Marion Dolezel, Marianne Miklau
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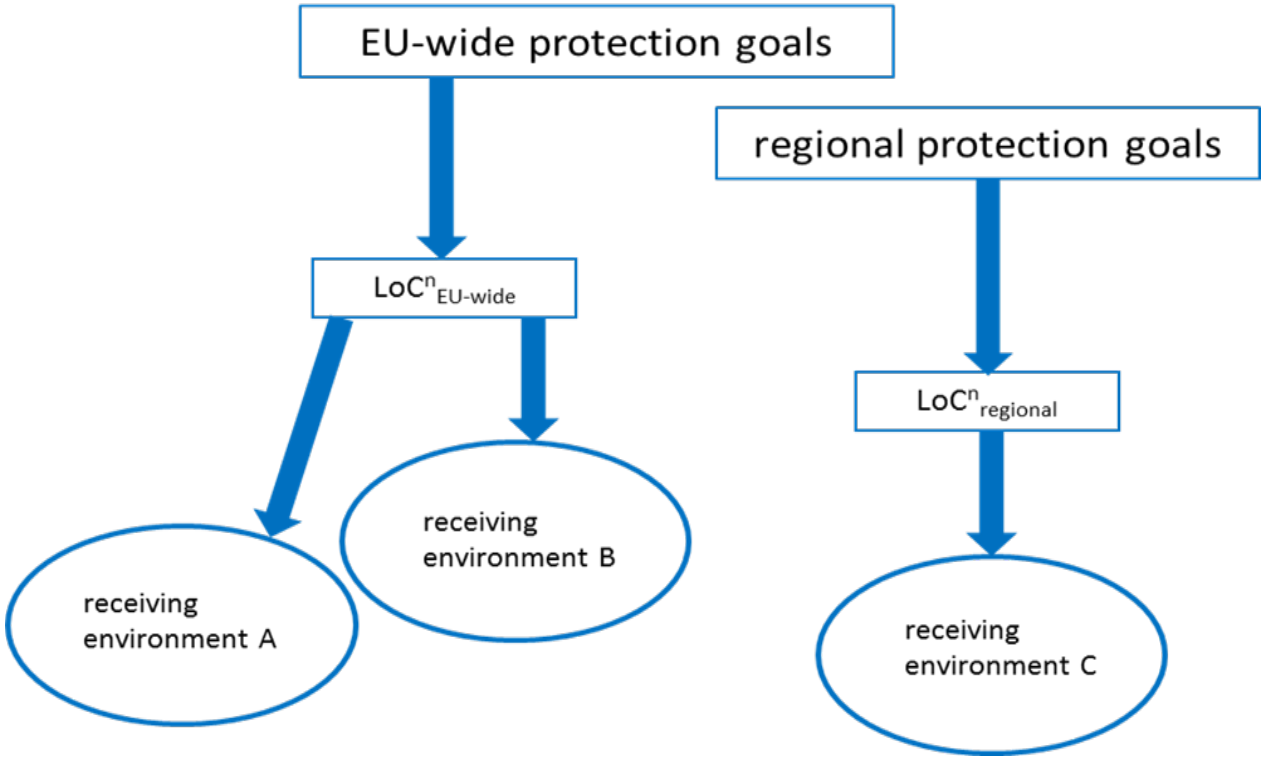
# Limits of Concern für die Risikobewertung von GVP

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# Limits of Concern für die Risikobewertung von GVP

## Endbericht zum gleichnamigen F+E-Vorhaben (FKZ: 3513 89 0200)

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BfN
Bundesamt für Naturschutz

**Titelbild:** Vorschlag zur Regionalisierung des Schadschwellenkonzepts (Limits of Concern, LoC). Possible regionalisation of the LoC concept. (M. Dolezel)

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## List of Abbreviations

| | |
|---|---|
| AF | assessment factor |
| EC | effect concentration |
| EFSA | European Food Safety Authority |
| ERA | environmental risk assessment |
| GMO | genetically modified organism |
| GMP | genetically modified plant |
| HT | herbicide tolerant |
| HQ | hazard quotient |
| IR | insect resistant |
| LoC | Limit of Concern |
| $LoC^{n}$ | sets of Limit of Concerns |
| $LoC_{EU\text{-}wide}$ | Limit of Concern for EU-wide protection goals |
| $LoC_{indicator}$ | Limit of Concern for indicators for ERA testing |
| LC50 | lethal concentration (50 % mortality) |
| LOEC | lowest observed effect concentration |
| LOEL | lowest observed effect level |
| MAF | maximum application factor |
| NTO | non-target organism |
| NOEC | no observed effect concentration |
| NOEL | no observed effect level |
| PPP | plant protection product |
| PEC | predicted environmental concentration |
| PNEC | predicted no effect concentration |
| PNED | predicted no effect density |
| SBA | soybean agglutinin |
| TER | toxicity exposure ratio |

# Zusammenfassung


## Hintergrund und Methoden

Im Jahr 2010 wurde von der Europäischen Lebensmittelbehörde EFSA ein neues Konzept für die Umweltrisikoabschätzung genetisch veränderter Organismen (GVO) eingeführt, das Konzept der „Limits of Concern“ (LoC). Ziel dieses Konzeptes ist es, Schwellenwerte und Kriterien für die Akzeptanz von negativen Effekten bzw. Risiken eines spezifischen GVO in der Risikoabschätzung festzulegen. Eine weitere Neuerung ist die notwendige Berücksichtigung von EU-weiten Schutzzielen bei der Bewertung von Umweltrisiken von GVO. Ziel der beiden Neuerungen ist es, das Vertrauen in die Schlussfolgerungen zu den möglichen Risiken von GVO zu verbessern. Seit der Einführung des Konzeptes im Jahr 2010 wurden von der EFSA weitere Leitliniendokumente zur Verbesserung der Umweltrisikoabschätzung veröffentlicht, im speziellen zur Einbindung von Schutzzielen, geschützten Arten sowie Ökosystemdienstleistungen in die Risikobewertung von GVO.

Vor diesem Hintergrund war es das Ziel dieser Studie, das LoC Konzept kritisch hinsichtlich seiner Anwendbarkeit und praktischen Umsetzung in der Risikobewertung von GVO, die für den Anbau in der EU beantragt werden, zu prüfen. Dabei wurden die folgenden Fragen bearbeitet: (i) Wie können LoCs von Schutzzielen abgeleitet werden? (ii) Welche Rolle spielen GVO im stufenweisen Testverfahren der Umweltrisikoabschätzung? (iii) Wie ist die Verbindung zwischen der vergleichenden Bewertung und LoCs? (iv) Sollen LoCs für Langzeiteffekte festgelegt werden? (v) Sind die von EFSA vorgeschlagenen LoC Werte für Nichtzielorganismen praktikabel und sinnvoll? (vi) Sollen für geschützte und gefährdete Arten verschiedene LoCs verwendet werden? Zusätzlich wurden relevante Aspekte für spezifische GVO und Risikobereiche diskutiert, die bei der Festlegung von LoC berücksichtigt werden müssen. Für verschiedene GVO wurden die relevantesten Schutzziele identifiziert und diskutiert und Vorschläge für notwendige Kriterien für LoCs für die unterschiedlichen Risikobereiche unterbreitet. Zudem wurden Vorschläge zur Verbesserung der praktischen Umsetzung des Konzeptes gemacht, sowie offene Fragen aufgezeigt, die vor der Anwendung des Konzeptes geklärt werden müssen.

Im Rahmen des FuE wurden Leitlinien der EFSA zur Risikoabschätzung von GVO analysiert und mit wissenschaftlichen Stellungnahmen und Leitlinien für Pflanzenschutzmittel, Chemikalien und Biozide verglichen, um Unterschiede und Gemeinsamkeiten zwischen dem LoC Konzept und vergleichbaren Konzepten bei der Umweltrisikobewertung verschiedener Umweltstressoren herauszuarbeiten. Um ein besseres Verständnis des LoC Konzeptes zu erreichen, wurden Interviews mit relevanten Stakeholdern durchgeführt. Ausgewählte GVO Anträge wurden hinsichtlich der Anwendung des LoC Konzeptes geprüft. Drei Fallstudien mit unterschiedlichen GVO, die verschiedene Risikobereiche darstellen, wurden ausgewählt und in Expertenworkshops diskutiert. Ein Treffen mit unterschiedlichen Stakeholdern der GVO Risikobewertung wurde durchgeführt, um Rückmeldung für die Bewertung des Konzeptes, aber auch für die Vorschläge von spezifischen LoC für die drei Fallbeispiele einzuholen.

**Ergebnisse**

Das von der EFSA im Jahr 2010 vorgeschlagene LoC Konzept ist ein nützliches Konzept, um Kriterien für die Akzeptanz nachteiliger Effekte bzw. Risiken, die in der Umweltrisikoabschätzung festgestellt werden, festzulegen. Allerdings muss das Konzept weiter ausgearbeitet und LoCs fallspezifisch festgelegt werden, um das Konzept praktisch anwendbar zu machen. Die für Schutzziele auf EU Ebene definierten Schadensschwellen müssen von festzulegenden Schwellenwerten für nachteiliger Effekte bzw. Risiken im Rahmen der Umweltrisikoabschätzung unterschieden werden. Schutzziele und Biodiversitätsniveaus innerhalb der EU sind nicht einheitlich, was durch eine Regionalisierung von LoC berücksichtigt werden kann. Eine große Herausforderung für die Umsetzbarkeit des LoC Konzeptes stellt die Frage dar, welche Konsequenzen die Überschreitung eines LoCs nach sich zieht. Dazu werden Vorschläge gemacht, wie eine Integration des LoC Konzeptes in das stufenweise Testverfahren der Umweltrisikoabschätzung erfolgen kann.

Es muss eine klare konzeptuelle Trennung zwischen der Risikobewertung von Futter- und Lebensmitteln und der Umweltrisikoabschätzung erfolgen. Während erstere sogenannte Äquivalenzlimits anwendet, die auf der natürlichen Variabilität von nicht GV-Vergleichspflanzen basieren, müssen für die Umweltrisikoabschätzung schutzzielabgeleitete LoCs angewandt werden. Die vergleichende Bewertung und das LoC Konzept müssen miteinander verbunden werden, indem relevante Ergebnisse der inhaltsstofflichen bzw. agronomischen Bewertung auf eine Überschreitung existierender LoCs geprüft werden. Durch die Verbindung können Umweltrisiken für relevante Schutzziele identifiziert und bewertet werden, die sich unter Umständen aufgrund von unbeabsichtigten Veränderungen in der Pflanzenphysiologie bzw. inhaltsstofflichen Zusammensetzungen ergeben.

Die Ergebnisse des FuE zeigen, dass LoCs auch für Langzeiteffekte relevant sind, sofern für sie Risikohypothesen formuliert werden können. Wenn LoCs für Langzeiteffekte festgelegt werden, dann müssen sie durch entsprechende Risikomanagementmaßnahmen begleitet werden, sowie durch eine Langzeitbeobachtung während der gesamten Anbauperiode eines GVO, idealerweise sogar länger.

Jegliche Festlegung von LoC sollte anhand von wissenschaftlichen Daten für die spezifische ökologische Einheit bzw. Ökosystemfunktion erfolgen. Allerdings ist das Wissen über sichere ökologische Schwellenwerte und das Mindestmaß an Biodiversität, das nötig ist, um wichtige Ökosystemfunktionen und -dienstleistungen in Agrarökosystemen zu bewahren, unzureichend. Dabei ist wichtig sich zu vergegenwärtigen, dass Entscheidungen zur Akzeptanz nachteiliger Effekte bzw. Risiken und damit Festsetzungen von LoCs immer einen normativen Aspekt enthalten. Es wird daher vorgeschlagen, die Definition des LoC entsprechend zu erweitern. Der LoC soll einen Schwellenwert darstellen, dessen Überschreitung nicht nur dann Bedenken und möglichen Handlungsbedarf auslöst, wenn das Potenzial zur Verursachung eines ökologischen Schadens besteht, sondern auch, wenn die beobachteten Effekte als wichtig für das spezifische Schutzziel erachtet werden.

Die Vorschläge für die drei Fallbeispiele zeigen, dass die für LoC zu berücksichtigenden Aspekte beträchtlich zwischen den verschiedenen GVO variieren. Daher ist eine fallspe-

zifische Festlegung von LoC notwendig. Für GVO, die mit wildverwandte Arten hybridisieren können, invasiv werden, bzw. in natürlichen Habitaten persistieren können (GV Raps), sind LoCs nicht für tatsächlich beobachtete Biodiversitätseffekte festzulegen, sondern für die biologischen Prozesse, wie beispielsweise Auskreuzung, Verbreitung oder Persistenz in der Umwelt. Die Gefährdung bzw. der Schutzstatus der betroffenen wildverwandten Art bzw. des natürlichen Habitats sind wichtige Kriterien bei der Festlegung von LoC. Präzisierungen bei der genetischen Konstitution der Schutzobjekte sind erforderlich. Andere wichtige Faktoren, die berücksichtigt werden müssen, sind der Status der aufnehmenden Umwelt, die Rückholbarkeit des GVO aus der Umwelt sowie die Umkehrbarkeit möglicher nachteiliger Effekte.

Für herbizidtolerante GVO muss bei der Festlegung von LoC berücksichtigt werden, dass die Umweltrisiken für diese Pflanzen im Rahmen zweier unterschiedlicher Rechtsvorschriften bewertet werden – jene für GVO und jene für Pflanzenschutzmittel. Deshalb müssen jegliche LoCs für herbizidtolerante GVO mit existierenden Schwellenwerten und Kriterien aus der Pflanzenschutzmittelbewertung abgeglichen werden. Eine Unterscheidung zwischen LoCs für Effekte innerhalb und außerhalb des Feldes wird als notwendig erachtet, da unterschiedliche Ökosystemdienstleistungen im Feld bzw. in angrenzenden Ökosystemen erhalten bleiben sollen. In diesem Zusammenhang kann die Anwendung von Schwellenwerten zur Bekämpfung von Beikräutern hilfreich sein; sie würden angeben wieviel Beikrautdiversität es im Feld braucht, um wesentliche Ökosystemdienstleistung zu erfüllen, jedoch ohne den Nutzpflanzenertrag wesentlich zu beeinträchtigen. Die Festlegung von LoCs für Organismen, die auf Beikräuter angewiesen sind (z. B. Nützlinge, Vögel), wird dadurch erschwert, dass die quantitativen Beziehungen und Abhängigkeiten zwischen Beikräutern und höheren trophischen Ebenen nur begrenzt bekannt sind.

Für insektenresistente GVO wie *Bt* Mais haben beobachtete Effekte auf Nichtzielschmetterlinge als Repräsentanten der Biodiversität, aber auch als geschützte und gefährdete Arten in Agrarökosystemen, bereits Diskussionen darüber ausgelöst, welche Risiken für diese Gruppe von Nichtzielorganismen akzeptabel sind. Sowohl das Ökosystemdienstleistungskonzept als auch das LoC Konzept können beide angewandt werden, um Schwellenwerte für Risiken für Nichtzielschmetterlinge festzulegen. Dabei ist jedoch zu beachten, dass auch für diese GVO eine Unterscheidung von LoCs innerhalb und außerhalb der Anbaufläche getroffen wird. Risiken für Nichtzielschmetterlinge durch *Bt* Mais Anbau außerhalb der Anbauflächen müssen geringfügig sein. Darüber hinaus werden für diese GVO auch Vorschläge zur Funktion von LoC für Laborstudien bei der Umweltrisikoabschätzung gemacht.

Die Ergebnisse des FuE zeigen auch, dass LoCs für Nichtzielorganismen je nach Art, Population und evaluierten Parameter differenziert festgesetzt werden müssen. Dabei müssen geschützte und gefährdete Arten notwendigerweise separat im LoC Konzept berücksichtigt werden. Es muss sichergestellt werden, dass Effekte und Risiken zusätzlicher Umweltstressoren wie GVO nicht die Schutzziele dieser Arten beeinträchtigen.

Die LoCs für alle drei diskutierten Fallbeispiele müssen nicht nur Auswirkungen des Anbaus der GVO auf die Biodiversität, sondern auch auf landwirtschaftliche Schutzziele berücksichtigen. Daher werden LoCs auch für landwirtschaftliche Schutzziele vorgeschla-

gen, die bisher in der Umweltrisikoabschätzung von GVO größtenteils vernachlässigt wurden.

**Schlussfolgerungen**

Das von der EFSA im Jahr 2010 eingeführte LoC Konzept ist ein nützliches Konzept, um mögliche Umweltschäden durch den Anbau von GVO zu bewerten. Allerdings muss das Konzept weiter entwickelt und spezifiziert werden. Auch sind weitere Vorgaben notwendig, um das Konzept praktisch umsetzen zu können. LoCs müssen auf wissenschaftlicher Grundlage festgelegt werden, sie beinhalten jedoch zugleich eine normative Komponente, da die EU Mitgliedstaaten mit den LoCs zugleich ihre Prioritäten bei agronomischen und umweltrelevanten Schutzzielen festlegen müssen. Das Fehlen einer ausschließlich wissenschaftlichen Begründung sollte jedoch nicht dazu führen, dass keine LoCs festgelegt werden. Jedenfalls müssen die politischen und wissenschaftlichen Begründungen, die hinter jeder LoC Festlegung stehen, transparent gemacht werden. Die Festlegung von LoCs muss eine Vielzahl an Stakeholdern einbinden, beispielsweise aus dem Risikomanagment, der Risikobewertung, der Antragstellung sowie die wissenschaftliche Gemeinschaft. Nur so können Risiken für spezifische Schutzobjekte in Agrarökosystemen aufgrund des Anbaus unterschiedlicher GVO und anderer Umweltstressoren so gering wie möglich gehalten werden. Dies kann zudem auch die Glaubwürdigkeit von Schlussfolgerungen über mögliche Umweltrisiken bestimmter GVO für alle Stakeholder erhöhen. Bei der Festlegung von LoC muss berücksichtigt werden, dass bereits die konventionelle Landwirtschaft gewisse nachteilige Effekte auf die terrestrische und aquatische Biodiversität in Agrarökosystemen hat, und dass diese zugleich als Vergleich für die Evaluierung möglicher nachteiliger Umwelteffekte durch GVO Anbau dient. Folglich sind keine zusätzlichen nachteiligen Effekte bzw. Risiken für die Biodiversität aufgrund von GVO Anbau zu tolerieren, damit sich die Biodiversität in europäischen Agrarökosystemen nicht weiter verschlechtert.

## Summary

### Background and Methodology

In 2010 the European Food Safety Authority EFSA introduced a novel concept for the environmental risk assessment (ERA) of genetically modified organisms (GMOs), the Limits of Concern (LoC). Aim of this concept was to define criteria and thresholds for the acceptability of adverse effects and risks posed by the specific GMO assessed during ERA. Another novel feature was the integration of EU-wide protection goals in the ERA. Both features intend to increase confidence in the risk conclusions made on the safety of GMOs by a quantitative evaluation of environmental harm. Since the introduction of this concept in 2010 a range of further guidance documents have been published by EFSA to improve the ERA, in particular with respect to protection goals, protected species and ecosystem services.

Against this background the aim of this report was to critically scrutinize the LoC concept regarding its applicability and the practical implementation in the risk assessment for GMOs intended for cultivation in the EU. The following questions were posed: (i) How can LoCs be derived from protection goals? (ii) Which role do LoCs play in the stepwise testing approach of the ERA of GMOs? (iii) What is the relationship between the comparative safety assessment and LoCs? (iv) Should LoCs be set for long-term effects? (v) Are the LoC values suggested by EFSA for non-target organisms practicable and reasonable? (vi) Should there be different LoCs for species of conservation concern and non-protected species? In addition, important aspects for specific types of GMOs and specific areas of risk, which need to be taken into consideration when defining LoCs, were discussed. Also, the most relevant protection goals for each type of GMO were discussed and the necessary criteria for the definition of LoCs for these specific risk areas and types of GMOs were proposed. Essential improvements were suggested for the practical operationalisation of the LoC concept, and open issues addressed which need to be solved before the LoC concept can be applied.

The EFSA ERA Guidance Document for GMP, Scientific Opinions and other relevant guidance documents were analysed in order to achieve a comprehensive understanding of the LoC concept. Guidance Documents for plant protection products, chemicals and biocides were screened in order to compare the LoC concept with concepts applied in the environmental risk assessment of other environmental stressors. In order to recognize the perception of the LoC concept, interviews with relevant stakeholders were conducted. Selected GMO applications were scrutinized regarding the application of the LoC concept. Three case studies with different types of GMOs representing different areas of risk evaluated in the ERA were selected and discussed in expert workshops. A stakeholder meeting was organised for feedback on the appraisal of the concept and the proposals for specific LoCs for the three case studies.

### Results

The LoC concept, as suggested by EFSA in 2010, is considered to be a useful concept for introducing thresholds and criteria for the acceptability of adverse effects or risks assessed in the ERA of GMOs. However, the concept still needs to be specified and different LoCs to be formulated on a case-by-case basis in order to make the concept opera-

tional for use in ERA practice. Harm thresholds for protection goals at EU level need to be differentiated from acceptability thresholds for adverse effects and risks used for ERA testing. It must be recognised that protection goals and biodiversity levels vary across the EU which must be accounted for by the possibility to regionalise LoCs. A major challenge for the operationalisation of the concept refers to the consequences in case LoCs are exceeded. A proposal is made on how to integrate the LoC concept into the stepwise ERA testing process.

A clear conceptual distinction is needed between the risk assessment for food and feed purposes, with equivalence limits based on the natural variability of non-GM comparators, and the environmental risk assessment of GMOs using protection goal-derived LoCs. The comparative safety assessment and the LoC concept need to be linked by scrutinizing whether relevant results from the compositional and agronomic analyses exceed existing LoCs. This allows identifying and assessing environmental risks for relevant protection goals that might be driven by e.g. unintentional changes in the plant's physiology and composition.

LoCs are also useful for long-term effects, if the latter can be substantiated by a risk hypothesis. If LoCs are defined for long-term effects they must be accompanied by corresponding risk management measures as well as long-term monitoring activities. Both shall be applied during the whole cultivation period of the GMP and ideally even longer.

Any LoC definition should be based on scientific data for the specific ecological entity or ecosystem function. However, due to the limited knowledge on safe ecological limits and the minimum level of biodiversity that is needed to sustain important ecosystem functions and services in agro-ecosystems, it is important to acknowledge that any decision on the acceptability of adverse effects and risks will contain also a normative aspect. Therefore the definition of the LoC should be extended. The LoC should represent a threshold that triggers regulatory concern not only if there is a potential to cause harm but also due to the decision that the effects observed are considered important for a specific protection goal.

The suggestions for the definition of LoCs for the three different types of GMOs and different areas of risk show that the specific aspects relevant for LoCs differ considerably between the different types of GMOs and require individual and case-by-case evaluation. For GMOs that are able to outcross into wild relatives, invade and persist in natural habitats (GM oilseed rape) LoCs will be based on biological processes such as outcrossing, spread or persistence rather than on the evidence for biodiversity threats. The conservation concern and the protection status of the affected wild relative or the natural habitat is an important criterion when determining the LoC. Further specifications are needed with respect to the genetic constitution of the protection objects. Other important factors to be considered are the status of the receiving environment, the retrievability of the GM crop from the environment and the reversibility of potentially adverse effects that may occur.

For herbicide tolerant GMOs any decision on LoCs must consider that environmental risks for these crops are assessed by two distinct authorization regimes – those relevant for GMOs and those relevant for plant protection products. Therefore, any LoC for GM herbicide tolerant crops must be aligned with existing acceptability thresholds and criteria for the non-selective herbicide which are applied in the ERA of plant protection products.

A distinction between LoCs for acceptable effects within and outside the field is necessary, as different ecosystem services are to be preserved in different spatial areas of the agro-environment. In this context the use of weed thresholds for weed control in fields can be helpful; they would define the minimum levels of weed biodiversity required in order to fulfil important ecosystem services in-field without compromising food production and crop yields. The determination of LoCs for organisms dependent on weeds (e.g. beneficial organisms, birds) will be impeded by the limited knowledge on quantitative links between weeds and weed-related biodiversity.

In case of insect resistant GMOs such as *Bt* maize non target butterflies have already triggered discussions on which risks are acceptable for this group of non target organisms. In the ERA of *Bt* maize common non target butterflies but also species of conservation concern represent part of the biodiversity in agroecosystems. Both, the ecosystem service concept and the LoC concept, can be used for defining thresholds for the acceptability of risks for non target butterflies. Importantly, also for these GM crops a differentiation of LoCs between in-crop and off-crop areas is needed, ensuring that risks for butterflies occurring outside the production area are negligible. For this type of GMO suggestions are also made for the potential role of LoCs for laboratory studies conducted during the ERA.

The results show that a differentiation of LoCs for non target organisms has to be made according to the respective species, population and parameter evaluated. In this context, species of conservation concern must be separately addressed by the LoC concept. It has to be ensured that pressure from additional environmental stressors such as GMOs must not compromise the protection objectives for these species.

LoCs for all three types of GMOs discussed in this study must take into account not only effects of their cultivation on biodiversity but also on agricultural protection goals. Suggestions are made for the determination of LoCs for agricultural protection goals which have so far been largely neglected in the ERA of GM crops.

**Conclusions**

The LoC concept as introduced by EFSA for the ERA in 2010 is a useful concept for the evaluation of environmental harm due to the cultivation of GMPs. However, the concept needs to be further developed and specified and further guidance is necessary for its practical implementation in GMO risk assessment. Clearly, LoCs must be founded on scientific grounds but at the same time will have to reflect a normative component which urges EU Member States to focus on their priorities regarding agro-environmental protection goals. Just because LoCs cannot be justified purely on scientific grounds shall not prevent them from being formulated, but in any case the political and scientific justifications behind the decisions on LoCs must be made transparent. The definition of LoCs will have to involve several stakeholders such as risk managers, risk assessors, applicants and the scientific community, in order to ensure that risks for particular protection objects in agro-environments due to different GMPs and other environmental stressors are kept as low as possible. This would also ensure confidence in risk conclusions by all stakeholders for a particular GMO. When defining LoCs it has to be considered that the current predominant type of agriculture is already exerting some adverse effects on terrestrial and aquatic agro-biodiversity and at the same time it is used as a comparator for the

evaluation of potential adverse effects due to GMO cultivation. Consequently, no additional impacts on biodiversity due to GMP cultivation should be considered acceptable in order not to further deteriorate biodiversity in European agro-ecosystems.

# 1 Background and Structure of the Report

In the European Union genetically modified organisms (GMOs) need to undergo an authorization procedure in which an environmental risk assessment (ERA) is performed in order to conclude on potential risks to the environment. The European Food Safety Authority (EFSA) and its GMO Panel play a crucial role in this authorization procedure as they issue, based on data provided in the GMO applications, Scientific Opinions on the safety of GMOs and provide advice for risk managers (i.e. the European Commission and the EU Member States). In addition, the GMO Panel produces guidance documents to specify certain aspects of GMO risk assessment and to provide guidance for the preparation and presentation of GMO applications.

For the risk assessment of GMOs and derived food and feed, EFSA suggests the use of a comparative safety assessment as a starting point for the whole risk assessment process (EFSA 2006, EFSA 2011a). In this concept, a comparator, usually a non-GM plant with a similar genetic background is used for comparison when assessing intended and possibly unintended effects of the GMO. This assessment comprises compositional parameters and other plant characteristics such as agronomic or phenotypic parameters. For the ERA the comparative safety assessment should also use information on plant-environment interactions of the GMO (EFSA 2010a). The concept behind the comparative safety assessment is the assumption that conventionally cultivated plants are safe for consumers, animals and the environment. Their `history of safe use´ should therefore assist the safety evaluation of a novel or GM food (EFSA 2011a, Constable et al. 2007, EFSA 2010c).

In 2010, EFSA published a guidance document for the ERA of GMOs (EFSA 2010a). The aim of this document was to further develop and update guidelines for the ERA previously available (EFSA 2006). In the guidance document EFSA requires that the biological relevance of statistically significant differences between the GMO and the non-GM comparator should be assessed, also considering potentially hazardous environmental implications (EFSA 2010a). EFSA clearly recognizes that such differences could be linked to morphological alterations or metabolic perturbations or may indicate unintended effects which may lead to environmental harm. Any identified potential adverse effect should be linked to assessment endpoints in order to quantitatively evaluate the potential environmental harm. These assessment endpoints are then translated into measurement endpoints for which a Limit of Concern (LoC) has to be defined. EFSA defines Limits of Concern as “the minimum ecological effects that are deemed biologically relevant and that are deemed of sufficient magnitude to cause harm” (EFSA 2010a). By testing whether the observed effect falls within the LoC, the biological relevance of the observed effect is determined. According to EFSA, LoCs can be derived from literature data, baseline data, modelling, existing knowledge, or policy goals and shall be explicitly stated and justified by the applicant (EFSA 2010a). The setting of the LoC and the definition of environmental harm are therefore considered crucial for the ERA of a GMO and in particular also for the assessment of potential effects on non-target organisms (EFSA 2010b). The necessity to set thresholds when determining acceptability of risks has also been recognized in international guidelines for the risk assessment of living modified organisms (CBD 2012).

An important aspect of the revised guidance document is the necessity to evaluate potential adverse effects of GMOs on the environment with regard to the potential harm they may pose for defined environmental protection goals. Protection goals are natural resources (e.g. arthropod natural enemies, bees) or natural resource services (e.g. regulation of arthropod pest populations, pollination) that are to be protected as set out by EU legislations (EFSA 2010a). Protection goals include biodiversity, protected species and habitats, but also ecosystem functions and ecosystem services as well as water, soil, and human and animal health. Examples of environmental protection goals and their legal basis in the European Union with relevance for GM plants are explicitly listed (EFSA 2010a).

According to EFSA relevant protection goals should be identified in the problem formulation step of the ERA and should be translated into assessment and measurement endpoints in order to be able to assess potential adverse effects by the GMP on natural resources or natural resource services. The LoC should be set for each measurement endpoint representing the level of protection. Further reference with respect to examples on how to consider these protection goals in the ERA is made in the Guidance Document on non-target organisms (EFSA 2010b). In this Guidance Document also the relevance of the ecosystem services (MEA 2005) in the context of the ERA of GMOs is emphasized (EFSA 2010b).

