European soil database or the map of Organic Carbon in Topsoils in Europe (Jones et al. 2004) may provide a useful basis to select relevant and representative soil conditions for GMP cultivation within Europe.

Develop criteria for the assessment of effects of the GMP on the abiotic environment

Risk assessment practice in the GMP notifications reviewed in this report does not consider effects of the GMP on the abiotic environment. Changes of the abiotic environment by the use of GMPs will depend largely on the introduced trait, and may be relevant for GMPs with altered tolerance of certain environmental conditions, such as climate, abiotic soil fractions or gases (EFSA 2006a). Although the level of assessment will have to be decided on a case-by-case basis, criteria are currently needed for the decision for which GMPs such an assessment will be relevant.

4.3.9 Assessment of effects related to land use and cultivation techniques

From an environmental point of view changes in land use or cultivation techniques when cultivating GMPs are of high relevance when the cultivation of the novel crop involves changes in pesticide or herbicide use or if the GMP itself produces some kind of pesticidal gene product (e.g. a *Bt* toxin). Changes in cultivation techniques, land use and/or pesticide applications are major driving forces for intensification in many cases affecting biodiversity in European agricultural landscapes (e.g. Krebs et al. 1999, Chamberlain et al. 2000, Robinson & Sutherland 2002) with GMHT crop adoption probably further contributing to adverse impacts of agriculture to biodiversity (Butler et al. 2007). In this context the pre-release assessment of a GMP should concern in particular the poten-

tial to use a herbicide more frequently or at other times during the growing season, thus affecting different weed species, controlling weeds more effectively and consequently affecting non-target organisms which depend on these weed species during any of their life stages. Thus the description of the current baseline and an evaluation of identifiable changes when growing a GMP can give a first insight into future developments of agriculturally used areas.

Include information derived from the national assessments of the relevant PPP to be used with the GMHT crop

According to Directive 91/414/EEC, Annex III, requirements exist for the provision of information on a range of aspects to be covered in the assessment of a PPP at a national level. These aspects are also of relevance for the environmental risk assessment of a GM crop according to Directive 2001/18/EC. The information included in the national assessments of the PPP should provide the basis for the evaluation of a GMHT crop when potential effects on cultivation and management techniques according to Directive 2001/18/EC are to be assessed:

Details on the **application** of a PPP on a GMHT crop such as application rate, number and timing of applications as well as proposed instructions for use developed for farmers and printed on labels and leaflets should be included. An assessment of the time, amount and frequency of herbicide applications in the GMHT crop compared to the non-GM crop in a specific agronomic context is necessary for the assessment of effects of the management technique of a GMHT crop compared to conventional crops.

Information on **metabolites** of the PPP and their potential ecotoxicological concern should be included which is relevant for an assessment of potential effects of newly formed metabolites on non-target organisms of GMPs. Depending on the time of application and the dosage of the PPP, newly formed metabolites or residues in the GMHT crops may be different from those anticipated in existing registrations of the non-selective herbicide used in conventional, non-GM crops.

Data on the **efficacy** of the PPP should be included. The efficacy evaluation is a central aspect in the assessment of a PPP at national level. Efficacy data have to be provided to permit an evaluation of the nature and extent of benefits of the PPP in comparison to suitable reference products and damage thresholds and to define its conditions of use. It comprises the following information:

- Information on **direct efficacy** (effectiveness). For GMHT crops data on the effectiveness of the herbicide can be used to evaluate potential effects of the GMHT crop on target and non-target organisms (weeds).
- Information on **resistance risk** composed of laboratory data and, if existent, also field information. Resistance development of target organisms has also to be considered in the ERA of GMHT crops. Information provided should also include the potential for the development of multi-resistance of weed species and the respective consequences.
- Information on the absence of **unacceptable effects** such as effects of the PPP on yield and quality, potential phytotoxicity to target plants, succeeding or adjacent crops as well as observations on unintended side-effects, e.g. on beneficial and other non-target or-

ganisms, especially effects on wildlife and beneficial organisms. This information may give an indication on indirect effects of the management practice of the GMHT crop.

- If included in national assessments, information on **direct effects of the herbicide** on biodiversity off the crop field, e.g. in neighbouring areas, field margins, etc. (e.g. spray drift).

Information submitted to national registration authorities including data from field trials for a given product use should be available also to the Authorities under Directive 2001/18/EC when notifying a GMHT crop either under this Directive or according to Regulation (EC) 1829/2003 in case cultivation of this crop is envisaged.

Conduct additional assessments of indirect effects of the GMHT crop on biodiversity

Indirect effects of the herbicide use on the GMHT crop on biodiversity in the crop field (e.g. the loss of weed species, the loss of herbivores feeding on weed species, seed eating species etc.) are generally not assessed in national assessments of the PPP. As the British Farm Scale Evaluations have shown, indirect effects of the herbicide use can be relevant for certain GM crop/GM trait combinations (see chapter 3.3.9). Hence, it is crucial to include information on:

- Weed management in conventional crops and potential changes in the weed management of GMHT crops as well as potential consequences of such changes on the reproductive success of the weeds (e.g. seed production).
- The ecology of weed-associated fauna, e.g. the relevance of weeds as host-plants for arthropods, trophic binding of arthropods to specific weed species etc.

Assess the potential of the GM trait conferring herbicide tolerance to be transferred to other plant species

The transfer of a herbicide tolerance trait to volunteer plants or related weed species of the GMP may create agricultural management problems (Hall et al. 2000). This risk is special to GM crops and should be separately considered in the ERA (see also chapter 3.3.5).

Choose a relevant baseline for the assessment of potential effects of the management and cultivation techniques of the GMHT crop

Potential changes in the agricultural practice, the soil management strategy or use pattern (e.g. crop rotation) are the basis for the assessment of environmental effects of GMHT crops. In conventional oilseed rape cultivation pre-sowing and pre-emergence herbicides are dominating while in conventional maize cultivation also post-emergence applications are relevant (see references in Hilbeck et al. 2008c). The introduction of GMHT varieties will give farmers the opportunity to use herbicides more frequently or even exclusively on emerged crops with higher efficiency as weeds are controlled which are not or poorly controlled in conventional crops (see also Devos et al. 2008). As the British Farm-scale Evaluations have shown, in herbicide-tolerant GM crops the choice of the comparator, i.e. the conventional herbicide applied to the non-GM crop, as well as the time of application of a herbicide is crucial for the evaluation of the effect of a particular crop management system on weed communities (Heard et al. 2003a, 2003b, 2003c, Perry et al. 2004).

Baselines to be included should thus comprise current conventional and/or organic crop management systems. Their effects can then be compared to predicted or recommended GMHT management systems. When testing effectiveness of a PPP normally trials are conducted to evaluate level, duration and consistency of control and the intended effects of the PPP. Hence, a suitable reference product must be used where this exists (Dir. 91/414/EEC, Annex III). Efficacy must be tested under conditions where the target harmful organism has been shown to have been present at a level causing an adverse effect on an unprotected crop. Thus, additionally to the test product and the reference product, an untreated control is to be included.

The information provided in the national assessments of a PPP (see above) refers to the management technique of the new herbicide on a GMHT crop. Recommended applications of the herbicide for GMHT crops will be those indicated as the application rate, concentration of active substance in the material used, the method of application, as well as the maximum number and timing of applications. The recommended application is specified during the national authorization of the PPP. For instance, for the use of glyphosate in herbicide-tolerant maize at maximum one to two post-emergence applications until the six or eight leaf-stage of maize are allowed with a maximum of six weeks in between the applications (**www.bvl.bund.de**).

Consider different environments in the assessment of potential environmental effects of the management and cultivation techniques of the GMHT crop

As weed communities differ significantly between continents, countries or even agricultural regions, an assessment should consider environmental differences and include weed species of national or regional conservation concern.

In the context of the efficacy assessment of a PPP during the national registration the range of conditions including the variability in plant health conditions, climatic differences, the ranges of agricultural practices, the application mode and the types of harmful organisms have to be considered (Directive 91/414/EEC, Annex III, point 6). The number of trials to be conducted and reported must reflect these differences. In addition, data have to be provided from regions and the range of conditions for which the use of the PPP is recommended. Also seasonal differences have to be addressed to confirm the performance of the PPP in each agronomically and climatically different region for a particular crop / harmful organism combination. Normally, at least two growing seasons should be chosen for effectiveness evaluations (Directive 91/414/EEC, Annex III).

The EPPO Guideline Nr. 223 on the efficacy evaluation of plant protection products specifies that trials should be conducted in locations which represent the range of agricultural, plant health and environmental conditions including climatic conditions, likely to be encountered in practice in the area of proposed use. With respect to climatic conditions EPPO has issued a guidance on comparable climates (EPPO Standard Nr. 241), defining 4 zones: the Mediterranean, the Maritime, the North-East and the South-East zone. However, EPPO emphasizes that other factors than climate will have to be considered, such as edaphic conditions (e.g. for soil applied products), agronomic conditions (e.g. crop rotation, etc.) and differences in the biology and pressure of target pests. In GMP notifications it should also be reported which environmental conditions were taken into consideration for the PPP registration of the non-selective herbicide used in the GMHT crop. The question whether the four zones proposed by EPPO will suffice for the evaluation of potential ad-

verse effects of the management regime of GMPs in different environments will need further evaluation and possibly further guidance.

4.3.10 Proposed risk management and monitoring

Monitoring of GMPs must fulfil the requirements as outlined by Directive 2001/18/EC – to trace and identify any effects on the environment of GMOs after they have been placed on the market - and to meet the objectives as stated in the Guidance Notes to Annex VII of the above mentioned Directive (EC 2002b). In order to fulfil the intention of Directive 2001/18/EC that any action should be based on the principle that preventive action is taken (Preamble, point 6), GMP monitoring shall serve as an early-warning system for adverse environmental effects when GMPs are placed on the market. Hence, GMP monitoring needs science-based concepts and models as well as a thorough implementation.

There is considerably discussion among stakeholders on how monitoring of GMPs shall be carried out. Major disagreements relate, for instance, to the necessity to monitor particular environmental risks if the conclusions of the ERA consider them negligible or if monitoring is only necessary in case adverse effects of a GMP were confirmed during the ERA. Also the distinction between CSM and GS is currently still under discussion. Areas have been identified which fall between CSM and GS (ACRE 2004) and there is also considerable dispute on the responsibilities assigned for each ‘type’ of monitoring.

These and other problems were also addressed at EU level by the “EU Monitoring Working Group” established by the Competent Authorities which have elaborated several checklists for the monitoring of certain GM crops, focussing on environmental aspects of GMO monitoring (**http://ec.europa.eu/environment/biotechnolgoy/monitoring.htm**). In separate papers also coordination and harmonization of monitoring data at EU level and General Surveillance have been addressed by this group. The proposals outlined in these checklists may be used as guidance for the preparation and submission of monitoring plans for GMPs intended for cultivation.

Cleary distinguish monitoring from risk management

The monitoring plan constitutes an explicit and separate condition in any GMO notification (Directive 2001/18/EC). While the decision on risk management measures will depend on the risks concluded in the ERA, a monitoring plan is obligatory for any GMO notification. For example, in case of IR crops, an IRM plan may be proposed as a risk management strategy, covering the risk that target organisms develop resistance to the GMP. However, also other risks may be covered by specific risk management measures. This has been shown, for instance, in the notification of oilseed rape Ms8xRf3 where specific risks such as control cross-pollination, weeds and volunteers were intended to be covered under risk management (agricultural guidelines; see also chapter 2.11). The following recommendations are largely based on the legal requirements as outlined in Directive 2001/18/EC and the Guidance Notes to Annex VII (EC 2002b).

Consider shortcomings, limitations and uncertainties in the ERA when deciding on CSM

According to Directive 2001/18/EC the monitoring plan constitutes an explicit and separate condition in any GMO notification. While CSM may not be required, a plan for GS has in any case to be submitted. The question of whether CSM is applicable or not for a specific notification has there-

fore to be decided on a case-by-case basis. Methodological shortcomings as well as uncertainties in the ERA may result in the necessity to conduct CSM (Lövei & Arpaia 2005, Marvier 2002). This relates to:

- The inability to transfer the results from small scale or laboratory experiments to large-scale releases of GM crops.
- Inter- and intraspecies variations of effects.
- Spatial and timely differences in the exposure of organisms and processes, or cumulative exposures due to interactions of GMOs and other stressors in the field.
- Limitations in the experimental method itself such as flaws in the experimental design or complete data gaps.

Furthermore current ERA methods must cover a variety of issues which might emerge from the development and introduction of new GMPs. The increasing complexity due to the insertion of a variety of genes and a combination of genes lead to severe limitations in the ERA process (SSC 2000). Thus authorities may conclude that a certain risk has to be monitored by CSM even if the conclusions of the ERA indicate no or a negligible risk due to the ERA's inherent uncertainties and limitations. This is clearly recognized in the Risk Assessment guidelines of Directive 2001/18/EC (EC 2002a) stating that 'CSM serves to **confirm** that scientifically sound assumptions in the ERA regarding potential adverse effects arising from a GMO and its use are correct' as well as 'where the conclusion of the risk assessment identifies an absence of risk or negligible risk, however, then CSM **may not** be required'. These assumptions to be confirmed may also comprise the evidence of no risk, e.g. if uncertainty from the ERA has to be taken into account. Alternatively, the ERA and the specific formulations of risks in the ERA may have to be reconsidered.

For a decision whether CSM has to be applied or not, criteria such as the level of uncertainty, the amount and quality of data available for a specific risk evaluation, the level of release, the potential consequence of an adverse effect or the level of (ir)reversibility of an adverse effect may be taken into consideration.

In this context it has to be emphasized that an ERA may be re-addressed or updated in case new information on the GMO and its effects on human health or the environment become available, e.g. during GS, further studies or due to scientific progress related to a specific GMO. This could be the case for effects which were not expected to happen based on the outcome of the ERA. This could in fact mean that the assumptions made in the ERA were incorrect. Such effects could be due to:

- The detection of new characteristics of a GMO which cause a potential adverse effect (e.g. higher expression levels under certain conditions)
- The change of consequences of an adverse effect (e.g. higher expression levels are more toxic to non-target species)
- The change of the likelihood of occurrence of an adverse effect (e.g. outcrossing to a wild relative)
- The detection of completely new adverse effects

Consider CSM for GMHT plants if risks were not addressed in the ERA

The assessment of potential adverse effects of GMHT crops and their associated management technique on the weed community is currently not contained in GMP notifications. The reasons for this practice have been explored in depth in chapter 3.3.9. This practice is mainly due to the notifiers' views that weeds do not constitute target organisms of the GMHT plant and that the associated plant protection product is separately evaluated under other regulatory frameworks. However, it is currently unclear to what extent the assessment of effects of the specific management technique of GMHT crops is actually covered by the regulatory framework for the authorisation of the complementary, non-selective herbicide. Risks due to the application of non-selective herbicides, at least for some GM crops, cannot be considered as negligible since the publications of the results of the British Farm-Scale Evaluations. Weed communities may be affected by large-scale or long-term changes in herbicide regimes with effects on higher trophic levels such as seed-eating carabids or birds. Thus these assumptions must be confirmed by CSM. This is also in accordance with the view of the Spanish Competent Authority evaluating the ERA of maize NK603 which repeatedly requested the inclusion of the weed communities in the CSM of the respective monitoring plan.

Risk-analysis based methods to identify plant and insect indicators for the monitoring of HT oilseed rape or HT/*Bt* maize based on an event tree and fault tree analysis have been proposed (Meier & Hilbeck 2005). By the selection of relevant weed species, the consideration of their sensitivity towards non-selective herbicides, the strength of their association with certain biotope types and the strength of association of the insect species to these weed species, relevant Lepidoptera were identified dependent on a certain amount of weed species which are at high risk and were thus proposed for monitoring in GMHT maize in Germany (Hilbeck et al. 2008c).

Ensure monitoring of relevant aspects in GS

In the sense of the aims outlined by Directive 2001/18/EC – to identify non-anticipated effects on the environment – monitoring and in particular GS need to consider environmental aspects. GS plans primarily focussing on questionnaires and on the likely involvement of external networks or monitoring programs, will not suffice for detecting environmental effects of a particular GMP (see also chapter 3.3.10). When drafting a GS plan, as a first step the monitoring objects and parameters should be fixed and consequently also the methods by which these will be monitored, including monitoring frequency, area(s) and the length of the monitoring activity. This will ensure that those aspects will be covered by GS which are of highest relevance and that unexpected effects may be detected. There are concepts for how to select the monitoring objects and parameters for GMO monitoring which are useful starting points for GS (see for example Traxler et al. 2001).

Only after these decisions have been made, it may be concluded that certain monitoring tasks and activities can be shifted to existing monitoring programs or networks, if existent and appropriate. In this context the document on GS issued by EU member state experts of the EU Working Group on Monitoring (see **http://ec.europa.eu/environment/biotechnology/monitoring.htm**) may be helpful. In addition, standardizing of monitoring methods is particularly useful in order to achieve comparable results. Certain monitoring efforts have been standardized already at national level in Germany (see **www.vdi.de/gmo**) and standardisation at EU level is currently in progress.

Specify existing monitoring networks if included in the GS plan

If existing monitoring networks or programs are a component of the GS plan proposed by the notifier, then a thorough evaluation of these networks and programs in individual EU member states with regard to their availability and suitability should be carried out by notifiers. The notifier should identify and specify existing monitoring systems and networks in the individual EU member states which could be suitable for and compatible with GS. When evaluating existing programs the notifier should describe which criteria were used for the selection and evaluation of these programs. In this context the following criteria should be considered:

- Coverage of ecosystems relevant for GMP monitoring (agro-ecosystems, natural habitats, semi-natural habitats)
- Coverage of relevant media or indicators for GMO monitoring (soil, water, biotic indicators)
- Geographic area covered by the monitoring network/program (nationwide/regionally)
- Intervals / frequency of observations and total time period of the respective monitoring program(s)
- Availability of reference sites (baseline) to GMP monitoring sites
- Availability of / access to data from the network/program

The notifiers should consequently describe which of the existing monitoring programs or networks in the individual member states would actually fulfil the above-mentioned criteria for GS or could be subject to adaptation. Remaining gaps of GS which cannot be covered by existing programs then need to be identified and suggestions made how these gaps could be covered e.g. by additional surveys and monitoring activities.

5 OVERALL CONCLUSIONS AND RECOMMENDATIONS

The analysis of the GMO notifications has shown significant deficits and shortcomings in the environmental risk assessments as provided by the notifiers. The submitted data and studies were often not considered sufficient in order to carry out an ERA which satisfies scientific and technical standards and the requirements as outlined in Directive 2001/18/EC and its Annexes (EC 2002a, 2002b).

However, in many cases the submitted data were regarded as sufficient by the evaluating authority at EU level (EFSA GMO Panel). In the last years different member states have addressed data gaps and uncertainties in the ERAs provided by notifiers. This apparent discrepancy between the the EFSA, the European Commission and the individual member states with respect to the quality and quantity of data currently provided in the ERA of GMO notifications can only be resolved if a common understanding of the way how an ERA should be performed and which data should be included will be achieved. This will need standardization and harmonization efforts, however, without neglecting the case-by-case principle and the individual assessment of each individual GMO. However, this also implies strict scrutinity of the data submitted for ERAs with respect to the scientific, technical or legal requirements already during the completeness check of the dossiers. As a result notifications should only be open to comments and evaluation by member states if these basic requirements are fulfilled.

The recommendations as outlined in this chapter focus on both, general aspects of the ERA (concept, data generation and presentation) and on specific aspects related to individual assessments of potential environmental risks of GMOs. General improvements are particularly necessary with respect to the general provisions as specified in Directive 2001/18/EC, such as the consideration of the whole GM crop in the ERA which is of high relevance for all assessments, but in particular for the assessment of non-target effects. Such improvements also comprise the consideration of target organisms in GMHT plants. Another general provision which needs more focus is the consideration of the receiving environment of the respective GMP, which is a part of the ‘case-specificity’ and the ‘region-by-region’ principle in the ERA process. But also other basic principles of Directive 2001/18/EC such as step-by-step principle need further guidance with respect to its specific implementation in the individual assessments. Particular need for improvement and possibly guidance has been identified with respect to the conclusions drawn by notifiers. This holds in particular for the assessment of environmental risks and the interpretation of results gained from testing and its consequences for the resulting conclusions for the risk management strategy and monitoring efforts. Also the handling of uncertainty as such in the ERA needs more focus. Development of guidance is further needed for the consideration of long-term and cumulative effects.