Further guidance on how to operationalise protection goals for ERA purposes is contained in a Scientific Opinion for the ERA of plant protection products (PPPs, EFSA 2010d). In this Scientific Opinion general protection goals are differentiated from specific protection goals. General protection goals refer to “overall goals to be achieved as required by the EU legislation to protect human health and the environment from unacceptable impacts of pesticides” (EFSA 2010d). Specific protection goals are “the entities that need to be protected, the attributes and/or functions of those entities, as well as the magnitude, temporal and spatial scales of effects on these attributes and/or functions that can be tolerated without impacting the general protection goals and the required degree of certainty with which the protection goal defined should be achieved” (EFSA 2010d). For the operationalisation of the protection goals it is suggested to use the ecosystem service concept. Relevant ecosystem services affected by plant protection products and their key drivers, i.e. taxa or functional groups, were identified and specific protection goals (SPGs) for each of the key driver/ecosystem service combinations proposed. These SPGs are composed of the following five dimensions: the ecological entity (e.g. biological organisation), the attribute (e.g. survival, nutrient cycling), the magnitude of effect that can be tolerated for the attributes and the temporal and spatial scales of the effects that can be tolerated for the attributes as well as the degree of certainty required that the effects will not exceed the specified levels (EFSA 2010d). This ecosystem service concept has recently been proposed for ERA use for other environmental stressors than PPPs, such as GMOs, feed additives and invasive alien species (EFSA 2016a). Additionally, two further Scientific Opinions have been issued by EFSA that address the coverage of endangered species and the ecological recovery of non-target organisms in the ERA, also relevant for different environmental stressors including GMOs (EFSA 2016b, EFSA 2016c). These documents indicate that EFSA strives for a common level of protection for agro-ecosystems independent of the stressor (EFSA 2014a).

In 2013, the German Federal Agency for Nature Conservation commissioned the project `Limits of Concern for the Risk Assessment of GM plants´. The aim of this project was the critical evaluation of the concept of Limits of Concern introduced by EFSA, as well as suggestions for improvements for the practical implementation in GMO risk assessment.

Open issues were identified and discussed in order to encourage the scientific discussion and make further progress in the practical implementation of the LoC concept in the ERA of GMOs. During the course of the project, using *inter alia* expert workshops and interviews the following key questions were identified:

- How can LoCs be derived from protection goals?
- Which role do LoCs play in the stepwise testing approach of the ERA of GMOs?
- What is the relationship between the comparative safety assessment and LoCs?
- Should LoCs be set for long-term effects?
- Can specific LoCs be formulated for certain areas of risk and which factors affect the formulation of LoCs for these areas of risk?
- Are the LoC values for non-target organisms suggested by EFSA (2010a) practicable and reasonable?
- Should there be different LoCs for species of conservation concern and non-protected species?

The suggestions made for LoCs are based on a conceptual framework for assessing environmental harm due to the cultivation of GMOs elaborated by Kowarik and co-workers (KOWARIK et al. 2008). However, where necessary, links are made to the ecosystem service concept and current developments in the ERA, in particular if related to discussions on acceptability thresholds and criteria for adverse environmental effects.

The project comprised several work packages: the evaluation of relevant EFSA guidance documents with respect to the LoC concept, the analysis of environmental acceptability criteria and thresholds applied for regulated products other than GMOs, the realisation of stakeholder interviews and expert workshops for feedback and critical discussions of the concept as well as the suggestions for LoCs exemplarily for three different types of GMO and areas of risk for the ERA of GMPs.

This report is the final report of the project in which the final results and conclusions of the individual work packages are presented. The results presented in the individual chapters represent the single steps of the project that led to the final suggestions for the improvement and operationalisation of the LoC concept in general and in particular for the three different types of GMO and areas of risk.

# 2 Methodology

In this chapter the methodology used in this study is described.

## 2.1 The `Limits of Concern´ concept according to EFSA

The LoC concept was described in detail by analysing several EFSA Guidance Documents and Scientific Opinions with relevance for the ERA of GMOs published since 2010. In addition, unclear aspects and inconsistencies between the documents were addressed.

The following EFSA documents were screened:

- Guidance on the environmental risk assessment of genetically modified plants (EFSA 2010a)
- Assessment of potential impacts of GM plants on non-target organisms (EFSA 2010b).
- Statistical considerations for the safety evaluation of GMOs (EFSA 2010c)
- Guidance on selection of comparators for the risk assessment of GM plants (EFSA 2011a)
- Guidance for risk assessment of food and feed from GM plants (EFSA 2011b)
- Scientific Opinion on statistical significance and biological relevance (EFSA 2011c)
- Guidance to develop specific protection goal options for environmental risk assessment in relation to biodiversity and ecosystem services (EFSA 2016a)
- Coverage of endangered species in environmental risk assessments at EFSA (EFSA 2016c)

## 2.2 Understanding the LoC concept – Interviews with stakeholders

To complement the analysis of the LoC concept as described by EFSA in the relevant guidance documents and Scientific Opinions, interviews with selected stakeholders were conducted. The aim of the interviews was to deepen the understanding of the LoC concept, in particular regarding its specific application for different types of GMOs (e.g. herbicide tolerant and insect resistant) and in different areas of risks (e.g. impacts on non-target organisms, persistence and invasiveness including plant-to-plant gene transfer and impacts of cultivation, management and harvesting techniques) and to identify open issues and inconsistencies or ambiguities. Also specific questions concerning statistical aspects as well as aspects regarding the implementation of the concept within the framework of the EU legislation relevant for GMOs were addressed.

In total 33 questions were identified for the interviews. These questions were grouped according to specific topics (e.g. regarding the application of the concept for certain risk areas, statistical aspects of the concept or aspect of its implementation). For each individual interview the questionnaire was adapted according to the expertise and field of activity of the interviewee. Each potential interviewee received the questions and a short project description in advance, together with the request for an interview. The questions therefore served as interview guidelines and for the preparation for the interview.

The interviews were conducted by telephone between September 23rd and October 14th 2014. The answers given by the interviewees were documented in interview protocols. The interviews lasted between one and two hours and were not recorded on any electronic device.

Stakeholders who were expected or known to be familiar with the LoC concept and the environmental risk assessment of GMPs were selected for the interviews. Some of these experts were involved in the development of the EFSA Guidance Document and the concept of LoCs. Others were known to deal with the LoC concept in the context of the ongoing EU project 'Assessing and Monitoring the Impacts of Genetically Modified Plants on Agro-ecosystems' (AMIGA) funded by the European Commission under the Framework Programme 7. One aim of this project was to generate scientific data related to the possible environmental and economic impacts of cultivation of genetically modified plants (GMPs) relevant to European environments useful for the derivation of LoC (ARPAIA et al. 2014). In addition to experts from the EFSA GMO unit dealing with the implementation of LoC, experts from the industry and representatives from NGOs were also contacted. As far as the experts from the GMO Unit are concerned it was agreed with them that the interview with one of their members should be sufficient to reflect the position of EFSA regarding this topic. Non-disclosure of the identity was requested by some of the interviewees. The following stakeholders were interviewed for the LoC concept:

- Member of National Competent Authority, former GMO Panel Member
- Scientific Officer, EFSA, GMO Unit
- Scientist, University, expert for Lepidoptera
- Independent Researcher, GMO Panel Member
- Scientist, Syngenta Crop Protection AG, Switzerland
- NGO representative

## 2.3 The use of the LoC concept used in GMO applications

### 2.3.1 Analysis of GMO applications

GMO applications according to Regulation (EC) No. 1829/2003 were scrutinized with the focus on the application of the LoC concept. This could be expected because all applications were submitted in 2010 and later and should therefore follow EFSA's revised ERA guidance (EFSA 2010a). The following criteria were applied when selecting the applications for the analysis:

- Adherence to the new structure of the EFSA Guidance Document (EFSA 2010a)
- Application for cultivation of the GMO within the EU

At the time of the analysis of the applications (start of 2014) there was only one GMO application which fulfilled the above mentioned criteria: the application of Bayer CropScience (2012) for the cultivation of herbicide tolerant cotton GHB614 (ES-2012-04). However, in this application no equivalence tests according to EFSA (2010a) were carried out, hence this application could not be used for the analysis. By end of July 2014 the application was withdrawn by the applicant. Instead, GMO applications for import and processing were evaluated whether the LoC concept was applied for environmentally rel-

evant plant compounds in the comparative safety assessment. Table 1 gives an overview of the GMO applications initially screened.

Table 1. GMO applications according to Regulation (EC) No. 1829/2003 selected for the analysis of the use of the LoC concept.

| Application number | GMO | Applicant | Scope of application | New structure |
|---|---|---|---|---|
| NL-2011-100 | Soybean MON 87705 x MON 89788 | Monsanto | food, feed, import, processing | yes |
| ES-2012-104 | Cotton GHB614 | Bayer | cultivation | yes |
| NL-2012-106 | Soybean DAS-44406-6 | Dow | food, feed, import, processing | yes |
| NL-2012-108 | MON 87708 x MON 89788 Soybean | Monsanto | food, feed, import, processing | yes |
| NL-2012-109 | Oilseed rape 73496 | Pioneer | food, feed, import, processing | yes |
| BE-2012-110 | Maize MON 87427 | Monsanto | food, feed, import, processing | yes |
| DE-2012-111 | Soybean SYHT0H2 | Syngenta | food, feed, import, processing | yes |
| NL-2013-114 | Cotton MON88701 | Monsanto | food, feed, import, processing | yes |
| NL-2013-116 | Soybean DAS-81419-2 | Dow | food, feed, import, processing | yes |

The analysis of the applications focussed on the plant composition assessed in the comparative safety assessment. Those compositional parameters were selected for which no equivalence could be shown. This referred to the following equivalence categories according to EFSA (2010):

- Category iii (equivalence less likely than not)
- Category iv (non-equivalence)

From the applications listed in Table 1, two dossiers were selected for detailed analysis based on the non-equivalence of plant compositional parameters with environmental relevance, namely the two anti-nutritiva lectins and gossypol. The two dossiers are:

- Soybean DAS-44406-6 (application NL-2012-106, herbicide tolerance)
- Cotton GHB 614 (application ES-2012-104, herbicide tolerance)

Using these two examples it was scrutinized whether the LoC concept was applied for the selected non-equivalent compositional parameters.

### 2.3.2 **Literature search**

The environmental relevance of the selected compositional parameters (lectins, gossypol) was evaluated using data from the scientific literature. This was done by evaluating whether adverse effects of the selected parameters have been described in the scientific literature. The databases Scopus (Advanced Search Tool) and Google Scholar were searched for by using the following search terms (in different combinations):

"SBA", "SBL", "soybean agglutinin", "soybean lectin", "toxicity" or "toxic effect(s)" or "effect(s)", "arthropod(s)", "gossypol", "phytoalexin(s)", "terpenoid(s)"

## 2.4 The use of thresholds in the ERA of other regulated products than GMOs in the EU

In this chapter thresholds and criteria used for the decision on the acceptability of adverse effects and risks in the ERA of regulated products other than GMO are described and analysed regarding aspects which may be also relevant for the LoC concept. The regulatory areas addressed were: plant protection products (PPPs), chemicals and biocides and ambient air quality. In addition, the Environmental Liability Directive (Directive 2004/35/CE), the FFH Directive (Council Directive 92/43/EEC) and the Impact Regulation of the German Nature Protection Law (BNatSchG, clause 13) were evaluated with respect to definitions of environmental harm and harm thresholds.

### 2.4.1 **Plant protection products**

The ERA of PPPs was described and relevant acceptability thresholds analysed using the relevant ERA requirements and related Guidance Documents. By using the case-study of the insecticide Chlorpyrifos, the relevant trigger values for the first tier and higher tier assessments were reported. The following legislative documents were screened for the analysis:

- Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.
- Council Directive 2005/25/EC of 14 March 2005 amending Annex VI to Directive 91/414/EEC as regards plant protection products containing micro-organisms
- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
- Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market
- Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

In addition the following guidance documents were analysed:

- SANCO (2002a). Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC. Draft Working Document. SANCO/10329/2002rev2final, 17 October 2002.
- SANCO (2002b). Guidance document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC. Working Document. SANCO/3268/2001rev.4(final), 17 October 2002.
- EC (2000). Guidance Document on persistence in soil. Working Document. DG Agri 9188/VI/97rev.8 12 July 2000.
- Candolfi M. P., Barrett K. L., Campbell P. J., Forster R., Grandy N., Huet M.-C., Lewis G., Oomen P.A., Schmuck R. & H. Vogt (2000). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop held at Wageningen International Conference Centre, 21-23 March 2000, Wageningen, The Netherlands. pp 50
- Campbell P. J., Brown K. C., Harrison E. G., Bakker F., Barrett K. L., Candolfi M. P., Canez V., Dinter A., Lewis G., Mead-Briggs M., Miles M., Neumann P., Romijn K., Schmuck R., Shires S., Ufer A. & A. Waltersdorfer (2000). A hazard quotient approach for assessing the risk to non-target arthropods from plant protection products under 91/414/EEC: hazard quotient trigger value proposal and validation. Journal of Pest Science 73, 117-124
- EFSA (2013). Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 11 (7): 3295
- EPPO (2003a). Environmental risk assessment scheme for plant protection products. Chapter 4: Soil. OEPP/EPPO Bulletin 33: 147-149
- EPPO (2003b): Environmental risk assessment scheme for plant protection products. Chapter 8: Soil organisms and functions. OEPP/EPPO Bulletin 33: 195-209
- EPPO (2003c): Environmental risk assessment scheme for plant protection products. Chapter 12: non-target terrestrial higher plants. OEPP/EPPO Bulletin 33: 239-244
- EPPO (2010a). Environmental risk assessment scheme for plant protection products. Chapter 10: honeybees. OEPP/EPPO Bulletin 40: 323-331
- EPPO (2010b). EPPO Standards PP1/170(4) Efficacy evaluation of plant protection products. Side-effects on honeybees. Bulletin OEPP/EPPO Bulletin 31, 323-330

### 2.4.2 **Biocides and Chemicals**

Trigger values and decision criteria used in the ERA of biocides and chemicals were evaluated from the relevant documents. A focus was put on the use of safety factors when adverse effects are assessed on soil organisms. As a case study the risk assessment of the biocidal product *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52 was used and the risk characterisation outlined. In addition, an example of the setting of an acceptability threshold in order to restrict the placing on the market of a chemical was outlined.

The following legislative documents and guidance documents were analysed:

- Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethosylate and cement).
- Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market.
- Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.
- Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (REACH)
- Commission Directive 2011/78/EU of 20 September 2011 amending Directive 98/8/EC of the European Parliament and of the Council to include *Bacillus thuringiensis* subsp. *israelensis* Serotype H14, Strain AM65-52 as an active substance in Annex I.
- ECB (2003). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment of existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II, Chapter 3: Environmental Risk Assessment. European Chemical Bureau.
- ECHA (2008). Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment.
- ECHA (2012). Guidance on information requirements and chemical safety assessment. Part E: risk characterisation
- ECHA (2013). Guidance on information requirements. Guidance on Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (BPR). Version 1.0. www.echa.europa.eu
- Swedish Chemicals Agency (2013). Restriction report. Proposal for a restriction of Nonylphenol and Nonylphenolethoxylates in textiles. http://echa.europa.eu/

### 2.4.3 **Ambient Air Quality**

Environmental quality standards in the EU for ambient air were evaluated. Thresholds for the acceptability of air pollutants were presented and analysed.

The following documents were used for the analysis:

- Directive 2001/81/EC of the European Parliament and of the Council of 23 October 2001 on national emission ceilings for certain atmospheric pollutants.
- Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe.

- Directive 2004/107/EC of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air.
- WHO (2000). Air Quality Guidelines for Europe. Second Edition. WHO Regional Publications, European Series, No. 91.

#### 2.4.4 Environmental Liability Directive and FFH Directive

The EU Directive on Environmental Liability (Directive 2004/35/EC) and the FFH-Directive (Council Directive 92/43/EEC) were analysed with respect to their definitions of environmental damage and damage thresholds.

#### 2.4.5 German `impact regulation´

The impact regulation under the German Nature Protection Law (BNatSchG, clause 13) was analysed regarding its definition of significant impacts on the environment.

## 2.5 Limits of concern for three areas of risks – conclusions from Expert-Workshops

In 2015 three workshops were held at the Environment Agency Austria in Vienna. The aim of the workshops was to discuss several aspects of the LoC concept with experts from different fields of expertise. Another aim was to evaluate the possibilities for operationalisation of the LoC concept and to identify problematic aspects for the implementation of the concept. Therefore, the three workshops discussed different examples for the operationalisation of the concept. The following topics were discussed:

- Protection goals
- Indicators for ERA testing
- Calibration of adverse effects
- Criteria for acceptability thresholds/LoCs
- LoCs for tritrophic and sublethal effects
- Differentiating LoCs in the stepwise testing approach of the ERA
- Consideration of protected species
- Consideration of uncertainties
- LoCs for stacked event GMPs
- LoCs for long-term effects

The results of the workshops were summarized in workshop reports and sent to the participants for commenting. Received comments were fed into the final workshop reports. The final workshop reports were used as the basis for the development of suggestions for the operationalisation of LoCs for three different areas of risk.

Workshop dates and participants are outlined in Annex I.

## 2.6 Suggestions for LoCs for three different risk areas

Suggestions for the definition of Limits of Concern for three different areas of risk were made. Using case studies of different GMOs (GM oilseed rape, herbicide tolerant GM crops and insect resistant *Bt* maize) the relevant aspects when defining LoCs were outlined. The evaluations were based on a conceptual framework for the assessment of eco-

logical damage for GM crops (KOWARIK et al. 2008). KOWARIK et al. (2008) suggested a methodology for assessing ecological damage due to GMO cultivation, considering not only the potential adverse effect by the GMO, but also taking the protection goal(s) that may be affected into account.

Adverse environmental effects of GMOs on protection goals are not always testable in the ERA, in particular if these are indirect effects, e.g. effects occurring through a causal chain of events, delayed effects or effects that occur in novel environmental contexts not encountered during the ERA. KOWARIK et al. (2008) recommended the use of indicators in order to assess adverse effects of the GMO in the ERA. These indicators were used as the basis for discussions on LoCs. Indicators were discussed with respect to their usefulness when setting LoCs and – if necessary – additional indicators were suggested. Indicators may be selected from the chain of adverse effects at different levels. Indicators can be selected at the level of triggers or processes or at the effect level (Figure 1). The results provide a basis for the definition of LoCs in the environmental risk assessment of GMOs for the three areas of risk.

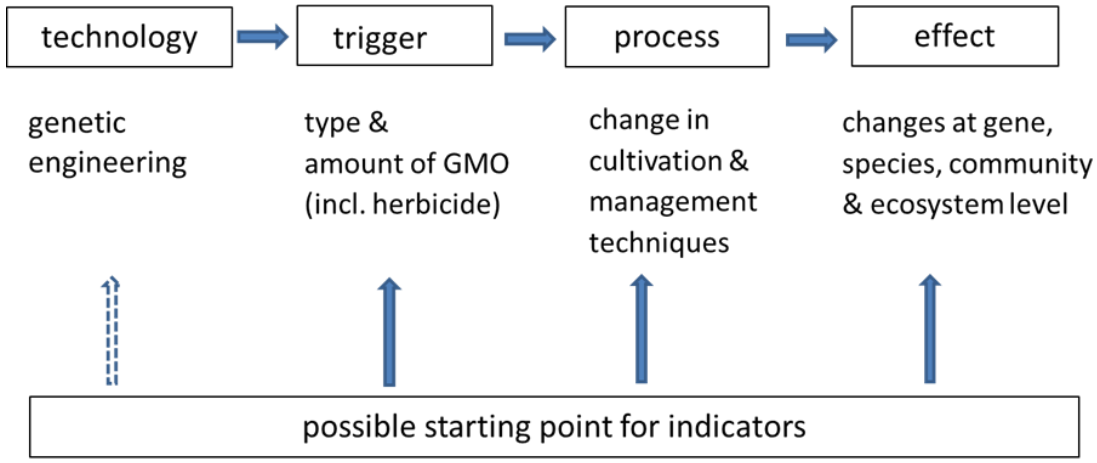


Figure 1. Chain of adverse effects of GMOs and possible starting points for the choice of indicators (KOWARIK et al. 2008).

The suggestions for LoCs for the three different types of GMOs and risk areas were sent to the experts who participated in the three workshops for commenting. Four experts sent their comments. At least one expert commented each of the thematic LoC suggestions.

## 2.7 Feedback workshop

On 25th October 2016 a workshop was held at BfN, Bonn, from 10:00 hrs to 16:30 hrs. The aim of the workshop was to discuss

- the general aspects proposed in order to make the LoC concept operational
- the results of the suggestions for the Limits of Concern for three types of GMOs and areas of risk

The workshop was organised by the Environment Agency Austria. Experts from EFSA, NGOs, the industry, competent authorities, national advisory bodies on GMO safety and the funding organisation were invited. For the list of participants see Annex II).

# 3 The `Limits of Concern´ concept according to EFSA

## 3.1 The EFSA guidance document on the risk assessment of GMPs

In 2010, the European Food Safety Authority (EFSA) issued a new guidance document on the environmental risk assessment (ERA) of genetically modified plants (GMPs; EFSA 2010a). The aim of this document was to further develop and update the guidance for the ERA of GMOs. One of the aims of the new guidelines was “the development of criteria for field trials to assess the potential ecological effects of the GMP in the receiving environment…” (EFSA 2010a).

The hitherto practice by the applicants of GMPs in the ERA showed that the compositional analyses of the GMP were used as a starting point for the whole ERA process. In this `comparative safety assessment´ a comparator (usually a non-GM plant) is used for comparison purposes with the GMP in question when assessing several characteristics of the GMP and when screening for intended and possibly unintended effects of the GMP. This comprises, among compositional parameters also other plant characteristics of the GMP (e.g. agronomic characteristics).

The statistical comparison usually comprises the values from the GMP versus the non-GMP pooled across all field trial sites and, if necessary, a single site comparison. Usually, any statistically significant difference which are not intended by the genetic modification are classified by the applicants as being `biologically not significant´ with no further evaluation of the results, a practice that has been criticised already earlier (e.g. DOLEZEL et al. 2011).

In the new guidance document EFSA (2010a) requires that the biological relevance of such statistically significant differences should be assessed, also considering “potentially hazardous environmental implications” (EFSA 2010a, p 13). Thus, the comparative approach should identify those characteristics of a GMP which may cause adverse environmental effects thereby structuring the whole ERA process (EFSA 2010a). In step 1 of the ERA (the problem formulation) these identified differences should then be the focus of the evaluation, discriminating between differences that are deemed irrelevant and differences which need to be assessed for their potential to cause harm.

In the ERA any identified potential adverse effect has to be linked to `assessment endpoints´ in order to quantitatively evaluate the potential harm. These assessment endpoints are then translated into measurement endpoints (e.g. mortality) for which a `Limit of Concern´ (LoC) has to be expressed. Thus, the LoC needs to be related to each measurement endpoint by testing whether the observed effect falls within the LoC in order to determine the biological relevance of the observed effect (EFSA 2010a, p 19). It can be derived from the literature data, baseline data, modelling, existing knowledge or policy goals (EFSA 2010a). For each measurement endpoint the LoC shall be explicitly stated and justified by the applicant.

For controlled environmental studies the applied LoCs are supposed to be `trigger values´ which, if exceeded, lead to further studies on higher tiers (EFSA 2010a, p.27). For field studies they should lay down the minimum effect that is ecologically or biologically relevant or is considered potentially to lead to environmental harm. Effect sizes of 20 % for laboratory studies, of 30 % for semi-field testing and of 50 % for field studies are sug-

gested as a starting point for defining LoCs (EFSA 2010a, p 27). A justification is given only for the 50 % value for field testing, based on the results of the British Farm Scale Evaluations (FSE, HEARD et al. 2003a). In the FSE effects of genetically modified herbicide tolerant crops on weeds were assessed. If the values exceed the LoC, then risk conclusions, further assessments at higher tiers or modelling are considered necessary (EFSA 2010a, p 14-16).

EFSA also particularly points to the relation between the effect size of each variable studied and the LoC set for the respective variable (EFSA 2010a, p 28). EFSA assumes that the effect size and the LoC will usually be identical, but requires full justification by the applicant regarding the effect size chosen, particularly if it is not identical with the LoC applied.

## 3.2 Other EFSA guidance documents with relevance for the LoC concept

Since the publication of the guidance document for the ERA of GMPs in 2010, EFSA has issued other guidance documents and Scientific Opinions to further improve the risk assessment process. Some of these documents also refer to the concept of LoC and environmental harm. In the following the aspects relevant for the LoC concept mentioned in these documents are outlined.

### 3.2.1 Guidance on the selection of comparators

In the guidance document on the selection of comparators for the risk assessment of GM plants (EFSA 2011a) a reference is made to the LoC concept. In this document EFSA discriminates between the risk assessment of the GMP intended for food and feed use and the environmental risk assessment (ERA). The requirement of an equivalence test in the food-feed risk assessment is justified by the need to verify "…whether the agronomic, the phenotypic and the compositional characteristics of the GMP fall within the range of natural variation" (EFSA 2011a, p 7). This range of natural variation should be estimated from non-GM reference varieties. In contrast, for the ERA LoCs should be used as limit which, if exceeded, may indicate environmental harm.

### 3.2.2 Scientific Opinion on the assessment of potential impacts of GMPs on NTOs

In the Scientific Opinion on the assessment of potential impacts of GMPs on non-target organisms (EFSA 2010b) applicants are asked to relate the results of the non-target organism testing to environmental damage in form of specification of `limits´ or `thresholds´ of concern for each assessment endpoint (EFSA 2010b, p 20 ff).

### 3.2.3 Guidance for the risk assessment of food and feed from GMPs

For food-feed risk assessment the comparative assessment of the GMP and its non-GM counterpart has to be evaluated using two complementary tests: the test of difference and the test of equivalence (EFSA 2011b). The comparative assessment refers to the compositional, phenotypic and agronomic assessment. The test of equivalence includes the assessment whether the GMP is equivalent or not to the non-GMP within bounds which are defined by the range of natural variation estimated from concurrently grown non-GM reference varieties. In case significant differences or a lack of equivalence is observed, "…further analysis should be done to assess whether there are interactions between any of the test materials and site…" (EFSA 2011b). EFSA emphasizes that the

outcome of the comparative analysis “will further structure the risk assessment”, but relates the possible impact of intended or unintended effects only to human and animal health such as allergenic and nutritional impact. For environmental aspects the standalone ERA guidance document (EFSA 2010a) is referred to.

### 3.2.4 Scientific Opinion on statistical considerations for the safety evaluation of GMOs

The Scientific Opinion on statistical considerations for the safety evaluation of GMOs (EFSA 2010c) emphasizes that due to the methodological diversity in GMO applications with respect to field trials, data analysis and statistical approaches new guidance has become necessary. Hence the document focuses in particular on the use of statistical models for the design and data analysis of field trials for the comparative safety assessment (e.g. compositional, agronomic and phenotypic studies) as well as for animal feeding trials. Statistical aspects of the data analysis of field trials for the ERA are not covered by this Scientific Opinion.

In detail the guidance covers the experimental design for field trials, the power of field experiments and levels of replication to be met when conducting field trials. Additionally, the document guides on how to set the equivalence limits for single and multiple endpoints and on how to select data for the estimation of equivalence limits. This includes data from commercial crop varieties, in particular if these are included in the experimental design of the field trials. These data are then used in order to estimate equivalence limits reflecting the range of natural variation of a particular endpoint assessed.

The document provides also guidance on how to interpret the results of comparisons based on the equivalence tests. The outcome of the equivalence test can be classified into four equivalence categories: category i (equivalence), category ii (equivalence more likely than not), category iii (non-equivalence more likely than not) and category iv (non-equivalence). For the category ii EFSA (2010c) recommends that further evaluation may be required, for category iii and category iv further evaluation is definitely required. EFSA (2010c) points out that the results should be placed into context and interpreted within a risk assessment framework if the GMO differs from the commercial varieties. EFSA (2010c) establishes a link between the non-equivalence observed in the compositional analyses and the LoC concept as it points out that further assessments of the results of the equivalence tests should “…focus on biological/toxicological relevance, taking safety limits into account when available”.

### 3.2.5 Guidance to develop specific protection goal options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services

In this document EFSA aims to harmonize the operationalisation of protection goals across different ERA frameworks and independent of different environmental stressors in the agro-environment (EFSA 2016a). EFSA suggests using the MEA Ecosystem Service approach (MEA 2005) to operationalize general protection goals for ERA purposes. General protection goals have to be broken down into specific protection goals for which six dimensions have to be defined. Specific protection goals (SPGs) are composed of the following five dimensions: the ecological entity (e.g. biological organisation), the attribute (e.g. survival, nutrient cycling), the magnitude of effect that can be tolerated for the attrib-

utes and the temporal and spatial scales of the effects that can be tolerated for the attributes as well as the degree of certainty required that the effects will not exceed the specified levels.

#### 3.2.6 Scientific Opinion on the coverage of endangered species in environmental risk assessments at EFSA

This Scientific Opinion addresses the relevance of endangered species in the ERA of regulated products such as plant protection products, feed additives, invasive alien species but also GMOs (EFSA 2016c). The opinion evaluates to what extent endangered species are covered in the current ERA schemes. It evaluates whether endangered species are more vulnerable than other species to potential stressors such as GMOs. The concept of ecological vulnerability comprises the factors sensitivity and exposure to a stressor as well as the potential for recovery. Several issues relevant for the ERA are addressed, such as the lack of effect and exposure data for endangered species and the use of assessment factors when assessing effects on these species. Also the relevance of modelling tools to cover endangered species in current ERA schemes is discussed. For the ERA of GMOs endangered species should be considered in the problem formulation phase of the ERA and pathways to harm for these species have to be identified. Due to their higher vulnerability conservative assumptions (e.g. consideration of worst-case conditions) should be made.

#### 3.2.7 Scientific Opinion on statistical significance and biological relevance

In this Scientific Opinion the difference between statistical significance and biological relevance is explored (EFSA 2011c). The ERA Guidance of EFSA requires that if statistically significant differences are observed between the GMP and the non-GM comparator the biological relevance of such differences has to be evaluated (EFSA 2010a). The Scientific Opinion defines biological relevance as an effect "…considered by expert judgment as important and meaningful for human, animal, plant or environmental health" (EFSA 2011c).