Specific recommendations for individual assessments have been made across all assessment categories. While standardization is certainly possible for certain categories and different GM crop species, other recommendations cannot result in final standardized prescriptions but need to follow a common, scientific procedure and are more prone to an individual case-specific approach. Recommendations for the improvement of the ERA need to account for a balance between standardization of assessments and keep hold of the case-by-case approach. The case-by-case principle

must not be overruled by across-GMO generalisation leaving case-specific factors and effects unconsidered. However, as shown in this report, a common and improved basis with respect to the underlying scientific models and approaches in the ERA as well as form and content of the ERA, is urgently needed.

6 REFERENCES

ABEL, C. A. & ADAMCZYK, J. J., JR. (2004): Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) on maize whorl leaf profiles. J Econ Entomol 97: 1737-1744.

ABSTC (2002): Field surveys of Non-target Invertebrate Populations in Bt Maize. Report submitted March 14, 2002. Author: Agricultural Biotechnology Stewardship Technical Committee – Non-Target Organism Subcommittee. MRID No. 45652001.

ACCINELLI, C.; SCREPANI, C.; VICARI, A. & CATIZONE, P. (2004): Influence of insecticidal toxins from Bacillus thuringiensis subsp. kurstaki on the degradation of glyphosate and glufosinate-ammonium in soil samples. Agriculture, Ecosystems and Environment 103: 497-507.

ACRE (2004): Guidance on Best Practice in the design of post-market monitoring plans in submissions to the Advisory Committee on Releases to the Environment. Guidance for Applicants seeking permission to release genetically modified crops into the environment (under Directive 2001/18/EC). ACRE Guidance Note 16. Department for Environment, Food and Rural Affairs, London, pp 21. **www.defra.gov.uk/environment/acre/pubs.htm**

AGES (2002): Sorten und Saatgutblatt 2002. Methoden für Saatgut und Sorten gemäß §5 Abs. 1 Saatgutgesetz 1997, BGBl. I Nr. 72 zgd. BGBl. I Nr. 109/2001. Richtlinien für die Sortenprüfung. In: Schriftenreihe 59/2002 des BFL, 10. Jahrgang, Sondernummer 13.

ANDOW, D. A. & HILBECK, A. (2004): Science-based risk assessment for non-target effects of transgenic crops. BioScience 54: 637-649.

ANDOW, D. A.; SOMERS, D. A.; AMUGUNE, N.; ARAGAO, F. J. L.; GOSH, K.; GUDU, S.; MAGIRI, E.; MOAR, W. J.; NJIHIA, S. & OSIR, E. (2004): Transgene locus structure and expression of *Bt* maize. In: Environmental Risk Assessment of Genetically Modified Organisms; Hilbeck A. & D. Andow (Eds); Volume 1: A cases study in Kenya;.CAB International, UK, p 83-116.

ANDOW, D. A. & ZWAHLEN, C. (2006): Assessing environmental risks of transgenic plants. Ecology Letters 9: 196-214.

ANDOW, D. A.; LÖVEI, G. L. & ARPAIA, S. (2006): Ecological risk assessment for Bt crops. Nature Biotechnology 24: 749-751.

ANDOW, D. A.; HILBECK, A. & NGUYEN, V. T. (2007): Environmental Risk Assessment of Genetically Modified Organisms. CAB International, Wallingford, UK.

ANDOW, D. A.; HILBECK, A.; NGUYEN VAN TUAT (eds.) (2008): Environmental Risk Assessment of Genetically Modified Organisms: Challenges and Opportunities with Bt Cotton in Vietnam. Volume 4, CAB International, Wallingford, UK.

ARCHER, T. L.; SCHUSTER, G.; PATRICK, C.; CRONHOLM, G.; BYNUM, E. D. & MORRISON, W. P. (2000): Whorl and stalk damage by European and Southwestern corn borers to four events of Bacillus thuringiensis transgenic maize. Crop Protection 19: 190-

ARCHER, T. L.; PATRICK, C.; SCHUSTER, G.; CRONHOLM, G.; BYNUM, E. D. & MORRISON, W. P. (2001): Ear and shank damage by corn borers and corn earworms to four events of Bacillus thuringiensis transgenic maize. Crop Protection 20: 139-144.

ASTOIN, M.F.; CHAMPOLIVIER, J.; MESSEAN A. (2000): Evaluation des impacts du flux de transgènes de tolerance à differents herbicides à large spectre, OCL, Vol 7 (4), July/August 2000, 345-349. (French; English Summary)

BAKER, H.G. (1974): The evolution of weeds. Ann. Rev. Ecol, Systematics, 5, pp.1-24.

BARTEL, A.; SCHWARZL, B.; DVORAK, M.; FRÜHAUF, J. & BIRDLIFE ÖSTERREICH (2008): Agrar - Umweltindikator "High Nature Value Farmland". Verifizierung der Gebietskulisse für Österreich. Forschungsbericht im Auftrag des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft (BMLFUW) Wien. 118 S.

References

BAUMGARTE, S. & TEBBE, C. C. (2005): Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol.Ecol. 14: 2539-2551.

BERENBAUM, M. R. (1995): Turnabout is fair play: Secondary roles for primary compounds. Journal of Chemical Ecology 21: 925-940.

Bily, A. C.; Reid, L. M.; Taylor J. H.; Johnston, D.; Malouin, C.; Burt, A. J.; Bakan, B.; Regnault-Roger, C.; Pauls, K. P.; Arnason, J. T. & Philogène, B. J. R. (2003): Dehydrodimers of ferulic acid in maize grain pericarp and aleurone: resistence factors to *Fusarium graminearum*. Phytopathology 93 (6): 712-719.

BINNING, R. R. & RICE, M. E. (2002): Effects of transgenic Bt corn on growth and development of the stalk borer Papaipema nebris (Lepidoptera: Noctuidae). Journal of Economic Entomology 95: 622-627.

BIRCH, A. N. E.; GEOGHEGAN, I. E.; GRIFFITHS, D. W. & MCNICOL, J. W. (2002): The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solanum tuberosum*). Annals of Applied Biology 140: 143-149.

BIRCH, A. N. E.; WHEATLEY, R.; ANYANGO, B.; ARPAIA, S.; CAPALBO, D.; GETU DEGAGA, E.; FONTES, E.; KALAMA, P.; LELMEN, E.; LÖVEI, G. L.; MELO, I. S.; MUYEKHO, F.; NGI-SONG, A.; OCHIENDO, D.; OGWANG, J.; PITELLI, R.; SÉTAMOU, M.; SITHANANTHAM, S.; SMITH, J.; VAN VON, N.; SONGA, J.; SUJII, E.; QUANG TAN, T.; WAN, F.-H. & HILBECK, A. (2004): Biodiversity and non-target impacts: A case study of Bt-maize in Kenya. In: Environmental Risk Assessment of Genetically Modified Organisms; Hilbeck A. & D. Andow (eds); Volume 1: A Case Study of Bt Maize in Kenya: CAB International, Wallingford, UK, p 117-185

BIRCH, A. N. E.; GRIFFITH, B. S.; CAUL, S.; THOMPSON, J.; HECKMANN, L.-H.; KROGH, P. H. & CORTET, J. (2007): The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project. Pedobiologia 51: 251-260.

BLANCO-LABRA, A.; CHAGOLLA-LOPEZ, A.; MARTINEZ-GALLARDO, N. & VALDES-RODRIGUES, S. (1995): Further characterization of the 12 kDa protease/alpha amylase inhibitor present in maize seeds. Journal of Food Biochemistry 19: 27-41.

BODET, J.M.; STRAEBLER, M.; BROUCQSAULT, L.M. (1994): Type de jachère et couvert Receuil des communications du colloque „Jachères 94“, p.19-41.

BOGOMOLOV, D. M.; CHEN, S.-K.; PARMELEE, R. W.; SUBLER, S. & EDWARDS, C. A. (1996). An ecosystem approach to soil toxicity testing: a study of copper contamination in laboratory soil microcosms. Applied Soil Ecology 4 (2): 95-105.

BRODERICK, N. A.; RAFFA, K. F. & HANDELSMAN, J. (2006): Midgut bacteria required for Bacillus thuringiensis insecticidal activity. Proceedings of the National Academy of Sciences 103: 15196-15199.

BROOKS, D. R.; BOHAN, D. A.; CHAMPION, G. T.; HAUGHTON, A. J.; HAWES, C.; HEARD, M. S.; CLARK, S. J.; DEWAR, A. M.; FIRBANK, L. G.; PERRY, J. N.; ROTHERY, P.; SCOTT, R. J.; WOIWOD, I. P.; BIRCHALL, C.; SKELLERN, M. P.; WALKER, J. H.; BAKER, P.; BELL, D.; BROWNE, E. L.; DEWAR, A. J.; FAIRFAX, C. M.; GARNER, B. H.; HAYLOCK, L. A.; HORNE, S. L.; HULMES, S. E.; MASON, N. S.; NORTON, L. R.; NUTTALL, P.; RANDLE, Z.; ROSSALL, M. J.; SANDS, R. J.; SINGER, E. J. & WALKER, M. J. (2003): Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. Philos.Trans.R.Soc.Lond B Biol Sci 358: 1847-1862.

BRUCK, D. J.; LOPEZ, M. D.; LEWIS, L. C.; PRASIFKA, J. R. & GUNNARSON, R. D. (2006): Effects of transgenic Bacillus thuringiensis corn and permethrin on nontarget arthropods. Journal of Agricultural and Urban Entomology 23: 111-124.

BRUINSMA, M.; KOWALCHUK, G. A. & VAN VEEN, J. A. (2003): Effects of genetically modified plants on microbial communities and processes in soil. Biol.Fertil.Soils 37: 329-337.

BRUNS, H. A. & ABEL, C. A. (2003): Nitrogen fertility effects on Bt delta-endotoxin and nitrogen concentrations of maize during early growth. Agronomy Journal 95: 207-211.

References

BRUNS, H. A. & ABEL, C. A. (2007): Effects of nitrogen fertility on Bt endotoxin levels in maize. Journal of Entomological Sciences 42: 35-43.

BRUSETTI, L.; FRANCIA, P.; BERTOLINI, C.; PAGLIUCA, A.; BORIN, S.; SORLINI, C.; ABRUZZESE, A.; SACCHI, G.; VITI, C.; GIOVANETTI, L.; GIUNTINI, E.; BAZZICALUPO, M. & DAFFONCHIO, D. (2004): Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (Zea mays) and its non transgenic counterpart. Plant & Soil 166 (1-2): 11-21.

BSA (2000): Richtlinien zur Durchführung von landwirtschaftlichen Wertprüfungen und Sortenversuchen. Bundessortenamt; **http://www.bundessortenamt.de/internet30/fileadmin/Files/PDF/Richtlinie_LW2000.pdf**

BUNDESAMT FÜR NATURSCHUTZ (2007): Das Step-by-Step Verfahren: Anforderungen an ein gestuftes Konzept zur ökologischen Begleitforschung im Rahmen von Labor-, Gewächshausversuchen sowie von GVO-Freisetzungen als Grundlage für eine spätere Marktzulassung. Laufendes Forschungsvorhaben FKZ. 806 84 020 http://www.bfn.de/4261.html

BUTLER, S. J.; VICKERY, J. A. & NORRIS K (2007): Farmland biodiversity and the footprint of agriculture. Science 315: 381-383.

BÜHLER, C. (2006): Biodiversity Monitoring in Switzerland: What can we learn for general surveillance of GM crops? Journal für Verbraucherschutz und Lebensmittelsicherheit 1: 37-41.

CANADIAN FOOD INSPECTION AGENCY (1994): Regulatory Directive 94-11: The Biology of *Zea mays* L. (Corn/Maize). CFIA, Variety Section, Plant Health and Production Division, Plant Biotechnology Office, Ottawa.

Capalbo, D. M. F.; Simon, M. F., Nodari, R. O.; Valle, S.; dos Santos, R. F.; Coradin, L.; Duarte, J. de O.; Miranda, J. E.; Dias, E. P. F.; Le Quang Quyen; Underwood, E. & Nelson, K. C. (2006). In: Hilbeck, A. and D. A. Andow (eds.). Environmental Risk Assessment of Genetically Modified Organisms, Volume 2: Methodologies for Assessing Bt Cotton in Brazil. CAB International, Wallingford, UK. pp 57-82.

CARRIÈRE, Y.; ELLERS-KIRK, CH.; BIGGS, R.; HIGGINSON, D. M.; DENNEHY, T. J. & TABASHNIK, B. E. (2004). Effects of gossypol on fitness costs associated with resistance to Bt cotton in pink bollworm. Journal of Economic Entomology 97 (5): 1710-1718.

CHAMBERLAIN, D. E.; FULLER, R. J.; BUNCE, R. G. H.; DUCKWORTH, J. C. & SHRUBB, M. (2000): Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. Journal of Applied Ecology 37: 771-788.

CHEN, M.-S.; FENG, G.; ZEN, K. C.; RICHARDSON, M.; VALDES-RODRIGUES, S.; REECK, G. R. & KRAMER, K. J. (1992): Alpha-amylases from three species of stored grain Coleoptera and their inhbition by wheat and corn prteinaceous inhibitors. Insect Biochemistry and Molecular Biology 22: 261-268.

CHEN, Z.-Y.; BROWN, S. L.; LAX, A. R.; CLEVELAND, T. E. & RUSSIN, J. S. (1999): Inhibition of plant-pathogenic fungi by a corn trypsin inhibitor overexpressed in Escherichia coli. Applied and Environmental Microbiology 65: 1320-1324.

CHEVRE, A. M.; EBER, F.; DARMENCY, H.; FLEURY, A.; PICAULT, H.; LETANNEUR, J. C. & RENARD, M. (2000): Assessment of interspecific hybridisation between transgenic oilseed rape and wild radish under normal agronomic conditions. Theoretically and Applied Genetics 100: 1233-1239.

CHILCOTT, C. N. & ELLAR, D. J. (1988): Comparative toxicity of Bt var. israelensis crystal proteins in vivo and in vitro. Journal of General Microbiology 134: 2551-2558.

CLAESSEN, D.; GILLIGAN, C. A.; LUTMAN, P. J. W. & VAN DEN BOSCH, F. (2005a): Which traits pormote persistence of feral GM crops? Part 1: Implications of environmental stochasticity. Oikos 110: 20-29.

CLAESSEN, D.; GILLIGAN, C. A. & VAN DEN BOSCH, F. (2005b): Which traits promote persistence of feral GM crops? Part 2: Implications of metapopulation structure. Oikos 110: 30-42.

CLARK, B. W.; PHILLIPS, T. A. & COATS, J. R. (2005): Environmental fate and effects of Bacillus thuringiensis (Bt) proteins from transgenic crops: a review. Journal of Agricultural and Food Chemistry 53 (12): 4643-4653.

References

CODEX ALIMENTARIUS COMMISSION (2001): Report of the Seventeenth Session of Codex Committee on Fats and Oils (ALINORM 01/17) 2001, pp. 26, United Kingdom

COLLONIER, C.; BERTHIERM, G.; BOYER, F.; DUPLAN, M. N.; FERNANDEZ, S.; KEBDANI, N.; KOBILINSKY, A.; ROMANUK, M. & BERTHEAU, Y. (2003): Characterization of commercial GMO inserts: a source of useful material to study genome fluidity. Poster presented at VIIth International Congress for Plant Molecular Biology, Barcelona, 23-28th June 2003.

CONNER, A. J.; GLARE, T. R. & NAP, J.-P. (2003): The release of genetically modified crops into the environment. The Plant Journal 33: 19-46.

CORBET, S.A.; CUTHILL, I.; FALLWOS, M.; HARRISON, T.; HARTLEY, G. (1981): Why do Nectar-Forgaging Bees and Wasps Work Upwards on Inflorescenses? Oecologia (Berl), 51, 79-83. (English)

COVIELLA, C. E.; MORGAN, D. J. W. & TRUMBLE, J. T. (2000): Interactions of elevated CO2 and nitrogen fertilization: Effects on production of Bacillus thuringiensis toxins in transgenic plants. Environmental Entomology 29: 781-787.

CRAIG, W.F. (1977): Production of hybrid corn seed. Pp.671-719. In: Corn and Corn Improvement, Second Edition. G.F. Sprague, ed. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Madision, Wisconsin (U.S.A.)

CRAIG, W.; TEPFER, M.; DEGRASSI, G. & D. RIPANDELLI (2008): An overview of general features of risk assessments of genetically modified crops. Euphytica DOI 10.1007/s10681-007-9643-8.

CRAWLEY, M. J. & BROWN, S. L. (2004): Spatially structured population dynamics in feral oilseed rape. Proceedings of the Royal Society London B 271: 1909-1916-

CRESPO, A. L. B.; SPENCER, T. A.; NEKL, E.; PUSZTAI-CAREY, M.; MOAR, W. J. & BLAIR, D. S. (2008): Comparison and validation of methods to quantify Cry1Ab toxin. Applied and Environmental Microbiology 74: 130-135.

CROON, K.A.; SANDERS, P.R.; KECK P.J.; LEE, T.C. PARKER, G.B. (1995): Petition for Determination of Nonregulated Status: Insect-Protected Corn (*Zea mays* L.) with the cry1Ab Gene from *Bacillus thuringiensis* subsp. kurstaki. USDA, No. 95-093-01P.

DARMENCY, H. (2002): Transgenic herbicide-resistant crops: What makes the difference? In: Ecological Impact of GMO dissemination in agro-ecosystems. International OECD Workshop, Eds: Lelley, T.; Balázs, E. & M. Tepfer; Grossrussbach, Austria, 27-28 September 2002.

DEVOS, Y.; COUGNON, M.; VERGUCHT, S.; BULCKE, R.; HAESAERT, G.; STEURBAUT, W. & REHEUL, D. (2008): Environmental impact of herbicide regimes used with genetically modified herbicide-resistant maize. Transgenic Res. DOI 10.1007/s11248-008-9181-8.

D`HERTEFELD, T. (2008): Long-term persistence of GM oilseed rape in the seedbank. Biology Letters

DIMAS, S. (2006). Co-existence of genetically modified, conventional and organic crops: Freedom of choice. Conference on GMO Co-existence. Vienna, 5 April 2006, SPEECH/06/224.

DEMAAGD, R.; WEEMEN-HENDRIKS, M.; MOLTHOFF, J. W. & NAIMOV, S. (2003): Activity of wild-type and hybrid Bacillus thuringiensis d-endotoxins against Agrotis ipsilon. Archives of Microbiology 179: 367

DOLEZEL, M.; HEISSENBERGER, A. & GAUGITSCH, H. (2005): Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Bundesministerium für Gesundheit und Frauen, Forschungsberichte der Sektion IV Band 6: pp 64.

DONEGAN, K. K.; PALM, C. J.; FIELAND, V. J.; PORTEOUS, L. A.; GANIO, L. M.; SCHALLER, D. L.; BUCAO, L. Q. & SEIDLER, R. J. (1995): Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the Bacillus thuringiensis var. kurstaki endotoxin. Applied Soil Ecology 2 (2): 111-124.

DOUVILLE, M.; GAGNÉ, F.; MCKAY, J.; MASSON, L. & BLAISE, C. (2005): Tracking the source of Bacillus thuringiensis Cry1Ab toxin in the environment. Biochem.Syst.Ecol. 33: 219-222.

References

DOUVILLE, M.; GAGNÉ, F.; BLAISE, C. & ANDRÉ, C. (2006): Occurrence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Safety 66: 195-203.

DUBELMAN, S.; AYDEN, B. R.; BADER, B. M.; BROWN, C. R.; JIANG, CH. & VLACHOS, D. (2005): Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environmental Entomology 34 (4): 915-921.

DUTTON, A.; OBRIST, L.; D`ALESSANDRO, M.; DIENER, L.; MÜLLER, M.; ROMEIS, J. & BIGLER, F. (2004a): Tracking Bt-toxin in transgenic maize to assess the risks on non-target arthropods. 27 (3): 57-63.IOBC/WPRS Working Group "GMOs in Integrated Production",

DUTTON, A.; D`ALESSANDRO, M.; ROMEIS, J. & BIGLER F. (2004b): Assessing expression of Bt-toxin (cry1Ab) in transgenic maize under different environmental conditions. 27 (3): 49-55.IOBC/WPRS Working Group "GMOs in Integrated Production",

DUTTON, A.; ROMEIS, J. & BIGLER F. (2005): Effects of Bt maize expressing Cry1Ab and Bt spray on Spodoptera littoralis. Entomologia Experimentalis et Applicata 114: 161-169.

EEA (2001): Late lessions from early warnings: the precautionary principle 1896-2000. Environmental issue report No 22. European Environment Agency, Luxembourg: Office for Official Publications of the European Communities 2001, pp 210.

EPA (1998): Guidelines for Ecological Risk Assessment; Published on May 14, 1998, Federal Register 63(93):26846-26924; US Environmental Protection Agency. EPA/630/R-95/002F; April 1998, pp 188.

ESSL, F.; EGGER, G.; POPPE, M.; RIPPEL-KATZMAIER, I.; STAUDINGER, M.; MUHAR, S.; UNTERLERCHER, M.; MICHOR, K. (2008): Rote Liste der gefährdeten Biotoptypen Österreichs. Binnengewässer, Gewässer- und Ufervegetation. Technische Biotoptypen und Siedlungsbiotoptypen. Umweltbundesamt Wien, Report Nr. 0134.

ETC/BD (2006): Indicative Map of European Biogeographical Regions: Methodology and Development. European Topic Centre on Biological Diversity under contract with the European Environment Agency.

FARIA, C. A.; WACKERS, F. L.; PRITCHARD, J.; BARRETT, D. A. & TURLINGS, T. C. J. (2007): High Susceptibility of Bt maize to aphids enhances the performanceof parasitoids of lepidopteran pests. PLOS online 7: 1-11.

FAO/WHO (2001): Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. 22 – 25 January 2001, FAO, Rome, Italy, pp 29. **http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf**.

FINKE, C.; MÖLLER, K.; SCHLINK, S.; GEROWITT, B., ISSELSTEIN, J. (1999): The environmental impact of maize cultivation in the European Union: practical options for the improvement of the enviroenmental impact. Case study Germany. Research Centre for Agriculture and Environment. Göttingen. Germany

FLORES, S.; SAXENA, D. & STOTZKY, G. (2005): Transgenic Bt plants decompose less in soil than non-Btplants. Soil Biology & Biochemistry 37: 1073–1082.

GARCIA-ALONSO, M.; JACOBS, E.; RAYBOULD, A. F.; NICKSON, T.; SOWIG, P.; WILLEKENS, H.; VAN DER KOUWE, P.; LAYTON, R.; AMIJEE, F.; FUENTES, A. M. & TENCALLA, F. (2006): A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environmental Biosafety Research 5: 57-65.

GIAMOUSTARIS, A. & MITHEN, R. (1997): Glucosinolates and disease resistance in oilseed rape. Plant Pathology 46: 271-275.

GLARE, T.R.; O'CALLAGHAN, M. (2000): Bacillus thuringiensis: Biology, Ecology and Safety. New York: John Wiley and Sons.

GREENPEACE (2007): Wieviel Gift ist im Gen-Mais, wie wirkt es und wie wird es gemessen? [How much poison is in GE maize, what is its impact and how is it measured?] **www.greenpeace.de**.

References

GRIFFITHS, B. S.; CAUL, S.; THOMPSON, J.; BIRCH, A. N. E.; SCRIMGEOUR, CH.; CORTET, J.; FOGGO, A.; HACKETT, C. A. & KROGH, P. H. (2006): Soil microbial and faunal community responses to Bt maize and insecticide in two soils. J. Environ. Qual. 35: 734-741.

GRIFFITHS, B. S.; HECKMANN, L.-H.; CAUL, S.; THOMPSON, J.; SCRIMGEOUR, CH. & KROGH, P. H. (2007): Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. Plant Biotechnology Journal 5: 60-68.

GUERRIERI, E.; LINGUA, G.; DIGILIO, M. C.; MASSA, N. & BERTA, G. (2004): Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? Ecological Entomology 29: 753-756.

HAIDER, M. Z.; KNOWLES, B. H. & ELLAR, D. J. (1986): Specificity of Bacillus thuringiensis var. colmeri insecticidal δ-endotoxin is determined by differential proteolytic processing of the protoxin by larval gut proteases. European Journal of Biochemistry 156 (3): 531-540.

HAILS, R. S. (2002): Assessing the risks associated with new agricultural practices. Nature 418: 685-688.

HALL, L.; TOPINKA, K.; HUFFMANN, J.; DAVIS, L. & ALLEN, A. (2000): Pollen flow between herbicide-resistant Brassica napus is the cause of multiple-resistant B. napus volunteers. Weed Science 48 (6): 688-694.

HARTMAN, TH. (1996): Diversity and variability of plant secondary metabolism: a mechanistic view. Entomologia Experimentalis et Applicata 80: 177-188.

HARWOOD, J. D.; WALLIN, W. G. & OBRYCKI, J. J. (2005): Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol.Ecol. 14: 2815-2823.

HARWOOD, J. D.; SAMSON, R. A. & OBRYCKI, J. J. (2007): Temportal detection of Cry1Ab-endotoxins in coccinellid predators from fields of *Bacillus thuringiensis* corn. Bulletin of Entomological Research 97: 643-648.

HAUGHTON, A. J.; CHAMPION, G. T.; HAWES, C.; HEARD, M. S.; BROOKS, D. R.; BOHAN, D. A.; CLARK, S. J.; DEWAR, A. M.; FIRBANK, L. G.; OSBORNE, J. L.; PERRY, J. N.; ROTHERY, P.; ROY, D. B.; SCOTT, R. J.; WOIWOD, I. P.; BIRCHALL, C.; SKELLERN, M. P.; WALKER, J. H.; BAKER, P.; BROWNE, E. L.; DEWAR, A. J.; GARNER, B. H.; HAYLOCK, L. A.; HORNE, S. L.; MASON, N. S.; SANDS, R. J. & WALKER, M. J. (2003): Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. Philos.Trans.R.Soc.Lond B Biol Sci 358: 1863-1877.

HAWES, C.; HAUGHTON, A. J.; OSBORNE, J. L.; ROY, D. B.; CLARK, S. J.; PERRY, J. N.; ROTHERY, P.; BOHAN, D. A.; BROOKS, D. R.; CHAMPION, G. T.; DEWAR, A. M.; HEARD, M. S.; WOIWOD, I. P.; DANIELS, R. E.; YOUNG, M. W.; PARISH, A. M.; SCOTT, R. J.; FIRBANK, L. G. & SQUIRE, G. R. (2003): Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philos.Trans.R.Soc.Lond B Biol Sci 358: 1899-1913.

HAYES, K. R. (1998): Ecological risk assessment for ballast water introductions: A suggested approach. ICES Journal of Marine Science 55, 201–212.

HAYES, K. R. (2003): Robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for Genetically modified Organisms. **www.deh.gov.au/settlements/publications/biotechnology/pubs/summary-report.pdf**

HEAD, G.; BROWN, C.R.; GROTH, M.E.; DUAN, J.J. (2001) Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol Exp Appl 99:37–45

HEARD, M. S.; HAWES, C.; CHAMPION, G. T.; CLARK, S. J.; FIRBANK, L. G.; HAUGHTON, A. J.; PARISH, A. M.; PERRY, J. N.; ROTHERY, P.; ROY, D. B.; SCOTT, R. J.; SKELLERN, M. P.; SQUIRE, G. R. & HILL, M. O. (2003a): Weeds in fields with contrasting conventional and genetically modified herbicide-toerlant crops. II: Effects on individual species. Philosophical Transactions of the Royal Society of London B Biological Sciences 358: 1833-1846.

References

HEARD, M. S.; HAWES, C.; CHAMPION, G. T.; CLARK, S. J.; FIRBANK, L. G.; HAUGHTON, A. J.; PARISH, A. M.; PERRY, J. N.; ROTHERY, P.; SCOTT, R. J.; SKELLERN, M. P.; SQUIRE, G. R. & HILL, M. O. (2003b): Non-crop plants in fields with contrasting conventional and genetically modified herbicide-tolerant crops. I Main effects of treatments. Philos.Trans.R.Soc.Lond B Biol Sci 358: 1819-1832.

HEARD, M. S.; HAWES, C.; CHAMPION, G. T.; CLARK, S. J.; FIRBANK, L. G.; HAUGHTON, A. J.; PARISH, A. M.; PERRY, J. N.; ROTHERY, P.; ROY, D. B.; SCOTT, R. J.; SKELLERN, M. P.; SQUIRE, G. R. & HILL, M. O. (2003c): Non-crop plants in fields with contrasting conventional and genetically modified herbicide-tolerant crops. II Species abundance and seasonal dynamics. Philosophical Transactions of the Royal Society of London B Biological Sciences 358: 1833-1846.

HECKEL, D. G.; GAHAN, L. J.; BAXTER, S. W.; ZHAO, J.-Z.; SHELTON, A. M.; GOULD, F. & TABASHNIK, B. E. (2007): The diversity of Bt resistance genes in species of Lepidoptera. Journal of Invertebrate Pathology 95 (3): 192-197.

HENRY, C. (2006): Cumulative long-term effects of genetically modified (GM) crops on human/animal health and the environment: risk assessment methodologies. Central Science Laboratory, York, UK. Final report, 31.3.2006, reference no 07-0402/2005/414455/MAR/B4.

HERMAN, R.A.; SCHERER, P.N.; YOUNG, D.L.; MIHALIAK, C.A.; MEADE, T.; WOODSWORTH, A.T.; STOCKHOFF, B.A.; NARVA, K.E. (2002): Binary insecticidal crystal protein from *Bacillus thuringiensis*, strain PS149B1: effects of individual protein components and mixtures in laboratory biosassays. J. Environ. Entomol., 95, pp.635-639.

HERMAN, R.A.; SCHERER, P.N.; WOLT J.D. (2002b): Rapid degradation of a binary PS 149B1 delt-endotoxin of *Bacillus thuringiensis* in soil, and a novel mathematical model for fitting curve-linear decay. Environ. Entomol. 31, pp.208-214.

HICKS, D.A. & THOMISON, P.R. (2004): Corn Management. Corn: Origin, History, Technology and Production, Chapter 3.2., 481-522.

HILBECK, A.; BAUMGARTNER, M.; FRIED, P. M. & BIGLER, F. (1998): Effects of transgenic Bt corn-fed prey on immature development of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environmental Entomology 27(2): 480-487.

HILBECK, A.; MEIER, M. & RAPS, A. (2000): Review of non-target organisms and Bt plants. Ecostrat Report, Commissioned by Greepeace International, Amsterdam (**www.greenpeaceusa.org/media/press_release/gmo-report-complete.pdf**

HILBECK, A. (2001): Implications of transgenic, insecticidal plants for insect and plant biodiversity. Perspectives in Plant Ecology, Evolution and Systematics 4: 43-61.

HILBECK, A. (2002): Transgenic host plant resistance and nontarget effects. In: D. Letourneau and B. Burrows (eds.): Assessing environmental and human health effects, CRC Press LLC, Boca Raton, USA, pp 167-185.

HILBECK, A.; NELSON, K.; ANDOW, D.A.; UNDERWOOD, E. (2004). Problem Formulation and Options Assessment (PFOA) to Assess the Ecological Risks Associated with a GM Crop. In: Breckling, B. and R. Verhoeven (eds.), Risk, Hazard, Damage - Specification of Criteria to Assess Environmental Impact of Genetically Modified Organisms. Naturschutz und Vielfalt, 1: 131 - 143.

HILBECK, A. & ANDOW, D. A. (2004): Environmental Risk Assessment of Transgenic Organisms.Volume 1: A Case Study of Bt Maize in Kenya; CAB International, Wallingford, UK.

HILBECK, A. & SCHMIDT, J. E. U. (2006): Another view on Bt-proteins - how specific are they and what else might they do? Biopesticides International 2: 1-50.

HILBECK, A.; FONTES, E. & ANDOW (2006): Environmental Risk Assessment of Transgenic Organisms. Volume 2: A Case Study of Bt Cotton in Brazil, CAB International, Wallingford, UK.

References

HILBECK, A.; ANDOW, D. A.; ARPAIA, S.; BIRCH, A. N. E.; CHEN, Y.; FONTES, E.; FITT, G.; LANG, A.; THI THU HONG LE; LÖVEI, G. L.; MANACHINI, B.; NGUYEN, T. T. C.; NGUYEN, V. H.; NGUYEN, V. T.; VAN LAM PHAM; VAN TOAN PHAM, P. C.; SUJII, E.; KHUONG LAI TRAC, U. E.; WHEATLEY, R.; WILSON, L. & ZWAHLEN, C. (2008a): Non-target and Biological Diversity Risk Assessment. In: Environmental Risk Assessment of Transgenic Organisms. Eds: D.A. Andow, A. Hilbeck & Nguyen V. T.; Volume 4: Challenges and Opportunities with Bt Cotton in Vietnam. CABI Publishing, Wallingford, UK.

HILBECK, A.; JÄNSCH, S.; MEIER, M. & RÖMBKE, J. (2008b): Analysis and validation of present ecotoxicolgocial test methods and strategies for the risk assessment of genetically modified plants. BfN Skripten 236, pp 116 + Appendices. Bundesamt für Naturschutz, Bonn, Germany.

HILBECK, A.; MEIER, M. & BENZLER, A. (2008c): Identifying indicator species for post-release monitoring of genetically modified, herbicide resistant crops. Euphytica 164 (3): 903-912.

HILL, R.; JOHNSTON, S. & SENDASHONGA, C. (2004): Risk assessment and Precaution in the Biosafety Protocol. Reciel 13: 263-269.

HILL, R. A. (2005): Conceptualizing risk assessment methodology for genetically modified organisms. Environmental Biosafety Research 4: 67-70.

HINTERMANN & WEBER AG (2003): Mögliche Synergien zwischen einem GVO-Monitoring und anderen Monitoringprogrammen in der Schweiz. Bericht im Auftrag des BUWAL, Abt.Biotechnologie und Stoffflüsse pp 53-

HORNER, T. A.; DIVELY, G. P. & HERBERT, D. A. (2003): Development, survival and fitness performance of Helicoverpa zea (Lepidoptera: Noctuidae) in MON810 Bt field corn. Journal of Economic Entomology 96: 914-924.

ILSI (2006): ILSI Crop Composition Database. **www.cropcomposition.org**. Version 3.0 http://www.cropcomposition.org

ICOZ, I. & STOTZKY, G. (2008): Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559-586.

JAY, S.C. (1986): Spatial Management of honey bees on crops. Ann. Rev. Entomol., 31, 49-65. (English)

JIANG, L.; DUAN, L.; TIAN, X.; WANG, B.; ZHANG, H.; ZHANG, M. & LI, Z. (2005): NaCl salinity stress decreased *Bacillus thuringiensis* (Bt) protein content of transgenic Bt cotton *Gossypium hirsutum* (L.) seedlings. Environmental and Experimental Botany 55: 315-320.

JOHNSON (2002): Gene flow and transgenic crops - how can potential impacts on fitness be assessed? 392-27-30 November 2001, Raleigh-Durham, USA.

JONES, R. J. A.; HIEDERER, R.; RUSCO, E.; LOVELAND, P. J. & L. MONTANARELLA (2004). The map of organic carbon in topsoils in Europe. Version 1.2., September 2003: Explanation of special publication Ispra 2004 No. 72. European Soil Bureau Research Report No. 17, EUR 21209 EN, 26 pp and 1 map in ISO B1 format. Office for Official Publications of the European Communities, Luxembourg.

JUGENHEIMER, R.W. (1976): Corn: Improvement, seed production and uses. Ch. 3,, pp. 25-42. John Wiley &Sons. New York.

KERSCHNER, F. & WAGNER, E. (2003): Koexistenz zwischen Gentechnik, Landwirtschaft und Natur. Juristische Schriftenreihe Band 201, Verlag Österreich, Wien 2003. 196 S.

KLUN, J. A.; GUTHRIE, W. D.; HALLAUER, A. R. & RUSSELL, W. A. (1970): Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy 2H-1,4-benzoxazin-3(4H)-one and resistance to the European corn borer in a ciallel set of eleven maize inbreds. Crop Science 10: 87-90.

KNOWLES, B. H.; FRANCIS, P. H. & ELLAR, D. J. (1986): Structually related *Bacillus thuringiensis* δ- endotoxins display major differences in insecticidal activity in vivo and in vitro. Journal of Cell Science 84: 221-236.

KOWALCHUK, G. A.; BRUINSMA, M. & VAN VEEN, J. A. (2003): Assessing responses of soil microorganisms to GM plants. Trends in Ecology and Evolution 18: 403-410.

References

KOZIEL, M. G.; BELAND, G. L.; BOWMAN, C.; CAROZZI, N. B.; CRENSHAW, R.; CROSSLAND, L.; DAWSON, J.; DESAI, N.; HILL, M.; KADWELL, S.; LAUNIS, K.; LEWIS, K.; MADDOX, D.; MCPHERSON, K.; MEGHIJI, M. R.; MERLIN, E.; RHODES, R.; WARREN, G. W.; WRIGHT, M. & EVOLA, S. V. (1993a): Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis. Bio/Technology 11: 194-200.

KOZIEL, M. G.; CAROZZI, N. B.; CURRIER, T.; WARREN, G. W. & EVOLA, S. V. (1993b): The insecticidal crystal proteins of Bacillus thuringiensis: past, present and future uses. Biotechnol.Gen.Eng.Rev. 11: 171-228.

KRANTHI, K. R.; NAIDU, S.; DHAWAD, C. S.; TATWAWADI, A.; MATE, K.; PATIL, E.; BHAROSE, A. A.; BEHERE, G. T.; WADASKAR, R. M. & KRANTHI, S. (2005): Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera). Current Science 89: 291-298.

KREBS, J. R.; WILSON, J. D.; BRADBURY, R. B. & SIRIWARDENA, G. M. (1999): The second Silent Spring? Nature 400: 611-612.

LAMB, R. J. (1989): Entomology of Oilseed Brassica Crops. Annual Review of Entomology 34: 211-229.

LANG, A.; LAUBER, E. & DARVAS, B. (2007): Early-tier tests insufficient for GMO risk assessment. Nature Biotechnology 25: 35-36.

LATHAM, J. R.; WILSON, A. K. & STEINBRECHER, R. A. (2006): Mutational consequences of plant transformation. J. Biomed. & Biotech Article ID 25376: 1-7.

LEVIDOW, L. (2003): Precautionary risk assessment of Bt maize: what uncertainties? J.Invertebrate Pathology 83: 113-117.

LILLEY, A. K.; BAILEY, M. J.; CARTWRIGHT, C.; TURNER, S. L. & HIRSCH, P. R. (2006): Life in earth: the impact of GM plants on soil ecology? Trends in Biotechnology 24: 9-14.

LOPEZ, M. D.; PRASIFKA, J. R.; BRUCK, D. J. & LEWIS, L. C. (2005): Utility of ground beetle species in field tests of potential nontarget effects of Bt crops. Environmental Entomology 34: 1317-1324.

LÖVEI, G. L. & ARPAIA, S. (2005): The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114: 1-14.

LUTZ, B.; WIEDEMANN, S.; EINSPANIER, R.; MAYER, J. & ALBRECHT, C. (2005): Degradation of Cry1Ab protein from genetically modified maize in the bovine gastroinstestinal tract. Journal of Agricultural and Food Chemistry 53: 1453-1456.

MAMAROT, J.; RODRIGUEZ, A. (1994): Etude du salissement des sols par la jachère en région Midi-Pyrénées. Recueil des communications du colloque „Jachères“ p.107-111.