### 3.3 Other documents relating to the LoC concept

#### 3.3.1 PERRY et al. (2009)

In their paper the authors explain in detail the differences between the statistical approaches for food-feed risk assessment and the ERA. They argue that for food-feed risk assessment the concept of history of safe use is paramount which is not relevant for the ERA where environmental harm is the focus of the assessment. Therefore, the equivalence limits for the ERA are not based on the natural variation of non-GMPs, but on ecological effects that are deemed of sufficient magnitude to cause harm (i.e. Limits of Concern).

PERRY et al. (2009) also provide recommendations for the experimental design of the field trials with respect to choice of comparators, statistical power, etc. They recommend that for each endpoint tested the size of the effect to be detected should be explicitly stated in the ERA. This detectable effect size should be directly linked to the LoC, i.e. the minimum ecological effect that is deemed biologically significant. For each endpoint at least one pair of LoC needs to be set which reflects the desired effect size. If more than

one pair of LoC is set then an equivalence test should be performed for each pair (PERRY et al. 2009).

### 3.3.2 VAN DER VOET et al. (2011)

The authors describe statistical methods for 1) the assessment of differences between the GMO and its non-GM counterpart and 2) the assessment of similarities (equivalences) between the GMO and concurrently grown reference varieties representing natural biological variability (i.e. natural background variation). They propose setting the equivalence limits (EL) from the results obtained in field trials and define tests to classify results into four equivalence classes. In addition, they present an adjusted scale which allows the simultaneous presentation of the results from both the comparison of the GMO with the counterpart and the comparison of the GMO with the reference lines.

In the proposed difference testing the null hypothesis is one of equality ($H_0$= equality or: the difference between GM and comparator = 0). If this null hypothesis can be rejected, then significant differences are identified – regardless of their biological significance. However, absence of a significant difference does not represent a proof of equivalence of the GMO and its counterpart. Thus difference testing should therefore be complemented by equivalence testing. In equivalence testing the null hypothesis is one of inequality ($H_0$= inequality or: the difference between the GMO and the reference mean is greater than the equivalence limit). If this null hypothesis can be rejected, equivalence can be established (the observed difference between the GMO and the reference mean is small).

The authors recommend that equivalence limits have to be set prior to performing statistical equivalence tests. They emphasize that the typical variations between reference varieties in the field trails are not true safety limits but only specifications of limits on natural background variation.

The equivalence assessment results in the identification of four equivalence categories (i = equivalence, ii = equivalence more likely than not, iii = non-equivalence more likely than not, iv = non-equivalence). For any characteristic there are seven possible types of outcome. For four of them the mean value of the GMO lies between the equivalence limits (outcome types 1-4) and for three of them it lies outside the equivalence limits (outcome types 5-7).

The authors state that significant differences (outcome types 2, 4, 6 and 7) should be checked for biologically relevant signals. Cases with a clearly established non-equivalence (outcome type 7) and cases, where non-equivalence is found more likely than not (outcome types 5 and 6), require further evaluation. They stress that these statistical approaches are only tools providing the appropriate context for the final biological interpretation of the results.

### 3.3.3 GOEDHART et al. (2014)

The authors describe a framework for simulating data of GMO field testing of non-target organisms. Specifically they consider statistical aspects encountered in this context, such as count data or presence/absence data of non-target organisms, different distributions of non-target organism data, a large number of zero values or sampling at different time points. They address the need to set Limits of Concern for equivalence testing. They

suggest using the variability in counts of non-target organisms derived from reference varieties to set such Limits of Concern.

### 3.3.4 **ANDOW et al. (2016)**

The authors use a 20 % effect size as a LoC for demonstrating the value of equivalence testing for laboratory toxicity data. The authors conducted equivalence tests on eight examples of toxicity data of *Cry* toxins obtained with various non-target arthropods (lady beetles and aphids). While no statistical difference was found in all the data sets, equivalence could only be demonstrated in six of the eight examples. However, the authors provided no scientific rationale for the use of the 20 % effect size as a trigger value.

# 4 Understanding the LoC concept – Interviews with Stakeholders

## 4.1 General perception of the concept

The usefulness of the LoC concept was judged very differently by the interviewed experts. Some welcomed it referring to the difficulties of designing adequate studies as the LoC directly relates to the effect size of an experiment and thus assists the determination of adequate statistical power and sample size. It was appreciated that with this concept more focus is put on the environment rather than on the GMO itself and that certain effects can less easily be dismissed as irrelevant. However, other experts expressed scepticism and considered the LoC concept to be a rather vague and theoretical. Concern was raised that it might lead to ambiguities regarding the borderline between ERA and risk management. Also the issue was raised that by applying LoCs the consideration of uncertainties could be neglected. The concept was also considered to be deficient in considering potential large-scale and combinatorial effects of GMPs.

## 4.2 Purpose of and idea behind the concept

One of the major drawbacks in ERA studies currently submitted in GMO applications for cultivation purposes is the lack of demonstrating the adequacy of the study design. According to some experts the main purpose of the concept was therefore to increase the conclusiveness of the ERA and to enable quantitative rather than qualitative statements in the risk characterisation. LoCs shall help providing a more quantitative basis, in particular at the field scale. As LoCs should relate to environmental harm they could help operationalizing and specifying protection goals. The setting of LoCs should serve to clarify study designs as there is a direct relationship between the design of an experiment including the ability to detect an effect of a given size and the LoC.

In the development of the concept, the focus was further put on the question how potential risks on non-target organisms could better be evaluated. Therefore it was meant to primarily define LoCs as ecological limits for populations of NTOs at the ecosystem level. While some experts primarily considered it adequate for toxicological questions concerning potential hazards for NTOs, others clearly stated that in principle the concept applies to all areas of risk. However, experts claimed that for certain areas of risk this would be more feasible (e.g. NTOs), while for others (e.g. cultivation & management) it would be very difficult to apply. One expert was of the opinion that the concept of LoC was not to be understood as a cooking recipe, but should be applied wherever possible and reasonable. There was awareness that for some risk areas the setting of LoC might still only be possible in qualitative and not in quantitative terms. With respect to the role of the LoC for the comparative safety assessment different views exist among experts. Some experts expressed their view that a LoC could be set only if a hazard was identified derived from an established non-equivalence (e.g. of a compositional parameter). For other experts the LoC should be set before starting the ERA, based on clear definitions on what should be protected, and thus influences also the comparative assessment.

## 4.3 Definitions and characteristics of LoC

According to EFSA the LoC shall determine the level of environmental protection to be preserved. Therefore a LoC will always include a normative and a scientific dimension. There was agreement among the experts that the setting of LoC is a value judgement

which cannot be delivered by scientists alone but needs to involve the European Commission and the EU Member States. Thus the concept has a strong political dimension which entails implications for decision making. Some experts remarked that in decision making some kind of decision criteria are always implicitly applied. In some cases these may be based rather on economic rather than on environmental concerns, e.g. as was the case for the non-approval of GM wheat in Canada due to concerns that its adoption could compromise the use of well-established crop rotations.

It was recognized that value judgements (for instance on protection goals) differ considerably between regions and EU Member States, but also among individual experts: some consider outcrossing *per se* to be considered as environmental damage while for others this is a natural process which may lead to damage e.g. if it displaces a naturally occurring species. Such differences can be taken into consideration by defining LoCs prior to the ERA.

It was recognized that it will be difficult and probably not always possible to define LoCs of general validity in the EU. The decisive question is what should be protected, where and when. However this problem needs to be tackled independently of the ERA and requires the operationalization of protection goals (e.g. biodiversity) set in EU legislation. In principle generally valid LoCs may also be set before the problem formulation (deriving from well-defined protection goals) which can then be further specified on a case-by case basis with regard to specific species which may be at risk in a particular case.

At the same time, the LoC concept also has a scientific dimension. However, relevant empirical data (e.g. concerning the effect hypothesis and/or regarding the population dynamics of NTOs) are often missing or deficient, so in certain cases the scientific basis for the setting of LoCs may be weak. One expert pointed out that without a reasonable scientific basis for LoCs no final risk conclusions could be drawn in the ERA. Hence, political decisions on LoCs in the risk management following the ERA would be necessary.

As far as protection goals are concerned, the respective LoC needs to consider the protection status of a certain species. There was agreement among the interviewees that a LoC for an endangered or protected species must be set differently than for a “common” species occurring in agro-environments. The experts acknowledged that the concept may be of help in taking different protection goals or different environments into account.

It was also recognized that the LoC should be set species-specific and should take into account the population size of the respective species. While it is considered to be of importance to take into account the natural variation of the population size (which for some Lepidoptera for instance may be highly fluctuating), the question arose on which basis a population shall be defined. For certain species it may make sense to define a LoC at the species level while for others different populations and/or subpopulations need to be taken into consideration. Some experts mentioned that depending on the question different types of entities would have to be considered when setting LoCs (e.g. different for functional groups, individual species, populations or even sub-populations). For microorganisms it was proposed that the LoC should rather be based on functions (e.g. nitrification) than on the species level.

There was agreement that the LoC has to be linked to a measurement endpoint, which ideally should contain both elements – hazard and exposure (e.g. mortality at a certain level of exposure to the GMP). In this respect some experts favoured sub-lethal parameters in order to conclude more accurately on effects of the population as a whole. There was no agreement among experts concerning the use of measurement endpoints, which are exclusively concerned with exposure (e.g. pollen production, flowering time). Some experts considered it possible if the relationship between exposure and effects is well established, for others this was deemed solely a risk management decision.

## 4.4 The role of LoC in tiered testing approach of the ERA

In the discussion on the LoC concept two fundamentally different approaches to tiered testing in the ERA of GMO became apparent. Most experts agreed that, if a LoC is exceeded, this demonstrates a risk or a safety concern. Some experts were of the opinion that LoCs were to be seen as trigger values which, if exceeded, require further considerations or actions. For example, higher tier studies are necessary in case LoCs for laboratory studies are exceeded. According to these experts the exceedance at higher tier levels has to be put into context (e.g. by upscaling or modelling) before decisions on the risk can be made. Others stressed that even if the results fell below a certain LoC set for lab studies, laboratory results always have to be combined with field experiments and a thorough exposure assessment in the field is a necessary prerequisite for the ERA of GMOs. Concern was expressed by one expert that if results from the ERA fell below a LoC this could be interpreted as a proof of safety and higher testing may be considered unnecessary. Thus any potential remaining risk would not be followed up in higher tier testing. Using the LoC as a stop criterion in the ERA will open the ERA for elements which are rather part of a risk management, blurring the border between ERA and administrative risk management and thus lead to the ignorance of uncertainties.

Some experts considered that if a risk was considered to be too high (e.g. the LoC was exceeded), the consequence might be the termination of the assessment. Other experts had doubts about the use of LoC as a stop criterion for the ERA process as then the LoC values would have to be set at relatively high level which, in practice, would rarely be exceeded. In this respect one expert pointed out that in principle two LoCs could be set for the same measurement endpoint requiring different actions: one that immediately stops the assessment and another that leads to further studies.

Other experts assumed that the exceedance of a LoC will most likely lead to risk management actions, such as risk mitigations measures or conditions laid down in the authorisation decision, monitoring activities. Depending on the relevant protection goals in different EU Member States (or even in different regions), the outcome of a risk assessment, i.e. the conclusions reached with respect to these protection goals, may be different and consequently entailing different risk management actions.

Most experts acknowledged that it will be difficult to link LoC values from lab studies with those for field studies. One expert suggested that such a link would also depend on the size of the release. For local effects this link will probably be tighter than for large scale effects. It was clear that the setting of LoCs for field trials has to take into account the final scale of the GMP cultivation, as risks may be different for large scale cultivation of GMPs within the EU.

## 4.5 Consideration of uncertainties in the LoC concept

There was agreement among the experts that the setting of LoCs will involve uncertainties depending on the scientific knowledge available at the time of setting the respective LoC. As any LoC will be based on the views of different stakeholders, it has to be acknowledged that the normative aspect when setting LoCs will be a larger compared to equivalence limits used in food-feed risk assessment. EFSA did not explicitly discuss the consideration of uncertainties when introducing the LoC concept for the ERA of GMPs. Even though it was acknowledged that the introduction of the equivalence testing contributes to the reduction of uncertainties, the experts unanimously stated that for reasons of transparency uncertainties should be explicitly considered as far as possible when setting the LoC. It was agreed that a LoC might also include safety margins, as for instance established in the risk assessment of chemicals in the form of assessment factors. However, the use of safety factors may mask whether existing uncertainties are due to lack of data, the experimental design, the model used or other causes. It was also recognized that uncertainties can only be reduced but never be eliminated. Thus it was considered paramount to know whether a LoC is solidly founded and based on scientific data or rather the results of expert judgement due to lack of knowledge and data. Some experts pointed out that uncertainties might be cut off, if the concept is applied in a way that further studies are not required if a LoC is not exceeded.

# 5 The use of LoCs in GMOs applications

The implementation of the LoC concept according to the provisions by EFSA (2010a) in GMO applications was evaluated. GMO applications were screened for statistically significant differences as well as non-equivalences in the comparative safety assessment. The identification of non-equivalences in the comparative safety assessment serves to identify potentially unintended effects in the GMP for which the biological relevance is to be determined and thus represents a case-study for the implementation of the LoC concept in the ERA of GMPs.

Two GMP applications were selected in which statistically significant differences and non-equivalences had been detected for environmentally relevant compounds: GM soybean DAS-44406-6 and GM cotton GHB614 (see Methodology). The results obtained from the analysis of the applications and the information gained from the literature search regarding the potential adverse effects of the selected compounds on non-target organisms were evaluated with a respect to the use of LoCs in the ERA of these two GMPs. The possibility of setting LoCs for lectins (for GM soybean) and gossypol (for GM cotton) as environmentally relevant compounds for the ERA is discussed.

## 5.1 Lectins in GM soybeans

Lectins are a complex and heterogenic group of glycoproteins that can specifically bind the sugar moiety of complex carbohydrates, called glycoconjugates. Their ability to reversibly bind to carbohydrates in cells and cell membranes allows them to induce a variety of biochemical reactions. They not only occur in plants, but also in viruses, bacteria, vertebrates and invertebrates (CARLINI & GROSSI-DE-SÁ 2002). Lectins (from legumes) are also known as `(phytohem)agglutinins´, a term which refers to their ability to agglutinate red blood cells which is used for testing.

According to various carbohydrate binding sites and structural similarities different classes of lectins can be distinguished in plants (e.g. chitin-binding lectins, legume lectins, amaranthin lectins) (VAN DAMME et al. 2004, VANDENBORRE et al. 2009). Classical plant lectins, e.g. wheat germ agglutinin (WGA), *Galanthus nivalis* lectin (GNA) and jackbean lectin (ConA) are expressed constitutively and occur mainly in cell vacuoles of storage tissues. Some lectins are toxic to mammals, like e.g. (soy)bean lectins and wheat lectin (CARLINI & GROSSI-DE-SÁ 2002). When plant hormones (e.g. jasmonic acid, salicylic acid) are produced under stress, upon injury or attack by pathogens, they may induce lectin synthesis. Contrary to the classical lectins these induced lectins are found in the cytoplasm and in the cell nucleus in low concentrations. Inducible lectins in plants are ascribed an important role in cell regulation and communication (VAN DAMME et al. 2004). Lectins do not only serve as storage proteins, but also play an important role in the plants' defence system (VANDENBORRE et al. 2009).

In soybean mainly the soybean lectin, also referred to as soybean agglutinin (SBA) occurs. In addition vegetative lectins also exist in soybean. For instance the vegetative soybean lectin (VSP) can be found in the whole plant and serves as a temporary reservoir for nitrogen and assimilates. The content of VSP and another vegetative lectin (SVL) increased, when pods were removed or when the plant was exposed to a gaseous plant hormone (SPILATRO et al. 1996). Soybeans also contain other secondary plant metabo-

lites, for some of which (e.g. trypsin inhibitors) stronger insecticidal effects on e.g. *Helicoverpa armigera* have been demonstrated than for lectins (SHUKLA et al.2005).

### 5.1.1 Definition of soybean lectins

Lectins in soybeans are generally termed as soybean agglutinin (SBA). However, the term SBA is used for at least four molecules with different structure (http://glyco3d.cermav.cnrs.fr/). In general lectins are located in the vacuole of storage tissues such as the seed but there are other lectins in soybean such as the vegetative soybean lectin (VSP) which occurs throughout the whole plant (leafs, stalk and seed) where it serves as a temporary reservoir for nitrogen and other assimilates. In addition another vegetative soybean lectin has been described (SVL; SPILATRO et al. 1996). Lectins are generally expressed constitutively but their synthesis may also be induced upon contact with pathogens or plant injury (VANDENBORRE et al. 2009) thereby playing a crucial role in the defence system against herbivores and pathogens.

In the ERA of the screened GM soybean application DAS44406-6 the terminus used in the application is `lectin´, thereby not differentiating between different soybean lectin types. Lectins occurring in plant organs other than seeds were not considered by the applicant. While this approach is relevant for the food-feed risk assessment where only soybean seeds are used for import and processing, for the environmental risk assessment also other plant organs, in which plant lectins can occur, are relevant as they may have ecological relevance (VODKIN & RAIKHEL 1986).

### 5.1.2 Lectin levels in GM soybean

Lectin levels in soybean DAS44406-6 were statistically higher than in the non-GM isoline, independent of the herbicide-spraying regime. In addition, the mean values and their confidence intervals were outside the equivalence limits set by the values derived from the reference varieties thus leading to a classification into equivalence category iv (non-equivalence) according to EFSA (2010c). The applicant argued that the 95 % confidence interval of the mean lectin value of all GM soybeans was within ranges reported in literature. The reported literature data refer to OECD (2001). The OECD refers to the publication of KAKADE et al. (1972). Other data, e.g. data reported by ILSI or other scientific literature were not mentioned. These values differ significantly from the values used in the GM soybean application (see also 5.1.3 and Table 2).

### 5.1.3 Determination and detection methods for soybean lectins

The values for lectins in GM soybean reported in the GMO application and those reported in the scientific literature differ considerably (Table 2). While the mean lectin level in GM soybean is approximately 100 HU (haemagglutination units)/mg protein, the values reported by ILSI (2014) are 10-fold to 1000-fold lower. Other reported literature values are either much higher (up to 4-fold higher, see KAKADE et al. 1972) or much lower (up to 1000-fold lower, BECKER-RITT et al. 2004) than the reported GM mean values.

Although all lectin values refer to the haemagglutination units, differences in the detection method used by different authors may explain the differences observed. Also some authors do not indicate whether the protein used was measured as dry weight or wet weight, which may considerably influence the outcome (see Table 2).

In the application of the GM soybean the determination of lectins was based on their ability to agglutinate red blood cells. The applicant refers to the method described by LIENER et al. (1955). LIENER et al. (1955) measured the ability of soybean extracts to agglutinate rabbit erythrocytes. According to the applicant of GM soybean this is done via measuring the absorbance at 620 nm of GM soybean samples which were defatted and extracted with a saline solution (LEPPING et al. 2011).

Also the values reported by ILSI (2014) were measured by using the agglutination method, however, quantifying the bioactivity of lectins in soybean oil.

BECKER-RITT et al. (2004) used dialysed crude extracts of soybeans determining the haemagglutination activity using human erythrocytes.

In addition, KAKADE et al. (1972) and BECKER-RITT et al. (2004) lacks the indication whether the haemagglutination is based on a protein weight basis using dry weight or wet weight.

Table 2. Comparison of reported values of soybean lectins in seeds. DW = dry weight; HU = haemagglutination unit

| Source | Mean | Range | Unit |
|---|---|---|---|
| GM soybean (Lepping et al. 2011) | 92-107[1] | 31 - 228 | HU/mg protein DW |
| ILSI (2014) [2] | 1.7 | 0.11 – 9.04 | HU/mg protein DW |
| Kakade et al. (1972) | | 37 - 323 (60 - 426) | HU/mg protein |
| Becker-Ritt et al. (2004) | | 0.03 – 0.13 | HU/mg protein |

[1]means of GM soybean treated with different herbicide regimes; [2]Online query of the ILIS Crop Composition Database on May 20th 2014: https://www.cropcomposition.org/query/index.html

### 5.1.4 **Content of lectins in soybean seeds**

Lectins are known to occur at about 1 % of total seed protein in the seeds of many legumes (SHUKLE & MURDOCK 1983). It is assumed that about 1 % - 10 % of the total soluble seed protein in legumes is lectin (VAN DAMME et al. 2004).

The protein content of the GM soybean seed was determined to be 38 % approximately (dry weight) which is in line with the reference range (35.1 - 44.9 % dry weight) and the literature range (32 – 48.4 % dry weight) and corresponds to the average protein content in soybean seed of about 40 % (MATEOS-APARICIO et al. 2008).

Data from various plant species indicate a soybean lectin content of 300 mg/100 g seeds (RÜDIGER & GABIUS 2001). The soybean agglutinin (SBA) content in seeds of various soybean cultivars (resistant and susceptible to *Phythopthora megasperma*) was quantified using a radioimmunoassay (GIBSON et al. 1982). On average the resistant cultivars contained approximately twice as much SBA per mg protein as the susceptible cultivars.

The SBA content varies considerably among cultivars ranging from 0.0019 to 0.0355 mg SBA/mg protein or 0.0121 to 4.091 mg SBA/ g seed (GIBSON et al. 1982). One study presents SBA data calculated on a seed protein basis. All other values are given on a unit weight basis (Table 3). Data for soybean seed show an average SBA content of up to 4 mg/g. None of these studies determined the haemagglutination activity of lectins. Nutritional studies report lectin values on a weight basis ─ either as absolute values (mg/g seed) or as relative values (% w/w). However, toxicological studies usually present lectin concentrations on a volume basis – either as absolute values (µg/ml) or as relative values (% w/v). The applicants of GM soybean (DAS44406-6) as well as ILSI use activity values (haemagglutination unit) only and do not provide absolute lectin concentrations. Therefore, the data provided by the applicant cannot be correlated with the literature data. While measuring the ability of lectins to agglutinate blood cells probably has proven advantageous for food-feed risk assessment, its usefulness for the environmental risk assessment has to be questioned.

Table 3. Lectin values in soybean seeds reported in the literature. n.i. = not indicated, SBA = soybean agglutinin

| **SBA content** | **In relation to seed weight** | **In relation to seed protein** | **Source** |
|---|---|---|---|
| 135-112 µg/g defatted soybean meal | | n.i. | Ahmed (1986) |
| 6.5 g/kg defatted soybean meal | | 1-2 % | Gonzalez de Meja et al. (2003) |
| up to 4 mg/individual seed | | n.i. | Vodkin & Raikhel (1986) |
| 3.6 mg/raw seed<br>2.5-12.2 mg/g defatted seed meal | 3.6 mg SBA/g seed | n.i. | Pull et al. (1987) cited by Calderon de la Barca (1991) |
| 300 mg/100g | 3 mg SBA/g seed | n.i. | Rüdiger & Gabius (2001) |
| 0.01 - 4.1 mg/g | 0.01- 4.1 mg SBA/g seed | 0.0019–0.0355 mg SBA/mg protein | Gibson et al. (1982) |

### 5.1.5 **Effects of soybean lectins on arthropods**

The insecticidal properties of lectins have received attention as they have often shown to specifically bind carbohydrates outside the plant's organism, e.g. to receptors located in insects' guts or on the cell surface of microorganisms. The binding of the lectins to the carbohydrate moiety of the glycoconjugates is a prerequisite for their insecticidal effects

(MICHIELS et al. 2010). Irrespective of their specificity, lectins can affect mortality, fertility, growth and development of insects (MICHIELS et al. 2010). Entomotoxic lectins are rather stable in insects and can be passed on to predators and parasitoids along the food chain and induce tri-trophic interactions. In general, the effect of a certain class of lectins is limited to one or a few insect orders. For instance for the *Galanthus nivalis* lectin (GNA) entomotoxic effects have been established for Lepidoptera, Coleoptera and Homoptera (SHUKLA et al. 2005). An overview of insecticidal effects of plant lectins can be found in CARLINI & GROS-SI-DE-SÁ (2002). There are lectins which are active against major agronomic pests such as lentil lectins, GNA, ConA or phytohemagglutinin (SHUKLA et al. 2005, KAUR et al. 2009, and THAKUR et al 2013).

In feeding studies the concentration of lectins used is indicated by presenting relative values of lectins in a culture medium (Table 4). However, one study indicated lectin activity (HU/g medium) in addition to a relative value did not specify the amount of medium fed to the larvae (SHUKLE & MURDOCK 1983, see Table 4). In most feeding studies profound effects of lectins on larval development have been detected (Table 4).

Since the reported values of lectin in soybean seeds and SBA concentrations used in feeding studies vary considerably, it is difficult to correlate both data. Consequently, it is hardly possibly to derive concentrations that trigger a specific effect on arthropods. Only one study states that the concentration of 1 % (w/v) used in the experiments with larvae of the tobacco hornworm is comparable with natural occurring lectin concentrations in soybean seed (SHUKLE & MURDOCK 1983).

Table 4. Effects of soybean lectins on arthropods (SBA = soybean agglutinin; SBL = soybean lectin; n.i. = not indicated).

| Lectin | Test design | Concentration | Test organism | Effects observed[1] | Source |
|---|---|---|---|---|---|
| SBL | Culture medium | 1% | Larvae of pest species (*Manduca sexta*) | Growth retardation | Shukle & Murdock 1983 |
| SBL | Culture medium | 2% | Larvae of pest species (*Ostrinia nubilalis*, *Diabrotica undecimpunctata howardi*) | No adverse effect after 7 days[2] | Czapla & Lang 1990 |
| SBA | Culture medium | 10 - 250 µg/ml | Larvae of pest species (*Acyrthosiphon pisum*) | After 7 days:<br>mortality: $LC_{50}$ > 500 µg/ml<br>growth: $IC_{50}$ > 500 µg/ml | Rahbé et al. 1995 |
| SBL | Culture medium | 0,1% | Pest species *Helicoverpa armigera* | No effect on Survivals of larvae or weight of pupae<br>Lower pupation and hatch rates | Shukla et al. 2005 |
| SBL | Culture medium | 0; 0,62; 1,25; 2,5 und 5 µg | Eggs of pest species (*Bactrocera cucurbitae*) | Statistically not significant reduction of hatched larvae | Singh et al. 2006 |
| SBL | Culture medium | 0; 1,56; 3,12; 6,25; 12,5 und 25 mg per 50 ml | Larvae of pest species (*Bactrocera cucurbitae*) | Development retardation, reduction of numbers of pupae and adults hatched | |
| SBL | GM tobacco | n.i. | Larvae of pest species (*Spodoptera exigua*) | Growth retardation and effects on development and metamorphosis | Guo et al. 2013 |

[1]Statistically significant effects (or otherwise indicated; [2]Adverse effects defined as 25 % mortality, 40 % weight loss, or both

## 5.2 Gossypol in cotton

Gossypol is a terpenoid which occurs mainly in pigment glands of leaves, stems, floral organs, roots and bolls of cotton. It is also induced in response to attack by herbivores and microbial infections, thus protecting the plant from both insects and pathogens. The major pest species of cotton are summarized in e.g. HAGENBUCHER et al. (2013). Gossypol occurs in a free or bound form, although only free gossypol is considered to be toxic (OECD 2009). There are different thresholds for gossypol in food or feed. For example, for food use the FAO and WHO permit up to 0.6 µg/mg free gossypol in edible cottonseed products. For use as animal feed the maximum level of gossypol in cottonseed is set at 5000 mg/kg, corresponding to 5 µg/mg (EFSA 2008).

The synthesis and content of gossypol in the plant is influenced by weather conditions but also varies between cotton species. Average gossypol values in cottonseed reach 10 µg/mg (SUNILKUMAR et al. 2006). The levels reported by OECD (2009) and ILSI (2014) for free gossypol in cottonseed range from 0.2 - 0.8 % (dry matter) and for total gossypol from 0.5 - 1.4 % (dry matter). However, cottonseed may contain concentration greater than 7000 mg/kg (corresponding to 7 µg/mg) free gossypol. For instance, the seed of *G. barbadense* may contain up to 34 g/kg, corresponding to 34 µg/mg (GADHELA et al. 2014). In leaves gossypol levels are lower with approximately 0.1 % dry matter (HAGENBUCHER et al. 2014).

In the application of GM cotton GHB614 a statistically significant difference for total gossypol in cottonseed has been detected between the GMO and the conventional counterpart. No equivalence test was conducted. As mean values were inside the reference ranges calculated from the commercial cotton varieties and the respective data derived from the literature the applicant considers the relevance of the statistically significant differences found between the conventional counterpart and the GHB614 cotton, from a biological and nutritional standpoint, negligible.

The gossypol content in GM cotton is indicated as % dry matter of seed by the applicant of GM cotton as well as in the scientific literature. The applicant of GM cotton distinguishes between total gossypol (TG) and free gossypol (FG, Table 5). Total gossypol is needed to calculate the concentration of bound gossypol which is formed during food processing. Free gossypol comprises gossypol and gossypol derivatives which are soluble and are physiologically active. Total gossypol is defined as the amounts of free and bound gossypol and gossypol derivatives extracted during hydrolysis (HRON et al. 1990). In the GM cotton application, gossypol is only considered from a food safety perspective and thus considered relevant for consumption by humans and livestock, while from an environmental point of view the amount of free gossypol is more relevant. Additionally gossypol content is only tested in seeds and not in other plant organs (e.g. bolls, leaves, squares).

Studies examining the effects of gossypol on arthropods either aim at the investigation of effects of gossypol on an important pest species in cotton, such as the generalist herbivore *Helicoverpa armigera*, or on organisms not directly associated with cotton cultivation (see Table 6). Data for non-target organisms are largely lacking. As far as target organisms are concerned it has to be considered that in the field Lepidoptera may prefer feeding on plant tissue with lower Gossypol content. For *H. armigera* a hormesis effect of

gossypol has been proposed (i.e. a beneficial effect of a compound at low concentrations which is detrimental at higher concentrations). When assessing the gossypol effects on arthropods, the studies do generally not differentiate between free and total gossypol in the plant material as is done in the GM cotton application (Table 6). In most cases the percentage of gossypol used in the experimental feeding tests is indicated. One study tested concentrations which are comparable to those found in natural cotton seed (see Table 6). This study found mortality rates in dipteran larvae between 30 and 50 % for gossypol concentrations between 0.2 and 0.6 % DM (XU et al. 2006). In one study absolute values of gossypol were presented demonstrating severe growth retardation of cotton bollworm larvae at a concentration of 3 mg/g diet (MAO et al. 2007).