MARTINS, C. M.; BEYENE, G.; HOFS, J. L.; KRÜGER, K.; VAN DER VYWER, C.; SCHLÜTER, U. & KUNERT, K. J. (2008): Effect of water deficit stress on cotton plants expressing the Bacillus thuringiensis toxin. Annals of Appl.Biol. 152: 255-262.

MARVIER, M. (2002): Improving risk assessment for nontarget safety of transgenic crops. Ecological Applications 12: 1119-1124.

MARVIER, M. & VAN ACKER, R. C. (2005): Can crop transgenes be kept on a leash? Front Ecol Environ 3: 99-106.

MASSON, L.; SCHWAB, G.; MAZZA, A.; BROUSSEAU, R.; POTVIN, L.; SCHWARTZ, J.-L. (2004): A Novel *Bacillus thuringiensis* (PS149B1) Containing a Cry34Ab1/Cry35Ab1 Binary Toxin Specific for the Western Corn Rootworm *Diabrotica virgifera virgifera* LeConte Forms Ion Channels in Lipid Membranes. Biochemistry, 2004, 43 (38), p. 12349-12357

MATTHEWS, D.; JONES, H.; GANS, P.; COATES, S. & SMITH, L. M. J. (2005): Toxic secondary metabolite production in genetically modified potatoes in response to stress. Journal of Agricultural and Food Chemistry 53: 7766-7776.

References

MEIER, M.S.; HILBECK, A. (2005): Faunistische Indikatoren für das Monitoring der Umweltwirkungen gentechnisch veränderter Organismen (GVO) – Verfahren zur Beurteilung und Auswahl. Bundesamt für Naturschutz, Heft 29

MEIER, M. (2007): Consequences of crop to wild plant transgene flow for biodiversity of associated organisms and implications for hybrid fitness. Dissertation submitted to the Swiss Federal Institute of Technology, Zurich, DISS. ETH. NO 17200.

MENDOCA-HAGLER, L. C.; DE MELO, I. S.; VALADARES-INGLIS, M. C.; ANYANGO, B. M.; SIQUEIRA, J. O.; PHAM VAN TOAN & WHEATLEY, R. E. (2006): Non-target and biodiversity impacts in soil. In: Environmental Risk Assessment of Genetically Modified Organisms. Volume 2: Methodologies for assessing Bt Cotton in Brazil. Ed.: A. Hilbeck, D. A. Andow & E. M. G. Fontes, CAB Interantional, Wallingford, UK.

MESSEAN, A.; ANGEVIN, F.; GÓMEZ-BARBERO, M.; MENRAD, K. & RODRÍGUEZ-CEREZO, E. (2006): New case studies on the coexistence of GM and non-GM crops in European agriculture. JRC IPTS, Technical Report EUR 22102 EN.

MESQUIDA, J. & RENARD, M. (1982): Etude de la dispersion du pollen par le vent et de l'importance de la pollinisation anémophile chez le colza (*Brassica napus* L., Var. *Oleifera* metzger). Apidologie, 13 (4), 353-366

MIDDELHOFF, U.; HILDEBRANDT, J. & BRECKLING, B. (2006). Die ökologische Flächenstichprobe als Instrument eines GVO Monitorings. BfN Skriptum Nr. 172, Bundesamt für Naturschutz, Bonn, Bad Godesberg, 110 S.

MONSANTO (2002): Safety assessment of YieldGard insect-protected event MON810. Published by agbios as Product Safety Description. **http://agbios.com/docroot/decdocs/02-269-010.pdf**.

NATIONAL RESEARCH COUNCIL (1983) Risk Assessment in the Federal Government: Understanding the Process, National Academy Press, Washington DC.

NATIONAL RESEARCH COUNCIL (2002): Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation. National Research Council, National Academy Press, Washington, DC.

NAVON, A.; HARE, J. D. & FEDERICI, B. A. (1993): Interactions among *Heliothis virescens* larvae, cotton condensed tannin and the CryIA(c) delta-endotoxin of Bacillus thuringiensis. Journal of Chemical Ecology 19: 2485-2499.

NELSON, K. C.; KIBATA, G.; MUHAMMAD, L.; OKURO, J. O.; MUYEKHO, F.; ODINDO, M.; ELY, A. & WAQUIL, J. M. (2004): Problem formulation and options assessment (PFOA) for genetically modified organisms: the Kenya case study. In: Hilbeck, A. and D.A. Andow (eds.). Environmental Risk Assessment of Genetically Modified Organisms, 2004, Volume 1: A Case Study of Bt Maize in Kenya. CAB International, Wallingford, UK. pp 57-82.

NELSON, K. C. & BANKER, M. J. (2007): Problem formulation and options assessment handbook. A publication of the GMO ERA Project. http://www.gmoera.umn.edu.

NGUYEN, H. T. & JEHLE, J. A. (2007): Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. Journal of Plant Diseases and Protection 114: 82-87.

NGUYEN, H. T.; HUNFELD, H.; MEISSLE, M.; MIETHLING-GRAFF, R.; PAGEL-WIEDER, S.; RAUSCHEN, S.; ZURBRUEGG, C.; EBER, S.; GESSLER, F.; ROMEIS, J.; TEBBE, C. C.; NENTWIG, W. & JEHLE, J. A. (2008): Round robin quantitation of Cry3Bb1 using the qualitative PathoScreen ELISA. IOBC/WPRS Bulletin. 33: 59-66, in print.

NORRIS, C.E.; SIMPSON, E:C:; SWEET J.B.; THOMAS, J.E. (1999): Monitoring weediness and persistence of genetically modified oilseed rape (Brassica napus) in the UK. In: Lutmann, P.J.W. (ed.) Gene flow and Agriculture: Relevance for Transgenic Crops. BCPC Symposium Proceedings No. 72, pp. 255-260.

OECD (1999a): Consensus document on general information concenrning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. OECD Series on Harmonization of Regulatory Oversight in Biotechnology Nr. 11.

OECD (1999b): Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. OECD Series on Harmonization of Regulatory Oversight in Biotechnology Nr. 10.

References

OECD (2001): Consensus document on key nutrients and key toxicants in low erucic acid rapeseed (Canola). OECD Series on the Safety of Novel Foods and Feeds Nr. 1.

OECD (2002a): Consensus document on compositional considerations for new varieties of maize (Zea mays): key food and feed nutrients, anti-nutrients and secondary metabolites. OECD Series on the Safety of Novel Foods and Feeds Nr. 6.

OECD (2002b): Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants. OECD Series on the Safety of Novel Foods and Feeds Nr. 4.

OECD (2002c): Module II: Herbicide Biochemistry, Herbicide metabolism and the residues in glufosinate ammonium (Phosphinotricin)-tolerant transgenic plants. OECD Series on Harmonization of Regulatory Oversight in Biotechnology Nr. 25.

OECD (2003): Consensus Document on the biology of *Zea mays* subsp. *mays* (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 27, ENV/JM/MONO (2003)11

OGER, P.; MANSOURI, H. & DESSAUX, Y. (2000): Effect of crop rotation and soil cover on alteration of the soil microflora generated by the culture of transgenic plants producing opines. Molecular Ecology 9: 881-890.

OLSEN, K. M. & DALY, J. C. (2000): Plant-toxin interactions in transgenic Bt cotton and their effect on mortality of Helicoverpa armigera (Lepidoptera: Noctuidae). Journal of Economic Entomology 93: 1293-1299.

PALM, C. J.; DONEGAN, K. K.; HARRIS, D. & SEIDLER, R. J. (1994): Quantification in soil of Bacillus thuringiensis var. kurstaki delta-endotoxin from transgenic plants. Molecular Ecology 3: 145-151.

PALM, C. J.; SCHALLER, D. L.; DONEGAN, K. K. & SEIDLER, R. J. (1996): Persistence in soil of transgenic plant produced Bacillus thuringiensis var. kurstaki endotoxin. Can. J. Microbiol. 42: 1258-1262.

PASCHER, K. & GOLLMANN, G. (1999): Ecological risk assessment of transgenic plant releases: an Austrian perspective. Biodiversity and Conservation 8: 1139-1158.

PERRY, J. N.; FIRBANK, L. G.; CHAMPION, G. T.; CLARK, S. J.; HEARD, M. S.; MAY, M. J.; HAWES, C.; SQUIRE, G. R.; ROTHERY, P.; WOIWOD, I. P. & PIDGEON, J. D. (2004): Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. Nature 428: 313-316.

PESSEL, F. D.; LECOMPTE, J.; EMERIAU, V.; KROUT, M.; MESSEAN, A. & GOUYON, P. H. (2001): Persistence of oilseed rape (Brassica napus) outside of cultivated fields. Theoretically and Applied Genetics 102: 841-846.

PETERSEN, B.; ELLWANGER,G.; BIEWALD,G.; HAUKE,U.; LUDWIG,G.; PRETSCHER,P.; SCHRÖDER,E. & A. SSYMANK,A. (2003): Das Europäische Schutzgebietssystem Natura 2000. Ökologie und Verbreitung von Arten der FFH-Richtlinie in Deutschland. Band 1: Pflanzen und Wirbellose. Bundesamt für Naturschutz, Bonn – Bad Godesberg; Schriftenreihe für Landschaftspflege und Naturschutz 69 (Band 1), pp 743+Anhang.

PHELAN, P. L.; MASON, J. F. & STINNER, B. R. (1995): Soil-fertility management and host preference by European corn borer, Ostrinia nubilalis (Hübner), on Zea mays L.: A comparison of organic and conventional chemical farming. Agriculture, Ecosystems and Environment 56: 1-8.

PILCHER, C. D. & RICE, M. E. (2001): Effect of planting dats and Bacillus thuringiensis corn on the population dynamics of European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology 94: 730-742.

PRASIFKA, J. R.; HELLMICH, R. L.; DIVELY, G. P. & LEWIS, L. C. (2005): Assessing the effects of pest management on nontarget arthropods: the influence of plot size and isolation. Environmental Entomology 34: 1181-1192.

PRASIFKA, J. R.; HELLMICH, R. L.; DIVELY, G. P.; HIGGINS, L. S.; DIXON, P. M. & DUAN, J. J. (2008): Selection of nontarget arthropod taxa for field research on transgenic insecticidal crops: using empirical data and statistical power. Environmental Entomology 37: 1-10.

PRESCOTT, V. E.; CAMPBELL, P. M.; MOORE, A.; MATTES, J., ROTHENBERG, M. E.; FOSTER, P. S.; HIGGINS T. J. V. & HOGAN, S. P. (2005): Transgenic Expression of Bean r-Amylase Inhibitor in Peas Results in Altered Structure and Immunogenicity. J. Agric. Food Chem. 53: 9023-9030.

References

PURRINGTON, C. B. & BERGELSON, J. (1995): Assessing weediness of transgenic crops: industry plays plant ecologist. TREE 10: 340-341.

PÜNTENER, W. (1981): Manual für Feldversuche im Pflanzenschutz. Documenta CIBA-GEIGY AG Basel, 145 pp.

RAPS, A.; KEHR, J.; GUGERLI, P.; MOAR, W.J.; BIGLER, F.; HILBECK, A. (2001) Immunological analysis of phloem sap of Bacillus thuringiensis corn and of the nontarget herbivore Rhopalosiphum padi (Homoptera: Aphididae) for the presence of Cry1Ab. Mol Ecol 10:525–533

RAYBOULD, A. F. & MOYES, C. L. (2001): The ecological genetics of aliphatic glucosinolates. Heredity 87: 383-391.

RAYBOULD, A. F. & GRAY, A. J. (1993): Genetically modified crops and hybridization with wild relatives: a UK perspective. Journal of Applied Ecology 30: 199-219.

RAYNOR, G.S.; OGDEN, E.C.; HAYES, J.V. (1972): Dispersion and Deposition of Corn Pollen from Experimental Sources. Agronomy Journal, 64: 420 – 427.

RISSLER, J. & MELLON M. (1993): Perils Amidst the Promise: Ecological Risks of Transgenic Crops in a Global Market. Union of Concerned Scientists, Cambridge, MA, pp.22

ROBINSON, R. A. & SUTHERLAND, W. J. (2002): Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39: 157-176.

RODRIGO-SIMÒN, A. ; DE MAAGD, R. A.; AVILLA, C.; BAKKER, P.L.; MOLTHOFF, J.; GONZALEZ-ZAMORA, J.E.; FERRE, J. (2006): Lack of Detrimental Effects of *Bacillus thuringiensis* Cry Toxins on the Insect Predator *Chrysorperla carnea*: a Toxicological, Histopathological, and Biochemical Analysis. Applied and Environmental Microbiology, Vol. 72, No.2, p.1595-1603

ROMEIS, J.; MEISSLE, M. & BIGLER, F. (2006): Reply. Nature Biotechnology 24, 751-753.

ROMEIS, J.; MEISSLE, M. & BIGLER, F. (2007): Reply. Nature Biotechnology 25, 36-37.

ROMEIS, J.; BARTSCH, D.; BIGLER, F.; CANDOLFI, M. P.; GIELKENS, M. M. C.; HARTLEY, S. E.; HELLMICH, R. L.; HUESING, J. E.; JEPSON, P. C.; LAYTON, R.; QUEMADA, H.; RAYBOULD, A. F.; ROSE, R. I.; SCHIEMANN, J.; SEARS, M. K.; SHELTON, A. M.; SWEET, J.; VAITUZIS, Z. & WOLT, J. D. (2008): Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nature Biotechnology 26: 203-208.

ROSATI, A.; BOGANI, P.; SANTARLASCI, A. & BUIATTI, M. (2008): Characterisation of 3'transgene insertion site and derived mRNAs in Mon810 YieldGard maize. Plant Molecular Biology in press: http://www.ncbi.nlm.nih.gov/pubmed/18306044-

ROSI-MARSHALL, E. J.; TANK, J. L.; ROYER, T. V.; WHILES, M. R.; EVANS-WHITE, M.; CHAMBERS, C.; GRIFFITHS, N. A.; POKELSEK, J. & STEPHEN, M. L. (2007): Toxins in transgenic crop byproducts may affect headwater steam ecosystems. PNAS 104: 16204-16208.

ROVENSKA, G.; ZEMEK, R.; SCHMIDT, J. E. U. & HILBECK, A. (2005): Effects of transgenic Bt-eggplant expressing Cry3Bb toxin on host plant preference of Tetranychus urticae and prey preference of its predator Phytoseiulus persimilis (Acari: Tetranychidae, Phytoseiidae). Biological Control 33: 293-300.

ROY, D. B.; BOHAN, D. A.; HAUGHTON, A. J.; HILL, M. O.; OSBORNE, J. L.; CLARK, S. J.; PERRY, J. N.; ROTHERY, P.; SCOTT, R. J.; BROOKS, D. R.; CHAMPION, G. T.; HAWES, C.; HEARD, M. S. & FIRBANK, L. G. (2003): Invertebrates and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philos.Trans.R.Soc.Lond B Biol Sci 358: 1879-1898.

RUHLAND, M.; ENGELHARDT, G. & PAWLIZKI, K. (2002): A comparative investigation of the metabolism of the herbicide glufosinate in cell cultures of transgenic glufosinate-resistant and non-transgenic oilseed rape (Brassica napus) and corn (Zea mays). Environmental Biosafety Research 1: 29-37.

References

RUHLAND, M.; ENGELHARDT, G. & PAWLIZKI, K. (2004): Distribution and metabolism of D/L-, L- and D-glufosinate in transgenic, glufosinate-tolerant crops of maize (Zea mays L ssp mays) and oilseed rape (Brassica napus L var napus). Pest Manangement Science 60: 691-696.

SANTIAGO, R.; MALVAR, R. A.; BAAMONDE, M. D.; REVILLA, P. & SOUTO, X. C. (2005): Free phenols in maize pith and their relationship with resistance to Sesamia nonagrioides (Lepidoptera: Noctuidae) attack. Journal of Economic Entomology 98: 1349-1356.

SANTOS, M. O.; ADANG, M. J.; ALL, J. N.; BOERMA, H. R. & PARROTT, W. A. (1997): Testing transgenes for insect resistance using *Arabidopsis*. Molecular Breeding 3: 183-194.

SAXENA, D. & STOTZKY, G. (2001): Bt corn has a higher lignin content than non-Bt corn. Am. J. Bot. 88: 1704-1706.

SAXENA, D. & STOTZKY, G. (2002): Bt toxin is not taken up from soil or hydroponic culture by corn, carrot, radish or turnip. Plant and Soil 239: 165-172.

SCHEFFLER, J.A. & DALE, P.J. (1994): Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Research 3, 263-278.

SCHEIRS, J.; DE BRUYN, L. & VERHAGEN, R. (2003): Host nutritive quality and host plant choice in two grass miners: primary roles for primary compounds? Journal of Chemical Ecology 29: 1373-1389.

SCHNEPF, H. E.; LEE, S.; DOJILLO, J.; BURMEISTER, P.; FENCIL, K.; MORERA, L.; NYGAARD, L.; NARVA, K. E. & WOLT, J. D. (2005): Characterisation of Cry34/35 binary insecticidal proteins from diverse Bacillus thuringiensis strain collections. Applied and Environmental Microbiology 71: 1765-1774.

SHAW R.H. (1988): Climate requirements. In: Sprague G.F., Duley J.W., eds. Corn and corn improvement. Agron. Monogr. 18. p.609-638, Madison, WI: ASA, CSSA, and SSSA.

SINGH, C. K.; OJHA, A.; KAMLE, S. & KACHRU, D. N. (2007): Assessment of cry1Ab transgene cassette in commercial Bt corn MON810: Gene, Event, Construct & GMO specific concurrent characterization, http://www.natureprotocols.com/2007/10/23/assessment_of_cry1ab_transgene.php

SNOW, A. A.; PILSON, D., RIESEBERG, L. H.; PAULSEN, M.; PLESKAC, N.; REAGON, M. R.; WOLF, D. E. & SELBO, S. M. (2003). A *Bt* transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecological Applications 13: 279-286.

SNOW, A. A.; ANDOW, D. A.; GEPTS, P.; HALLERMAN, E. M.; POWER, A.; TIEDJE, J. M. & WOLFENBARGER LAREESA, L. (2004): Genetically modified organisms and the environment: Current status and recommendations. Ecological Applications 15: 377-404.

SPÖK, A.; HOFER, H.; VALENTA, R.; KIENZL-PLOCHBERGER, K.; LEHNER, P. & GAUGITSCH, H. (2002): Toxikologie und Allergologie von GVO-Produkten. Umweltbundesamt Monographien, Band 109, Wien. 214 S.

SPÖK, A.; HOFER, H.; VALENTA, R.; KIENZL-PLOCHBERGER, K.; LEHNER, P.; STIRN, S. & GAUGITSCH, H. (2003a): Toxikologie und Allergologie von GVO-Produkten. Teil 2A. Umweltbundesamt Monographien, Band 164A, Wien.

SPÖK, A.; KARNER, S.; STIRN, S. & GAUGITSCH, H. (2003b). Toxikologie und Allergologie von GVO-Produkten. Teil 2B. Umweltbundesamt Monographien, Band 164B, Wien.

Spök, A.; Dolezel, M.; Eckerstorfer, M.; Freigassner, M.; Gaugitsch, H.; Heissenberger, A.; Karner, S.; Klade, M.; Proksch, M.; Schneider, L.; Treiber, F. & Uhl, M. (2008): Assessment of toxic and ecotoxic properties of novel proteins in GMOs. Bundesministerium für Gesundheit, Familie und Jugend, Forschungsberichte der Sektion IV, Band 1/2008, pp 189.

SSC (2000): Risk assessment in a rapidly evolving field: the case of genetically modified plants (GMP). Scientific Opinion of the Scientific Steering Committee expressed on 26/27 October 2000. pp 17.

STOTZKY, G. (2004): Persistence and biological activity in soil of the insecticidal proteins from Bacillus thuringiensis, especially from transgenic plants. Plant and Soil 266: 77-89.

References

SWAIN, T. (1977): Secondary compounds as protective agents. Annual Review of Plant Physiology 28: 479-501.

TABASHNIK, B. E.; GASSMANN, A. J.; CROWDER, D. W. & CARRIÉRE, Y. (2008): Insect resistance to Bt crops: evidence versus theory. Nature Biotechnology 26: 199-202.

TAPP, H.; CALAMAI, L. & STOTZKY, G. (1994): Adsorption and binding of the insecticidal proteins from Bacillus thuringiensis subsp. Kurstaki and subsp. Tenebrionis on clay minerals. Soil Biol. Biuochem. 26: 663-679.

TAPP, H. & STOTZKY, G. (1998): Persistence of the insecticidal toxins from Bacillus thuringiensis subsp. Kurstaki in soil. Soil Biol. Biochem. 30: 471-476.

THEN, CH. & LORCH, A. (2008): A simple question in a complex environment – how much Bt toxin do genetically engineered MON810 maize plants actually produce? A review. In: Breckling B., Reuter H. & Verhoeven R. (Eds): Implications of GM-crop cultivation at large spatial scales. Theorie in der Ökologie 14. Frankfurt, p 5.

TRAXLER A., HEISSENBERGER A., FRANK G., LETHMAYER CH. & H. GAUGITSCH (2001): Ecological Monitoring of Genetically Modified Organisms. Umweltbundesamt Wien, Monographs, Issue 147.

TRAXLER A. MINARZ, E., HÖTTINGER H., PENNERSTORFER J., SCHMATZBERGER A., BANKO G., PLACER K., HADROBOLEC M. & H. GAUGITSCH (2005a): Biodiversitäts-Hotspots der Agrarlandschaft als Eckpfeiler für Risikoabschätzung und Monitoring von GVO. Bundesministerium für Gesundheit und Frauen, Forschungsberichte der Sektion IV, Band 5/2005, pp 182.

TRAXLER, A.; MINARZ, E., ENGLISCH,T.; FINK,B.; ZECHMEISTER, H.; ESSL, F. (2005b) Rote Liste der gefährdeten Biotoptypen Österreichs. Moore, Sümpfe und Quellfluren; Hochgebirgsrasen, Polsterfluren, Rasenfragmente und Schneeböden; Äcker, Ackerraine, Weingärten und Ruderalfluren; Zwergstrauchheiden; geomorphologisch geprägte Biotoptypen. Umweltbundesamt Wien, Monographien, Band 174.

UPOV (2002): General introduction to the examination of distinctiveness, uniformity and stability and the development of harmonized descriptions of new varieties of plants. International Union for the Protection of New Varieties of Plants TG/1/3; **http://www.upov.int/en/publications/tg-rom/tg001/tg_1_3.pdf**

USSUF, K. K.; LAXMI, N. H. & MITRA, R. (2001): Proteinase inhibitors. Plant derived genes of insecticidal protein for developing insect-resistant transgenic plants. Current Sci. 80, 7(10): 47-853.

WANDELER, H.; BAHYLOVA, J. & NENTWIG, W. (2002): Consumption of two Bt and six non-Bt corn varieties by the woodlouse Porcellio scaber. Basic and Applied Ecology 3: 357-365.

WANG, S; JUST, D.R. & PINSTRUP-ANDERSEN, P. (2008): Bt-cotton and secondary pests. International Journal of Biotechnology 10: 113 – 121.

WARWICK, S. I.; LEGERE, A.; SIMARD, J. & JAMES, T. (2007): Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy Brassica rapa population. Molecular Ecology xx: xx-xx.

WATSON, S.A. (1982): Corn: Amazing Maize. General Properties. Pp. 3-29. In: CRC Handbook of Processing and Utilization in Agriculture, Vol. II, Part 1 Plant Products. I.A. Wolff (ed.). CRC Press, Inc., Florida

WATSON, S.A. (1987): Structure and Composition. In: “Corn: Chemistry and Technology”. Pp. 53-82. Ed. Watson, Ransted. Amreican Ass. of cereal chemists, Inc. Minnesota.

WILKINSON, M. J.; DAVENPORT, I. J.; CHARTERS, Y. M.; JONES, A. E.; ALLAINGUILLAUME, J.; BUTLER, H. T.; MASON, D. C. & RAYBOULD, A. F. (2000): A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. Molecular Ecology 9: 983-991.

WINDELS, P.; TAVERNIERS, I.; DEPICKER, A.; VAN BOCKSTAELE, E. & DE LOOSE, M. (2001): Characterization of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213, p.107-112.

WINTER, G. (2007a): Naturschutz bei der Ausbringung von gentechnisch veränderten Organismen - Teil 1. Natur und Recht 29: 571-587.

WINTER, G. (2007b): Naturschutz bei der Ausbringung von gentechnisch veränderten Organismen – Teil 2. Natur und Recht 29: 635-641.

References

WOLFENBARGER L. L.; NARANJO, S. E.; LUNDGREN, J. G.; BITZER, R. J. & WARTRUD, L. S. (2008): Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLOS online 3: 1-4.

YOSHIMURA, Y.; BECKIE, H. J. & MATSUO, K. (2006): Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environmental Biosafety Research 5: 67-75.

ZWAHLEN, C.; HILBECK, A.; GUGERLI, P. & NENTWIG, W. (2003a): Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Mol. Ecol. 12: 765-775.

ZWAHLEN, C.; HILBECK, A.; HOWALD, R. & NENTWIG, W. (2003b): Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Mol. Ecol. 12: 1077-1086.

ZWAHLEN, C.; HILBECK, A. & NENTWIG, W. (2007): Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. Plant Soil 300: 245-257.

Standards on efficacy evaluation of plant protection products (PPPs) issued by EPPO/OEPP (**www.eppo.org/STANDARDS/standards.htm**):

Nr. PP1/13(3): Ostrinia nubilalis

Nr. PP1/214(1): Principles of acceptable efficacy

Nr. PP1/223(1): Introduction to the efficacy evaluation of PPPs

Nr. PP1/226(1): Number of efficacy trials

Nr. PP 1/241: Guidance on comparable climates

Nr. PP1/152(3): Design and analysis of efficacy evaluation trials.

Nr. PP1/181(3): Conduct and reporting of efficacy evaluation trials, including good experimental practice

Documents issued by European Institutions:

Directive 79/409/EEC: Council Directive of April 2nd 1979 on the conservation of wild birds. OJ L103, 25.4.1979, p 1-18.

Directive 90/220/EEC: Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. OJ L7, 10.1.1991, p 39.

DIRECTIVE 91/414/EEC: Council Directive of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32.

DIRECTIVE 92/43/EEC: Council Directive of May 21st 1992 on the conservation of natural habitats and wild fauna and flora. OJ L 59, 8.3.1996, p 63.

DIRECTIVE 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. OJ L 106, 17.4.2001, p. 1–39.

REGULATION (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L268, 18.10. 2003, p 1-23.

REGULATION (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. OJ L 268, 18.10.2003, p. 24–28.

REGULATION (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms (Text with EEA relevance). OJ L 287, 5.11.2003, p. 1–10.

References

DECISION 1600/2002/EC of the European Parliament and of the Council of 22 July 2002 laying down the Sixth Community Environment Action Programme

COMMUNICATION COM/2006/0216: Communication from the Commission – Halting the loss of biodiversity by 2010 – and beyond- sustaining ecosystem services for human well-being

EC (2002a). Commission Decision of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L 200/22. 2002/623/EC.

EC (2002b). Council Decision of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L 280/27, 2002/811/EC.

EFSA (2006a). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99, 1-100.

EFSA (2006b). Guidance document of the Scientific Panel on Genetically Modified Organisms for the renewal of authorisations of existing GMO products lawfully placed on the market, notified according to Articles 8 and 20 of Regulation (EC) No. 1829/2003. The EFSA Journal 435, 1-4.

EFSA (2007). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512, 1-5.

7 ANNEX: TABLES A1-A14

Table A1. Notification of GMO notifications reviewed in this study.

C/BE/96/01 (oilseed rape Ms8xRf3)		
Date	Action by notifier	Action by authorities (EC, EFSA, CA)
December 1996	Submitted by the notifier (PGS) to Belgian CA (accord to Dir 90/220/EEC)	
January 1997		Submission of notification to EC+MS by Belgian CA including a statement of the Belgian CA
January/March 1997		Additional information on plasmids and vector used for transformation; Letter of the notifier on the voluntary labelling of OSR hybrids submitted by EC to MS
1998	Additional documents provided by the notifier (12 Annexes) "summary of data package submitted as annex to part C application" (Document C005583)	
19 May 1998		SCP Opinion
January 1999	Proposal for monitoring and stewardship plan by the notifier	
November 1999	Updated environmental risk assessment and monitoring plan, traceability, labelling (Document C005938)	
March 2000	Letter of the notifier (Aventis) with information regarding traceability, labelling	
Sept 2003/Feb 2004	Updated notification (consolidated version) accord to Art 35 of Dir 2001/18 (environmental risk assessment, monitoring plan, additional information and complements)	Notification submitted incl. assessment report of Belgian CA to EC (excluding cultivation)
2004	Additional information supplied by the notifier (incl. 2 CDs, no further data to support cultivation); 9 Annexes	Additional information sent from the Belgian CA to EC and MS
Sept 2005		EFSA Opinion
26 March 2007		Commission Decision
C/SE/96/3501 (potato EH92-527-1)		
Date	Action by notifier	Action by authorities (EC, EFSA, CA)
1996/April 1998	Notification submitted to Swedish CA (accord to Dir. 90/220/EEC)	
May 1998		Submission of notification to EC and MS by Swedish CA including a summary of the risk assessment by Swedish CA
May 1999	Information to questions of SCP (molecular characterisation)	
July 1998		Additional info to MS on the use of the product by Swedish CA (exclusion tubers as feed)
July 2002		SCP opinion
April 2004	Notification updated and re-submitted to Swedish CA (accord to Dir. 2001/18/EC)	
April 2004		Assessment report of Swedish CA
May 2004		Circulation of notification to MS including assessment report of Swedish CA

Oct/Nov 2004	Additional information to comments and objections of MS	
December 2005	Information to Swedish CA and EC for change of scope (feed use excluded)	
unknown		Additional info requested by EFSA (probably non-target organism studies)
December 2005		EFSA opinion
C/F/95/12-02 (maize MON810)		
Date	Action by the notifier	Action by authorities (EC, EFSA, CA)
Unknown	Notification submitted	
March 1996		Notification acknowledged by CA of France
June 1996		Notification distributed to member states by EC
May 1997	Additional information submitted to EC (labelling proposal)	
July 1997	Additional information submitted to EC (research on IRM management in Itay)	
September 1997	Additional information submitted to EC (confirmation of willingness to participate on Insect resistance project launche by EC; revisal of labelling proposal)	
C/F/96/05-10 (Maize Bt11)		
Date	Action by notifier	Action by authorities (EC, EFSA, CA)
March 1996	Notifcation submitted to French CA	
April 1999		Revised version circulated by EC to EU MS (including statement of French CA and the RA report of French CA)
Nov 2000		Opinion of the SCP
?? 2002	Update of Appendices (Appendix 16: Faust 1996, CBI)	
Jan 2003	Update submitted according to Directive 2001/18/EC (SNIF, ERA, Monitoring, etc.)	
Aug 2003		Additional study (Foster & Beavers 1997) circulated by EC to MS CAs (already reviewed by SCP in 2000)
Nov 2003	Response to MS CA (Molecular characterisation, ERA, Monitoring, Coexistence, etc.)	
Feb 2004	Additional information in response to Belgian Biosafety Advisory Council report regarding Bt11 insert	
Feb 2005	General Surveillance plan submitted	
April 2005		Opinion of the Scientific Panel on GMOs
C/ES/01/01 (Maize 1507)		
Date	Action by notifier	Action by authorities (EC, EFSA, CA)
July 2001	Notification submitted to Spanish CA	
Aug 2003		Assessment Report of Spanish CA
March 2004		Additional information requested by MS CAs
Dec 2004		Additional information provided upon request of EFSA
Jan 2005		EFSA Opinion adopted

Annex: Tables A1-A14

EFSA/GMO/NL/2005/22 (maize NK603)		
Date	Action by the notifier	Action by authorities (EC, EFSA, CA)
August 2005	Notification submitted	
October 2005		Acknowledgement receipt notification by EFSA
March 2006		Request for clarification and completion of the notification
April 2006	Updated Notification submitted	
May 2006		Statement of validity of application by EFSA
September 2006		Request for further information on risk assessment (non-target, agronomic traits and composition, impacts of cultivation and management techniques, general surveillance) by Spanish CA
December 2006	Additional information for Spanish requests	
February 2007		Additional information considered not satisfactory by Spanish CA
February 2007		Additional requests from EFSA (non-target studies)
August 2007	Additional information for EFSA requests	
October 2007	Additional information for Spanish requests	
November 2007		Additional information not considered satisfactory by Spanish CA; request for Case-specific monitoring (non-target effects, effects on weeds due to cultivation/management)
December 2007	Additional information in answer to Spanish requests	
EFSA/GMO/NL/2005/23 (maize 59122)		
Date	Action by the notifier	Action by authorities (EC, EFSA, CA)
October 2005	Notification submitted	
November 2005		Acknowledgement receipt notification by EFSA
November 2006		Information on lack of Completeness of dossier by EFSA (GS plan)
November 2006	Updated GS plan	
January 2007		Request for clarification regarding overlap of notifications (EFSA/GMO/NL2005/12 and EFSA/GMO/NL/2005/23) by EFSA
March 2007		Request for clarification, additional data by EFSA (compositional data)
March 2007	Additional data for composition	
March 2007		Statement of validity by EFSA
March 2007		Request for additonal information on molecular characterisation, environmental aspects by Dutch CA
March/April 2007	Response to requests of Dutch CA	
July 2007		Request for additionl information by Dutch CA (additional lab and field tests with non-target organisms)
October 2007		Rephrased questions by Dutch CA
December 2007	Additional info provided by notifier (non-target	

	studies)	
EFSA/GMO/UK/2005/17 (Maize 1507xNK603)		
Date	Action by notifier	Action by authorities (EC, EFSA, CA)
June 2005	Notification submitted	
Nov 2005		EFSA request for clarification
Feb 2006	Updated notifcation submitted to EFSA	
March 2006		Validity statement by EFSA
July 2006		Additional information requested by Spanish CA (molecular characterization, effects on NTOs and of the herbicide regime on the weed flora, compositional analysis & expression, general surveillance plan)
Nov 2006	Additional CBI info by the notifier (sequence information for NK603)	
Dec 2006	Response to the questions of the Spanish CA to EFSA submitted by Pioneer (No author: 2005 Spanish Field Study – summary report, Buffington 2005, Linderblood 2006)	
EFSA/GMO/NL/2005/26 (maize NK603 x MON810)		
Date	Action by the notifier	Action by authorities (EC, EFSA, CA)
October 2005	Notification submitted	
December 2005		Acknowledgement receipt notification by EFSA
September 2006		Request for clarifications from EFSA (summary on data of single events and cross reference, data & argumentation on NTOs)
October 2006	Updated version of notification	
November 2006		Further requests for clarification from EFSA
December 2006	Updated version of notification	
January 2007		Statement of validity of application
February 2007		Requests for further information from French CA

Table A2. Comparison of assessment categories as designated in this study and corresponding requirements derived from Directive 2001/18/EC or EFSA (2006)

	Assessment of …	Directive 2001/18, Annex III B, Section D. and Annex II, Section D.2.	EFSA (2006) Guidance Doc, D.
Assessment of the molecular characterisation			
1	Traits introduced	Description of traits/characteristics introduced or modified (Annex III, D.1)	description of the trait(s) and characteristics which have been introduced or modified (D.1.)
		Information on the sequences actually inserted/deleted: in case of deletion: size/function of deleted sequence (Annex III, D.2.b)	Information on the sequences actually inserted or deleted: in case of deletion: size/function of deleted region(s) (D.2.c)
2	number of integration sites within plant genome (insert number)		Information on the sequences actually inserted or deleted: size and copy number of all detectable inserts, complete and partial (D.2. a)
3	copy number	Information on the sequences actually inserted/deleted (Annex III, D.2) copy number of insert (D.2.c)	Information on the sequences actually inserted or deleted: size and copy number of all detectable inserts, complete and partial (D.2. a)
4	location of insert	Information on the sequences actually inserted/deleted: location of insert(s): chromosome, chloroplasts, mitochondria or non-integrated form; methods for determination (Annex III, D.2.d)	Information on the sequences actually inserted or deleted: sub-cellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) and methods for its determination (D.2.d)
5	insert characterisation (including flanking regions)	Information on the sequences actually inserted/deleted: size/structure of insert; methods for its characterisation; part of vector introduced or any carrier or foreign DNA remaining in GMP (Annex III, D.2.a)	Information on the sequences actually inserted or deleted: organisation of the inserted genetic material at the insertion site and methods used for characterisation (D.2. b); all sequence information incl. Location of primers used for detection (D.2. e)
6	genetic stability	Genetic stability and phenotypic stability of the plant (Annex III, D.5.)	Genetic stability of the insert and phenotypic stability of the GM plant (D. 5.)
Assessment of expression			
1	characterisation of expression of each transgene		
2	expression in different plant tissues	Information on the expression of the insert (Annex III, D.3.) parts of the plant where the insert is expressed (e.g. roots, stem, pollen etc.) (3b)	Parts of the plant where the insert is expressed (D.3.b)
3	expression during life cycle of plant (ontogenetic stages)	Information on the expression of the insert (Annex III, D.3.) developmental expression of the insert during life cycle of the plant (incl. Methods used for characterisation) (3a)	Information on developmental expression of the insert during the life cycle of the plant (D.3.a)
4	stability of expression over several generations (in tissues targeted)	Genetic stability and phenotypic stability of the plant (Annex III, D.5.)	Genetic stability of the insert and phenotypic stability of the GM plant (D. 5.)
5	potential fusion proteins and expression of unrelated gene products		Expression of potential fusion proteins (D.3.c)
6	expression in differ-		

	ent genetic backgrounds / varieties		
Assessment of agronomic behaviour			
1	agronomic parameters		Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed: agronomic traits (D.7.4.)
Assessment of composition			
1	compositional parameters (with relevance for the environment)		Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed: comparative assessment (D.7.1.) and Selection of material and compounds for analysis (D.7.3.)
Assessment of dissemination and related processes			
1	reproduction	Information on how the GMP differs from the recipient plant in mode(s) and/or rate of reproduction (Annex III, D.4.a)	Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability (D.4.)
2	dissemination	Information on how the GMP differs from the recipient plant in dissemination (Annex III, D.4.b)	Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability (D.4.)
3	survivability (dormancy)	Information on how the GMP differs from the recipient plant in survivability (Annex III, D.4.c)	Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability (D.4.)
4	persistence and invasiveness	Likelihood of GMHP becoming more persistent than recipient or parental plants in agricultural habitats or more invasive in natural habitats (D.2., 1.)	Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification: Persistence and invasiveness (D. 9.1.)
5	selective advantage / disadvantage of the GMP	Any selective advantage or disadvantage conferred to the GMHP (Annex II, D.2., 2.)	Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification (D.9.) selective advantage or disadvantage (D. 9.2.)
6	gene flow to same species	Any change to the ability of the GMHP to transfer genetic material to other organisms (Annex III, D.6.); Potential for gene transfer to the same or other sexually compatible plant species… and any selective advantage or disadvantage conferred to those plant species (Annex II, D.2.,3.)	Any change to the ability of the GM plant to transfer genetic material to other organisms (D.6.) and Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification: Potential for gene transfer (D.9.3.)
7	gene flow to wild relatives / other species	see point 6	see point 6
8	consequences of gene flow to same species or wild relatives and consequences for selective advantage/disadvantage/persistence/invasiveness	see point 6	see point 6
Assessment of effects mediated via target organisms			

1	mechanism and effect of GM plant on target organism	Mechanism of interaction between the GMP and target organism (if applicable) (Annex III, D.9.)	Mechanism of interaction between the GM plant and target organisms (if applicable) (D.8.) and Interactions between the GM plant and target organisms (D.9.4.)
2	food / prey availability effects mediated by the loss of the target organism	Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with target organisms such as predators, parasitoids and pathogens (Annex II, D.2., 4.)	
3	Resistance development		
4	occurrence of secondary pests		
Assessment of interactions of the GMP with non-target organisms and the biotic environment			
1	general: description of non-target organisms and ecological processes and criteria used for selection of species/processes	Potential changes in the interactions of GMHP with non-target organisms resulting from the genetic modification (Annex III, D.10.) and Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms ...(Annex II, D.2., 5.): population levels of competitors, herbivores, symbionts, parasites and pathogens	Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification: Interactions of the GM plant with non-target organisms (D.9.5.)
2	exposure		see also: D.9.8. Effects on biogeochemical processes; fate of gene products and exposure of soil biota
3	eco-toxicity of gene product and whole plant to non-target organisms		
4	species of conservation concern or human value		
5	assessment of effects on mammals/birds	Information on the safety of the GMHP to animal health… (Annex III, D. 8.)	Effects on animal health (D.9.7.) and Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed (D.7.)
Assessment of effects on biogeochemical processes and the abiotic environment			
1	general: description of possible adverse effecs	Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release (Annex II, D.2., 8.)	Effects on biogeochemical processes (D.9.8.)
		Potential interactions with the abiotic environment (Annex III, D.11.)	Potential interactions with the abiotic environment (D.10.)
2	persistence and spread in environmental media		
3	effects of the GMP on species and/or functional groups involved in biogeochemical processes		

Assessment of effects related to changes in land use or cultivation techniques			
1	description of techniques for GMP and identification of differences to non-GMP	Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs (Annex II, D.2., 9.)	Impacts of the specific cultivation, management and harvesting techniques (D.9.9.)
2	consequences of changes of cultivation techniques		
Proposed risk management and monitoring			
1	conclusions of the risk assessment		
2	proposed risk management measures		
3	proposed case specific monitoring	Monitoring plan (Annex VII) case specific monitoring (C.3.1)	Environmental Monitoring Plan: Case-specific GM plant monitoring (D.11.3)
4	proposed general surveillance	Monitoring plan (Annex VII) general surveillance (C. 3. 2)	Environmental Monitoring Plan: General surveillance for unanticipated adverse effects (D.11.4.)