Table 5. Gossypol content in cottonseed reported in different sources; DW = dry weight, FG = free gossypol, TG = total gossypol; n.i. = not indicated

| **Cited from:** | **GM cotton application ES-2012-104 (GHB614)** | | **OECD 2009** | | **ILSI 2014** | |
|---|---|---|---|---|---|---|
| | **FG** | **TG** | **FG** | **TG** | **FG** | **TG** |
| Mean value | 0,51 - 0.53 | 0,74 - 0,76 | n.i. | n.i. | 0.47 | 0.75 |
| Range | 0.23 - 1.4 | 0.46 - 1.99 | 0.47 - 0.70 | 0.51 - 1.43 | 0.23 - 0.85 | 0.46 - 1.01 |
| Unit | % DW | % DW | % DW | n.i. | % DW | % DW |

Table 6. Effects of gossypol on arthropods (DW = dry weight; n. e. = not evaluated)

| **Test substance** | **Test design** | **Concentration** | **Test organism** | **Effects observed** | **Source** |
|---|---|---|---|---|---|
| Gossypol | Culture medium | 0; 0.2; 0.4 and 0.6 % DW | Larvae of Diptera (*Lycoriella pleuroti*) | Increased mortality | Xu et al. (2006) |
| Gossypol | Culture medium | 0, 1, 2 and 3 mg/g | Larvae of pest species (*Helicoverpa armigera*) | Growth retardation | Mao et al. (2007)[1] |
| Gossypol (natural) | Leaves, buds and seed capsules of cotton | n.e. | Larvae of pest species (*Helicoverpa armigera*) | Effect on weight gain | De la Paz Celorio-Mancera et al. (2012) |
| Gossypol from seeds | Culture medium | 0%; 0.0004%, 0.0016%; 0.004%, 0.008%; 0.016% 0.04%; 0.16% (w/v) | Larvae of pest species (*Helicoverpa armigera*) | Hormesis effect[2] for larval and pupal weight | De la Paz Celorio-Mancera et al. (2011) |
| Cottonleaf extract | Water solution | 450 ppm 650 ppm | Larvae of mosquitoes (*Aedes aegypti*, *Anopheles stephensi*) | 30-40 % mortality | Patil et al. (2013) |

[1]Aim of the study was to evaluate the natural tolerance of *H. armigera* to Gossypol; [2]Hormesis: low doses of toxic substance can have positive effects on organism

# 6 The use of acceptability criteria in the ERA for the assessment of regulated products other than GMOs in the EU

The use of acceptability criteria in the ERA for the assessment of regulated products other than GMOs was scrutinized regarding their use as acceptability thresholds in the ERA of GMO (see Methodology). The evaluation comprised (i) EU-wide regulated products such as plant protection products, biocidal products or chemicals, (ii) EU-wide legal provisions for ambient air quality, protected species as well as habitats and (iii) provisions for environmental liability. In addition, the impact regulation under the German Nature Conservation Act ("Eingriffsregelung", § 13 ff BNatSchG; http://www.gesetze-im-internet.de/bundesrecht/bnatschg_2009/gesamt.pdf) was also analysed due to its relevance for the topic. The acceptability criteria as required by the respective legal provisions or guidance documents were analysed. In the following chapters selected aspects regarding these criteria are discussed. The focus is put on aspects that are of relevance for the LoC concept in the ERA of GMOs.

## 6.1 Plant protection products

This regulatory area is characterised by well-defined acceptability criteria in the ERA. In lower-tier risk assessment, decision criteria are based on the ratio between toxicity values (e.g. $EC_{50}$) and exposure of the test organisms in the relevant environmental compartment (e.g. the hazard quotient or TER-ratio), for which trigger values are defined. For the calculation of the trigger value the most sensitive organism tested is used. Individual assessments are made for different environments (e.g. soil, water) or organisms (e.g. non-target arthropods, bees etc.) and different calculations are made for in-field and off-field exposure.

The trigger values used in the lower tier risk assessment (e.g. laboratory studies) serve as decision criteria in order to decide whether the risk is considered acceptable or not for the specific ecological entity in the particular environmental compartment. A relevant aspect for the LoC concept of GMOs is that these trigger values used for the lower tier risk assessment of non-target organisms have been validated with realistic semi-field and field data (CAMPBELL et al. 2000).

In contrast, for higher tier risk assessment studies (field studies) generally no fix trigger values are used. In most cases expert judgment is considered necessary in order to determine whether the risk can be considered acceptable or not. In semi-field tests effects on non-target arthropods with an effect size of 50 % is considered acceptable, however, provided that the affected population recovers within one year. It is commonly recognized that the 50 % threshold is an arbitrary value and in most cases justified by the design of the field study which does not allow detecting smaller effect sizes. It is also recognized that acceptability criteria at higher tier tests need to consider differences between arthropod taxa. The recovery and the recolonization of the impacted habitat by the affected taxa plays is an important criterion when evaluating the acceptability of adverse effects observed in the field. Generally, a recovery period of one year is considered acceptable. In certain cases an impact classification scheme is applied with different categories, e.g. little to no impact, moderate effect with rapid recovery and reduction with recovery after one year or later. Such a classification scheme supports the decision on the acceptability

of adverse effects on non-target arthropods observed in field studies. The approach considers frequency and duration of adverse effects due to the application of the PPP and the range of taxa affected in the field. Results strongly depend on the limits of the applied test system, e.g. the definition of the population for which an effect is considered, and the starting point of the assessment. The recovery concept has been criticised as poor indicator for effects of PPPs when used under commercial conditions and the need for further research has been emphasized (ALIX et al. 2010). For the time being the potential for recovery of a species has been proposed as a better endpoint than its actual recovery. The recovery concept is certainly not useful for species of conservation concern since here even adverse effects on individuals may not be considered acceptable. For effects on species which occur outside the field (off-crop or off-field) it has been proposed that no effect or only transient effects are considered acceptable, therefore measuring recovery is not applicable (ALIX et al. 2010).

An important aspect in the ERA of PPPs is the use of assessment factors. Assessment factors are applied on the effect concentrations available from toxicity tests (e.g. NOEC) to derive predicted no effect concentration (PNEC) for a specific environmental compartment, but also assessment factors which are applied for the determination of acceptable effects on non-target organisms off-field.

An example for the application of decision criteria which are guided by protection goals is given in the guidance document for the ERA of PPPs for bees (EFSA 2013). Trigger values for the assessment of risks are adjusted to specific protection goals defined for bees. The scientifically validated thresholds partly replace the previously used hazard quotient approach. The protection goal centred approach not only considers different life stages of honey bees, but also other pollinators. It follows the suggestion to focus on ecological functions or ecosystem services when assessing PPPs (ALIX et al. 2010, NIENSTEDT et al. 2012, EFSA 2010d), thereby better reflecting protection goals in the ERA process.

## 6.2 Biocides and Chemicals

When assessing environmental risks of biocidal products and chemicals sensitivity distribution methods or assessment factors are used in order to derive the predicted-no-effect-concentration (the PNEC). The size of the applied assessment factor depends on the available toxicity data (type and amount of data), thereby covering existing uncertainties with respect to the extrapolation of the most sensitive organism in the lab to the most sensitive organism in the field. Currently, assessment factors are not applied within the environmental risk assessment of GMOs.

Generally, the regional and the local scale are differentiated in the risk assessment approach. It enables a differentiation of risks at different spatial scales. The approach is similar to the approach used in the ERA of PPPs where different scenarios are considered for in-field and off-field areas. It is also relevant for the definition of LoCs in GMO risk assessment.

In this context the case of the chemical substances nonylphenol and nonylphenol ethoxylate is remarkable. In 2006 these chemical substances were restricted in their use when placed on the EU market. In 2013 Sweden proposed a further restriction for use in textiles due to uncertainties regarding endocrine effects and due to effects on the aquatic

environment. The case shows that under certain circumstances remaining uncertainty over environmental effects may be a decision criterion for the ERA of a regulated product.

In chemical risk assessment the weight of evidence approach is used in order to take uncertainty in the ERA into consideration (ECHA 2010). This approach constitutes a formalised method to evaluate the evidence of an observed effect. It is mainly applied in case of contradicting study results, often to avoid testing on vertebrates. The weight of evidence approach tries to determine the significance of each piece of information using the following criteria: reliability (quality of the study), relevance (appropriateness of data and tests), adequacy (usefulness) and quantity. The overall weight of evidence refers to more than one piece of information. While in GMO risk assessment this is usually done by expert judgement, in chemical risk assessment the weight of evidence approach is a more formalised procedure to assess the value of each information piece. The approach is also used to determine subsequent testing steps. It provides a formalised and transparent method to evaluate the evidence of effects regarding one specific endpoint, considering criteria by which the evidence provided is scored (reliability, adequacy, relevance and quantity).

In 2017 EFSA drafted a guidance document on the use of the weight of evidence approach in scientific assessments (EFSA 2017). Although not specifically aimed at GMO risk assessment it aims to “provide a general framework for considering and documenting the approaches used to weigh the evidence in answering the main question of each scientific assessment or questions that need to be answered in order to provide, in conjunction, an overall answer” (EFSA 2017). It may therefore be also useful and applicable for GMO risk assessment as it encompasses aspects related to the reliability of the various pieces of evidence submitted in order to conclude on the environmental risks of GMOs.

## 6.3 Ambient air quality

The legal provisions put forward to define ambient air quality lay down different thresholds for air pollutants which trigger different actions. Thresholds above which adverse effects on the environment are likely, are differentiated from values below which no adverse effects are expected. These thresholds are established on a scientific basis. An analogy for the exposure of environmental compartments to GM pollen or GM plant debris may be evident, if the occurrence of adverse effects for certain organisms at a certain exposure level can be scientifically established. However, other thresholds for air pollutants aim at preventing harmful effects e.g. by triggering information actions.

## 6.4 Environmental Liability Directive and FFH Directive

Both Directives provide guidance on how to establish thresholds for environmental effects with respect to the species and habitats covered by the FFH Directive. In order to classify an adverse effect as damage, the significance of the adverse effect has to be determined. The recovery of the affected ecological entity to a baseline condition excludes an effect as being significant. Consequently, natural fluctuations of populations protected under the FFH Directive are excluded from being environmental damages. In this context it is important to note that for species and habitats covered by the FFH Directive the favourable conservation status has to be defined. For example, the conservation status of a

species is considered favourable if the population status is such that it can maintain itself on a long-term basis, its natural range is not reduced and there is a sufficiently large habitat to maintain the population in the long-term. These criteria can be useful when defining whether an adverse effect on the species is considered acceptable or not. Similarly, criteria for FFH habitats are available.

## 6.5 Impact regulation under the German Nature Conservation Act

The Impact Regulation as defined by the German Nature Conservation Act contains some criteria on how to assess the significance of environmental damage that may also be relevant when establishing LoCs for GMO risk assessment. These criteria include the assessment of consequences of adverse effects, effects due to the accumulation of projects, long-term effects and the occurrence of previous impacts. In addition, the significance of an intervention and its sustainability (up to 5 years from the intervention) must be assessed.

## 6.6 Conclusions on acceptability criteria in other regulatory areas

The analysis of five different regulatory areas for regulated products in the EU has shown that different aspects may be useful for the LoC concept in the ERA of GMPs. In particular, the use of trigger values (plant protection products) may be useful for lower tier testing of non-target arthropods if the trigger values are scientifically validated, used in standardized test systems and contain assessment factors that also cover uncertainties. Importantly, such trigger values do not decide on the biological relevance of adverse effects but on the acceptability of risks for a specific test organism. That means that trigger values already contain a normative aspect. The investigated regulatory areas apply methods to address uncertainties in their ERA methodology (biocides and chemicals). Not all of them may be relevant for the ERA of GMOs but need to be individually scrutinized for their potential applicability in GMO risk assessment. It is also evident that for higher tier risk assessment and the decision on the acceptability of risks expert judgment (plant protection products, biocides and chemicals) is always needed.

# 7 Limits of Concern– conclusions of expert workshops

## 7.1 General feedback on the LoC concept

The LoC concept for the ERA of GMOs was generally considered problematic by the experts. The necessity to use a broader approach and data basis for the ERA and the definition of a risk hypothesis before ERA testing, relevant for all three test levels in the tiered approach (lab-semi-field and field level), was considered to be a prerequisite before acceptability criteria for adverse effects can be defined.

Other difficulties were identified such as the lack of a definition of damage as well as damage thresholds for protection goals and the lack of scientific knowledge regarding acceptable adverse effects on biodiversity. The experts suggested that the level of acceptable adverse effects could either have an absolute or a relative value. An absolute value would have no reference value and define the minimum environmental quality to be preserved. Alternatively, a relative level of acceptable adverse effect could be defined which depends on a reference value, e.g. based on accepted effects in conventional or organic agriculture. The use of the LoC as a structuring tool for the tiered testing approach – in analogy to trigger values used for the ERA of PPPs – was critically viewed. This is due to the fact that the necessary prerequisites are not fulfilled to establish such trigger values for the ERA of GMOs. In addition this would counteract the step-by-step principle of Directive 2001/18/EC and the possibility to assess potential environmental risks comprehensively under different conditions of release of the GMO.

The question was raised regarding the relationship between the LoC and the statistical significance of a difference test. In current ERA practice, a statistically significant difference observed in a test carried out for the ERA does not necessarily trigger any consequence such as further ERA testing. The experts pointed out that any statistical test for differences is able to detect a certain effect size, depending on the specific test design and the number of replications. The use of a power analysis allows assessing the minimum number and size of the sample, in order to prove an effect of a certain size with a certain probability. The difference between the GMP and the control should be related to the LoC.

It was also discussed whether it is possible to define a Limit of Safety (LoS) in addition to the Limit of Concern (LoC). Falling below the LoS would result in the non-acceptability of an adverse effect and consequently in the termination of the ERA testing. Thereby, the LoS would correspond to the lowest safety limit. The LoC, in contrast, would not result in stopping the ERA testing. However, it was questioned whether the use of a double threshold of acceptability would be feasible from a technical point of view as knowledge on safe ecological limits is currently lacking. In addition the use of double limits would complicate the interpretation of the ERA results. Most likely, it would also weaken the LoC concept, since as long as the LoS was not reached, no consequences would be imposed. Therefore, the possibility to define a LoS was not further discussed.

In case the LoC concept is to be operationalised, the post-market environmental monitoring would have to be strengthened. Monitoring would have to be used as a control tool in order to assess whether the defined LoCs (at least for field test LoCs) would be not exceeded under realistic cultivation conditions. An assessment of adverse effects for certain

protection goals *ex-ante* in the context of the ERA was considered as hardly feasible but rather *ex-post* during monitoring. Monitoring activities can generate data by using higher replication numbers compared to ERA testing and this can help detecting smaller effects (e.g. effects on rare weed species). Generally, a methodologically adequate monitoring can help to provide relevant baseline data which is helpful for the definition of LoCs and the establishment of safe ecological limits. In this context, the LoC concept should not be seen as static. If results from the post-market environmental monitoring show that a defined LoC is not sufficient to protect a particular protection goal, then this LoC would have to be revised and re-defined. This can also be the case if the results of monitoring show that the assumptions made during the ERA are wrong (e.g. the LoC has be set too low or too high).

In certain cases the LoC has to be accompanied by risk mitigation measures. For example, for the resistance development of weeds a defined LoC (i.e. no further resistances) must be ensured by the use of resistance management measures during cultivation. Further examples are the definition of crop rotations or restrictions of GMO cultivation for a certain land area. The non-exceedance of the LoC has then to be assessed via a monitoring program.

A major problem for the operationalisation of the LoC concept was seen in the consideration of the variability of the receiving environments into which the GMO is introduced (different biotic and abiotic factors, different stressors, different protection goals etc.). Therefore also the use of standard values as LoCs was critically addressed. The need was stressed to use different LoCs for different species, populations, receiving environments and protected species.

It was also emphasized that defining environmental harm requires also considering the human benefit. This is in particular relevant if the ecosystem service concept is to be applied for the ERA of GMOs, pesticides and other environmental stressors, as addressed by EFSA in several guidance documents. The question, which and how much of a certain ecosystem function and service is needed and to be protected, can only be answered from an anthropocentric view.

## 7.2 Consequences of the exceedance of the LoC

The question of the consequences of an exceedance of the LoC was repeatedly addressed in the expert workshops. In particular for the assessment of effects for non-target organisms a system analogous to the ERA of PPPs using trigger values was not recommended (i.e. the use of the LoC as stop criterion for further testing if it is not exceeded). There was agreement that LoCs should not be used in analogy to trigger values, as several prerequisites are not fulfilled for the ERA of GMOs (e.g. lack of validation of values, lack of safety factors, no standardised test organisms and methods). The requirements for testing living organisms have to be seen differently from those for testing synthetic pesticides (e.g. non-linear dose-effect relationships, multiple stressors in plant material, no constant exposure). The use of LoCs similarly to trigger values would be problematic as the current ERA approach is considered to be too narrow (e.g. the use of acute toxicity tests only). Hence, it is questionable whether such an approach could be useful at all for the ERA of GMPs in its current form.

For the assessment of persistence, invasiveness and plant-to-plant gene flow of a GMP the use of the LoC as a stop criterion was, however, considered useful as it provides the opportunity to discontinue the ERA if the risk is considered too high already in the lower tier assessments. In contrast, it may be possible that due to specific biological or environmental conditions (no outcrossing, no compatible wild relatives) the risk in the lower tier assessment is considered so low that further testing in the field is considered unnecessary.

It was mentioned that an exceedance of the LoC should not be linked to specific legal consequences (e.g. restriction of approval). The decision whether a risk is considered acceptable or not or whether certain risk mitigation measures have to be implemented has to be made by the risk manager (i.e. the European Commission and the Member States). However, if the LoC is also used to structure the tiered ERA approach, then decisions on acceptable risks have to be made by risk assessors during the ERA approach (e.g. if further data are necessary or not). The risk assessor can suggest risk mitigation measures depending on the results of the ERA (e.g. the exceedance of the LoC).

## 7.3 LoCs and protection goals

As a fundamental problem for the operationalisation of the LoC concept the lack of specification of protection goals regarding definitions of damage and damage thresholds was identified. Defining what kind of effects constitutes an adverse effect for a specific protection object and what size of adverse effect is considered acceptable is a necessary prerequisite for the definition of a LoC for the ERA. This also refers to the lack of instruments in order to measure adverse effects on these protection goal (e.g. for ecosystem services). In practice, the assessment of environmental damage is conducted on a case-by-case basis by expert opinion (e.g. for FFH species and habitats). So far environmental damage has not been defined for many protection goals, also because the human benefit has not been evaluated in this context. When evaluating environmental damage, often different ecosystem services but also different protection goals have to be balanced (e.g. food production versus biodiversity). Also benchmarks have still to be defined, in order to evaluate environmental risks comparatively. In this context, the choice of the non-GM counterpart was emphasized. However, this comparative approach was also considered problematic when used for assessment of adverse effects on the environment.

Regionally different or even contrary protection goals were mentioned as problematic issue for the application of the LoC concept. The definition of the receiving environment is considered particularly relevant. For non-target organisms the biogeographic regions were considered the relevant reference area. For other risk areas (e.g. cultivation and management techniques) other reference areas will be useful, also depending on the type of crop. For the assessment of adverse effects on the protection goal also the recovery potential and the resilience of the receiving environment should be taken into consideration.

*Agricultural protection goals*

Agricultural protection goals have been defined at EU-level, such as the Directive 2009/128/EC on the sustainable use of PPPs (see EFSA 2010a), but their implementation is often accomplished at the national level. For example, the protection of the seed

diversity emphasized in the Biodiversity Action Plan for Agriculture (2001), however, seed production is carried out nationally or even regionally. The achievement of a particular protection goal cannot be accredited to a single GMO (e.g. pesticide reduction) as the entirety of the production methods is responsible for the overall outcome. In addition, the respective instruments and methods in order to assess the effects on the relevant protection goals at the national level are often missing.

Some agricultural protection goals were considered particularly important for GMOs. This referred to the genetic diversity of crop plants, which is not only covered by the EU seed catalogue but also by the use and production of old seed varieties and landraces as well as the artisanal seed production. However, it is difficult to define and locate these seed production areas. A comprehensive inventory of those seed varieties are currently lacking for the whole EU. A higher significance could be contributed to seed varieties that are not listed in EU seed catalogues. The workshop participants attributed a higher protection status to seed production, in addition to high nature value farmland and IPM methods. These must be addressed separately be the LoC concept.

*Biodiversity protection goals*

There was agreement that species and habitats of conservation concern have to be considered separately by the LoC concept. The protection or endangerment status (e.g. Red List) is a useful criterion in order to justify the higher vulnerability of natural resources towards environmental stressors such as PPPs or GMOs. For protected habitats it has to be considered that specific protection objectives and, possibly, damage thresholds are already defined in the national ordinances for protected areas (e.g. Natura 2000 areas). These have been defined independently of GMO cultivation, but are relevant for GMOs. In practice such provisions and thresholds cannot be addressed during the EU-wide notification procedure but must be assessed case-by-case. The protection goal biodiversity was generally considered to be difficult to be made operational as in many cases the instruments for assessing biodiversity are lacking.

## 7.4 Indicators for the ERA of GMPs

The different indicators suggested by KOWARIK et al. (2008) for which acceptability criteria can be applied were discussed in the workshops. In all workshops it was emphasized that the indicators chosen have to reflect the protection goals relevant for the GMP in question. The indicators can be on the effect level (measuring the adverse effect on the protection goal), however, indictors on the trigger- or process-level are also useful if adverse effects on the protection goal cannot be measured directly in the ERA. In addition, indicators should be independent in order to avoid double counts. Ideally, indicators are chosen which can be used in both, the ERA and monitoring. For long-term effects it should be considered that indicators should be defined if a risk hypothesis can be formulated (i.e. predictable adverse effects in relationship to the GMO). The choice of indicators and their combination should be flexibly used, depending on the problem formulation. Ideally, the indicator should be testable at all tiers (lab-greenhouse-field). If this is not possible, suitable indicators should be combined in order to test a specific hypothesis at all test levels. When assessing individual species, the reproductive success was considered the most important indicator.

Specific aspects for indicators for the different areas of risk are:

a) Indicators for the assessment of persistence and invasiveness, including plant-to-plant gene flow
   - The lack of definition of the terms `persistence´ and `invasiveness´ in EFSA Guidance Documents was emphasized. These terms can relate to different meanings (see CBD definition and definition according to the regulation of invasive species) and a common definition for the ERA of GMOs should be used.
   - For the assessment of invasiveness it was mentioned that prediction errors are not unlikely (in analogy to the prediction errors of invasive species).
   - Further indicators may be useful which are derived from invasion biology and the assessment of risks from invasive alien species.
   - Indicators which cannot be tested in the ERA (e.g. establishment of dominant populations) have to be assessed via data and experiences from other countries or continents.
   - For the calibration of indicators the protection status of compatible outcrossing partners was considered relevant and suitable.
   - Existing thresholds for certain indicators (e.g. growing through) should be considered for the classification of effects.
   - The receiving environment and its condition have to be taken into account. In this context the resilience, the persistence and the retrievability are relevant aspects. These aspects can be taken into account by the use of up- or downgrading factors when evaluating the strength of effects.

b) Indicators for the assessment of adverse effects due to changes in the cultivation and management techniques
   - The problem of non-linear relationships between indicators (e.g. weed species) and ecological functions (e.g. food web support) was mentioned. This means that it is difficult to define thresholds for the indicator that guarantees the conservation of a certain ecosystem function.
   - It was considered necessary to take functional aspects of weed species into account (e.g. differentiation between monocotyledonous and dicotyledonous species), in particular if effects on higher trophic levels are to be assessed. Thereby also conclusions on the selectivity of herbicides applied with the GMO can be drawn.
   - Many indicators are not sufficient if used alone, as they may not cover certain effects. Therefore indicators should be used complementary.
   - The yield of a crop is considered a useful indirect indicator, as there is a good data basis on the relationships between yield and weed biomass or density of different crops.
   - There is a need to develop suitable soil indicators.
   - Certain indicators will be useful for the post-market monitoring only (e.g. cumulative weed species numbers).
   - The calibration of indicators should be transparent and biologically justified.

c) Indicators for the assessment of impacts on NTOs
   - The use of the PEC/PNEC ratio as indicator was considered useful for the assessment of the toxicity and specificity of a *Bt* toxin. However, any further use for the ERA was considered problematic, due to non-linear dose-effect relationships, additional stressors in plant material, the lack of safety factors for the PNEC etc. This indicator was therefore not considered sufficient for the definition of LoCs.
   - Ecological functions should not be used as single indicators but only in combination with the assessment of individual species. As an exemption the assessment of soil functions was mentioned, if individual species cannot be assessed.
   - The suggested calibrations for the PEC/PNEC indicator by KOWARIK et al. (2008) were considered to be unrealistic for ERA practice.
   - Often it is not known if a minimum level for a certain ecological function can be determined and if yes, how this can be done. If such a level can be determined then the human benefit has to be taken into account.
   - For field tests the suggested indicator `reduction of populations´ was considered useful. However, the use of standardised protocols for this indicator was recommended.

## 7.5 Definition of LoCs for the ERA of GMPs

The definition of LoCs has to relate to the protection goals relevant for the GMP in question. The status of the receiving environment is also an important determinant of the LoC. In addition, implications due to future environmental changes, such as climate change, have to be considered (also in analogy to the risk assessment requirements of invasive species). Certain aspects of the receiving environment can be applied as upgrading or downgrading factors of adverse effects (e.g. retrievablity of the GMP, persistence of the GMP, resilience of the receiving environment). When determining the status of the receiving environment it should be kept in mind that historic data may be relevant (e.g. in case of the evaluation of soil seed banks). The environmental quality of a certain environmental compartment may experience a downward trend over time which is only evident if longer time scales are looked at (e.g. the phenomenon of `shifting baselines´). In the context of the LoC this requires to amend the LoC regularly (relative LoC) or the setting of an absolute LoC in order to define the minimum environmental quality that should be preserved (absolute LoC).

The role of conventionally cultivated plants as non-GM comparators was discussed controversially. Some experts considered that the LoC should reflect the absolute environmental quality independent of the effects due to conventional crops or cultivation systems. Other experts emphasized that the natural variability should be taken into account, which is usually defined by non-GM conventional crops. There was consensus among experts that any natural variability has to be defined in spatial and temporal terms. For the comparison of effects of GMOs with conventionally cultivated plants the use of historic data was not considered useful.

The definition of LoCs for ecological functions was considered problematic due to the necessity to define also the human benefit. The question of how much of an ecological function is necessary or sufficient cannot be answered scientifically.

It was emphasized that, in general, the LoC should be determined by biologically relevant effect sizes. Statistical criteria (e.g. effect sizes that can be detected due to a specific test design) should be subordinated.

Specific aspects for different areas of risk were addressed in the workshops:

a) Workshop on effects due to persistence and invasiveness including plant-to-plant gene flow
   - The LoC should be defined for indicators that are used for testing a common risk hypothesis at all testing levels.
   - When using different indicators either the average of the outcomes should be used (calculation of a mean value) or the highest values should be used for the estimation of the overall risk.

b) Workshop on effects due to changes in cultivation, management and harvesting techniques
   - As a major problem the lack of knowledge regarding the relationship between different indicators on different trophic levels was mentioned (e.g. changes in the weed flora and its effects on higher trophic levels). In many cases it is not practicable to define LoCs for higher trophic levels as effects at this level are more difficult to assess.
   - Using the case study of weeds showed that different protection goals and ecosystem services have to be balanced in the ERA (e.g. yield loss versus biodiversity loss). Here the use of weed thresholds and the IPM approach can support to define LoCs in order to support both ecosystem services sufficiently (yield and weed biodiversity).
   - The use of LoCs for weeds should consider the respective crop and its particularities. In particular, rotational aspects of the crop in question must be considered as crop rotations strongly influence weed diversity and composition. LoCs may have other effects on biodiversity if defined for weeds in break crops than for main crops as effects in break crops have stronger consequences for the protection goals.
   - The LoC should not counteract the provisions defined in the EU Common Agricultural Policy (e.g. for crop rotation, greening etc.).
   - For stacked event GMOs (e.g. multiple herbicide resistances) no separate LoCs were considered necessary. However, it has to be taken into consideration that the LoC may be exceeded earlier for stacked event GMPs. Relevant risk mitigation measures and monitoring should therefore be defined.

c) Workshop on impacts on non-target organisms
   - Standard LoC values as suggested by EFSA were not considered useful. LoCs must be defined for each taxon individually. If necessary, LoCs must consider specificities of individual populations of a certain taxon, considering the population size and development.
   - Different LoCs have to be defined for protected species, endangered species or organism groups which are in general in decline (e.g. bees, amphibians).

- Any LoC may require a different sampling effort, depending on the status of the non-target population. Available data from existing monitoring programs may help to define the sampling efforts.
- For effects on NTOs that are assessed in the lab, glasshouse or field the statistical significance was suggested to be an important decision criterion (or qualitative LoC) indicating the biological relevance of an adverse effect observed. However, it has to be considered that the relevance of the detected effect depends also on the tested parameter (e.g. parameters measuring acute toxicity may be of lower relevance than chronic toxicity). For field studies the use of standardised protocols has been emphasized.
- The use of existing criteria (e.g. IUCN criteria) and modelling approaches (e.g. minimum viable population) was considered useful when defining the LoC.

## 7.6 Spatial aspects of LoCs

A specific spatial reference area should be chosen when defining LoCs. This area should correspond to the area in which any organism or habitat could be exposed to the GMO or its products. For non-target organisms larger reference areas than the field or even the exposure areas may be relevant, if these organisms are highly mobile (e.g. carabids, birds). The necessity to differentiate between protection goals for the field (in-field) and the field margins (off-field) was recognized as these may be different between the two areas. The focus in the field lies on the conservation of the ecological functions and services while off field species-specific protection goals may be more relevant. Certain protection goals which cannot be achieved in the field could be achieved by separate management measures in certain areas of the field (e.g. different weed management in certain field areas).