Table A3. Overview of field trials conducted by the notifiers to generate data for the assessment of expression, agronomic parameters and plant composition of the respective GMO; (n.i….. not indicated)

GMO	Field trial (location, year)	Field trials for the assessment of			Source of information
		Expression analysis	Agronomics	Plant composition	
Oilseed rape Ms8xRf3	CAN/1994-1995		x	x	Technical dossier (1996), Annex, Part 1, Annex VI.5
	EU/BE+FR+SE+UK/ 1994-1995		x	x	Technical dossier (1996), Annex, Part 1, Annex VI.5
	EU/BE/2000-2002		x	x	Additional info 2004, Annex 5 (Oberdörfer 2003, Appendix B)
	n.i./greenhouse/n.i.	x			Technical dossier (1996), Annex, Part 1, Annex VI, L09 (De Beuckeleer &Vanderstraeten)
	n.i./greenhouse/n.i.	x			Additional info (2003), (C015156, Bautsoens 2001)
	EU/BE(greenhouse)/ 2004	x			Additional info (2004), Annex 2 (C039688, Van der Klis 2004)
Potato EH92-527-1	EU/SE/1993(-1997)[1]		x		Annexes 18-23 in technical dossier
	EU/SE/1996-1998			x	Annexes 25, 29, 32-34 in technical dossier
	n.i./greenhouse/n.i.	x			Technical dossier (1996), Annexes 11 (Larsson et al. 1996) and 12 (Hovenkamp-Hermelink et al. 1988)
	n.i./greenhouse/n.i.	x			Additional info 2004, Annex 26
Maize MON810	USA/1994	x			Sanders et al. (1995, Monsanto study, not attached); Info in technical dossier
	EU/FR+IT/1995	x			Info in technical dossier
Maize Bt 11	EU/F/1995		x		Annex 11
	EU/F/greenhouse/1996			x	Appendix B of Annex 13 (Grain)
	EU/F/1998			x	document missing, referred to in Appendix 5 of update 11/2003, info in table (Grain)
	USA/greenhouse/n.i	x			Annex 5 (Cry1Ab)
	USA/MN+IL/n.i.	x			Annex 5 (Cry1Ab)
	USA/MN/1995	x			Annex 6 (PAT)
	USA/NC/1995 US/WI+OH+IO/1995			x	Appendix C and D of Annex 13 (Grain)
	USA/TX+n.i./1995			x	Update 11/2003; Appendix 2 of Appendix 5 (whole plants)
	USA/CA/1996			x	Update 11/2003; Appendix 1 of Appendix 5 (Grain)

	USA/n.i./1996			x	document missing, referred to in Appendix 5 of update 11/2003, info in table (whole plants)
Maize 1507	EU/F+I/1999	x		x	Stauffer 2000 (Annex 4)
	EU/F+I+BG/2000		x	x	Pavely 2002 (Annex 19)
	EU/ES/2002		x		Info in technical dossier
	CL/1998/99	x			Stauffer & Rivas 1999 (Annex 2)
	CL/1998/99			x	Stauffer & Zeph 2000 (Annex 3)
Maize NK603	USA 1999		x		Info in technical dossier
	EU/FR+IT/1999	x		x	Info in technical dossier
	USA/1998, 2002	x		x	Info in technical dossier, Appendices II, III and IV of the dossier
	EU/DE: 2000, 2001+ FR: 2002		x		Jacobs et al. 2005
Maize 59122	EU/BG/2003	x	x	x	Buffington 2004 (Annex 3)
	EU/BG+ES/2004	x	x	x	Buffington 2005 (Annex 4)
	CL/2002/03	x	x	x	Essner & Coats 2003 (Annex 37)
	USA+CA/2003	x	x	x	Buffington 2004 (Annex 38)
Maize 1507xNK603	EU/BG+ES/2003	x	x	x	Buffington 2004 (Annex 3)
	EU/ES/2004	x			Buffington 2005 (Annex 6, addit. information requested by Spanish CA)
	EU/ES/2005	x			Linderblood 2006 (Annex 7, addit. information requested by Spanish CA)
Maize NK603 x MON810	EU/FR/2000	x		x	Information in technical dossier
	USA/2002		x		Information in technical dossier

[1]Data only provided for 1993 trials and one parameter from 1996/1997 trials

Table A4. Overview of the expression analysis of the notifications examined. n.d….no data presented/data missing; WOSR = winter oilseed rape, SOSR = summer oilseed rape; V1-V9 and R1-R6 represent developmental stages of maize; L = leaf, LY = leaf young; LM = leaf mature; G/S = grain/seed; R = root, FB = flower buds; T = tubers, P = pollen, WP = whole plant, F = forage

GMO	Tissues examined	Developmental expression	Years / Countries / Sites	Expression in different genetic backgrounds (varieties)	Expression over generations
Oilseed rape Ms8 x Rf3 (original notification, update 2003, add info 2004[1])	Barnase/ Barstar and Pat: LY and R (3-5 leaf stage) LM, F, P, G/S (immature, dry seeds)	LY, LM	n.d.	Yes: backcrossing to different WOSR and SOSR lines	Flower and herbicide segregation data of 2-3 generations F1, F2
Potato EH92-527-1	NptII L GBSS protein: L, T, R (tips), P (immature FB, stamina)	n.d.	n.d.	n.d.	n.d.
Maize MON810	Cry1A(b): L, G/S, WP (US), F (EU) NptII: no expression	L (3x, stages not specified)	1994/USA/6 1995/Europe/4 (FR)+1(IT)	Crosses to recurrent parent B73 or to unrelated inbred Mo17and commercial inbreds	n.d.
Maize Bt 11	Cry1F[3]: L, stalk, husk, G/S PAT: L, stalk, tassel, P, silk, R, G/S	n.d.[4]	n.d./USA/n.d. (2 locations)	Bt11 hybrid lines (X4334CBR, X4734CBR, X6534CBR, X7634CBR)	n.d.
			1995/USA/n.d. (1 location)	Bt11 hybrid lines (X4334CBR, X4734CBR)	
Maize 1507	Cry1F: L (V9), G/S (maturity), WP, stalk (R1), P (R1), silk (R1) PAT: See above	WP (R4, senescent),	1999/Chile/4	1507 maize inbred and hybrid lines (variety not specified)	n.d.
	Cry1F and PAT: L (V9), G/S (maturity), WP, stalk (R1), P (R1), silk (R1)	WP (V9, R1, R4, senescent)	1999/Europe/3 (FR)+3(IT)	1507 maize hybrid (variety not specified)	

Maize NK603	EPSPS: Europe: G/S, F USA: L, G/S (R6), F (without roots at R4-6), R, forage root, P (R1)	US field trials only: L (V2-3, V6-8, V10-13, at pollination) R (V2-3, V6-8, V10-13, at pollination)	1999/Europe (FR, IT)/4 2002/USA/4	n.d.	Segregation data for 9 generations
Maize 59122	Cry34/35Ab1 and PAT: L, G/S, WP, F, stalk, R, P	L (V9, R1, R4, R6) R (V6, V9, R1, R4, R6) WP (V9, R1, senescent)	2002-2003/Chile/6 2003/USA, Canada/5 2003/Europe/3 (BG) 2004/Europe/3 (BG)+3 (ES)	BC1 hybrid, BC4 hybrid (breeding tree indicated)	segregation data of BC2S1 generation
Maize 1507xNK603	Cry1F and PAT/EPSPS L, G/S (R6/maturity) WP, F (at R4[2]) stalk (R1), R, P (R1)	L (V9, R1, R4, R6) R (V9, R1, R4, R6); WP (V9, R1, R6)	2003/EU(ES,BG)/5 2004/EU(ES)/2 2005/EU(ES)/3	n.d. [5]	n.d.
Maize NK603xMON810	Cry1Ab and EPSPS: G/S, F	n.d. (reference to single events)	2000/France/3	n.d.	n.d.

[1] Only the presence of the respective proteins was checked; [2] Results only presented for forage (R4) and grain (R6, maturity); [3] In the greenhouse trial additional plant tissues were examined: cob, brace root, stalk pith, tassel, pollen, silk, ear shank, stalk epidermis, cotyledon, root, leaves; [4] In the greenhouse trial cotyledons were analysed twice, 2nd leaf six times, 5th leaf five times, 10th leaf three times, 15 th leaf twice, roots six times, stalk epidermis four times, stalk pith four times, tassel three times, pollen once, silk, ear shank, husk, cob, and brace root three times and kernels twice (indicated as “days post planting”); [5] In the trial of 2005 the maize lines with single events (1507 and NK603) were also evaluated

Table A5. GM lines and non-GM control lines used for the evaluation of agronomic parameters. Terms inbrackes indicate the breeding history in order to establish the respective variety. BC = backcross, n.i. = not indicated

GM plant	Location	GM plant line	Non-GM control line
Oilseed rape Ms8 x Rf3 (original notification)	Belgium 1994	Ms8, Rf3	variety Drakkar
	Belgium, France, Sweden, UK, 1995	Ms8, Rf3, Ms8xRf3	non-GM (control variety)
(add info 2004)	Belgium 2000-2002	Ms8xRf3	PP0005B
Potato EH92-527-1	Sweden 1993(-1997)	EH92-527-1	parental variety Prevalent
Maize MON810	USA 1994*	n.i.	n.i.
Maize Bt11	France 1995	H8540 Bt11	H8540
Maize 1507	Europe 2000	Line 35R57-TC1507[1]	Line 35R57
	Spain 2002	1507 maize hybrids from 4 different genetic backgrounds (no further details)	Respective non-GM control maize hybrids with comparable genetic background
Maize NK603	Europe 2000-2003	DE 2000: line CRR0501RR	DE 2000: line CRR502
		DE 2001: line TW812H-A (1 site), line CRR0501RR (3 sites)	DE 2001: variety Monumental (1 site), line CRR0502 (3 sites)
		FR 2002: line DKC4445	FR 2002: DK440
Maize 59122	Europe 2003	BC1 hybrid (T0 x inbred 09B x inbred 05F x 2x inbred 1W2 x inbred 3KP)	Pioneer brand commercial hybrid 36B08
	Europe 2004	BC4 hybrid (T0 x inbred 09B x 5x inbred 05F x self x inbred 581)	cross of inbred lines A and B
Maize 1507 x NK603	Europe 2003	Stacked hybrid 1507xNK603 (no variety indicated)	Genetic background representative of the test substance 1507xNK603 without the genetic modification
Maize NK603 x MON810	USA 2002	NK603xMON810	4 different traditional maize hybrids with similar background genetics at each location

[1]GM maize hybrid with a genetic background equally close to the non-GM elite line was obtained through several rounds of backcrossing, selection and selfing (one cross of GM line to elite line followed by 4 subsequent backcrosses to elite line)

*for 1 agronomic parameter only (“germination”)

Table A6. Overview of agronomic parameters assessed and methods used (OSR = oilseed rape; GDU = growing degree units; n. e. = no explanation given)

Parameter group	Parameters evaluated	Methods of evaluation
plant growth and development	germination/early population/early stand count/emergence number of plants (potato)/emergence and establishment (OSR)	Number of plants emerged per x (e.g. 60) seeds planted or estimation using a 1-5 or 1-9 scale (e.g. OSR)
	plant vigour at various growth stages (e.g. seedling vigour)	Visual estimate (1-9 scale or 1-5 scale)
	time to pollen shed/GDU 50% pollen shed; time to silking/GDU 50% silking; %	Number of accumulated heat units/growing degree units from time of planting to time when approx. 50% of plants produce silks/shed pollen)
	male/female flowering (maize)	Number of days to flowering
	flowering start (OSR)	scale 1-9 (OSR)
	maturity (potato, OSR)/ number of days to maturity (OSR)	maturity: 1-5 or 1-9 scale (OSR)
	Stalk/root lodging, lodging resistance at maturity (OSR)	Visual estimate (% plants broken below primary ear or % plants leaning approx. 30° or more in 1st half meter above soil surface); scale 1-9
	final population/final plant count	Number of plants at approx. R6 stage
	stay green	Visual estimate of overall plant health (1-9 scale)
plant morphology	plant height, ear height (ear: maize only)	cm from soil surface to tip of the tassel; to the base of the primary ear, 1-9 scale (OSR)
	Dropped ears (maize only)	Number of dropped ears per plot
	Leaf or ear deformities (maize only)	% leaf or ear deformities
	Leaf colour, leaf shape (maize only)	n. e.
	pollen shape, pollen colour (maize only)	% pollen grains with collapsed walls, % pollen grains with intense yellow colour
	Foliage size (potato only)	n. e.
	Flower colour (potato only)	n. e.
	flower phenotype segregation (OSR only)	number of male fertile/male sterile plants
plant health	insect damage	Visual estimate (1-9 scale)
		number of larvae per stalk or ear, length of tunnels (of stalk or ear)
		% ears damaged
		"stressor or symptom" (e.g. NK603xMON810)
	disease incidence	Visual estimate (1-9 scale, yes-no classification)
		% plants infected
		% infestation severity
		"stressor or symptom"
	Susceptibility to insecticides, fungicides, herbicides	Yes-no-classification
		"stressor or symptom"
		tolerance to herbicide / herbicide segregation (OSR only)
	Frost sensitivity (potato only)	Softness of tubers,
		sprouting after 10-24 hrs at -2°C or -5°C

yield characteristics	Yield	Harvest weight, test weight, yield (at or adjusted to 15,5% moisture)
		Fresh weight at harvest
		Total plant biomass (forage)
		% barren plant without ear
		number of tubers, kg, kg per plant, grams per tuber, % amylose (potato only)
		kg/plot or ha or 1000 kernel weight (oilseed rape)
		No of pods/raceme (OSR only)
	moisture	% moisture or dry matter at harvest
	grain density	weight in kg of a bushel of grain at 15.5% moisture
	Seed quality parameters (OSR only); % amylase in tubers (potato only)	% oil, protein in seeds/meal, glucosinolates in seed/meal

Table A7. Significant differences between GMPs and non-GMP in composition of secondary metabolites (or toxins in case of potatoes) and anti-nutrients (sites in brackets indicate that a significant difference was only detected at a specific site), n.r. = not relevant (parameter not analysed); "-" = no data provided.

GMO	Tissue	Secondary metabolites/toxins	Anti-nutrients	Baseline used for comparison
Oilseed rape Ms8xRf3	Seed (meal)	n.r.	Glucosinolate content[1]	-
Oilseed rape Ms8xRf3 (add info 2004)	Seed	n.r.	significantly higher alkenyl and total glucosinolate values in GM OSR	Non-GM control, literature data
Potato EH92-527-1	Tubers	Glycalcaloids: sign lower in GM potato); not sign different if yield as covariant	Nitrate (sign. higher in GM); not different if covariance with yield is taken into account	Non-GM parental variety, official statistics for starch potatoes
Maize MON810		n.r. (not tested)	n.r. (not tested)	n.r. (not tested)
Maize Bt 11		n.r. (not tested)	n.r. (not tested)	n.r. (not tested)
Maize 1507	Grain (1999)	Not sign. different	Not sign. different	Non-GM control, published range of values
	Grain (2000)	furfural, p-coumaric acid	Not sign. different	
Maize NK603	Grain (only tissue tested)	phytic acid sign higher in NK603 (1 site FR), at IT site not sign diff; other 2 sites no statistical evaluation	-	Non-GM parental control, non-GM conventional reference hybrids (grown at same site); commercial ranges from other trials (US 1998, 1993-1995)
Maize 59122	Grain	2003 (sprayed): inositol, p-coumaric acid	2003 (sprayed, unsprayed): Raffinose	Non-GM control; published literature references (Watson 1982, OECD 2002a, ILSI 2006)
		2004 (sprayed): p-coumaric acid	2004 (sprayed, unsprayed): phytic acid	
Maize 1507xNK603	Grain	Inositol (for 2 out of 3 treatment variants)[2]	Raffinose (for 2 out of 3 treatment variants)[2]	Non-GM control, publicly available data on commercial maize
Maize NK603xMON810	Grain	p-coumaric acid (site 1), inositol (site 2)	phytic acid (site 3)	Non-GM control, calculated tolerance levels of commercial hybrids

[1] There is no indication if and what statistical analysis has been carried out; the significant difference is mentioned in the conclusions of meal quality; [2] 3 different herbicide treatments were tested: see text for explanation

Table A8. Overview of the parameters evaluated in laboratory studies with non-target organisms. n.i. = not indicated

Test organism	Parameter evaluated	GM plant	Study author
Honey bees (larvae, adult)	Mortality	MON810	Maggi & Sims 1994a, b
	Mortality	Bt11	No author, study missing
	Mortality	59122	Maggi 2001
	Mortality, adult emergence	1507	Maggi 1999
	n.i.	Oilseed rape Ms8xRf3	Not indicated (study by LI-SEC Ecotoxicology)
	Mortality, signs of toxicity	NK603xMON810	Maggi & Sims 1994a, b
Earthworms	Mortality, health assessment, growth (weight), burrowing time	Bt 11	Garvey 1994
	Mortality, body weight, signs of toxicity	1507	Hoxter et al. 1999d
	mortality	NK603	Levine 2004/Levine 2007
	Mortality	59122	Bryan et al. 2000b
	Mortality, signs of toxicity	NK603xMON810	Palm & Beavers 1995
Coleoptera: Carabids	survival, development rate, adult weight	59122	Vinall 2005
Bugs: Anthocorids	Developmental time, adult weight	MON810 (MON801 used)	Appendix IV (no data provided, only description)
Aphids	Mortality	59122	Herman 2000
	Growth, progeny	NK603	Chamornman et al. 2002
Green Lacewings	Mortality	MON810	Hoxter & Lynn 1992a
	Mortality	Bt 11	No author, Study missing
	Mortality, signs of toxicity, effects on pupation	1507	Hoxter et al 1999a
	Development, consumption, reproduction	NK603	Chamornman et al. 2002
	Mortality	59122	Sindermann et al. 2001
	Mortality, signs of toxicity	NK603xMON810	Hoxter & Lynn 1992a
Coccinellids (Ladybird beetles)	Mortality, developmental time, adult weight	MON810 (in part MON801 used)	Hoxter & Lynn 1992c, Appendix IV (no data provided, only description)
	Mortality, signs of toxicity	Bt 11	No author, Study missing
	Mortality, signs of toxicity	1507	Hoxter et al. 1999b
	Mortality, weight reduction, developmental delay	59122	Bryan et al. 2000a, Higgins 2003
	Mortality, signs of toxicity	NK603xMON810	Hoxter & Lynn 1992b
Parasitic hymenoptera	Mortality	MON810	Hoxter & Lynn 1992b
	Mortality	Bt 11	Study missing
	Mortality, signs of toxicity	1507	Hoxter et al. 1999c
	mortality	59122	Porch & Krueger 2001
	Mortality, signs of toxicity	NK603xMON810	Hoxter & Lynn 1992c
Lepidoptera (pest species)	Mortality, growth inhibition	59122	Herman 2000, Dogillo 2002 (study not attached to notifi-

			cation)
	Mortality	1507	Evans 1998
	mortality	NK603	Uffman & Levine 2007
	Mortality	1507xNK603	Evans 1998
Lepidoptera (other)	Mortality, growth inhibition	1507	Bystrak 2000
	Mortality, weight gain, development, consumption	59122	Sears 2003 (study not attached to notification)
Coleoptera (Carabids)	mortality	NK603	Levine & Uffman 2007
Collembola	Mortality, health assessment, number of offspring	Bt 11	Collins 1994
	Mortality, progeny	1507	Halliday 1998a (study missing)
	Fecundity (no of eggs, egg viability), body length, development (instar duration)	NK603	Goldstein 2003
	Mortality	NK603xMON810	Halliday 1997
	mortality, lethargy, reproduction	59122	Teixeira 2001
Birds	Mortality, body weight, signs of toxicity	1507	Gallagher et al. 1999 (study missing)
	Food consumption, behaviour, body weight	Oilseed rape Ms8xRf3	LISEC Ecotoxicology
Fish	Mortality	59122	Marino & Yaroch 2002
Mammals (Rabbit, Rat)	Mortality, consumption, weight gain, feed efficiency, clinical symptoms,	Oilseed rape Ms8xRf3	LISEC Ecotoxicology
	Mortality, consumption, body weight, blood and urine analysis, microscopy of organs	NK603	Dudek 2001
Daphnids	Mortality	1507	Drottar & Krueger 1999 (study missing)
	Mortality	NK603xMON810	Graves & Swigert 1996
	Immobility	59122	Marino & Yaroch 2001
Micro-organsims	C/N transformation	NK603	Carson et al. 2004

Table A9. Details on field studies with non-target organisms carried out with the respective GM plant of the notification. n. i. = not indicated

GM plant	Study	Countries / Locations / years	Species evaluated	Sampling method	Plants used	Plot size, field size, etc.	Parameters assessed	Statistics	remark
Oilseed rape Ms8xRf3	Volkmar et al. (Martin Luther University, DE)	n.i./DE (n.i.)	Arthropods: Spiders, Carabids, Staphylinids	Barber traps	n.i. (probably not Ms8xRf3)	3 x 5 m plots , 3 different weed controls, 4 replicates	species spectrum, species count	n.i.	-
	Not indicated (PGS/BCS field trials)	n.i. (n.i.)	Pollinators	n.i.	Ms8, Rf3, Ms8xRf3	n.i.	foraging behaviour	n.i.	-
	Field observations BCS/AgrEvo/PGS	n.i. (n.i.)	Birds, mammals	n.i.	n.i.	n.i.	n.i.	n.i.	-
Potato EH92-527-1	Thieme 2005a	1/DE (2004)	Aranaeae Coleoptera (Carabidae, Staphylinidae, other predatory and pest groups) Hemiptera (Heteroptera, predatory and pest bugs) Stenorrhyncha (Homoptera, mainly aphids) Hymenoptera (parasitoid wasps) Diptera Collembola	pitfall, beating, yelly sticky, blue sticky traps	Potato EH92-527-1	15,3 m^2 plots (4 rows), 4 replicates	abundance	ANOVA, pair-wise-comparison (Dunnett-test), non-parametric test f.b. pair wise comparison (Mann-Whitney-U-test)	Several Pesticide applications (against Colorado beetle, aphids, *Phyt. infestans*)
	Thieme 2005b	1/NL (2004)		pitfall, beating, yelly sticky, blue sticky traps	Potato EH92-527-1	16,8 m^2 plots	abundance		As above
	Thieme 2005c, 2005d	2/SE (2004)		Pitfall, beating traps	Potato EH92-527-1	16 m^2 plots / 15,3 m^2 plots	abundance		As above
Maize MON810	Appendix IV	2/FR (1995)	"Beneficial arthropods" (Anthocoridae, Coc-	Visual sampling (n.i.; Au-	MON810, MON802 controls (with	Plot size: n.i.; 3 plots; 10 plants per plot sampled (total 30	abundance	n.i.	Purpose of trial: Insect control per-

		cinellidae, Nabidae, Staphylinidae, lacewings, spiders)	gust only?)	and without insecticide treatment	plants)			formance; results presented for "beneficial arthropods" Taxa not separately assessed
Appendix IV	2/USA (1993)	"key beneficial arthropods" (only Orius sp. In sufficient numbers present)	Visual sampling (15 July, 27 July, 11 August (IA); 20 July, 9 Aug (NE))	2 GM maize lines (MON801, line 523-06-1) 2 non-GM controls	Plot size not indicated; 8 rows: counts on 5 plants per row, no replication	abundance	no statistical evaluation	No MON810 used; Only *Orius* sp. results
Appendix IV	2/USA (1994)	Arthropods (only Orius)	Visual sampling (2x: 5 July, 27 July)	GM maize: maize line 523-06-1, 546-09-1, MON809 and MON801 non GM controls	Plot size not indicated, 4 replications	n.i. (probably abundance)	n.i.	No MON810 used; Only *Orius* sp. results
Appendix IV: USA 1995	3/USA (1995)	Bonnut location: spiders, Anthocorids	Visual sampling 12 Aug (FR), 11 July, 20 July, 7 Aug (KY), 1 Aug, 6 Aug, 18 Aug (IA)	GM maize: maize line 654-04-1, 600-14-2, MON810, MON809, MON801; non GM controls	Plot size n.i., 5 plants per row for sampling, 4 replications	abundance	n.i.	
		Lexington location: not indicated			As Bonnut	abundance	n.i.	No MON810 used, results for *Orius insidiosus* only
		Ames location: predators: coccinellid eggs, larvae and adults, chrysopid eggs, larvae and adults, nabids, arachnids, ECB masses			Plot size n.i., 6 plants per replication (4 replications)	abundance	n.i.	Results for "predators and ECB egg masses"

Maize Bt11 (2 publ. & 3 un-publ.)	Pilcher et al. (2002)	4/US (1997-8) two years after one pilot year (3 different planting times)	Focus on 5 predators and one parasitoid but censused foliage dwelling arthropods more broadly	Sticky traps	E-176 Bt11 Non-Bt near isoline (Insecticide/herbicide treatment not indicated)	70-100 x 60 ft plot size	abundance	n.i.	Submitted to Environ. Entomol.; presented at ESA meeting San Diego 2001
	Dowd (2000)	1/US (1998-9) (4-8-replications)	All species on ears,sap beetles on ears and leaf axils; aphids, lady beetles and other predators (1999 only)	Corn ears, visual counts of plants for sap beetles	Bt11 near isoline sweet corn both untreated	6 x 20 m per replication	distribution	n.i.	Publ. J.Econ.Entomol. 93: 1714-1720
	Wold et al. (2001)	1/US (1998-9) (4 replications)	Foliage-dwelling natural enemies	Visual sampling	Bt11 non-Bt near-isoline (insecticide/herbicide treatment not indicated)	3 x 5m (1998) 23 x 25 m (1999) per replication	n.i.		Publ. J. Entomol. Science 36 (2):177-187
	Dively & Rose (2002)	2/US (2000-2001)	Community census (everything present)	Pitfall traps, visual counts, sticky traps, litter sampling	Bt11 (untreated, treated 1x with pyrethroid spray) non-Bt near isoline (untreated, treated 5x with pyrethroid spray) no glufosinate applications	0.4-0.6 acres/site	Abundance, diversity	ANOVA to test for treatment & time effects on mean abundance Principle response curve analysis (PRC) Monte-Carlo permutation	Interim report
	Nuessly & Hentz 1999	4/US (1998-9) (one year per site)	Community census (everything present)	Destructive whole-plant sampling, whole-plant vacuum samples (at one site	Bt11 (treated 1-3 x with insecticide)) Non-Bt sweet corn – different conv. sweet corn at each	16-40 acres/field	Nr. of arthropods per plant & for some also proportion of	ANOVA	4 unpublished reports (one per site)

				also sweep net sampling)	site (treated 8-16 x with insecticide) No glufosinate applications		specific insect damaged plants		
Field trials with other Cry1Ab maize	Orr & Landis 1997, Pilcher et al. 1997, Lozzia 1999,								all published
Field trials with other Cry1Ab maize	Warren 1994, Venditti & Steffey 2002, Candolfi et al. 2002, Castanera & Ortega 2002, Kalthoff et al. 2002,								5 unpublished
Field trials with Cry1F maize	Higgins 1999, Vernier 2000, No author 2001								3 unpublished
Maize 1507	Lefko (2002)	2/ES (2002)	6 insect predator families (Coccinellidae, Nabidae, Anthocoridae, Chrysopidae, Pentatomidae, Lygaeidae)	Visual inspection of 20 plants per plot at V9, R1, R2 and R4 growth stages	1507 maize conventional non-GM hybrids with similar genetic background (no insecticides applied)	randomized block design, (2 replicates), 4 row plots, 50 seeds per row, row length = 7.5 m	Abundance	None ("experimental design precluded statistical comparison between GM and non-GM maize")	observational study overlaid on an existing small-plot experiment intended for agronomic purposes
	Vernier et al. (2001a)	1/FR (2000)	aphids, micro-hymenoptera, thrips *(*phytophagous & predaceous), green lacewings*, Orius sp,* leafhoppers	Visual observation (7x during season)	1507 maize non-GM maize: conventional maize treated with insecticide	randomized complete block design (4 replicates), 3 plots per replication field size 0.2 ha plot size 24 m x	abundance leaf damage caused by feeding (leafhoppers)	Statistics only for thrips and Orius sp. General Linear Model	not indicated but obviously the field from the management study evaluating the

						8 rows			efficacy was used here (Vernier et al. 2001b)
	Higgins 1999	1/USA (1999)	lady beetle (adult & larvae), predatory beetles (Carabidae), *Orius* sp., spiders, bugs (Reduviidae, Nabidae), spiders, parasitic hymenoptera, Odonata, Hemerobiidae (lacewings)	Visual counts (6 weeks), sticky traps (5 weeks)	1507 maize, 1306 maize) (comparator: non-GM isolines) (insecticide treatment not indicated)	randomized complete block design, (4 replicates), each block 27 x 3,75 m	abundance	n.i. (GENMOD software)	
Maize NK603	-								
Maize 59122	Higgins & Wright 2003*	2/USA (2001, 2002)	Key indicator species/taxa	Visual observations, sticky traps, pitfall traps, soil samples	Maize lines TC5639, TC15344	2800 square-foot	abundance	ANOVA (mixed linear model), Principal response curve (community level analysis)	Other events than 1507 maize
	Higgins & Hong 2007a	1/ES/2006	Key non-target arthropods	Pitfall traps, sticky traps, visual observation	59122, 1507x59122, 1507xNK603 (sprayed and unsprayed), 59122xNK603 (sprayed and unsprayed) , 59122x1507xNK603 (sprayed and unsprayed)	Plots of 675 m^2	abundance	1. community level analysis (Principal response curve) for all taxa of one sampling method 2. ANOVA (mixed linear model) for key taxa with sufficient abundance	

	Higgins & Hong 2007b	1/HU/2006	Key non-target arthropods: Rove beetles, spiders, centipedes/millipedes, ground beetles, Collembola Flea beetles, leafhoppers, aphids, thrips, Orius spp., Nabis spp., parasitic hymenoptera, Flea beetles, H. armigera, green lacewings, ladybird beetles	Pitfall traps, sticky traps, visual observation	59122, 1507x59122, 1507xNK603 (sprayed and unsprayed), 59122xNK603 (sprayed and unsprayed), 59122x1507xNK603 (sprayed and unsprayed)	Plots of 625 m^2	abundance	1. community level analysis (Principal response curve) for all taxa of one sampling method 2. ANOVA (mixed linear model) for key taxa with sufficient abundance
	Higgins & Hong 2007c	2/ES/2005	Key non-target arthropods: Carabids, rove beetles, collembolan, earwigs, spiders, centipedes/millipedes Parasitic hymenoptera, Chloropidae, flies, coccinellids, phytophagus thrips, *Orius* spp., leafhoppers, planthoppers, other Heteroptera, aphids	Pitfall traps, sticky traps, visual observation	59122, 1507x59122, 1507xNK603 (sprayed and unsprayed), 59122x1507xNK603 (sprayed and unsprayed) non-GM control: Hybrid A and Hybrid B (with and without soil insecticide application)	Randomized complete block design, 3 replications, plots of 30 m^2	abundance	mixed model analysis for key taxa, community level analysis pairwise comparison with treated and untreated control
Maize 1507x NK603	No authors (Answers to Spanish CA, 2005)	1/ES/2005	Carabids, rove beetle, globular and elongated Collembola, earwigs, spiders, centipeds/millipeds Parasitic hymenoptera, Chloropidae, flies, Coccinellidae, phytophagous trips, Orius spp., leaf-	Pitfall traps, sticky traps, visual observation	59122, 1507x59122, 1507xNK603 (gly) non-GM control (treated with soil insecticide)	randomized complete block design, (3 replicates), 900 m^2 per plot	abundance	Principle response curves for effects at community level; ANOVA for key taxa (but not for visual observation due to low

		hoppers, planthoppers Heteroptera, rove beetles, aphids		overall abundance)
		Orius spp., Nabidae, spiders, carabids, *Chrysopa*, Coccinellidae		
Maize NK603x MON810	-			

*study not attached to notification

Table A10. Details of the studies conducted by notifiers (unpublished studies) to assess potential effects of the GM plant on biogeochemical processes, except degradation studies; LC_{50}, EC_{50}, GI_{50} = the concentration required to kill or inhibit the growth of 50 % of a tested population; n.i. = not indicated.

GM plant	Study	Aim of the study	Methods used	Parameter assessed
Oilseed rape Ms8xRf3	Leyns (1994)	Comparison of the rhizobacterial flora	Bacterial counts, protein fingerprinttypes	Population density per gram root ; qualitative bacterial differences
	No author (no year)	Performance of GM OSR following crops	Biomass assessment of wheat plants grown on GM/non-GM fields	biomass
Potato EH92-527-1	Hofvander (Annex 31)	Evaluation of kanamycin resistant bacteria in soil	Plate counts of bacteria from four locations on kan+ medium	Colony-forming bacteria per gram soil
Maize MON810	-			
Maize Bt11	-			
Maize 1507	Halliday 1998a (study missing)	Effect of Cry1F protein on *Folsomia candida* (Collembola)	n.i.	n.i.
	Hoxter et al. 1999d (Annex 29)	Acute toxicity of Cry1F on the earthworm	Lab bioassay with artificial soil substrate	mortality, body weight
Maize NK603	Goldstein 2003	Effect of RR soybean and RR corn on Collembola	3 generations of Collembola fed RR soybean or corn	fecundity, instar duration, no of eggs per batch, egg viability percentage, body length
	Levine 2004	Effect of EPSPS proteins on earthworms	Acute toxicity study for 14 days	LC_{50} of EPSPS protein/kg dry soil
Maize 59122	Bryan et al. 2000b (Annex 27)	Effect of PS149B1 binary insecticidal crystal protein on Earthworms	Lab bioassay for 7 and 14 days (acute toxicity)	Body weight, survival (LC_{50} of PS149 B1 protein)
	Teixeira 2001 (Annex 28)	Effect of PS149B1 binary insecticidal crystal protein on Collembola	Lab bioassay for 28 days (chronic toxicity)	Survival, no of offspring
	no author; response to EFSA (Annex 32)	Effects on Dung beetles	theoretical exposure/effects assessment	PEC (faeces), NOEC (Coleoptera)
Maize NK603xMON810	-			
Maize 1507xNK603	-			

Table A11. Details of the studies conducted by notifiers (unpublished studies) to assess degradation of the protein or the GMP; DT_{50} = time until 50 % of the concentration of the toxin is not detectable; EC_{50}, GI_{50} = the concentration required to kill or inhibit the growth of 50 % of a tested population.

GM plant	Study	Aim of the study	Methods used	Parameter assessed
Oilseed rape Ms8xRf3	-			
Potato EH92-527-1	-			
Maize MON810	-			
Maize Bt11	Novartis seeds (orig. notification, Annex 12)	Fate of the Cry protein in degrading plant tissues and soil	soil spiked with Btk protein or Bt11 stalk and leaf tissue placed on soil surface or incorporated into soil; protein assay, (ELISA, Western Blot); insect bioassay (*O. nubilalis*)	half-life of Btk proteins in soil (DT_{50}) after 3 weeks
	Dubelman (2003)[1]	Persistence and accumulation of Cry1Ab protein in soil	n.i.	"bioactivity" in the soil 6 weeks after harvest
Maize 1507	Halliday 1998b (Annex 32)	Degradation of Cry1F in soil	incubation of purified Cry1F protein in soil for 28 days (lab); insect bioassays (*H. virescens*)	half-life of *Bt* proteins in soil (DT_{50}); growth inhibition of insect (EC_{50})
Maize NK603	Carson et al. 2004	C-, N-transformation of the EPSPS protein in soil	Incubation of EPSPS protein in soil for 28 days	carbon/nitrogen transformation
Maize 59122	Herman et al. 2000	Degradation of two endotoxins in soil	Incubation of isolated proteins in soil (lab) for 28 days; insect bioassay (*Diabrotica* spp.)	half-life of *Bt* protein in soil; growth inhibition of insect (GI_{50})
Maize NK603xMON810	-			
Maize 1507xNK603	-			

[1] unclear whether notifier study or not (see text); not attached to notification

Table A12. Details of the studies cited by notifiers (published studies) to assess potential effects of the GM plant on the biogeochemical cycles or on the abiotic environment; DT_{50} = time until 50 % of the concentration of the toxin is not detectable; LC_{50}, EC_{50}, GI_{50} = the concentration required to kill or inhibit the growth of 50 % of a tested population; n.i. = not indicated.