It was also suggested to adapt the LoC to the area in which risk mitigation measures are carried out. This may be relevant for areas larger than the field, e.g. if a certain crop rotation is concerned. In this context it is also important to define the area in which the adherence to the LoC should be evaluated by post-market monitoring. This is particularly important if the LoC has a strong regional context (e.g. for herbicide applications, weed resistances).

## 7.7 Time aspects of LoCs

The necessity to address the relevant time frame for the validity of the LoC was emphasized in all workshops. In this context also the time aspects of protection goals have to be considered when defining the LoC. The LoC may have to consider larger time scales than the GMO cultivation on a particular field, if crop rotations play a crucial role for the adherence to the LoC. In this case the role of monitoring was particularly emphasized. Also for non-target organisms the time scale of the LoC may have to be adapted to the biological particularities of the species (e.g. life cycle) which may not necessarily be in accordance with the single season cultivation of the GMP.

## 7.8 LoCs and uncertainties

Generally, existing uncertainties should not prevent the definition of LoCs. When assessing environmental risks, uncertainties derive from the biological variability but also from the variability due to modelling approaches. In order to achieve transparency in the

ERA, the source and extent of uncertainties should be indicated. This is also important when defining the LoC. The largest potential to reduce uncertainties in the ERA lies in the improvement of the data basis, in the use of alternative risk assessment methodologies and the inclusion of worst-case scenarios in the ERA. In addition, adherence to the step-by-step principle also improves the knowledge about the GMP and reduces uncertainty on potential effects under different release scenarios.

A possibility to reduce uncertainties for the LoC setting is to use indicators which are close to the protection goal, i.e. on the effect level (see KOWARIK et al. 2008). Also the use of assessment factors aims at the reduction of uncertainty, as they aim to compensate for the extrapolation of lab results to field conditions or the differences in the sensitivity of species towards a particular stressor. However, assessment factors were critically discussed, as their definition is mostly done by expert judgement rather than being scientifically based. The inclusion of a worst-case scenario in lab testing using the most sensitive species and the most critical endpoints is also an appropriate way to reduce uncertainty.

Further approaches in order to reduce uncertainties such as the `weight of evidence´ approach and the use of `systematic reviews´ were valued differently. On one hand their use was considered helpful in order to judge on controversial results and to reach objective conclusions. On the other hand the misuse in order to support biased study results was addressed (e.g. via the definition of certain criteria in order to selectively use studies). This would need to define specific requirements and criteria in order to make these approaches fit for purpose for the ERA of GMPs.

Last but least the possibility for a more conservative LoC setting was addressed in order to take uncertainties into account.

## 7.9 LoCs and long-term effects

Generally, there was agreement that no specific LoCs for long-term effects would be needed. Any threshold for the acceptability of adverse effects is valid for short-term and long-term effects. Ideally, the same indicators should be used in order to assess short-term and long-term effects. A possibility would be to use lower (more conservative) LoCs if long-term effects are difficult to assess (or if more uncertainty remains). Modelling approaches are useful tools in order to address long-term effects. A major problem are time lags of effects, in particular if these are mediated via social systems (e.g. GM contaminations affect seed producers and consequently the diversity of available seed). The problem of the chosen baseline and the phenomenon of `shifting baselines´ are particularly relevant for long-term effects (e.g. changes of seed compositions in soil seed banks over time).

Importantly, the terminology of long-term effects should be harmonised between different regulatory areas (e.g. PPPs, GMOs), in particular if the operationalisation of common protection goals for the ERA is to be harmonised.

# 8 Suggestions for the improvement and operationalisation of the LoC concept

## 8.1 Definition of the LoC

One of the aims of the LoC concept is to define the biological relevance of an adverse effect observed during ERA testing. One major aspect of the definition of biological relevance is its normative nature. This means that expert judgment is needed when deciding on the biological relevance of an observed adverse effect. This, however, implies that biological relevant effects are not necessarily synonymous with environmental harm. Against this background, in this report a Limit of Concern is understood as an acceptability threshold, either quantitatively or qualitatively, for adverse effects on entities, functions or processes that triggers regulatory concern either due to the possibility of the observed effects to cause harm to the relevant protection goal or because these adverse effects are valued as being important for a specific protection goal.

## 8.2 Operationalisation of protection goals when defining LoCs

The LoC concept, as proposed by EFSA in 2010, is a concept to integrate protection goals into the ERA of GMPs. LoCs should represent the level of environmental protection for a specific measurement endpoint assessed in the ERA and thereby help to operationalise protection goals during the ERA process.

However, two major questions remain in the context of the operationalisation of protection goals: 1) How are adverse effects defined for a specific protection goal and 2) What size of adverse effect on the specific protection goal is considered acceptable? In this context LoCs for EU-wide protection goals should ideally cover multiple environmental stressors which is also one of the aims of EFSA when suggesting the use of the ecosystem service concept for ERA purposes of GMOs and PPPs. The first question addresses the lack of definition of adverse effects for the relevant protection goals in the legislative texts at EU-level. No criteria for the definition of adverse effects are available, although examples have been provided, such as reduction of population size of species, impacts on native species or changes in the specific structure or function of a habitat or ecosystems (ESSL et al. 2011, KOWARIK et al. 2008). Even more, such definitions have also to be made for ecosystem services for which appropriate assessment instruments still have to be developed if they are to be used for ERA purposes. For the evaluation of effects on biodiversity this implies further specifications regarding the minimum level of biodiversity that is to be preserved in order to ensure the functioning of the ecosystem (EFSA 2010b) or to fulfil normative requirements of legislative provisions (e.g. conservation of species and habitats).

For the LoC concept it is important to acknowledge the difference between the environmental damage to EU-wide protection goals and the threshold level for acceptable adverse effects used in ERA testing. It is not feasible to assess *ex-ante*, i. e. during the premarket ERA, the potential harm for a relevant EU-wide protection goal due to GMO cultivation. Therefore, indicators are needed to test for potential effects on the relevant protection goals in the ERA. For these indicators LoCs can then be defined.

We therefore suggest a conceptual framework for the use of LoC in the ERA of GMPs, depicted in Figure 2. For this purpose several terminologies for LoCs are used. For each protected natural resource or resource service at EU level one LoC or several LoCs ($LoC^n$) should be defined ($LoC_{EU\text{-}wide}$). LoCs different from those at EU-level have to be specified for testing effects during the ERA by the use of indicators (KOWARIK et al 2008). These LoCs are termed $LoC_{indicator}$ and are used at laboratory, semi-field or field level. It is probably challenging to link the thresholds for environmental damage at EU level (the $LoC_{EU\text{-}wide}$) with those at the ERA testing level ($LoC_{indicator}$) due to differences in the spatial and temporal scales for the maximum tolerable adverse effects. If tolerable adverse effect sizes refer to EU-wide populations of a certain taxon then the spatial and temporal scales refer to the whole EU population. Thresholds for the acceptability of adverse effects at the ERA level need to relate to the meta-population of the same taxon affected when conducting laboratory tests or during field testing.

Once the damage thresholds for protection goals at EU-level have been defined, damage thresholds for the relevant indicators for ERA testing can be set. As soon as the results of the ERA testing are available, the outcomes for each indicator are compared with the $LoC_{indicator}$ for each testing level.

It has to be taken into account that changes in the type and magnitude of potential environmental effects depend on the scale of GMP adoption which cannot be reflected by small-scale and short-term risk assessment studies (BENBROOK 2016). Hence, ERA methodologies have to be applied such as modelling approaches which integrate the accumulation of adverse effects across larger spatial and temporal scale resulting in predictions of risks when large-scale and long-term cultivation (over the full authorization period of 10 years) of GMPs is envisaged (see e.g. BRECKLING et al. 2011).

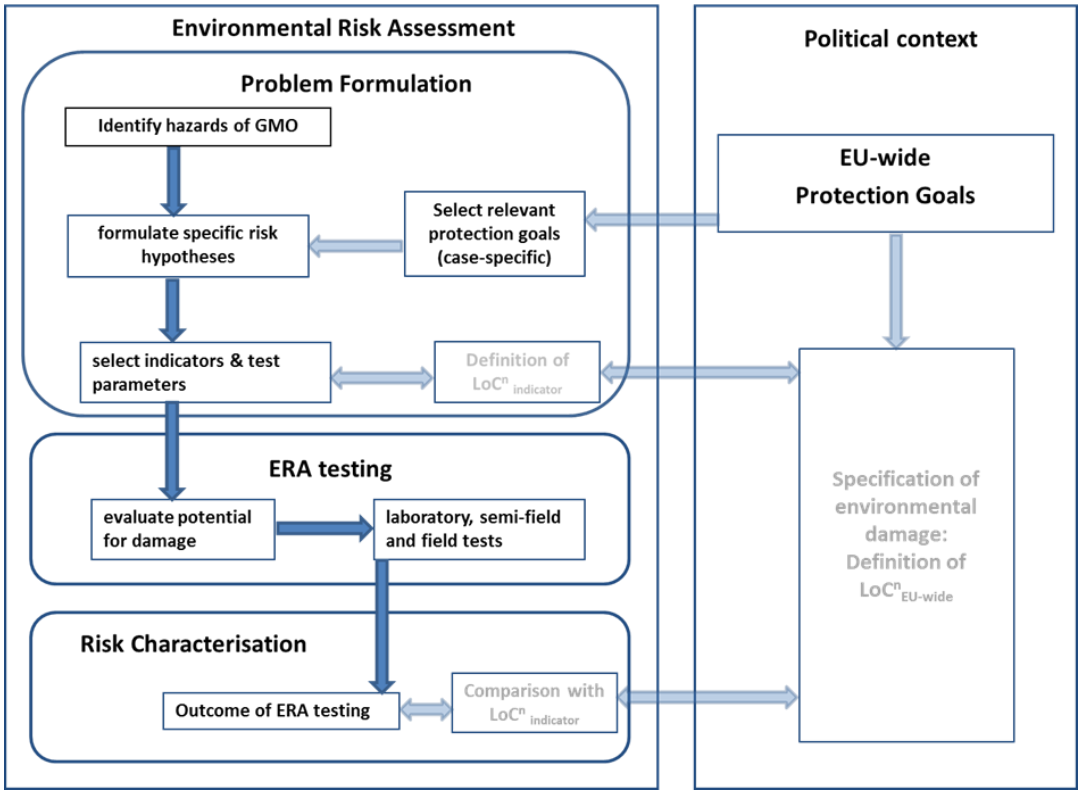


Figure 2. Integration of the LoC concept in the ERA of GMOs. Boxes and dark blue arrows = aspects and flow of information as currently done in the ERA. Transparent boxes and arrows = aspects and information flow that still need to be defined. $LoC^{n}_{EU\text{-}wide}$ = set of LoC for a natural resource or resource service at EU level. $LoC^{n}_{indicator}$ = set of LoC for indicators used for ERA purposes.

## 8.3 LoCs and the stepwise testing approach

In the context of the tiered testing approach, the role of a LoC and the consequences of its exceedance are still to be clarified.

EFSA points out that LoCs should act as trigger values within the tiered testing approach. If a LoC is exceeded at a specific tier, then further tests at higher tiers or modelling and scaling up of effects are considered necessary (EFSA 2010a). Consequently, the LoC indicates the necessity of further tests at higher tiers only in case the LoC is exceeded. As long as EFSA does not explicitly state the consequences in case a LoC is not exceeded, the present provisions in the guidance can be interpreted in a way that the non-exceedance of a LoC is a stop criterion for the ERA, meaning that no further testing at higher tiers is considered necessary in order to conclude on the environmental risk of the GMO (Figure 3).

Directive 2001/18/EC is the legal basis for the ERA of GMOs in the European Union and establishes the step-by-step principle. This principle allows for a gradual reduction of the containment of a GMO and the upscaling of its release only, if the evaluation of earlier steps indicates that the next step can be taken safely (Directive 2001/18/EC, Preamble 24 and 25). Although this principle is not a legal requirement for the authorisation, it is a

general principle for the GMO risk assessment. It indicates that the reduction of the containment of a GMO should be accompanied by the build-up of knowledge about the performance and risks of that GMO (VON KRIES & WINTER 2011). In addition, it can be viewed as an additional tool to structure the collection of information for GMOs (VON KRIES & WINTER 2011).

In accordance with the step-by-step principle of Directive 2001/18/EC, the role of the LoC in the ERA of GMOs should go beyond any type of stop criterion. As mentioned before, the idea and the purpose of the step-by-step principle are at least twofold: (i) to gradually decrease the containment of a GMO during testing and (ii) to simultaneously build up specific knowledge and reduce uncertainties about environmental risks related to the release of a GMO. These ideas would be compromised, if ERA testing was stopped in case the LoC was not exceeded already at a lower tier (e.g. a laboratory study), because then any uncertainties regarding environmental risks would be dismissed already at this early step of the ERA. This "trade-off between simplicity of the ERA and uncertainty" (LANG et al. 2007) should therefore not be reinforced by a concept that allows for using LoCs as stop criteria.

It also has to be considered that LoCs for different studies with different containments of a GMO may not necessarily be related to each other as the studies themselves serve different purposes (EFSA 2010a, EFSA 2010b). LoCs for field studies will be related to the assessment of changes in abundance of individual species or species guilds, the evaluation of multi-trophic interactions, or ecosystem services (e.g. biological control). Therefore LoCs defined for field studies reflect more directly environmental damage thresholds for protection goals. In addition, LoCs for field studies should consider that effects observed in ecologically more realistic scenarios will have stronger consequences with respect to their relevance for the protection goals in question. However, effects occurring at larger scales (e.g. at landscape or regional level) or combinatorial effects (e.g. via the interaction of two or more environmental stressors such as *Bt* toxins) cannot be anticipated at field testing level (BRECKLING et al. 2011). For example, effects and processes at landscape-scale may have profound consequences on biodiversity (see e.g. FAHRIG 2003) and such effects can only be accounted for by extrapolations using modelling or upscaling approaches in the ERA (see BRECKLING et al. 2011 and references therein). Therefore any LoC for field level testing has to allow for potential large-scale implications of the effects observed at small-scale.

Lower tier tests such as laboratory tests serve to identify hazards (e.g. the sensitivity to a *Bt* toxin), clarify exposure routes or assess the extent of severity of an effect at single-species level under worst case conditions of exposure and best-case environmental conditions, but often neglect ecological realism (LANG et al. 2007). Therefore, LoCs for laboratory tests have a more indicative value as their role is to put the risk posed by a hazard into a broader context (e.g. the toxicity of a specific *Cry* toxin in comparison with other *Cry* toxins). There is insufficient knowledge in how far effects seen in eco-toxicological laboratory studies can predict the likelihood of adverse effects in field experiments (KIMBALL & LEVIN 1985, CAIRNS 1983, CAIRNS 1986). In the ERA of plant protection products trigger values for the two standard test species *Typhlodromus pyri* and *Aphidius* spp., tested with standard protocols in laboratory tests, have been validated by (semi-) field data (CAMPBELL et al. 2000). For the ERA of GMOs such a validation has not been made for

any of the species tested in the laboratory. Also standard test protocols and criteria are not yet commonly used, although recommended (ROMEIS et al. 2011). As long as certain minimum requirements for laboratory and field testing for GMOs are not met (DOLEZEL et al. 2011, HILBECK et al. 2011), and validation of laboratory results in the field has not been done, LoCs should not be used as trigger values allowing risk conclusion from lower tier tests only.

Therefore a more precautionary approach to the LoC concept would be to use the results (exceedance and non-exceedance of the LoCs) from all test tiers to inform the risk characterisation and to conclude whether the observed effects tested in the different tiers fall below or above the set of $LoC^{n}_{EU\text{-}wide}$ relevant for the respective protection goal (Figure 4). Such a comprehensive approach would also correspond to EFSA`s description of the risk characterisation step in the ERA. In this step, which is carried out after ERA testing, an assessment should be made whether an observed effect falls within the LoC, thereby assessing its biological relevance (EFSA 2010a, EFSA 2010b). In this regard it has to be kept in mind that usually a range of indicators are assessed at different tiers during the ERA, using several different measurement endpoints (lethal and sub-lethal parameters, population parameters, etc.) for different taxa and possibly their different development stages (e.g. when assessing effects on non-target butterflies). Comparing results from testing with the $LoC^{n}_{indicator}$ for each of the measurement endpoints will give a first indication of the risks to a specific indicator used for testing. However, results from all studies carried out at all steps (exceedance and non-exceedance of all $LoC^{n}_{indicator}$) will have to be integrated in the risk characterisation in order to conclude on the environmental risk for a particular indicator and, consequently, for the related protection goal.

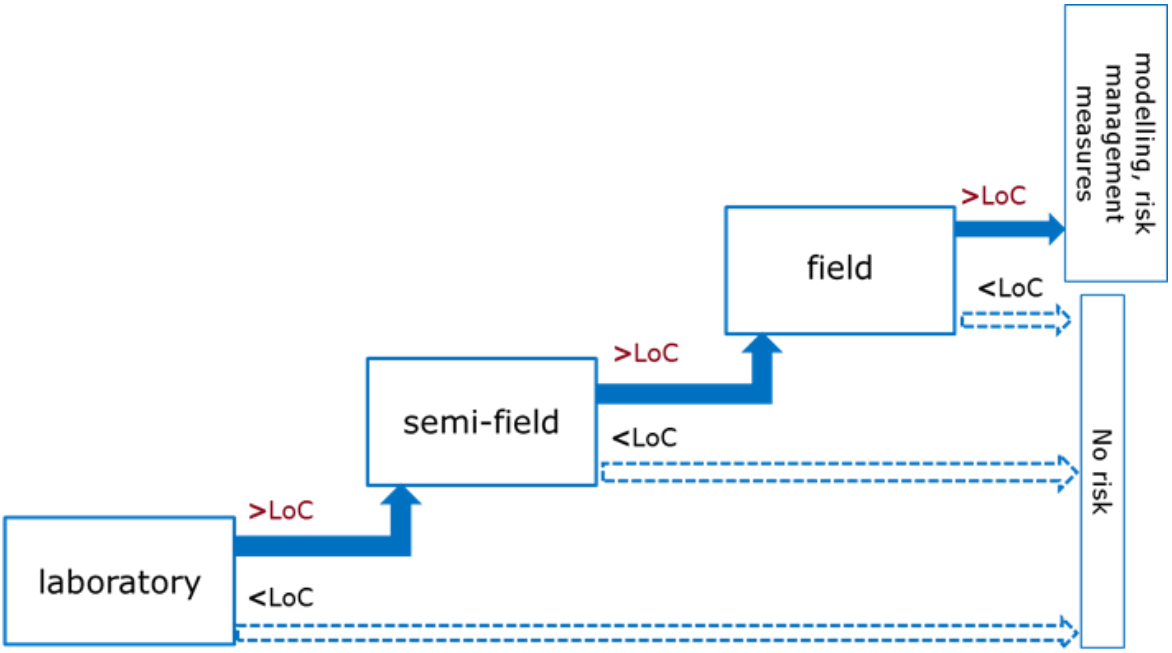


Figure 3. Limits of Concern and consequences for the stepwise testing strategy in the ERA of GMO as interpreted from the EFSA guidance. Filled arrows indicate proceeding to the next (higher) tier, if the LoC is exceeded at the preceding tier. Dashed arrows indicate the conclusion of no risk and testing ends, if the LoC is not exceeded.

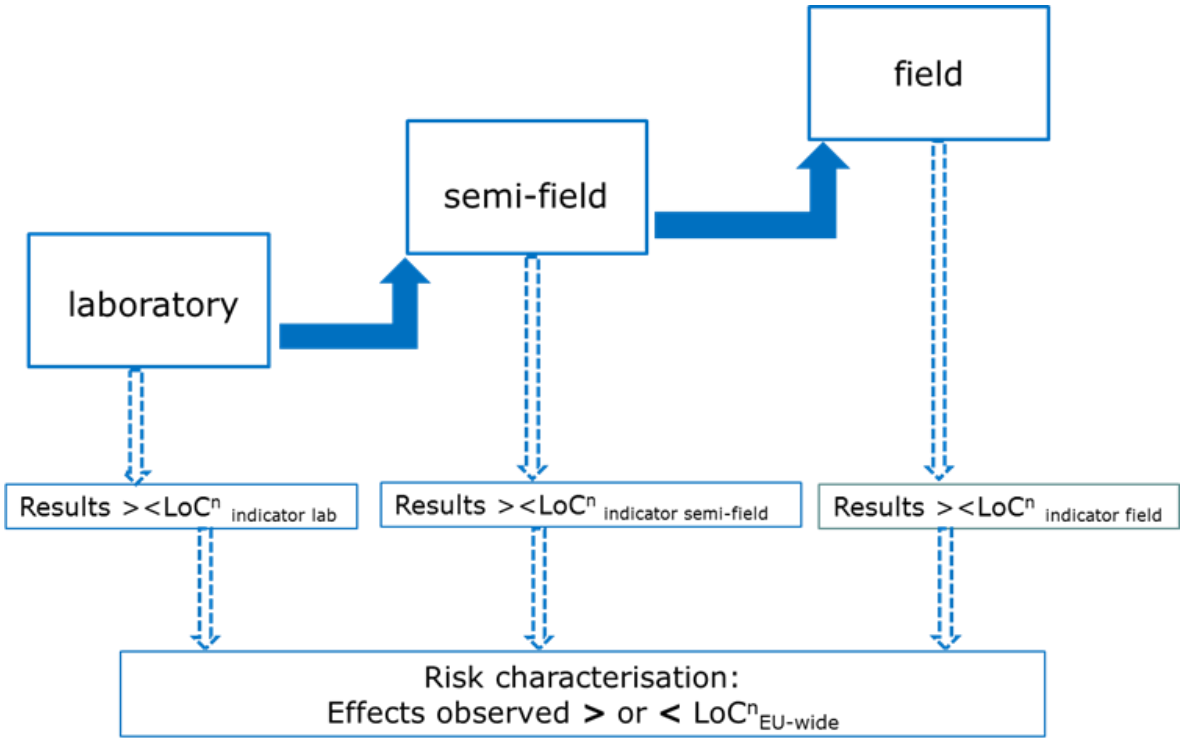


Figure 4. Limits of Concern and the consequence for the stepwise testing strategy in the ERA of GMO – own view. Filled arrows indicate the proceeding to the next (higher) tier, irrespective of whether or not the $LoC^{n}_{indicator}$ has been exceeded. Dashed arrows indicate that results from the testing are compared with the $LoC^{n}_{indicator}$ (exceedance/non-exceedance) at each tier and then integrated in the risk characterisation step of the ERA.

## 8.4 LoCs and the comparative safety assessment

A basic element of the ERA of GMPs is the comparative safety assessment. The level of difference between a GMP and its conventional non-GM counterpart is evaluated in view of its potential to cause harm (EFSA 2011a). For this purpose not only tests for difference but also equivalence tests are required in order to put any difference found into the context of harm (EFSA 2011a, PERRY et al. 2009). For food-feed risk assessment `equivalence limits´ have to be defined which are composed of the values from commercial plant varieties. Also for the ERA usually a conventional counterpart is used in test systems to assess potential adverse effects on e.g. non-target organisms. The underlying assumption behind the concept of the comparative safety assessment is that traditionally cultivated plants are considered safe due to a history of safe use for human and animal consumption (EFSA 2011a, CONSTABLE et al. 2007). This concept has been widened to include environmental effects as it is assumed that traditionally cultivated crops "…have gained familiarity for the environment" (EFSA 2010c). While the history of safe use is of relevance for traditional food and feed, a history of environmentally safe cropping cannot be established. Environmental impacts of conventional farming practices can be adverse and substantial, well documented e.g. by the decline in farmland bird populations in post-war Europe due to agricultural intensification (DONALD et al. 2001, but see also EC 2015a, EC 2015b). The introduction of provisions under the EU common agricultural policy, such as cross compliance requirements, compensations for voluntary measures in the

context of the rural development pillow as well as recently greening requirements, aims at the improvement of the environmental performance and increased sustainability of current agricultural practices in the EU. Consequently, a clear conceptual distinction is needed between the risk assessment for food and feed purposes with equivalence limits based on the natural variability of non-GM comparators, and the environmental risk assessment of GMOs using independent and protection goal-derived LoCs (PERRY et al. 2009).

If the LoC concept is to be made operational then a separate evaluation of the results of the comparative safety assessment for the ERA is needed, scrutinizing (i) whether the assessed components cover sufficiently environmentally relevant plant compounds for the GMO in question and (ii) whether detected differences between GMOs and their non-GM comparators are of environmental relevance. Any detected significant difference or non-equivalence in the concentration of compositional parameters in relevant plant tissues needs to be scrutinized whether they may affect relevant protection goals. This can be achieved by establishing a causal link between the environmentally relevant parameters and any existing LoCs for the protection goal in question. This approach is not only needed for compositional aspects of the GMO but also if differences in agronomic practices such as the application (e. g. numbers and frequency) of herbicides to be used with herbicide tolerant crops are identified. A risk hypothesis has to be formulated to test whether the identified hazard falls below the existing LoCs for a relevant protection goal. Otherwise it may be necessary to define a new LoC specifically adjusted to the hazards identified from the comparative assessment (Figure 5). Therefore, the LoC concept has to be further specified and the ERA guidance has to be further developed in order to provide sufficient guidance for applicants and risk managers.

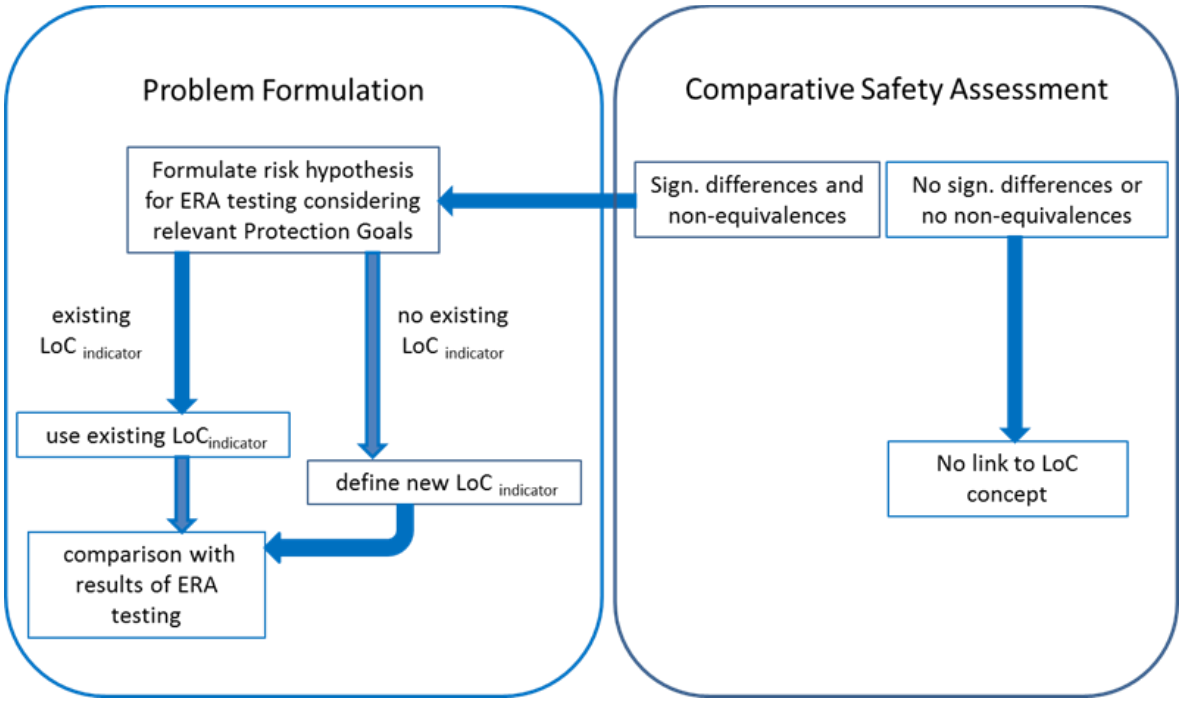


Figure 5. The relationship between the LoC approach and the comparative safety assessment in the ERA of GMPs. Arrows indicate consequences or necessary actions.

## 8.5 LoCs for long-term effects

The setting of LoCs is an inherent requirement of the problem formulation of the ERA, hence they are also of relevance for long-term effects. LoCs have to be defined for long-term effects if they are likely to occur and if appropriate risk hypotheses can be formulated. Long-term effects often occur under novel agro-ecological conditions, which were not encountered during the ERA or which are difficult to test in the ERA. An example is the resistance development of weed species to non-selective herbicides applied with the corresponding herbicide tolerant GMO. Resistance development does not occur immediately when herbicide tolerant GMOs are cultivated, but after several years of extended use of the complementary herbicide (POWLES 2008). Resistance development of target species is clearly recognized as an adverse effect in the ERA of insect-resistant GM maize requiring risk mitigation measures as well as case-specific monitoring (DOLEZEL et al. 2011). For insect-resistant GM maize the aim is to prevent the occurrence of resistance in the respective target organisms, thereby defining a damage threshold. In contrast, no such thresholds for the resistance development of weeds due to the application of the non-selective herbicide in herbicide tolerant crops have been defined yet (DOLEZEL et al. 2011).

In the ERA practice it will not be possible to assess the exact type and extent of the adverse effects that the novel management and cultivation practices associated with herbicide tolerant GMO cultivation may have. Nevertheless, the formulation of LoCs will be needed also for these types of effects (see also BENBROOK 2016). Risk management measures and a well-designed post-market environmental monitoring are needed in order to evaluate whether any LoC for long-term effects are exceeded during cultivation. The efficacy of specific risk management measures (e.g. weed resistance management measures) needs to be evaluated at least during the post-market monitoring period, usually 10 years after consent, and ideally longer. Any adverse effect observed during the post-market monitoring period (e.g. the occurrence of resistant weed species) needs to be scrutinized whether it has been caused by the cultivation of the respective GMO and whether it falls within the defined LoCs.

## 8.6 LoCs and receiving environments

An important aspect so far left unconsidered is the necessity to integrate regional specificities of the receiving environments into the LoC concept. A link between any damage threshold defined at the EU level (i.e. the $LoC^{n}_{EU\text{-}wide}$) with the specificities of the receiving environments needs to be established (Figure 6), thereby accounting for differences in environmental effects of the same GMO in different regions, e.g. due to large scale land-use changes (LANG et al. 2007) or because of interactions with GM plants that are already cultivated in a specific region (EFSA 2010a). This may be achieved by modifying the $LoC^{n}_{EU\text{-}wide}$ according to individual conservation priorities of EU Member States resulting in a regionally adapted damage threshold (the $LoC_{regional}$).

In this context it has to be kept in mind that the definition of the receiving environment depends on the area of risk that is considered during the ERA. Receiving environments may be defined differently if potential effects on the crop management system are considered or if potential effects on non-target organisms are assessed. For example, considering changes in management practices for GM cotton in Europe may require defining

different receiving environments in Greece and Spain, the two main cotton-producing countries in the EU: Although Greece and Spain belong to the same biogeographical region, differences between these countries with regard to the occurrence of pathogenic fungi, seed treatments, soil preparation and weed management have been reported (RÜDELSHEIM & SMETS 2012). Therefore regional differences in agricultural management measures such as irrigation intensity, crop rotation or weed and pest management practices have to be considered. In contrast, for the evaluation of non-target organisms the receiving environments will be rather based on geographical zoning concepts (JÄNSCH et al. 2011) in order to account for differences in their occurrence and their regional relevance (MEISSLE et al. 2012).

Alternatively, a regionalisation of damage thresholds may also be achieved after the authorization of the GMO at Member State level, e.g. via the adoption of national opt-out measures restricting or prohibiting the cultivation of a GM crop according to Directive (EU) 2015/412.

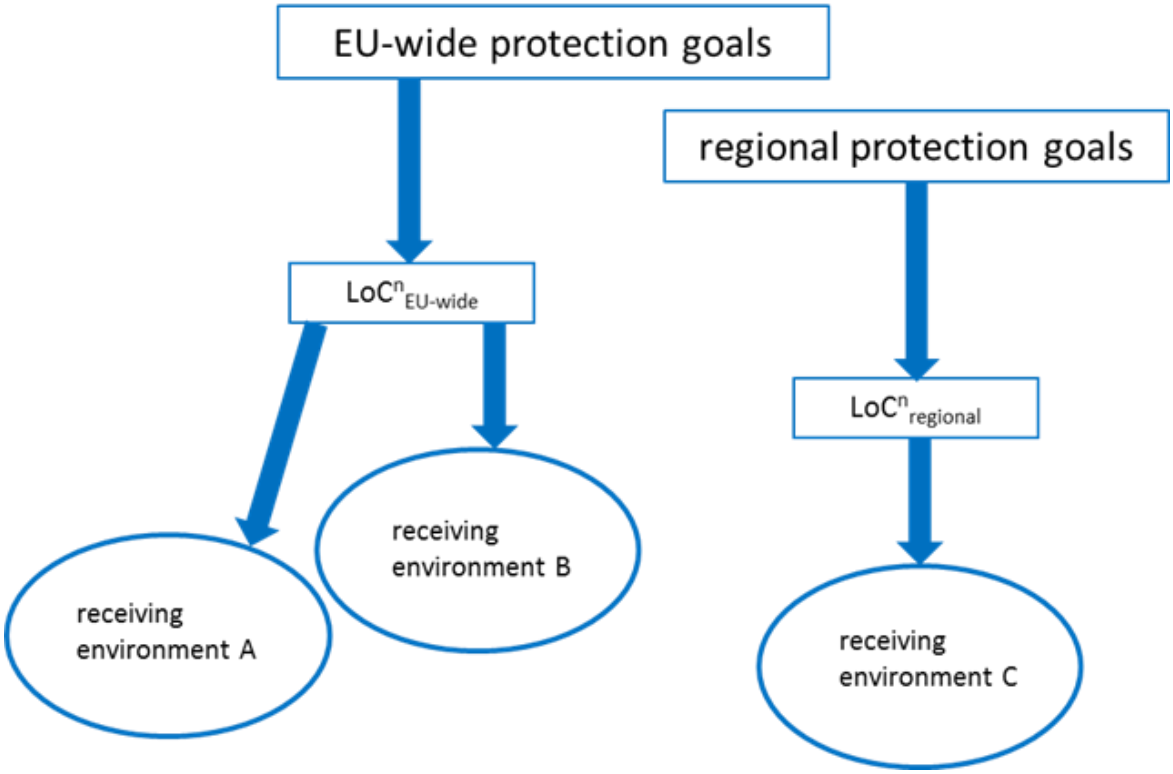


Figure 6. Possible regionalisation of the LoC concept. In this example two sets of LoCs (LoC$^{n}$) are defined for three receiving environments A, B and C. LoC$^{n}_{EU-wide}$ are valid for the receiving environments A and B, whereas LoC$^{n}_{regional}$ are valid for receiving environment C only.

# 9 Suggestions for LoCs for the risk area: persistence and invasiveness including plant-to-plant gene transfer

The following chapter contains suggestions for the definition of LoCs for the area of risk: persistence and invasiveness including plant-to-plant gene transfer. The analysis is based on the example of GM oilseed rape. For the discussions in this study the introduced GM trait is of minor relevance as general aspects of the topic are discussed. In the first subchapter the relevant protection goals for GM oilseed rape are discussed. The second subchapter discusses relevant aspects when setting LoCs for GM oilseed rape.

## 9.1 Protection goals relevant for GM oilseed rape

### 9.1.1 Biodiversity and Ecosystem Services

Protected or endangered species and habitats as well as ecosystem functions and services are relevant protection goals that may be affected by GM oilseed rape. Therefore all legislative provisions relating to the protection of biodiversity, of different environmental compartments and ecosystem services as mentioned by EFSA (2010a) are considered relevant for this GM crop.

One aspect of special relevance for GMPs that are able to outcross into wild plant species is the question whether the genetic constitution of a species or a species community is a specific characteristic that is particularly relevant for or explicitly protected by a specific protection goal. The FFH-Directive (Directive 92/43/EEC) does not refer to the genetic constitution when defining the favourable conservation status of a species or a habitat. However, certain national nature protection provisions aim to conserve the `characteristic features´ of nature (see German Nature Protection Law § 1) under which a specific genetic constitution may also be subsumed (BRECKLING & MENZEL 2005). Further, the concept of `national responsibility´ includes information on the genetic constitution of populations, and uses biogeographic proxies, because original genetic data are usually not available for wild populations (GRUTTKE et al. 2004, GRUTTKE & LUDWIG 2004; for plants see LUDWIG et al. 2007). The provisions of Regulation (EU) No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species specify that threats to biodiversity and ecosystem services by invasive alien species include, among others, genetic effects caused by the hybridisation of native species with invasive alien species. For example, the hybridisation of the invasive ruddy duck *Oxyura jamaicensis* with the endangered native white-headed duck *Oxyura leucocephala* has led to the listing of this species on the list of invasive alien species of Union concern (Commission Implementing Regulation (EU) 2016/1141).

Another important biodiversity protection goal with relevance for GMPs that are able to outcross and hybridise are crop-wild relatives. Certain crop-wild relatives that are able to hybridise with oilseed rape such as species within the genera *Brassica*, *Sinapis*, *Raphanus*, *Rapistrum*, *Hirschfeldia*, *Eruca* and *Erucastrum* (PASCHER & GOLLMANN 1997) have an EU-wide protection status (see Directive 92/43/EEC, Annex II). Also on a national scale wild relatives of oilseed rape may have a particular protection status or may be threatened, such as *Crambe tatarica* (ADLER 1994, PASCHER & GOLLMANN 1997). In addition, crop-wild relatives are important components of plant genetic resources for food and agriculture due to their ability to be potentially used for crop improvement (BILZ et al.

2011). About 18 % (i.e. 25 species) of the crop-wild relatives of the Brassica complex are considered to be threatened at the European level (BILZ et al. 2011).

Species that are sexually compatible with oilseed rape may also be assigned a higher protection level due to their status as old crop varieties which became feral. Some of these feral or wild species are still or have been cultivated in parts of Europe, such as *Eruca sativa* or *Brassica rapa*, *Raphanus raphistrum* and *Sinapis alba* (ADLER 1994) and thereby constitute important plant genetic resources for food and agriculture (see BILZ et al. 2011).

Protection goals for GM crops that are able to outcross, persist and invade refer not only to species but also to habitats that may be invaded by feral GM oilseed rape. Habitat conservation is one of the cornerstones of the conservation of biodiversity in Europe laid down by the FFH Directive (Directive 92/43/EEC). Populations of feral oilseed rape mainly occupy open and disturbed habitats, either naturally or by human intervention, where oilseed rape establishes as a pioneer plant (PASCHER & DOLEZEL 2005).

Beside protection of natural habitats at the European level, habitat conservation is important at the national level. Specific habitat types such as those frequently occupied by feral oilseed rape, e.g. ruderal habitats that occur in agricultural landscapes, may also be included in national Red Lists such as the Red List of threatened habitat types in Austria (TRAXLER et al. 2005a) or the German Red Data Book on endangered habitats (RIECKEN et al. 2006). For those habitats site-specific information and structural characteristics of the habitats are indicated as well as characteristic plant communities.

Nature conservation goals of these protected habitats may be compromised due to the spread and persistence of GM feral oilseed rape or GM oilseed rape-wild relative hybrids in these habitats, if the specific objectives of conservation are affected.

### 9.1.2 **Agricultural protection goals**

The Biodiversity Action Plan in Agriculture includes specific measures related to the maintenance of local, traditional and rustic breeds and varieties and the diversity of varieties used in agriculture in order to maintain the genetic variety and biodiversity of domesticated plants and animals in situ (EC 2001). The need to conserve agro-biodiversity is also recognized by the Convention on Biological Diversity (CBD 1992), the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2001) and the Global Plant Conservation Strategy (CBD 2011). In particular, the 2010 Biodiversity Targets address the conservation needs of the genetic diversity of crops and specific targets to halt the loss of genetic diversity of crops and other plant species (CBD 2002). Particularly the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2001) focusses specifically on agro-biodiversity and calls for the conservation and sustainable use of plant genetic resources for food and agriculture.

Diversity of plant genetic resources in agriculture is reflected by the modern seed varieties, cultivars and breeding lines listed in the EU common catalogues of plant varieties that can be marketed throughout the EU (http://ec.europa.eu/food/plant/plant_ propagation_material/plant_variety_catalogues_databases/index_en.htm) as well as those listed in the National seed catalogues. Apart from these modern cultivars plant genetic diversity for agriculture also encompasses crop landraces, ecotypes and crop-wild relatives (see

overview in VETELÄINEN et al. 2009) as well as threatened crop plant species (HAMMER & KHOSHBAKHT 2005). Landraces have been defined as `a set of populations or clones of a plant species which are naturally adapted to the environmental conditions of their region´ (Commission Directive 2008/62/EC). Also other traits have been attributed to be specific for landraces such as a local name, the lack of formal crop improvement and "…the close association with the uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow it" (LORENZETTI & NEGRI 2009). These landraces are not covered by the EU Common Catalogue of plant varieties or National Seed catalogues but represent an important component of threatened agro-biodiversity in Europe (NEGRI et al. 2009). However, Commission Directive 2008/62/EC on agricultural landraces and varieties accounts for the marketability of landraces with the aim to conserve and sustainably use these plant genetic resources. In this context also the genetic erosion risk of these conservation varieties is an important and recognized aspect. This risk is explicitly defined and refers to the "loss of genetic diversity between and within populations or varieties of the same species over time or reduction of the genetic basis of a species due to human intervention or environmental change" (Commission Directive 2008/62/EC).

A special aspect that needs attention in the context of agricultural protection goals for GMPs that are able to outcross are environmental effects that are mediated by socio-economic aspects. Socio-economic effects due to the cultivation of GM crops are outside the scope of Directive 2001/18/EC and the related ERA, but can be put into claim in the context of Directive (EC) 2015/412 regarding the possibility for the Member States to restrict or prohibit the cultivation of genetically modified organisms (GMOs) in their territory. Effects on protection goals may be mediated by socio-economic effects caused by the cultivation of GMPs which are able to outcross to conventional crop varieties. Outcrossing of GM oilseed rape may adversely affect seed producers which may abandon their business due to repeated contamination incidences or cost for seed testing. The number of small-scale and artisanal seed producers indirectly affects the diversity of seed varieties that are produced and available within a certain territory. Such indirect effects on agricultural protection goals – albeit mediated by socio-economic effects – may need special attention both during the ERA as well as post-market environmental monitoring.

Another priority of the Biodiversity Action Plan in Agriculture relates to the diversification of the types of production and of cultivated varieties together with all the aspects related to crop rotation as opposed to the specialisation of production systems and intensification of certain practices (EC 2001). Except seed production, also other production types such as organic or GMO-free production constitute agricultural production types with a specific sensitivity towards GMO contamination. Indirect effects on the diversity of these production types and cultivated varieties mediated by GM contamination may therefore also compromise agricultural protection goals.

Last but not least agricultural protection goals at the EU Member State level are relevant if they refer to the status of seed regarding the non-tolerance of GMO contaminations, such as the Austrian Ordinance on Genetically Modified Seed (Saatgut-Gentechnik Verordnung 2001).

## 9.2 Limits of Concern for GM oilseed rape

### 9.2.1 Introduction

Within the scope of this report the focus is on LoCs for effects of GMPs that are able to outcross, persist and invade natural or agricultural habitats using the example of GMHT oilseed rape. Recommendations for LoC definitions are structured according to the indicators as suggested by KOWARIK et al. (2008) for this area of risk:

The assessment of the vertical gene transfer using the following indicators

- Indicator 1: Presence of sexually compatible plants
- Indicator 2: Spontaneous hybridisation has been proved under field conditions
- Indicator 3: Hybrids are fertile
- Indicator 4: Backcrossing has been demonstrated

The assessment of the spread and persistence of the GMP using the following indicator:

- Indicator 5: Establishment of dominant populations of the GMP

These indicators were used as the basis for discussions on LoCs. Indicators were discussed with respect to their usefulness when setting LoCs and – if necessary – additional indicators were suggested.

KOWARIK et al. (2008) suggest a calibration of the selected indicators. Calibration in this context is understood as assignment of numerical effect categories to normative classifications of potential adverse effects (see Figure 7).

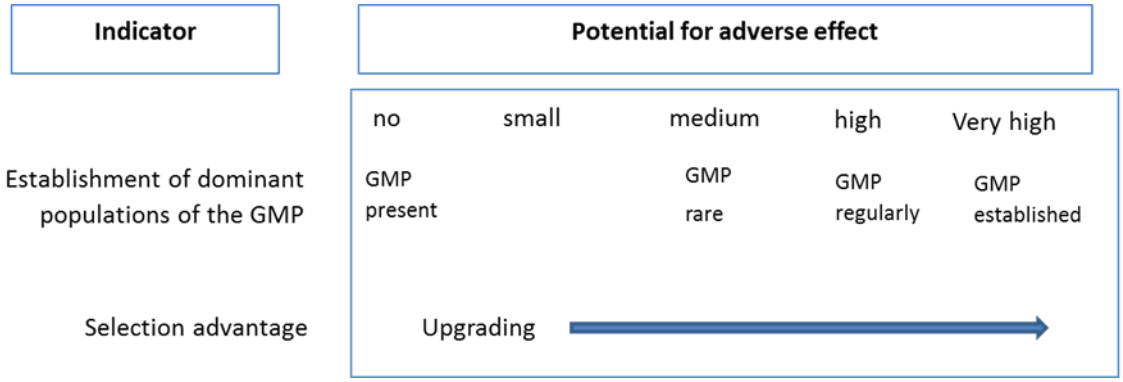


Figure 7. Example for the calibration of the indicator for spread and persistence of the GMP (KOWARIK et al. 2008).

### 9.2.2 Indicators for the assessment of effects on species due to vertical gene transfer

Effects on species (and biotopes) by vertical gene transfer are assessed by using the following indicators:

- Indicator 1: Presence of sexually compatible plants
- Indicator 2: Spontaneous hybridisation has been proved under field conditions

- Indicator 3: Hybrids are fertile
- Indicator 4: Backcrossing has been demonstrated

The more of these indicators are applicable for a particular crop, the higher the risk for vertical gene transfer (KOWARIK et al. 2008). Therefore the use of the indicators is consecutive and conditional. If the previous indicator applies, the next indicator is assessed. The highest potential for adverse effects on species or biotopes is given, if the GMP has sexually compatible wild relatives, is able to hybridise, produces fertile hybrids and hybrids are able to backcross.

### 9.2.3 LoCs suggested for effects on species due to vertical gene transfer

The indicators suggested for the assessment of vertical gene transfer (Indicators 1-3) are comparable to the stage 1 information requirements as outlined by EFSA (2010a), although EFSA additionally refers to the overwintering ability of the GMP. Also stage 2 information requirements include the assessment of hybridisation potential with compatible relatives outside the production system. If all indicators apply, the ERA proceeds to the next stage of information requirements (stage 2 or Stage 3, respectively). Therefore the ability of a GMP to outcross is used by EFSA (2010a) as a trigger value to proceed to the next step of information requirements within the ERA.

### 9.2.4 Aspects to consider for LoCs for effects on species due to vertical gene transfer

#### *Consider a different LoC for crop-wild relatives of conservation concern, landraces or rare crop varieties*

Gene transfer of GM oilseed rape to a wild relative plant and the establishment of GM crop-wild relatives constitute a controversial issue for the ERA of GM oilseed rape. The question whether the outcrossing of a GMP such as oilseed rape to wild relatives is considered as environmental damage and therefore exceeds an acceptability threshold such as a LoC or not, is not a scientific one and therefore answers are based on normative perceptions of environmental protection goals and may differ between EU Member States.

Considering regulation of and practical experience from invasion biology, the hybridisation of a non-native, alien species into a protected native species may also be considered a threat to biodiversity if effects on the population levels are evident (see Chapter 9.1). In analogy, hybridisation of GM oilseed rape with wild relatives of conservation concern could similarly represent an environmental effect which exceeds the threshold of acceptability due to the higher environmental value attributed to these species.

The definition of a LoC for the hybridisation of a population of a wild relative (of conservation concern) of oilseed rape requires setting criteria which determine the genetic baseline condition, i.e. the GM free status of the respective native populations of wild relatives, as well as the statement of the aim to preserve this (GM free) genetic condition of the natural (wild relative) species in the respective nature conservation laws and regulations.

Similarly, for rare seed varieties, crop-wild species or landraces which are considered relevant agricultural protection goals and are attributed higher relevance than `normal´ seed varieties (see Chapter 9.1) such provisions need to be made. Although genetic ero-

sion has been recognized as a potential risk for these varieties any genetic alteration due to the introgression of GM crops has so far not been characterised as risk. Whether the introduction of transgenes by outcrossing of a GMP such as oilseed rape into a landrace could constitute a reduction of the genetic basis due to human intervention and would thereby foster the genetic erosion of landraces is still to be clarified. Such a decision would imply that these varieties have a higher sensitivity towards hybridisation events due to GM crop cultivation which would call for a more conservative LoC than for conventional varieties.

### 9.2.5 Indicators for the assessment of effects on habitats due to the spread and the persistence of the GMP

The indicator suggested for the assessment of spread and persistence of the GMP is:

- Indicator 5: Establishment of dominant populations of the GMP

For GMPs with a trait that confers a selective advantage for the establishment and spread of the plant an upgrading of the adverse effect is suggested by KOWARIK et al. (2008). For this indicator it is recommended to use experiences and knowledge from other areas where the GMP has been cultivated (KOWARIK et al. 2008). It is suggested to assess effects on habitats only.

In general, several other indicators may also be useful to assess the potential for spread and establishment of the GMP. These include the potential to overwinter, the fitness, potential for expansion (also of GMO products), seed size, dormancy of seeds, seed losses, persistence etc. Indicators should be independent in order to avoid double counts. Ideally, indicators used can be assessed in all three tiers of the ERA (lab-greenhouse-field) in order to decrease the level of uncertainty of the effect tested.

### 9.2.6 LoC suggested for effects on habitats due to the spread and the persistence of the GMP

The indicator suggested for the assessment of the spread and persistence of the GMP is reflected by Stage 2 and Stage 3 information requirements and refers to agricultural production systems (the cropping area) as well as outside agricultural production systems, i.e. natural habitats (EFSA 2010a). A link to the LoC concept is made by EFSA in case the GMP is found to be more persistent than the conventional counterpart. In this case an evaluation has to be made if an agricultural or environmental impact is evident (in-crop) or if environmental damage will occur outside production systems (see EFSA 2010a). However, no further guidance is given with respect to the acceptability of these impacts. Hence, although in this ERA scheme a link is made to the LoC concept, no specific suggestions are made for LoCs.

### 9.2.7 Aspects to consider for LoCs for effects on habitats due to the spread and the persistence of the GMP

#### *Consider the conservation status of the natural habitat where the GMP occurs or establishes*

The indicator suggested by KOWARIK et al. (2008) refers to the occurrence of the GMP in a specific natural habitat. The suggested calibration of the indicator relates to whether the

GMP is absent (potential for no adverse effect), whether it occurs casually (potential for middle to high adverse effect) or whether the GMP is considered established and reproduces (potential for very high adverse effect) in the particular natural habitat. In this context `establishment´ refers to the occurrence of species with two spontaneous generations within at least 25 years (KOWARIK 2003 in KOWARIK et al. 2008). Any LoC for this indicator is therefore considered an exposure-based indicator as it does not include a measurement endpoint related to an adverse effect. For this indicator it is assumed that the potential to adversely affect a specific protection goal is higher the greater the spread of the GMP and the more regularly the occurrence of the GMP (KOWARIK et al. 2008). If the establishment of the GMP is predicted, this would have the highest potential for an adverse effect on the relevant natural habitat.

Defining a LoC therefore needs to consider whether any protection objective is relevant for the natural habitats where the GMP is predicted to establish. If habitats of conservation concern are considered, they are attributed a higher value due to their conservation status which must be accounted for by protecting them from likely adverse effects. This requires further specification of the protection objectives for these habitats in question, e.g. regarding their genetic status of their baseline condition in the relevant legislative provisions. If these specifications are made, then already the casual occurrence of GM oilseed rape may be considered an environmental effect that exceeds an acceptability threshold and therefore the LoC.

The setting of a LoC for such natural habitats can be justified by the fact that predictions on potential adverse effects of the GMP in a particular habitat are hardly feasible on a scientific basis. Experience from the introduction of alien species into European ecosystems shows that the invasiveness of alien species and their effects in the receiving environment are hardly predictable and often include high uncertainties (WILLIAMSON 2001, see also ESSL et al. 2011). Errors in the prediction of adverse impacts should be avoided when predicting the ability of a GMP to establish or spread in natural habitats. Similarly to the uncertainties in the *ex-ante* assessment of the future invasiveness and impact of alien species, invasiveness, persistence and impacts of GMPs are hardly predictable. The use of relevant indicators and criteria for the ERA process such as the `invaders elsewhere´ criterion as applied in the risk assessment for alien species as well as the recognition of the uncertainty of potential future adverse effects in the ERA greatly helps to reduce prediction errors (ESSL et al. 2011). For GM oilseed rape this means that experiences from the cultivation of the GMP from other areas is useful for a predictive assessment of risks although caution has to be applied with respect to the transferability of results from areas with different climatic or ecological characteristics (ESSL et al. 2011).

The predictability of the spread of GM oilseed rape into certain types of habitats is limited. Spread into and establishment in certain habitat types can occur in both, protected and non-protected habitat types. If the occurrence or establishment of GM oilseed rape in habitats of conservation concern is considered non-acceptable, this would require specific management measures (e.g. isolation distances to the respective habitats or the removal of the plants from the habitat). In contrast, if the occurrence or establishment of GM oilseed rape in non-protected habitats would not exceed an acceptability threshold, no management measures would be required. In practice the application of different LoCs for habitats with different conservation status will be difficult to manage.

### *Specifications for seed production can be a starting point for LoCs for GM oilseed rape in agricultural production systems*

The acceptability of adverse effects due to the establishment of GM oilseed rape in agricultural production systems (in-crop) depends on the specific agricultural protection goals (see Chapter 9.1.2). The persistence of the GM crop within the cropping area is usually evaluated by the use of the comparative approach using a conventional counterpart. However, this comparative approach cannot be further substantiated when applying the LoC concept due to the following reasons:

If conventional oilseed rape spreads and establishes on conventional fields, this does not compromise any agricultural protection goal such as the diversity of cropping systems, organic production, reduction of inputs, non-chemical weed strategies, maintenance of GM free seed etc. In contrast, the spread and establishment of GM oilseed rape in Europe will most likely affect agricultural practices as evidenced from experiences with GM oilseed elsewhere (e.g. MAURO et al. 2009) and therefore may also threaten agricultural protection goals such as low herbicide use and conservation tillage (MARVIER & VAN ACKER 2005, DEVOS et al. 2004). Also the maintenance of different production systems that have to comply with labelling thresholds for the adventitious presence of GM material in the product such as GM-free and organic production (DEVOS et al. 2004) as well as seed production (FRIESEN et al. 2003) may be put at risk. Therefore any decision on the acceptability of adverse effects should not be based on a comparative approach, but on an assessment whether the occurring GMP affects agricultural protection goals.

Decisions on acceptability thresholds and LoCs could be based on existing thresholds for volunteers in seed production. Thresholds for the acceptability of grow through of oilseed rape plants of other varieties differ depending on the sensitivity of the seed lot produced (e.g. basic seed or certified seed). According to the specifications of the Austrian Methods for Seed and Varieties 15 plants of other varieties are allowed on an area of 150 m² for certified seed of oilseed rape. Higher values lead to the non-approval of the seed plot for seed production (AGES 2004, BAES 2013). These thresholds aim at guaranteeing the purity of seed and consequently the crop varieties in the respective field when used in commercial cultivation. As these specifications relate to crop purity, it would be necessary to scrutinize whether non-exceedance of these values also ensures that the relevant agricultural protection goals are not compromised.

### *The status of the receiving environment into which the GMP spreads and establishes will influence the LoC*

The condition of the receiving environment as well as its resilience to environmental stressors is an important determinant for the acceptability of adverse effects. Ecosystem resilience is defined as the amount of disturbance a system can absorb and still remain within the same state including the ability to reorganize and renew itself (see discussion and references in ELMQUIST et al. 2003). In particular for plant species that are able to reproduce and disperse independent of human intervention, the resilience of the receiving environment is an important factor that may aggravate or lessen potential adverse effects mediated by the novel organism. For example, outcrossing rates of oilseed rape are depending on environmental factors such as geographical location (BECKER et al. 1992)

or the relative fitness of *Bt* oilseed rape hybrids (VACHER et al. 2004). Similarly, the spread and establishment of non-native, invasive species has been considered to depend on the prevailing conditions of the receiving environment (see references in KOWARIK et al. 2008).

Criteria for assessing the significance of an adverse impact should therefore include not only the cumulative impact of several similar projects in a specific environment (such as e.g. the occurrence of further feral GM oilseed rape plants or crop-wild hybrids), but also already existing pre-stressors relevant within a particular area (see BUNDESNATURSCHUTZGESETZ). The significance of an adverse effect and consequently any LoC defined may therefore also depend on the present status of the habitat, i.e. if it is pre-stressed or rather in a pristine condition. In both situations adverse effects are more likely to have significant impacts and require a more conservative LoC.

### *Consider the retrievability of the GM crop from the receiving environment and the reversibility of adverse effects*

The ability of oilseed rape populations to build up long persisting soil seed banks and to establish stable and self-dispersing feral populations (PASCHER et al. 2006) as well as the evidence that the distribution of feral oilseed rape populations is not only restricted to the cultivation context (BRECKLING & MENZEL 2004, CRAWLEY & BROWN 2004) indicate that feral GM oilseed rape populations cannot be retrieved easily from the receiving environment. The expected persistence has been suggested to be at least six to ten years (e.g. CLAESSEN et al. 2005a), up to 20 years (CLAESSEN et al. 2005b) or even longer (LUTMAN et al. 2005). The presence of such cultivation-independent populations of GM oilseed rape may therefore compromise the protection goals in question, in particular as their dispersal and establishment capacities cannot be easily controlled under cultivation conditions.

The ability to retrieve the GMP from the specific receiving environment where it spreads and establishes may be an additional factor that affects the acceptability of potential adverse effects when cultivating GM oilseed rape. Crop plants that are not able to outcross to wild relatives, spread or persist without human intervention in a particular environment can be removed from this environment by terminating cultivation. For crops like oilseed rape that are able to spread and persist, control and eradication measures would have to be applied once GM oilseed rape spreads and establishes. The availability of such eradication and control measures for non-native species has been suggested as a criterion for differences in the listing, and consequently the potential invasiveness of these species (ESSL et al. 2011). For self-spreading crops such as GM oilseed rape any occurrence in the receiving environment may be therefore be not acceptable.

In addition, the reversibility of adverse effects is an important aspect in the context of the determination of the significance of adverse effects. Invasive alien species with irreversible impacts (e.g. causing extinction of a native species) are classified in the highest impact category (see e.g. BLACKBURN et al. 2014). In the ERA of plant protection products the recovery of non-target species after an adverse effect has occurred is important to determine the acceptability of adverse effects in field studies (CANDOLFI et al. 2000a, CANDOLFI et al. 2000b). Recovery is defined as "….the return of damaged natural resources and/or impaired services to baseline condition…" (Directive 2004/35/EC). In the

context of spread and persistence of a GM crop the ability to recover also refers to the re-establishment of a certain baseline condition (e.g. without the GM plant in the receiving environment). When evaluating the impact in nature conservation practice the regeneration ability of protected biotopes or habitats is also an important criterion (RIECKEN et al. 2006, WULFERT 2016 and https://www.bfn.de/0322_biotope.html). The regeneration ability refers to the potential of a biotope to independently regenerate after adverse effects have occurred and considers the regeneration of the specific character of the respective biotope type. In this context the German Nature Protection Law (BUNDESNATURSCHUTZGESETZ) refers to a recovery time to the baseline condition of five years, thereby considering also the effect duration and sustainability (ILN 1996). For the setting of the LoC this implies that any threshold for the occurrence of the GMP in the respective habitat must consider the type and duration of potential adverse effects due to the occurrence of the GMP.