GM plant	Study	Title
Oilseed rape Ms8xRf3	-	
Potato EH92-527-1	Nap et al. 1993	Biosafety of kanamycin-resistant transgenic plants
	Bergmans 1993	Acceptability of the use of antibiotic resistance genes as markers in transgenic plants
	Kärenlampi 1996	Health effects of marker genes in genetically engineered food plants
	WHO 1993	Health aspects of marker genes in GM plants. Report of a WHO workshop
	Harding 1996	The potential for horizontal gene transfer within the environment
Maize MON810	-	
Maize Bt11	Griego & Spencer 1978	Inactivation of Bt spores by UV and visible light
	Ignoffo & Garcia 1978	UV-Photoinactivation of cells and spores of Bt and effects of peroxidase on inactivation
	Palm et al. 1994	Quantification in soil of Bt var. Kurtsaki delta-endotoxin from transgenic plants
	Palm et al. 1996	Persistence in soil of transgenic plant produced B. th. var. kurstakii delta-endotoxin
	West 1984	Fate of the insecticidal, proteinaceous parasporal crystal of Bt in soil
	Andrews et al. 1985	Protease activation of the entomocidal protoxin of Bt ssp. Kurstakii
	Entwistle et al. 1993	Bt and environmental Biopesticide: theory and practice
	Choma & Kaplan 1990	Folding and Unfolding of the protoxin from Bt: Evidence that the toxic moiety is present in an active conformation
	DeLucca 1981	Bt distribution in soils of the US
	Zwahlen et al. 2003a, b	Effects of transgenic Bt corn litter on the earth worm Lumbricus terrestris; Degardation of the Cry1Ab protein within transgenic Bt corn tissues in the field
	Saxena & Stotzky 2001a, b	Bt toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil; Bt corn has a higher lignin content than non-Bt corn
	Escher et al. 2000	Decomposition of transgenic Bt maize by microorganisms and Woodlice Pocellio scaber (Crustacea: Isopoda)
	Tapp & Stotzky 1995a, b	insecticidal activity of the toxins from Bt subsp. Kurtsakii and tenebrionis adsorbed abd bound opn pure soil clays; Dot blot enzyme-linked immunosorbent assay for monitoring the fate ofinsecticidal toxins from Bt
	Sims & Holden 1996	n.i.
	Venkateswerlu & Stotzky 1992	n.i.
Maize 1507	OECD 1999a	Consensus Document on general information concerning the genes and their enzymes that confer tolerance to phosphontricin herbicide

Maize NK603	-	- - -
Maize 59122	Herman et al. 2002b	Rapid degradation of a binary, PS149B1, delta-Endotoxin of Bt in soil, and a novel mathematical model for fitting curve-linear decay
Maize NK603xMON810	Koskella & Stotzky 2002	Larvicidal toxins from Bt subsp. Kurstaki, morrisoni and israelensis have no microbiocidal or microbiostatic activity against selected bacteria, fungi and algae in vitro
	Saxena & Stotzky 2001	Bt toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil.
	Sims & Holden 1996	Insect bioassay for determining soil degradation of Bt subsp. Kurstaki CryIA(b) protein in corn tissue
	Palm et al. 1994	Quantification in soil of Bt var. Kurstaki delta-endotoxin from transgenic plants
	Palm et al. 1996	Persistence in soil of transgenic plant produced Bt var kurstaki delta-endotoxin
	Pruett et al. 1980	Effect of exposure to soil on potency and spore viability of Bt
	West 1984	Fate of the insecticidal, proteinaceous parasporal crystal of Bt in soil
	West et al. 1984	Persistence of Bt parasporal crystal insecticidal activity in soil
Maize 1507xNK603	EFSA 2005	Opinion of the Scientific Panel of GMOs on a requeset from the EC related to the notification (C/ES/01/01) concerning the placing on the market of insect-tolerant GM 1507 maize for import, feed and industrial processing and cultivation

Table A13. Information provided on Part B or previous releases within or outside the EU in selected notifications. For EFSA notifications (maize NK603, 59122, 1507xNK603 and NK603xMON810) B-notification numbers are indicated. In bold: data provided in the notification which does not correspond to a field trial. See also table on field trials in the main document (review of notifications, Chapter 5); NTO = field study with non-target organisms

GMO	field trials	Purpose of release	Aim of post-release monitoring	Results/conclusions	remarks	Correspondance with EU field trials indicated by notifier for agronomic/ compositional/expression assessment
Oilseed rape Ms8xRf3	n. i.; contained use 1993	Basic phenotypical characterisation, identification of homozygous fertility restorer transformants			Original notification	
	n. i.; contained use, 1993, 1994, 1995	Seed production			Original notification	
	n.i., contained use, 1994, 1995	Backcross program, inheritance, expression			Original notification	Expression
	EU: BE 1994	Field evaluation (restoration, seed quality, yield)		Rf3 identified based on restoration capacity; no diff in seed quality data, yield	Original notification	Agronomics
	EU: BE, FR, SE, UK (1995) and CAN	Evaluation of stability/reliability of restoratin under diff climatic conditions, agronomic evaluation of male sterile and restorer lines and restored products		Several parameters satisfactory, all combinations were restored, some sign differences in seed quality	Original notification	Agronomics
	EU: BE 1995 and CAN	Glufosinate tolerance evaluation		No negative influence of glufosinate treatment on seed quality characteristics	Original notification	

EU: BE, UK 1995 and CAN	Restoration evaluation of male fertility		Restoration effective	Original notification	
n. i.; contained use	Seed germination ability		Not different	Original notification	
CAN n.i.	Environmental safety field trial (competitive advantage)		Similar performance of GM and non-GM	Original notification	
EU: BE 1990		Post trial monitoring study: seed dispersal, persistence		(Add info Oct 1998, Annex 2, Part 2, Annex II.2. (1 page)	
EU: BE 1990-1993		Post trial monitoring studies: emergence, recovery of volunteers, weed species		Add info Oct 1998, Annex 2, Part 2, Annex II.3.	
EU: UK 1990, 1991		Monitoring transgenic experimental sites: volunteers		Add info Oct 1998, Annex 2, Part 2, Annex II.4 (1 page)	
EU: BE 1992	Safety assessment: germination, survival, competition, fertility			Add info Oct 1998, Annex 2, Part 2, Annex II.5 (2 pages, results only for seed yield)	
EU: DK, BE (?)	Ecological evaluation of competitiveness			Add info Oct 1998, Annex 2, Part 2, Annex II. 6., not Ms8xRf3	
EU: DE 1994-1995	Epigean predatory arthropods			Add info Oct 1998, Annex 5, not Ms8xRf3	Volkmar et al. ? (NTO)
EU: DE 1995-1997	Pollen and seed dispersal			Add info Oct 1998 Annex 10, Unclear which OSR line	
EU: FR 1996-1997	Unknown			Add info Oct 1998, Annex 11, Report in french	
EU: UK 1990 (PROSAMO)	Pollen dispersal			Add info, 1999 (ERA)	
EU: BE 1992, 1993 (BRIDGE)	Pollen dispersal			Add info, 1999 (ERA)	

	EU: UK 1994 (BRIDGE)	Pollen dispersal			Add info, 1999 (ERA)	
	EU: BE 2001-2002	Agronomic performance, compositional/ nutritional analyses			Additional info 2004, Annex 5 (Oberdörfer 2003, Appendix B)	Agronom-ics/composition
	EU: BE green-house (2004)					expression
Potato EH92-527-1	SE 1993	Observation trial (mor-phology, plant develop-ment, maturity, flower col-our)		n. i.	Annex 40	Agronom-ics/compsition
	SE 1994	observation trials, seed production		Not different with respect to suscep-tibility to chemicals, late blight, frost, production capacity	Annexes 18, 19 and 21	Agronom-ics/composition
	SE 1995	DUS testing, official trial, starch, seed production		Variety is distin-guishable, uniform, stable no differ-ence to herbicide treatment, in pests, diseases or para-sites		Agronom-ics/composition
	SE 1996	DUS testing, official trial, starch, seed production, frost tolerance		Variety is distin-guishable, uniform, stable, frost toler-ance not different	Annexes 18, 19 and 21	Agronom-ics/composition
	SE 1997	official trial, starch, seed production, frost tolernace		frost tolerance not different	Annex 19	Agronom-ics/composition
	SE 1998	official trial, starch, seed production		n.i.		Agronom-ics/compsition
	SE 1999	Yield trials, starch, seed production		n.i.		
	SE 2000	Starch, seed production		n.i.		
	SE 2001	Seed production		n.i.		
	n. i. / greenhouse / n. i.					expression
Maize	USA (1993-	n.i.	n.i.	n.i.	Technical dossier (A.	Expression (1994)

MON810	1995)				general information)	NTOs (1993-1995)
	CL (1993-1995)	n.i.	n.i.	n.i.	Technical dossier (A. general information)	
	Canada (1995)	n.i.	n.i.	n.i.	Technical dossier (A. general information)	
	AR (1994)	n.i.	n.i.	n.i.	Technical dossier (A. general information)	
	ZA (1994-1995)	n.i.	n.i.	n.i.	Technical dossier (A. general information)	
	EU: FR (1994-1995) 94.02.11; 94.02.16, 94.03.02 95.03.06; 95.03.08-95.03.12	n. i.	n.i.	n.i.	Technical dossier (A. general information)	Expression (1995) NTOs (1995?)
	EU: IT (1995) B/IT/95-38; B/IT/95/23	n. i.	n. i.	n. i..	Technical dossier (A. general information)	Expression (1995)
Maize Bt 11	USA, CAN, CL, UY, PR (1992-98)	n.i.	n.i.	n.i.	In technical dossier (Annex 15)	
	EU: ES (1996-2003)	- seed production for development purposes - testing Bt11- maize lines and hybrids for tolerance to corn borers - yield and other agronomic characteristics	Maintained one year after the year of the trail aiming at the control of volunteers (the trails were each conducted for one growing season!)	- efficacy against corn borers demonstrated, - no difference observed in other agronomic or phenotypic characteristics, inlcuding persistence as volunteer plants - no negative impact on human health or the environment was observed		

	EU: FR (1994-2003)	See above (some trials in France were part of an insect resistance monitoring programme -ICTIA)	See above	See above		Agronomics/composition (1995-1998)
	EU: IT (1995-98)	See above	See above	See above		
	EU: PT (1998)	See above	See above	See above		
Maize 1507	EU: ES (2002)	agronomic performance	One season to control potential volunteers	-no evidence of any unintentional morphological or phenotypical characteristics - no evidence of enhanced weediness - no adverse effect on human health and the environment observed	In technical dossier, point 13	Agronomics NTOs (Lefko 2002)
	EU: IT (1998-2002)	agronomic performance	See above	See above		Expression/agronomics/composition (1999, 2000)
	EU: FR (1999-2000)	agronomic performance	See above	See above		Expression/agronomics/composition NTOs (Vernier 2001a)
	Argentina (n.i.)	efficacy trials and hybrid registration	See above	See above		
	Brazil (n.i.)	research	See above	See above		
	Chile (n.i.)	research	See above	See above		
	Bulgaria (n.i.)	research	See above	See above		Agronomics/composition (EU: BG 2000)?
	South Africa (n.i.)	research	See above	See above		

	USA (n.i.)	research	See above	See above		
Maize NK603	EU: DE (2000, 2001) EU: FR (2002)[1]	Phenotypic and agronomic characterization trials in the EU	n. i.	Not different from traditional maize, except for introduced herbicide tolerance	details see Jacobs et al. 2005	agronomics
	EU: FR B/FR/99/04/06; B/FR/00/03/05; B/FR/01/01/01; B/FR/04/02/02	Agronomic performance, growth, developmental, morphological and other phenotypic characteristics, yield potential, residues determination, protein expression, compositional analysis, variety testing	n.i.	No evidence that the maize would cause any adverse effects on human or animal health or to the environment	Part II (summary)	expression/composition (FR 1999) B trials: agronomics (FR 2002) missing!
	EU: IT B/IT/99/17; B/IT/02/01					expression/composition (IT 1999)
	EU: BE B/BE/00/WSP13					
	EU: DE B/DE/00/115; B/DE/03/148					agronomics (DE 2000) B-trial: agronomics (DE 2001) missing!
	EU/ES B/ES/00/06; B/ES/01/05; B/ES/02/03; B/ES/04/17; B/ES/04/19					
	USA/CAN (since 2001)	Commercial release		No evidence of adverse effects to human or animal health or to the environment	Part II (summary)	Expression/composition (USA 1998/2002)?
Maize 59122	EU: BG (2003-2004)	Regulatory trials	Control of potential volunteers	Performance as expected, no evidence of any unin-	Annex III (2001/18/EC) and Part II (Summary)	Expression/ agronomics/composition

				tentional morphological and phenotypical characteristics		
	EU: ES (2004) B/ES/04/01	n.i.	n.i.	See above	Annex III (2001/18/EC) and Part II (Summary)	Expression/ agronomics/composition
	EU: FR (2003) B/FR/03.01.05	n.i.	n.i.	See above	Annex III (2001/18/EC) and Part II (Summary)	
	EU: HU (2004)	Regulatory trials	Control of potential volunteers	See above	Annex III (2001/18/EC) and Part II (Summary)	
	USA (2001-2004)	Research and/or regulatory	Control of potential volunteers	See above	Annex III (2001/18/EC) and Part II (Summary)	Expression/agronomics/composition (2003)
	CL (2002-2003)	Research and/or regulatory	Control of potential volunteers	See above	Annex III (2001/18/EC) and Part II (Summary)	Expression/agronomics/composition
	AR (2003)	Research	Control of potential volunteers	See above	Annex III (2001/18/EC) and Part II (Summary)	
	CAN (2003-2004)	Research and/or regulatory	Control of potential volunteers	See above	Annex III (2001/18/EC) and Part II (Summary)	Expression/agronomics/composition (2003)
Maize 1507x NK603	EU: ES (2003-2005) B/ES/03/10; B/ES/04/03; B/ES/05/04; B/ES/05/10	n.i.	n.i.	Performance as expected, no evidence of any unintentional morphological and phenotypical characteristics, no evidence of enhanced weediness no negative impact on human health or the environment	Annex III (2001/18/EC) and Part II (Summary)	Expression/ agronomics/composition (2003) Expression (2004-2005) NTOs (no author, ES 2005?)
	EU: FR (2003-2005)	n.i.	n.i.	See above	Annex III (2001/18/EC) and	

	B/FR/03/02/02; B/FR/04/06/01; B/FR/05/01/04				Part II (Summary)	
	EU: HU B/HU/05/02/1	No field trials carried out	-	-	Annex III (2001/18/EC)	
	EU: PT B/PT/05/02	No field trials carried out	-	-	Annex III (2001/18/EC)	
	EU: BG (n.i.)	Regulatory trials	Control of potential volunteers	See above	Annex III (2001/18/EC)	Expression/ agronomics/composition (2003?)
	CAN (n.i.)	research	n.r. [2]	See above	Annex III (2001/18/EC)	
	CL (n.i.)	research	Control potential volunteers	See above	Annex III (2001/18/EC)	
	USA. (n.i.)	research	n.r. [2]	See above	Annex III (2001/18/EC)	
Maize NK603x MON810	EU: FR (since 2000) B/FR/00/02/06; B/FR/04/02/01	agronomic performance, phenotypic and morphological characteristics, yield, residues determination, protein expression and compositional analysis.	n.i.	no significant evidence that this maize would likely cause any adverse effects to human or animal health or to the environment.	Part II (Summary)	Expression, composition (2000)
	EU: ES B/ES/04/18; B/ES/04/20	See above	n.i.	See above	Part II (Summary)	
	EU: DE B/DE/04/163	See above	n.i.	See above	Part II (Summary)	
	USA (since 2002)	Commercial release		See above	Part II (Summary)	Agronomics (2002)

[1]German trials were designed as part of larger crop safety (selectivity) trials by addition of plots with conventioanl maize hybrids; French trials were specifically designed to assess phenotypic/agronomic characteristics; [2] the release was not regulated, so no post-release monitoring was performed

Table A14. Examples of EFSA risk assessment requirements as specified in EFSA (2006a) and lack of compliance or interpretation by notifiers in GMP notifications reviewed in this report.

	Risk assessment guidance as specified in EFSA (2006a)	(Non-)compliance by notifiers	Shortcoming of current ERA guidance (EFSA 2006a)
Expression	Developmental expression during the life cycle of the plant (considered on case-by-case basis)	Inconsistently considered Different plant organs Different plant stages	Only obligatory with relevance for food/feed safety (plant parts) Lack of specification (organ, stage)
Difference in reproduction, dissemination, survivability	Information on biological features that affect fitness and environmental sensitivity (e.g. …)	None - General reference to agronomic traits without evaluation whether these traits were relevant for the specific assessment	Unclear which 'biological features' relevant for each crop
Ability of GMP to transfer genetic material to other organisms (e.g. plant-plant transfer)	Evaluation of change in biology of GMP that might lead to increase or decrease of the potential gene transfer (e.g. extended flowering period) or experimental evidence; assessment of potential consequences	General reference to agronomic traits e.g. general lack of assessment of flowering period	Unclear what traits should be assessed or which experimental evidence should be provided
Comparative assessment:	comparator = non-GM line of comparable genetic background	Only indication of 'comparator with a comparable genetic background' (or at the most: name of line used)	'comparable genetic background' not specified, no need for indication of breeding history
	Soil composition should be taken into consideration when comparing field and literature data	None – generally not addressed	
	data for commercial varieties may be used in the comparison of the GMP – may be compiled from the literature	Several literature resources addressed differ for different plant compounds	Databases not specified for each compound Environmental relevance not considered
	Field trials: adequate description of field experiments (treatments, etc.); in case of HT use of herbicide-treated and untreated GMP	Lack of indication of herbicide treatments Only one variant (herbicide treated or untreated) used	
	field trials: more than one representative growing season, multiple geographical locations representative of various environments in which GMP will be cultivated	Varying number of locations non-EU locations only EU locations often dismissed Rarely more than one season No indication if locations used are representative	Lack of criteria for representativeness
	Field trial design: sufficient statistical power to detect differences	No power analysis carried out; no indication of effect size to be detected with respective field design	
	statistically significant differences should trigger further	Argumentation-based dismissal of statistically signifi-	Lack of guidance on the interpretation of statistically

	investigations as to the relationship between the differences and the genetic modification	cant differences No follow-up of differences by additional testing/experiments	significant differences (e.g. biological significance)
Agronomic traits	Plant biology and agronomic traits including common breeding parameters	Varying number and types of parameters Varying methodology to assess plant traits	Lack of specification for each crop plant which parameters are relevant including relevant methodology
Interactions between GMP and target organisms	- likely effects on target organism and population dynamics - Interaction between traits and effects on target organisms - comparative susceptibility of GMP to pests and diseases/agronomic performance	Rough description of mechanism of action (e.g *Bt* crops) no efficacy data provided on target or non-target pests/diseases	No specific assessment required Lack of specification of methods
Changes in interactions of GMP with the biotic environment	Data from field experiments from representative geographical locations	Few or no field data under European conditions	Lack of specification of criteria for representativeness
	Persistence, invasiveness: likelihood assessment	theoretically assessed, no specific data provided or reference to agronomic traits	Lack of specification of methods
	Selective advantage or disadvantage: comparison with non-GMP and similar phenotypes	theoretically assessed, no specific data provided or reference to agronomic traits	Lack of specification of methods
	Gene transfer assessment	theoretically assessed, no specific data provided or reference to agronomic traits	Lack of specification of methods
	Risk assessment for each different environmental compartment that are exposed to the GMP	Lack of scientific risk assessment model no exposure analysis carried out	
	Non-target organisms: tiered approach	reference to published literature conclusion on no risk only from acute tox lab studies using the toxin and not the GMP	Lack of specification of data requirements by the notifier
	Design of studies in order that sufficient statistical power is obtained to detect possible effects on NTOs	lack of power analysis in field studies (if conducted)	
	Non-target organisms: impact assessment on NTOs, if appropriate, from the aquatic environment	Reference to acute tox lab studies with Daphnia	Lack of criteria when appropriate and methods
	Non-target organisms: Duration of experiments should be sufficient to reflect pattern and duration of exposure that these organisms are	No exposure assessment – thus no other than short term, acute tox tests in the lab	

	likely to experience under field conditions		
	Biogeochemical processes: long-term or sustainable deleterious effect on soil microbial communities and associated functional activities; fate of gene products in environmental compartments which result in exposure of NTOs	Only short-term degradation of protein addressed No exposure assessment of soil organisms Reference to acute-tox tests with soil organisms in NTO assessments	Lack of specification of data requirements
	Biogeochemical processes: exposure estimation of relevant soil biota	no exposure assessment carried out	
	Impacts of cultivation, management, harvesting techniques: description of intended commercial management regimes (incl. changes in applications of PPPs, rotations, etc.) where different from non-GMP	Argumentation: management regime of GMP not different than of non-GMP No indication of intended applications of PPP (e.g. HT crops) No assessment of differences	Lack of specification of data requirements
	Impacts of cultivation, management, harvesting techniques: assessment of effects of management of GMP including biodiversity within GM crop and adjacent habitats	No assessment of biodiversity – reference to Pesticide Directive 91/414/EEC	
	Abiotic environment: on a case-by-case basis	Generally not considered relevant	Lack of indication when such an assessment relevant (criteria)

*PPPs: plant protection products