## 9.3 Conclusions for LoCs for GMPs that are able to outcross, spread and persist

In the discussion on the ERA requirements and adverse effects caused by GMPs that are able to outcross, spread and persist in the environment the analogy with invasive alien species has often been made (SUKOPP & SUKOPP 1993). This is due to the fact that alien species and GMPs like oilseed rape share many common features such as the unpredictability of their spread and possible adverse environmental impacts. Proposed risk assessment approaches for invasive alien species are based on the assessment of potential threats to biodiversity by the respective species, such as the threatening of native species populations or alteration of ecosystem processes or properties. The classification and listing of alien species and related control or management actions are achieved by mostly qualitative decisions on the potential threat to native biodiversity. If, according to the state of knowledge, no negative impacts to biodiversity are evident, the species is considered as non-invasive and no management measures are required. In analogy for GMPs like GM oilseed rape this would mean that biodiversity risks would have to be clearly evident before any action is required. If applying similar benchmarks for GM oilseed rape, the establishment of a GMO in a protected habitat without documented adverse impacts on biodiversity would not constitute a threshold for acceptability. Applying a more precautionary approach for the ERA of GMOs would have to consider that GM oilseed rape is non-retrievable from the environment once released and that any potential adverse effect on relevant protection goals may also be non-reversible.

In many cases potential effects of the GMP which are due to plant-to-plant gene flow, spread and persistence cannot be tested *ex-ante* in the ERA. By using a precautionary approach the assessment of adverse effects of GM oilseed rape on biodiversity must be based on indicators for biological processes rather than on documented effects on the protection goal(s). For the indicators suggested in this report the potential adverse effects are considered more likely the more of the criteria fit (for outcrossing) or the further the spread and establishment of the GMP has advanced (for establishment and persistence). Any LoC for these indicators which evaluate the process in the ERA that may lead to an adverse environmental effect (e.g. the spread and establishment of the GMP) is therefore not effect-based but exposure-based.

The decision whether the outcrossing of GM oilseed rape into wild species or agricultural cultivars is considered acceptable or not, should be based on the vulnerability of these species, i.e. by considering if these species or cultivars are protection objects. While outcrossing to wild relatives of oilseed rape may be considered acceptable, this may not be the case for wild relatives of conservation concern or in protected habitats. Plant genetic resources, comprising landraces, conservation varieties as well as crop-wild species, are of particular relevance when defining LoCs for GM oilseed rape. So far these agricultural protection goals have not been recognized in the ERA of GMPs that are able to outcross in the receiving environments. The conservation of agricultural biodiversity is specifically mentioned as a protection goal in the ERA guidance document and adverse effects on agricultural biodiversity are likely when cultivating GM oilseed rape in Europe. However, specifications of the genetic constitution of species and varieties which are able to interbreed with GM oilseed rape are necessary in order to justify decisions on the non-acceptability of gene flow and hybridisation between these species and cultivars.

For spread and establishment of GM oilseed rape in agricultural or natural habitats, the potential adverse effects that may occur in the respective habitat need to be considered. Relevant factors for determining a LoC are the particular conservation status of the natural habitat or the agricultural protection goals that may be compromised by the presence of the GMP in agricultural production systems. In this context the use of the comparative approach in the way it is currently done in the ERA process is clearly not useful to define LoCs as it does not take potential adverse effects for the protection goals in question into consideration.

The different perceptions of EU Member States on the significance and acceptability of the establishment and spread of GM oilseed rape in natural and agricultural habitats cannot be resolved during the ERA, but should be disclosed beforehand. Here, the use of the LoC concept can be helpful as it pins down different value judgements on the environmental significance of GMPs that are able to hybridise with wild relatives before any risk assessment is conducted. Focussing on the relevant protection goals can therefore support decisions on the acceptability of potential adverse effects by GM oilseed rape.

# 10 Suggestions for LoC for the risk area: impacts of the specific cultivation, management and harvesting techniques

The following chapter contains suggestions for the definition of LoCs for the area of risk: impacts of the specific cultivation, management and harvesting techniques. The analysis uses the example of genetically modified herbicide tolerant (HT) crops. For the general discussions on LoCs in this study the specific crop (e.g. maize, soybean) is of minor relevance, although the case-by-case principle in the ERA of GMOs shall not be dismissed. In the first subchapter the relevant protection goals for GM oilseed rape are discussed. The second subchapter discusses relevant aspects when setting LoCs for HT crops.

## 10.1 Protection goals relevant for GMHT crops

### 10.1.1 Biodiversity and Ecosystem Services – in-field and off-field

In the agro-ecological context the aim of herbicide use is to control weeds in the field where the crop is grown. So far, common plant species growing in the field have not been protected by legislation; they have not been considered biological resources requiring any form of protection. No specific protection goals or risk assessment requirements were previously defined for in-field plants in the regulation of plant protection products (SANCO 2002a, EPPO 2003c). In the regulation of plant protection products, in-field weeds were considered target plants, while plants growing outside the field were considered non-target plants. Hence, potential adverse effects on non-target plants considered in ERA requirements of plant protection products so far considered only "non-crop plants located outside the treatment area" (EC 2002, EPPO 2003c). For terrestrial plants outside the cropping area (i.e. the non-target plants) the risk assessment requirements covered risks e.g. through spray drift. This was based on the implicit protection goal defining that effects on these plants at a certain distance from the treated crop area are less acceptable than in the field (EPPO 2003c, SANCO 2002a). Protection goals for in-field terrestrial plants in agro-ecosystems were therefore absent from EU regulations (SANCO 2002a, EPPO 2003c).

Certain weeds constitute protection goals in agricultural landscapes, and the necessity to protect also common – in addition to rare or protected – species in the agro-environmental context has been called for (SANVIDO et al. 2012). Recently it has been recognized that also for in-field plants specific protection goals are needed as in-field plant species are important for the provision of certain ecosystem services (EFSA 2014b). Therefore the importance of weeds and non-target terrestrial plants for the support and maintenance of farmland biodiversity has been acknowledged (see EFSA 2010a, EFSA 2014b). Weeds and non-target plants were identified as key drivers for certain ecosystem services such as the provision of resources and supporting food webs at higher trophic levels (EFSA 2014b). Weeds are part of the biodiversity, of the `other living organisms´ (EFSA 2010a). Also the definition of non-target terrestrial plants was reformulated as `all terrestrial plants affected by pesticides although they are not the intended target of the pesticides´ (EFSA 2014b). In addition, it has been also recognized that certain in-field species need specific protection due to their rarity or endangerment (EFSA 2014b). Consequently, new data requirements for the ERA of plant protection products

take non-target plants not only off-field but also in-field into consideration (Regulations (EU) No. 283/2013 and No. 284/2013).

Due to the fact that protection goals are only broadly described in EU legislation, EFSA has developed a methodology to derive specific protection goals for the ERA of plant protection products by the use of the ecosystem services concept (NIENSTEDT et al. 2012, EFSA 2010d). In the ecosystem service concept non-target plants were differentiated according to their role as key drivers for different ecosystem services in-field and off-field (EFSA 2014b). While non-target plant populations in the off-field area should not be affected by plant protection products, for in-field plant populations different specific protection goals were considered necessary. It was recognized that a further differentiation of the specific protection goals for non-target plants between in-crop and off-crop areas is still needed (EFSA 2014b). The maintenance of biodiversity was highlighted as overarching protection goal for terrestrial plants in agricultural systems (EFSA 2014b).

### 10.1.2 **Species of conservation concern**

Rare and endangered plant species occur in arable fields and are subject to further pressures due to intensification of agricultural activities. The role of rare and endangered species as driver of aesthetic values and cultural ecosystem services in agroecosystems has been recognized in the ERA of plant protection products (EFSA 2010a, EFSA 2014b). Due to the trade-off between crop productivity and the conservation of certain species within the field, separate protection goals for endangered plants are needed (EFSA 2014b).

Around 40 species of European policy plants (i.e. plant species that are listed under European or international policy instruments) are subject to major threats by intensive arable farming in Europe (BILZ et al. 2011). At national scale red lists specifically for weed species (e.g. ELIAS et al. 2005 for SI, BOMANOVSKA 2010 for PL) or red lists of threatened biotope types including arable and fallow land (e.g. RIECKEN et al. 2006 for DE; TRAXLER et al. 2005a for AT) have been developed or important areas for the conservation of the biodiversity of segetal plant species were identified (TRAXLER et al. 2005b for AT). In addition, conservation priorities for communities of arable vegetation have been suggested in certain EU Member States (BYFIELD & WILSON 2005 for UK, MEYER et al. 2013 for DE). An important aspect of the conservation efforts for rare arable weeds is the *in-situ* protection of weeds and weed communities by the establishment and definition of “important arable plant areas” (BYFIELD & WILSON 2005, UK), “protection fields” (VAN ELSEN & LORITZ 2013, MEYER & LEUSCHNER 2015, DE) or “biodiversity hotspots” (TRAXLER et al. 2005b, AT).

### 10.1.3 **Agricultural production and management methods**

A specific feature of GMHT crops is that they introduce a novel flexibility in the use of the non-selective herbicide on the crop and during the cropping season and therefore may change the commonly used agricultural production and management methods. Such changes can relate to changes in the timing of the herbicide application or to changes in the crop rotation. For example, the occurrence of weeds resistant to ALS-inhibitor-based herbicides triggered the use of GMHT soybean in some regions in the USA (FREUDLING 2004) which in turn affect production and management methods. Such indirect effects

due to the use of a GMHT and the related herbicides are not covered by the regulation of plant protection products (i.e. Regulation (EC) No. 1107/2009).

EFSA (2010a) lists legislative documents not only relevant for the protection of biodiversity but also for achieving agricultural protection goals when carrying out the ERA of GMPs. These documents comprise e.g. the Biodiversity Action Plan for Agriculture (EC 2001), Directive 2009/128/EC on the sustainable use of pesticides as well as Regulation (EC) No 1107/2009 concerning the placing on the market of plant protection products.

Directive 2009/128/EC on the sustainable use of pesticides aims to promote integrated pest management with the focus on non-chemical methods. It aims at the reduction of risks and impacts of pesticide use on human health and the environment. According to Directive 2009/128/EC EU Member States are requested to define reduction targets for pesticide use for particular crops. Another aim of the Directive is to limit the levels of pesticide use in order not to increase the risk for resistance development in populations of harmful organisms (Directive 2009/128/EC; Annex III).

Priorities for the integration of biodiversity and sustainability considerations into agricultural production practices have been outlined by the Biodiversity Action Plan for Agriculture (EC 2001):

- The diversification of production types and cultivated varieties including crop rotation
- Less intensive use of inputs (including plant protection products) in certain situations
- Promotion of organic farming and integrated crop management
- The conservation of the biodiversity of domestic (animals and) plants *in situ*
- Support of measures to maintain the diversity of varieties used in agriculture (e.g. the use of local, traditional and rustic breeds and varieties)
- Encouraging the marketing of landraces and varieties adapted to local and regional conditions

In addition, Regulation (EC) No. 1107/2009 provides for the placing on the market of so-called low-risk plant protection products and the use of plant protection products which are significantly safer for human or animal health or the environment when evaluating candidates for substitution.

The agricultural protection goals and proposed measures to achieve these goals which are relevant for the ERA of GMHT crops can be summarized as follows:

- Promotion of integrated pest management methods including low-pesticide input pest management systems and priority to non-chemical methods (i.e. agronomic techniques or physical, mechanical or biological pest control methods)
- Diversification of production types, crop varieties and crop rotation
- Avoidance of resistance in target organisms
- Reduce dependency on use of pesticides
- Substitution of plant protection products with products with a lower risk to human and animal health and the environment

## 10.2 Limits of Concern for GMHT crops

### 10.2.1 Introduction

The focus of this report is on LoCs for effects of herbicide tolerant GMPs and their specific cultivation, management and harvesting techniques. The recommendations for LoCs are structured according to the indicators as suggested by KOWARIK et al. (2008) for this area of risk. KOWARIK et al. (2008) recommended the use of the following indicators in order to assess adverse effects of the GMP on species and biotopes due to changes in cultivation and management systems:

- Indicator 1: The use of non-selective herbicides
- Indicator 2: Reduction in the degree of coverage of plant populations
- Indicator 3: Reduction of the plant species diversity
- Indicator 4: Changes of the biotopes into biotopes with decreased conservation value

These indicators were used as the basis for discussions on LoCs for plant populations as well as for faunal species associated with weed communities.

Indicators for potential effects due to changes in cultivation and management techniques on agricultural protection goals were not considered by KOWARIK et al. (2008). Hence, additional indicators were suggested to cover agricultural protection goals (see Chapter 10.2.8). Effects of the GMHT cropping systems on soil ecosystems are not covered by this report.

KOWARIK et al. (2008) suggests a calibration of the selected indicators. Calibration in this context is understood as assignment of numerical effect categories to normative classifications of potential adverse effects (see Figure 8). Figure 8 shows the calibration of indicator 2, the reduction in the degree of coverage of plant populations.

| Indicator | Potential for adverse effects on populations of plants (weeds) | | | | |
|---|---|---|---|---|---|
| | none | small | medium | high | very high |
| Reduction in degree of coverage | 0% | > 0 to < 5% | ≥ 5 to < 10 % | ≥ 10 to < 15 % | ≥ 15 to 100 % |

Figure 8. Example for the calibration of the indicator for the assessment of potential for adverse effects on plant populations; in this case the reduction in the degree of the plant coverage (KOWARIK et al. 2008).

### 10.2.2 **Indicators for the assessment of effects of the GMHT crop on plant populations**

Four indicators were suggested by KOWARIK et al. (2008).

- Indicator 1: The use of non-selective herbicides

This indicator is considered useful for the ERA of herbicide tolerant GMPs. It considers the spatial occurrence of affected plant populations which allows differentiating in-field and off-field effects of non-selective herbicides on plant communities. Such effects may be valued differently according to different protection goals for in-crop and off-crop plant communities.

The use of this indicator is recommended by KOWARIK et al. (2008) only, if no further indicators are applied. If data for other indicators are available (e.g. for indicators 2 or 3), these should be preferentially used as they indicate adverse effects directly at the level of the impact on the relevant protection objects.

However, when used as single indicator, it is considered insufficient to assess effects on weed communities due to GMHT crop cultivation and therefore, it was not further discussed in the context of the assessment of effects on plant populations. However, indicator 1 is considered useful for assessing effects on agricultural protection goals and is discussed in detail in Chapter 10.2.8.

- Indicator 2: Reduction in the degree of coverage of plant populations
- Indicator 3: Reduction of the plant species diversity

Indicators 2 and 3 relate to in-field effects of the herbicide use on plant populations. These indicators aim to assess effects on the emerged weed flora and are important in order to predict within-field management intensity (HAWES et al. 2010). Calibrations for the proposed indicators 2 and 3 made by KOWARIK et al. (2008) were based on the assumption that the potential effects on the plant communities are higher the more the degree of coverage of a particular plant population is reduced or the more species are affected.

It is important to recognize that the way how in-field weed diversity is assessed may affect the outcome (FAGÚNDEZ 2014). Parameters that could be used include α-diversity (species richness), β-diversity (species turnover) and γ-diversity (total species number). Weed diversity was found to be a useful indicator at field scale, in particular weed species richness and weed diversity, in order to assess effects due to management intensity (HAWES et al. 2010). However, effects of GMHT crop management on weed diversity as observed by the British Farm Scale Evaluations were only small and transient (HEARD et al. 2003a). The use of species accumulation curves has been proposed as indicator when the loss of individual species is considered relevant (SQUIRE et al. 2009). Effects due to GMHT cropping on rare species can be inferred by using data at regional scale (SQUIRE et al. 2009). Such effects on biodiversity are not necessarily evident at local scale (HEARD et al. 2003a, HEARD et al. 2003b).

- Indicator 4: Changes of the biotopes into biotopes with decreased conservation value

Indicator 4 is relevant for GMPs with novel GM traits that allow cultivation of GMOs in areas or habitats which were so far not used for crop cultivation (e.g. GMPs with tolerance against abiotic stress). This indicator aims at assessing land use changes (KOWARIK et al. 2008).

Indicator 4 is currently not considered relevant for GMHT crops for two reasons: (a) at present the only GMO with abiotic stress tolerance (against drought) is maize MON87460 and (b) without stacking of traits GMHT crops can be grown on arable land only. The agronomic resource requirements for growing a GMHT crop are not different than for conventional crop (e.g. regarding soil quality, soil nitrogen, soil moisture, and climate). However, GM crops tolerating certain environmental stress such as cold stress or drought may change fundamentally agronomic requirements of that crop and widen the possibilities for cultivation on land outside of what is currently considered to be arable for the respective crop in question. Depending on the development of GM plants with novel traits this indicator may become relevant. For the purpose of this study, however, this indicator is omitted from further discussions.

Additional indicators suggested

Differences in weed biomass (either total or of the individual weed species) represented the most prominent and significant effect of GMHT crops observed by the British Farm Scale Evaluations (HEARD et al. 2003a, HEARD et al. 2003b). Depending on the type of crop, weed biomass and plant density, in particular final plant density before harvest, were shown to be sensitive parameters to indicate differences in weed management. Therefore, in addition to the indicators suggested by KOWARIK et al. (2008), a further indicator for assessing effects on in-field plant populations is suggested:

Additional indicator: reductions in weed biomass ($g/m^2$) or weed density ($individuals/m^2$)

### 10.2.3 **LoCs suggested for effects of the GMHT crop on plant populations**

#### *LoC for in-field plant populations*

The necessity to define protection goals for plant populations in the field, particularly when assessing herbicides, has been recognized by EFSA (EFSA 2014b). Plants that are not the intended target of the pesticide but affected by the pesticide in the field are considered non-target plants (see Chapter 10.1). These in-field non-target plants have been recognized as drivers of provisioning and cultural ecosystem services (e.g. food web support). For in-field non-target plants the functional aspect of the plants such as the provision of food or habitat for higher trophic levels has to be evaluated as being more important than the biodiversity of the in-field plant community (EFSA 2014b). In consequence, the magnitude of acceptable effects for in-field non-target plants is differentiated depending on which ecosystem services they provide (EFSA 2014b). Medium effects are considered acceptable for plants, if they act as key drivers of food web support services, while for plants as key drivers supporting aesthetic values and genetic resources medium to large effects are tolerated (EFSA 2014b).

Observed effects on weed communities can range from negligible or very small to very large effects over years. Effect sizes on total weed biomass due to GMHT crop management as observed by the British Farm Scale Evaluations showed an almost six fold de-

crease in GMHT sugar beet compared to conventional beet while an almost threefold difference was observed between GMHT and conventional spring oilseed rape (HEARD et al. 2003a). For weeds in spring oilseed rape an approximately 20 % decrease in plant number per $m^2$ was reported (HEARD et al. 2003a). For individual weed species the reductions in biomass or density were larger than 90 % for certain species, such as *Polygonum aviculare* or *Persicaria maculosa* in beet, which lead to significant reductions of the weed seed bank of these particular species (HEARD et al. 2003a, HEARD et al. 2003b).

#### *LoC for off-field plant populations*

For the ERA of plant protection products protection goals are clearly defined for off-field non-target plants. These should also apply to the ERA of GMHT crops in order to achieve consistency in protection goals due to different agricultural stressors which is one of the aims of EFSA's undertaking on protection goals. In the off-field area non-target plant populations are considered drivers for a range of ecosystem services and should not be affected by the use of herbicides (EFSA 2014b). Therefore, only negligible effects are considered acceptable in the ERA (EFSA 2014b).

### 10.2.4 **Aspects to consider for LoCs for effects of the GMHT crop on plant populations**

#### *Discrimination of LoCs between in-field and off-field areas*

Generally, the aim of any non-selective herbicide use with a GMHT crop is to eliminate plant populations and communities other than the crop in the field, thereby controlling the weed populations and, inevitably, the biodiversity related to them. For the farmer the aim is to reduce competition between the crop and non-crop plants and to maximise crop yield and quality. However, it has been recognized that weed populations in the field are not only deleterious to the crops, but provide important ecosystem services (provision of resources and supporting food webs at higher trophic levels, see 10.1.1) that benefit the overall goal of the farmer when cultivating a particular crop. In addition, in-crop and off-crop areas provide different ecosystem services and those taxa that provide certain ecosystem services in the field may derive from off-crop areas (NIENSTEDT et al. 2012). Spray drift of non-selective herbicides can also affect non-target plant and invertebrate communities in field margins and off-crop areas (ROY et al. 2003, GOVE et al. 2007). While the focus of protection for in-crop plant communities is on the support of the food-web, for off-crop areas the aim is to protect biodiversity and minimize effects due to the cultivation and management practices in-field (EFSA 2014b).

Consequently, different protection goals between in-field and off-field areas as well as different acceptable effect thresholds for in-field and off-field plant populations are necessary when defining LoCs for GMOs. While specific off-field protection goals for off-field non-target plants have been proposed, no such in-field protection goals have been defined yet (EFSA 2014b). In practice it will be hardly feasible to differentiate – as proposed – non-target plants in the field according to their role as key drivers supporting different ecosystem services. A range of plant taxa certainly fulfil several ecosystem services, thereby different protection goals for different ecosystem services may be difficult to achieve for a particular species. In addition, there are currently no specific requirements to protect in-field non-crop plants according to current EU agricultural policies, leaving room for individual Member States to define the relevant protection level (EFSA 2014b).

However, consistent protection goal levels and acceptable effect thresholds for ERA purposes of plant protection products and GMOs need to be achieved. In ERA practice it will not be feasible to consider different protection goals and separate effects and effect levels for these two types of stressors.

### *Consider weed thresholds*

Agricultural management decisions based on pest thresholds is an important principle in Integrated Pest Management (IPM), as outlined by Directive 2009/128/EC on sustainable use of pesticides (Annex III, Principle 3). In arable fields the trade-off between different ecosystem services such as the provision of food or feed has to be balanced against regulating or cultural services, if certain protection goals are to be met (POWER 2010, see also NIENSTEDT et al. 2012). Such trade-offs are not necessarily inevitable – conservation of ecosystem services by relevant agricultural practices do not necessarily result in a yield penalty (see discussion in POWER 2010). In this context, the use of threshold levels for weeds in arable fields may help balancing the necessary trade-off between maximising yield and quality and sustaining necessary ecosystem services as provided by plant communities in the field.

Low weed densities do not significantly affect crop yield and certain weed densities resulting in yield reductions of 10 - 20 % are not considered unacceptable by farmers (OLIVER 1988, SWANTON et al. 1999). Yield losses of approximately 5 - 10 % correspond to the costs for weed control in many crops (SWANTON et al. 1999). Weed-yield relationships are available as agronomic management tools in order to define the density of weeds at which the control costs equal the economic return and the application of herbicides is economically worthwhile (ZANIN & SATTIN 1988, O`DONOVAN 1996). Decisions on the reduction of yield losses due to pests depend on the yield loss potential and the productivity of the crop itself (OERKE 2006). The relationships between yield loss and weed density can be described, by a rectangular hyperbolic function (COUSENS 1985, O`DONOVAN 1996, SWANTON et al. 1999), although also sigmoidal models were proposed (see discussion in COUSENS 1985).

Most data for establishing weed-yield relationships were obtained from artificial contexts, but there are also data from large scale experiments in the UK that assessed weed-yield relationships at realistic agricultural conditions (see references in SQUIRE et al. 2005). Although the definition of weed thresholds as a management tool is highly dependent on the context under which they were derived, they are considered useful, in particular in fields with low weed infestation (SWANTON et al. 1999). Examples of weed threshold levels for different crops and weeds are provided by SWANTON et al. (1999) and in the Talisman experiments from the UK (YOUNG et al. 2001, SQUIRE et al. 2005), and range from fewer than 1 plant $m^2$ in less competitive crops like sugarbeet or potato to 30 - 50 plants $m^2$ for highly competitive crops like cereals (SQUIRE et al. 2005, GEROWITT & HEITEFUSS 1990).

Weed threshold values have to be determined for a particular crop, a particular weed or weed community and in a particular environmental and agronomic context. Weed-yield relationships are known to differ between crops and locations (LUTMAN et al. 2000) as well as cropping systems (e.g. organic and conventional, RYAN et al. 2009).

In the European Union IPM programs and strategies have become important due to Directive 2009/128/EC on the sustainable use of pesticides which requires careful monitoring of harmful organisms and the use of warning and forecast systems as well as the use of threshold values for decision making for plant protection measures. Consequently, a range of countries currently develop or plan to develop crop-specific IPM guidelines (BARZMAN et al. 2015). The use of weed thresholds is therefore a useful tool for balancing ecosystem services of in-field weed populations and, consequently, the definition of LoCs for in-crop weed communities.

### *Consider functional traits of the plant populations*

There is evidence that plants with specific functional traits can be differently affected by the use of non-selective herbicides and/or different types of crop management. In GMHT oilseed rape monocotyledons were not significantly affected by the non-selective herbicide treatment, while dicotyledonous plants were significantly affected (HEARD et al. 2003a). Further analyses explained the increases in monocotyledons due to the specific response of the particular functional group (HAWES et al. 2009). Therefore, if indicators for assessing effects on weed communities such as degree of coverage, dominance, weed biomass or plant density are used (in particular for weeds in-field) functional aspects of the relevant plant taxa should be taken into account, e.g. by discriminating between monocotyledonous and dicotyledonous species. In addition, discrimination between reproductive and non-reproductive weed species, particularly at the end of the growing season, give an indication whether the seed bank of a particular species will be replenished at the end of the growing season. These aspects would have to be taken into account, if criteria for the acceptability of effects are to be defined.

### *Consider the role of the GMHT crop in the crop rotation*

Crop rotation significantly affects weed communities and suppresses weed seed densities in comparison to monocultures (see references in KREMEN & MILES 2012). Traditionally, in many European countries maize, sugar beet and oilseed rape are used as break crop for summer or winter cereals (SQUIRE et al. 2005). Break crops are important for sustaining certain weed populations, in particular dicotyledonous weed species, if the crop rotation is dominated by cereals (HEARD et al. 2003b and references therein). If GMHT crops are used as break crops, then the effect on the weed communities is of particular relevance as in particular dicotyledonous species would be adversely affected. The British Farm Scale Evaluations have shown that effects due to the GMHT management for the break crops maize and spring oilseed rape on the weed seed bank was traceable for at least two seasons after GMHT crops were cultivated (FIRBANK et al. 2006). Small differences in seed return in one cropping year can have large impacts if sustained over a longer time period.

Consequently, when defining thresholds of acceptability of effects on weed communities the role of the crop in the crop rotation should therefore be taken into consideration. This may be achieved by an upgrading of adverse effects when calibrating adverse effects on weed communities (see Methodology of this Report) or by applying a more conservative LoC for GMHT crops that are intended for cultivation as break crop.

### *Consider the assessment endpoint/ecological entity to protect*

Potential adverse effects on individual species or populations may be valued higher than potential adverse effects on biotopes due to the assumption that biotopes are only indirectly affected by adverse effects (KOWARIK et al. 2008). Consequently, it is important to consider the assessment endpoint, i.e. the natural resource or natural resource service that needs protection (EFSA 2010a), when evaluating the adverse effect. In the context of the ecosystem service concept to be applied in the ERA this circumstance is recognized by the definition of the relevant ecological entity to be protected (Nienstedt et al. 2012, EFSA 2010d, EFSA 2016a). For in-field plants exposed to non-selective herbicides it is important to consider that a specific weed community is the affected entity rather than specific plant populations. However, if considering higher trophic levels and food-web related effects the importance of a particular species may be valued higher than the importance of the weed community, as exemplified by the weed species *Chenopodium album* which is an important food resource for the skylark *Alauda arvensis* (WATKINSON et al. 2000, MARSHALL et al. 2003). Another example, although not relevant for European ecosystems, is the Monarch butterfly, a species of conservation concern which is an obligate herbivore of milkweed species growing on agricultural land (LUNA & DUMROESE 2013). The loss of breeding habitats due to significant decreases of milkweed caused by the cultivation of glyphosate resistant soybean and maize in the US has been identified as a major cause for the decline of the North American Monarch population (BROWER et al. 2012).

### *Different LoCs needed for species of conservation concern*

Species of conservation concern, i.e. rare and endangered species pose a considerable challenge in the ERA of GMPs. Due to the fact that rare weed species are not commonly present in arable fields; it is difficult to assess potential adverse effects on these species during ERA testing. The field scale level may not be sufficient to detect adverse effects on these species. It has been shown that the loss of less common weed species was not apparent when comparing field sites, but only if an indicator for regional upscaling was used (SQUIRE et al. 2009). In addition, any abundance-based selection of surrogate or focal species for ERA purposes will underrepresent rare and protected species (HILBECK et al. 2014).

When assessing adverse effects on biodiversity, the level of endangerment is considered as one aspect that can justify a particular magnitude of acceptable effect (EFSA 2016a). For plant protection products it has been suggested that for endangered species in the field no adverse effects should be tolerated, also recognizing that specific measures will be necessary to reach this protection goal (EFSA 2014b).

Adverse effects on rare or threatened arable plants should be avoided in order to not further deteriorate their population status or to increase the risk for their extinction (STORKEY et al. 2012). The selection criteria for these species should be based on the nomination in the following listings:

- Annex II of Council Directive 92/43/EEC on the conservation of natural habitats of wild fauna and flora
- Red List on regional, national (e.g. LUDWIG & SCHNITTLER 2009, ELIAS et al. 2005, BOMANOVSKA 2010), European (IUCN 2010a) or international (IUCN 2010b) level

- Listings for the national responsibility for the conservation of species (LUDWIG et al. 2007)
- Listing of declining arable species (see STORKEY et al. 2012)

The occurrence of rare and endangered or threatened segetal species is in many cases linked to arable sites with specific environmental or agronomic conditions which represent important retreat areas for these species (TRAXLER et al. 2005a, TRAXLER et al. 2005b, VAN ELSEN et al. 2006), underlining the requirement to define protection goals at Member State level. Endangered segetal species in Austria are clustered in their occurrence indicating a centralisation of endangered weeds in the Austrian Pannonian basin (TRAXLER et al. 2005b). Therefore, Red Lists for endangered biotope types are also an important instrument for the conservation of arable plants (e.g. RIECKEN et al. 2006, TRAXLER et al. 2005a).

As a consequence for the ERA of GMOs and the definition of the LoC, rare and endangered species need to be considered differently from `common´ in-field plant species and require a different, more conservative LoC. Even small effects on these species should not be tolerated and will require specific risk mitigation measures. In this context it has been already emphasized that individual Member States will need to apply specific management measures in order to protect endangered non-target plant species in-field (EFSA 2014b).

### 10.2.5 Indicators for the assessment of effects of the GMHT crop on faunal species associated with weed communities

In the ERA of GMOs adverse effects on the biodiversity of higher trophic levels due to changes in the weed flora caused by changes in the crop management method need to be assessed (Directive 2001/18/EC, EFSA 2010a). Also, the new data requirements for active ingredients of plant protection products demand an assessment of the “potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web” (Regulation (EU) No 283/2013).

For assessing effects on weed-related biodiversity due to changes in cultivation and management techniques the following indicator is suggested by KOWARIK et al. (2008):

- Indicator 5: Decrease in the abundances of selected faunal species

This indicator is useful for evaluating indirect effects of GMHT cultivation practices on weed-related biodiversity. The indicator is based on the assumption that the potential for an adverse effect is higher the more the population size of affected species decreases (KOWARIK et al. 2008). The effect categories used for the calibration of this indicator are based on the assessment of the condition of species according the FFH Habitats Directive, but amended for non-protected species. According to these normative specifications a reduction of the population of 6 % within six years refers to an unfavourable to bad conservation status of the respective species. Transferring this value to a time frame of 10 years, a population reduction of 10 % would be the respective cut-off value. Converting this to non-protected species results in `very high´ effect classes for effects larger than 10 % and 15 %, respectively, for the assessment of faunal species in the ERA, depending on the accuracy of the available data basis. If effects on biotopes are assessed,

effects of 10 - 100 % are considered as `medium´ effects and no `high´ effects are classified.

Weed-based food webs of arable fields are well described, e.g. regarding the major taxonomic groups of vertebrates and invertebrates, also for GMHT-relevant crops (see e.g. HAWES et al. 2003, HAUGHTON et al. 2003). Also trophic interactions and functional relationships between arthropods and weeds in agro-ecosystems are described (see e.g. NORRIS & KOGAN 2000, NORRIS & KOGAN 2005, HAWES et al. 2009, BÀRBIERI et al. 2010, CABALLERO-LOPEZ et al. 2010).

The British Farm Scale Evaluations have shown that effects of non-selective herbicides on the weed flora (e.g. weed biomass) can translate into effects on arthropod consumer groups such as detritivores and herbivores (HAWES et al. 2003). It was also evident, that generalists and mobile arthropod taxa such as Carabidae and Araneae were not significantly affected (HAUGHTON et al. 2003). Consequently, a selection of relevant taxa for the use of this indicator will depend on the specific GMHT crop and the relevant agronomic environment. Suggestions for the selection of arthropod as non-target test species for ERA purposes, also considering ecological functions, have been made by HILBECK et al. (2014).

### 10.2.6 LoCs suggested for effects of the GMHT crop on faunal species associated with weed communities

#### *LoC for indirect food-web effects and biodiversity **in-field***

The results of the British FSE have shown that certain invertebrates are sensitive to changes in weed communities due to GMHT crops (HAUGHTON et al. 2003, BROOKS et al. 2012). Weed-dependent and sedentary taxa were more likely to be affected by the effects due to GMHT cultivation. Changes in the weed community of GMHT crops triggered whole-season increases in detritivores of approximately 50 % in GMHT maize (R = 1.56), decreases in predators and parasitoids in GMHT spring oilseed rape in the range of 20 % - 30 % (R= 0.8 and 0.85, respectively), as well as 30 - 40 % decreases in parasitoids (R = 0.61) and herbivores (R = 0.73), respectively, in GMHT sugar beet (HAWES et al. 2003, see review in SQUIRE et al. 2005).

When relating these observed effect sizes to the suggested calibrations suggested by KOWARIK et al. (2008), then the observed reductions in species abundances are evaluated as `very high effect´, as they constitute reductions of more than 10 – 15 %. This would be relevant, if effects on individual species were considered. If evaluating these effects for the biotope in question (i.e. the arable field), then these effects would be considered `medium´ according to the calibration suggested by KOWARIK et al. (2008). The less stringent calibration of effects for the biotope is based on the assumption, that biotopes are only indirectly affected, in contrast to the individual taxon.

Direct effects of herbicides on non-target arthropods are generally considered to be low and therefore harmless for these species. The ERA requirements for assessing effects of plant protection products on non-target arthropods provide acceptability thresholds for lower tier testing and thresholds for higher tier testing which are largely based on expert judgement (CANDOLFI et al. 2000a, 2000b). While effects on non-target arthropods due to the application of the product are taken into consideration in the ERA of plant protection

products, the depletion of food resources of higher trophic groups such as birds and mammals has so far been left unconsidered (UMWELTBUNDESAMT 2015). Suggestions have been made to add the arthropod biomass as an additional endpoint in arthropod risk assessment in order to cover these food web effects (see BOADMAN et al. 2004 in UMWELTBUNDESAMT 2015). The exclusion of phytophagous insects from current ERA testing regimes of plant protection products has also been criticised (UMWELTBUNDESAMT 2015). Hence, although indirect effects need to be addressed in the ERA by the new Regulation (EU) No 283/2013, no specific threshold values for indirect food web effects are available yet for the ERA of plant protection products.

Certainly, any LoC derived for food-web related effects on in-field biodiversity will have to be related to acceptable thresholds defined for in-field non-target plants. As already mentioned, effects on in-field non-target plants have recently been recognized as important for ecosystem services relating to the support of higher trophic levels and in-field food webs, in particular when assessing effects of herbicides (EFSA 2014b). However, any acceptable effect for higher trophic levels also depend on the strength of the trophic link between plants and e.g. arthropod taxa as well as the specific protection goal that need to be addressed for individual taxa or even species (e.g. for birds).

#### *LoC for indirect food-web effects and biodiversity **off-field***

In the ERA of plant protection products there are no fixed acceptability criteria for off-field effects on non-target arthropods when assessed in field tests (CANDOLFI et al. 2000a, 2000b). The suggested trigger values for semi-field trials for lethal or sub-lethal effects for in-field habitats (50 % effect thresholds) are also suggested for off-field habitats (CANDOLFI et al. 2000a, 2000b). Also the recolonization of the habitat by non-target arthropods is a decision criterion, but there are no fixed acceptability values. In off-field habitats `ecologically relevant times´ have to be considered, although also the duration of the effect and the range of affected taxa have to be taken into consideration (CANDOLFI et al. 2000a, 2000b).

Changes in the agricultural management methods such as changes in the herbicide regimes when cultivating GMHT crops, can also affect food-webs in field margins. In field margins butterfly density consistently decreased in GMHT oilseed rape field margins with a magnitude of effects of approximately 24 % (ROY et al. 2003). Effects on other functional arthropod groups were less pronounced or recorded only at certain times of the year (ROY et al. 2003).

Specifically indirect effects on non-target arthropods, e.g. by the destruction of off-field host plants, have been recognized in the ERA of plant protection products (ALIX et al. 2010). Specific protection goals for off-field non-target plants have been suggested by EFSA (EFSA 2014b). These require that non-target plants in the off-field area are not affected by the use of plant protection products. Consequently, adverse effects on the related food-web arthropod community must be considered unacceptable.

### 10.2.7 Aspects to consider for LoCs for effects of the GMHT crop on faunal species associated with weed communities

#### *Differentiate LoCs between in-crop and off-crop habitats*

In the ERA of plant protection products risks to non-target arthropods in off-crop habitats are assessed by extrapolation of in-crop effects (CANDOLFI et al. 2000a). It has been argued that the arthropod community of field margins and off-crop habitats are different in their composition as well as their sensitivities to plant protection products from in-field habitats (UMWELTBUNDESAMT 2015). The underestimation of effects for biodiversity in off-crop habitats in the current ERA schemes of plant protection products has been criticised and suggestions to overcome these shortcomings include the use of assessment factors for extrapolation from in-crop situations or the use of off-crop field studies (ALIX et al. 2010). Such a differentiation of adverse effects should therefore not only consider direct effects of the plant protection product, but also relate to indirect effects of a non-selective herbicide, e.g. via the effect on non-target plants in off-crop habitats.

#### *Consider different LoCs for different functional groups of faunal species*

The British Farm Scale Evaluations have shown distinct functional group responses of invertebrates to GMHT management (HAWES et al. 2009). Detected adverse effects of GMP cultivation may relate to different taxonomic levels such as orders, genera and species (see e.g. HAUGHTON et al. 2003, ROY et al. 2003, and CHAMBERLAIN et al. 2007). However, it has to be considered that for taxa at higher trophic levels (e.g. birds) adverse effects at the population level of the species may be more relevant than for the functional group. In this context the usefulness of the ecosystem service concept is emphasized, which allows the definition of the ecological entity which is to be protected, depending on the relevant protection goal defined (EFSA 2016a). Consequently, a differentiation of the LoC may be necessary according to the ecological entity selected for the risk assessment.

In addition, the importance of the individual species for the affected biotope has to be considered. It has been suggested that effects on a species should be assessed differently depending on whether it is typical for a particular biotope or whether it occurs irregularly or spontaneously (KOWARIK et al. 2008). A different assessment may be relevant, if effects on a particular biotope are evaluated. In this case, the importance of the species for the whole weed community or for higher trophic levels (e.g. for a particular bird functional group) is relevant and for this species a different LoC may be relevant than for other species.

#### *Consider separate LoCs for species of conservation concern*

The reports on significant reductions of larval populations of the Monarch butterfly due to large reductions of host plants growing in agricultural habitats in the US (PLEASANTS & OBERHAUSER 2012 and references therein) show that indirect effects through agricultural practices on individual species may be severe. Over a time period of eleven years a considerable reduction in host plants in agricultural fields was recorded as well as a significant decline of approximately 80 % of the egg production of the US Midwest Monarch butterfly population (PLEASANTS & OBERHAUSER 2012). Also the ecosystem service concept to be applied for GMO ERA purposes considers species of conservation concern or

of special aesthetic value as representative of cultural ecosystem services (EFSA 2016a). The level of endangerment is considered as one aspect that can justify a particular magnitude of acceptable effect (EFSA 2016a). In this context specific legal requirements with regard to the maximum tolerable effect for protected species, either on EU-level or nationally, should be taken into account.

#### *Consider long-term and large-scale data when determining LoCs*

Effect sizes are valued differently, if observed in field trials (short-term, small-scale) or if knowledge is derived from large-scale experiments or even commercial cultivation (see also KOWARIK et al. 2008). In this context the role of post-market environmental monitoring has to be emphasized, because it can provide a data basis to be used for further decisions on the acceptability of effects. The observed reductions in larval food plants and larval populations of the Monarch butterfly in agricultural habitats in the US (HARTZLER 2010, PLEASANTS & OBERHAUSER 2012) show that data from prolonged observation periods under realistic agricultural conditions are more valid than data from short-term and small-scale experiments. If data on effects due to agricultural cultivation and management regimes from large-scale and long-term experiments or commercial cultivation are available, they should be used to support decisions on acceptable effect sizes and the definition of the LoC.

### 10.2.8 Indicators for the assessment of effects of the GMHT crop on agricultural protection goals

Agricultural protection goals may be negatively affected by the use of GMHT crops and their related specific cultivation and management decisions. Potential adverse effects by the use of GMHT crops a) depend on specific agricultural conditions that are regionally different (e.g. regional crop diversity, tillage systems) and b) cannot be assessed *ex-ante* during the ERA with field-scale based assessments. Assessments must therefore include larger-scale and multi-dimensional aspects which may result in several adverse-effect scenarios depending on the baselines used and projections made.

- Indicator 1: use of non-selective herbicides

So far no indicators have been suggested for the ERA in order to assess effects of the GMHT crop on agricultural protection goals. KOWARIK et al. (2008) suggested the use of the non-selective herbicide as an indicator at the trigger level reflecting changes in cultivation and management techniques which, in turn, may affect biological entities and their functions (see 10.2.2).

When considering agricultural protection goals, not the effect of the use of non-selective herbicides on biological entities needs to be assessed, but on the relevant agricultural protection goal(s). Hence, assessment endpoints are required which reflect relevant agricultural protection goals such as the sustainable use of pesticides, reduced inputs of chemical herbicides, and the diversity of production types. When using these indicators, it is important to note that the adverse effect on the agricultural protection goal(s) is not just mediated by the non-selective herbicide itself, but as well by decisions on weed management made and the biological and agricultural consequences thereof. Changes in weed management decisions are difficult to assess during the pre-market ERA of GMOs as they involve a comparison with the weed management decisions in conventional crops

that differ regionally and temporally throughout the EU. Current herbicide regimes in soybean or maize include the application of pre-emergence and/or post-emergence residual or foliar herbicides (EFSA 2012a, EFSA 2012b, DEVOS et al. 2008). The numbers of post-emergence applications of herbicides in e.g. conventional maize range from 0.4 to 2.3 depending on the country (MEISSLE et al. 2010).

Additionally, predictions of management decisions together with the use of the non-selective herbicide in conjunction with the GMHT crop are also likely to vary, depending on regional specificities such as local weed control or the occurrence of resistant weeds. The predicted herbicide regimes for GMHT soybean or maize in the EU include two scenarios: the substituted post-emergence herbicide application (see e.g. substitution scenario by EFSA 2012a, EFSA 2012b) and worst-case scenarios with different non-selective herbicide applications combined with residual herbicides (EFSA 2012a, EFSA 2012b, DEVOS et al. 2008).

For the indicator `use of non-selective herbicides´ the following assessment endpoints are proposed which reflect potential effects on agricultural protection goals:

### *Potential for effects on the diversity of crops in the crop rotation due to the use of non-selective herbicides*

Diversity of crops refers to the use of different crop types within a specific crop rotation. The indicator should assess whether changes in the sequence of crops or sequential application of GMHT crops are predicted (e.g. HT maize after HT maize).

For the calibration of the indicator it is assumed that if no changes in the current crop rotation sequence of the conventional crops are predicted the adverse effect is estimated to be low. Any shortening of the crop rotation sequence, e.g. a reduction of a 4- or 3-year rotation to a 3- or 2-year rotation, is considered a high adverse effect if the GMHT crop is still used in one particular crop of the sequence (see Table 7). Similarly, increases in the frequency of GMHT crops in the crop rotation sequence (i.e. more than one GMHT crops in the sequence) constitute a high adverse effect for crop diversity).

Table 7. Possible calibration of the indicator `use of non-selective herbicide´ for adverse effects on crop rotations and crop diversity due to the cultivation of GMHT crops

| | **Potential for adverse effect on diversity of crops in the crop rotation** | | |
|---|---|---|---|
| | Low | Medium | High |
| Use of non-selective herbicide | No change in crop rotation sequence | - | Reduction of crop rotation sequence or<br>GMHT crop increase in sequence |

### *Potential for increase in pesticide use/increased reliance on chemical pesticides*

One adverse effect on agricultural protection goals is the increase in pesticide use and the increased reliance on chemical pesticides (see Chapter 10.1). Increases in glyphosate use due to the commercialisation of GMHT crops have been documented from the US, reflected by more applications per hectare and crop as well as higher rates of applications (BENBROOK 2016). Also the use of additional herbicidal active ingredients together with the non-selective herbicide has been reported (BENBROOK 2016). The indicator should focus on the number of applications and the application rates for the GMHT crop as well as the use of additional active ingredients used for GMHT crops (e.g. an additional selective herbicide to control problematic resistant weeds). Assessments of effects on quantitative pesticide reduction targets, as required by Directive 2009/128/EC may be necessary if such targets exist on a Member State level.

The assessment outcome for this indicator depends on a range of management decisions to be made with the use of the GMHT crop in comparison to management decisions currently made in the non-GM crop. In this context the use of scenario analyses is considered useful (see also EFSA 2010a).

Calibrations of this indicator with respect to pesticide use are based on the assumption that the more often the non-selective herbicide is used and the higher the application rates are the higher is the adverse effect on the protection goal. If no specific quantitative values for pesticide use or reductions are defined as a limit (e.g. via national Pesticide Reduction Targets), any increase in pesticide use is considered a high adverse effect (Table 8). In the light of the current discussions on sustainable use of pesticides it is recognized that the current use level of pesticides in Europe is not sustainable (UMWELTBUNDESAMT 2016) and any change in pesticide use that do not result in decreased levels of pesticide applications and numbers cannot be considered acceptable. However, a change of the calibration should be envisaged if reduction targets or obligations for minimal pesticide use e.g. via IPM are legally binding.

Table 8. Possible calibration of the indicator `use of non-selective herbicide´ for adverse effects on pesticide use due to the cultivation of GMHT crops

| | **Potential for adverse effects on pesticide use** | | |
|---|---|---|---|
| | Low | Medium | High |
| Use of non-selective herbicide | no increase in application rates or numbers | - | increase in application rates or numbers |

### *Resistance development in weeds*

The avoidance of resistance development in weeds is an overall agricultural protection goal relevant for the ERA of plant protection products and GMOs. The prevention of resistance development in weeds ensures the sustainable use of currently successful weed control strategies. Weed species resistance affects agricultural protection goals indirectly as established weed control strategies cannot be used sustainably or due to the need for additional herbicides. The assessment outcome for this indicator depends on the predict-

ed management decisions applied with the GMHT crop. Therefore experience from other continents and regions as well as the use of scenarios is considered useful for ERA purposes.

Risk scenarios were used for the assessment of glyphosate resistance occurring in GMHT soybean cropping systems (EFSA 2012a). The evaluation of the relative weed resistance risk was based on different crop rotation and weed management option scenarios (EFSA 2012a). It has to be assumed that the resistance risk to glyphosate-based non-selective herbicides evaluated by EFSA (2012a) refers to the development of resistance in one particular weed species that has so far not been resistant to the respective non-selective herbicide in the region of commercialisation.

The calculated resistance risks for the development of glyphosate-resistant weeds by EFSA (2012a) can also be used as a basis for calibration of the indicator (see Table 9). Risk scenarios are based on the frequency of glyphosate herbicide regimes applied on the GMHT crop, the adoption of no or reduced tillage systems as well as crop rotational aspects (see EFSA 2012a for details). The lowest risks were identified for 4-year crop rotations and the single substitution of soybean with RR soybean and a single glyphosate application. The highest risks were identified for GMHT crop rotations (either continuous GMHT soybean or GMHT soybean-GMHT maize rotation) and double glyphosate applications as well as the adoption of no- or reduced tillage systems in GMHT soybean.

Table 9. Possible calibration of the indicator `use of non-selective herbicides´ for resistance development risk in weeds based on the assessment of Roundup Ready soybean according to EFSA (2012a). For details on scenarios see EFSA (2012a).

| | **Risk scenarios for weed resistance** | | |
|---|---|---|---|
| | Very low-Low | Medium | High-very high |
| Use of non-selective herbicide | Best-case scenario (2-3-4 year rotation) **and** substitution scenario (4 year rotations) | Substitution scenario (3 year rotation) **and** worst-case scenario (4 year rotation) | Substitution scenario (2 year rotation) **and** worst-case scenario (2- and 3 year rotation) **and** very worst case scenarios 1 and 2 |

### 10.2.9 **LoCs suggested for effects of the GMHT crop on agricultural protection goals**

Currently, no specific suggestions for acceptability thresholds for potential effects on agricultural protection goals are available.

The avoidance of development of resistance in target organisms is a clear policy goal in the ERA of plant protection products and GMOs. Experience from the ERA with insect-tolerant GMOs shows that even small risks for the development of resistance of the target organism result in the non-acceptability of the effect and, consequently, in the application of risk mitigation measures, such as the need for Insect Resistance Management plans for insect-tolerant maize 1507 (see EFSA 2012b). Although the threshold for acceptability applied for insect-resistant GMOs is not further specified with respect to the

spatial and temporal scope, it can be assumed that the aim is to avoid any further occurrence of weed resistance within the territory of the EU and that this requirement is, in principal, temporally not limited.

For the ERA of plant protection products information on the possible occurrence of the development of resistance in target organisms is a data requirement according to Regulations (EC) No. 283/2013 and No. 284/2013, respectively. Appropriate risk management strategies are required if resistance development is likely. Acceptability of resistance risks is based on the probability of the resistance occurring and the possible consequences thereof (EPPO 2015). A risk is considered acceptable only if no resistance avoidance strategy and no limitations on the conditions of use need to be applied (EPPO 2015). Management measures can be used in the resistance management strategy in order to reduce the risk and come to an acceptable risk conclusion, e.g. specifications of a good plant protection practice, limiting the number of applications, dose rates and restrictions for the application timing (EPPO 2015).

Analogous to the non-acceptability of resistance risks for target organisms in insect-tolerant GMPs, any resistance risk of weeds due to GMHT crop cultivation would result in an exceedance of the acceptable threshold. Such decisions require the GMHT crop to be assessed together with the non-selective herbicide and an alignment of the ERA of GMHT crops and the corresponding non-selective herbicide in this aspect. Risk managers may then decide on risk mitigation measures to be applied in order to avoid the development of weed resistance to herbicides.

#### 10.2.10 Aspects to consider for LoCs for effects of the GMHT crop on agricultural protection goals

*Consider the status of the receiving environment*

LoCs for potential effects on the diversity of crop production systems, pesticide use and resistance development need to take regional differences of the receiving environment into consideration. For example, in regions where crop diversity is already low or where crop rotations are already short, adverse effects will be assessed differently than for regions with specific production patterns in which adverse effects due to changes in crop sequences or in pesticide use patterns may have stronger consequences. In this context it has to be kept in mind that certain production systems (e.g. organic, IPM) have a higher vulnerability to changes in the weed flora due to the fact that their ability to apply additional and alternative herbicides is more restricted than in conventional systems. This must be accounted for in the selection of the LoC for these production systems.

## 10.3 Conclusions

When discussing GMHT crops and related acceptability of adverse effects it has to be kept in mind that the ERA of these crops is `divided´ between two distinct ERA requirements – one of the GMO according to the requirements as laid down by Directive 2001/18/EC or Regulation (EC) 1829/2003 and one of the related plant protection product to be used with the GMHT crop under Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market. At this point it is also relevant to note that the application for commercial cultivation of the GMHT crop and the related assessments occur at EU-level, the authorisation for placing on the market and use of the plant

protection product to be used with the GMHT crop will granted at Member State level (Regulation (EC) No. 1107/2009). Experience with the application of herbicide tolerant GMPs has shown that applicants did not include information on the assessment of potential adverse environmental effects due to the use of the complementary herbicide in the dossier submitted for the authorisation of the GMHT crop (DOLEZEL et al. 2011). However, many of the adverse effects and, consequently, relevant acceptability thresholds, relate to the use of the herbicide applied with the GMHT crop. Therefore the determination of LoCs for GMHT crops will largely depend on existing acceptability thresholds defined for the assessment of adverse effects of plant protection products. This is important, in particular, if protection goals for the ERA in relation to biodiversity and ecosystem services are to be harmonized among different environmental stressors, as intended by EFSA (see EFSA 2014a, EFSA 2016a).

The efforts made by EFSA with respect to the use of the ecosystem service concept for the ERA of both, plant protection products and GMOs, include the operationalisation of general protection goals (EFSA 2016a, DEVOS et al. 2015) and provide a useful basis for the discussion on LoCs. The formulation of specific protection goals (SPGs) in the ecosystem service concept includes the definition of the magnitude of the effect that is considered to be acceptable. However, a major challenge when defining acceptable thresholds for effects on agricultural biodiversity is the necessity to define the effect categories (e.g. negligible, small, medium or large effects) for each SPG. This definition of effect categories should be transparent and relate to the specific protection goal which is relevant for each indicator or SPG in question. The respective legislative acts for the protection and conservation of biodiversity lack benchmarks for biodiversity in arable production systems and the definition of a `good ecological status´ for dynamic and resilient ecosystems like agricultural fields. In addition, knowledge regarding the necessary ecological processes behind a particular ecosystem service is limited (ZHANG et al. 2007). Although it has been postulated that any minimum level of diversity to sustain certain ecosystem functions can be maintained by a certain set of functionally distinct species (see discussion in SWIFT et al. 2004) knowledge on which and how many species and functions are necessary to sustain a certain ecosystem service is incomplete.

In the discussion of defining acceptable thresholds for adverse effects of GMPs due to impacts of the specific cultivation and management and harvesting techniques it is important to focus on the relevant protection goals. Although it has been recognized that weeds are important drivers of ecosystem services, relevant minimum levels of weed biodiversity in different crops in different receiving environments in order to sustain these services still have to be defined. It has also been postulated that the aim should be to maintain specific weed communities that benefit specific beneficial higher trophic levels or the biodiversity generally (MARSHALL et al. 2003). However, questions on the environmental quality to be preserved in agro-ecosystems will also depend on the baselines defined as benchmarks for future changes in environmental parameters (ANDREASEN et al. 1996).

Specifically weed communities play a crucial role in the assessment of adverse effects of GMHT crops and their related cultivation and management methods and useful guidance has been provided by EFSA for the definition of acceptable effects in-crop and off-crop. However, specific protection goals are still lacking for in-field non-target plants. Conse-

quently, LoCs for in-field weed-related biodiversity must be aligned to acceptability thresholds for in-field plants but also need to take into consideration that the knowledge on quantitative links between weeds and higher trophic levels is largely limited (SQUIRE et al. 2005).

For the definition of acceptable effects on weeds in the crop production area it is evident that there is a need for balancing different ecosystem services such as food production and other ecosystem services (MARSHALL et al. 2003). At smaller scales it has been shown that such trade-offs are manageable (e.g. KEMPENAAR et al. 2007). Also for crop production systems the use of weed thresholds may be a tool to define thresholds for the acceptability of effects on different ecosystem services. In contrast to the needed balancing of ecosystem services in the crop production area it is evident that off-crop effects of herbicides used with GMHT crops need to be largely avoided.

When defining LoC the importance of the regional `background´ level of farmland biodiversity in the receiving environment has to be considered. For example, the presence of diverse field margins and natural landscape elements, the regional crop diversity and the presence of alternative food sources for higher trophic levels influence the observed magnitude of effects on biodiversity (see e.g. EFSA 2014b). Due to the high variability of biodiversity in agricultural landscapes and crop management systems across Europe any decision on the acceptability of adverse impacts during the EU-wide ERA should consider worst-case scenarios as the baseline, for which decisions are made. The rejection of adverse impacts in biodiversity-poor receiving environments would therefore avoid adverse impacts of biodiversity in all other receiving environments that are richer or more diverse in their natural resources or resource services.

In contrast to environmental protection goals, agricultural protection goals are not reflected by provisions for the ERA of plant protection products, but are specifically addressed in the ERA of GMOs. These protection goals have so far not been fully addressed in the ERA of GMOs although for insect-resistant GMOs LoCs have been implicitly set for the development of resistance in target organisms. The definition of LoCs for agricultural protection goals will require further specifications e.g. with respect to the particular crop rotations to be preserved and the relevant baseline to be considered on a regional scale. In addition, national targets for pesticide use of EU Member State may be used to derive respective LoCs for the use of GMHT crops.

# 11 Suggestions for LoC for the risk area: interactions of the GMP with non-target organisms

The following chapter contains suggestions for the definition of LoCs for the area of risk `interactions of the GMP with non-target organisms´. The analysis is based on the example of insect resistant (*Bt*) maize and non-target Lepidoptera. In the first subchapter the relevant protection goals for *Bt* maize are discussed. The second subchapter discusses relevant aspects when setting LoCs for *Bt* crops in general.

## 11.1 Protection goals relevant for *Bt* maize

Considering the legislative documents as outlined by EFSA (2010a) the following general protection goals are relevant for *Bt* maize:

- Biodiversity (of NTOs living and occurring in and nearby crop fields)
- Ecosystem services and functions supported and delivered by NTOs in agricultural landscapes (e.g. pollination, pest & disease limitation and regulation)
- Specific agricultural production and management methods (e.g. sustainable pest management practices)

EFSA (2010b) gives examples for relevant protection goals in agro-ecosystems which might be adversely affected by *Bt* maize cultivation (Table 10).

Table 10. Examples for protection goals potentially affected by *Cry* protein-expressing GM maize (EFSA 2010b)

| In agro-ecosystems | In adjacent (non-managed) habitats |
|---|---|
| Natural regulatory mechanisms controlling pest populations | Protected and endangered species in protected areas |
| Pollination | Pollination |
| Soil biodiversity and ecosystem services | Water bodies |
| Healthy plant stands | Breeding sources (e.g. for birds) |
| Biodiversity in ecological infrastructure (e.g. field margins) | |
| Sustainability of pest management practices | |

### 11.1.1 Biodiversity and Ecosystem Services

As representatives of biodiversity and ecosystem services non-target Lepidoptera are addressed in the ERA of insect resistant *Bt* maize (EFSA 2011d, 2011e, 2012b, 2012c, 2012d, 2012e, 2012f and 2015b). In several Scientific Opinions effects on non-target Lepidoptera by *Bt* maize at local scale (i.e. on larvae within fields and their margins) and at larger scales (over a landscape and growing season) were modelled. Conclusions and risk management recommendations referred to two different protection goals, to non-target Lepidoptera occurring within fields and their margins as well as to non-target Lepi-

doptera of conservation concern occurring in protected habitats (EFSA 2012b, 2012d, 2015b).

Before EFSA published its ERA guidance document in 2010 (EFSA 2010a) species of conservation concern were considered in the ERA of *Bt* maize only by assessing adverse effects on a surrogate species, the Monarch butterfly, which has no relevance for European agro-ecosystems (DOLEZEL et al. 2011). The ERA guidance document (EFSA 2010a) introduced the necessity to consider potentially affected protection goals in the problem formulation of the ERA of GMOs. These protection goals refer to e.g. species and habitats listed in Annexes II and IV of the FFH Directive (Council Directive 92/43/EEC). Not only protected species but also the necessity to consider endangered species in the ERA of GMOs has been recognized (EFSA 2014a) as well as the necessity to implement risk management measures for protected non-target Lepidoptera occurring in protected habitats in the agricultural landscape (EFSA 2015b). Specific aspects of how to consider endangered species when conducting ERAs have been outlined in detail by EFSA (EFSA 2016c). Species were considered to be endangered if they are listed on Red Lists (globally, nationally or regionally) or they are rare (EFSA 2016c).

Suggestions have been made for a better consideration of nature conservation aspects when selecting non-target focal species for the ERA of GMOs, based on the legal protection status, the red list status and the national responsibility for the conservation of species (HILBECK et al. 2014). For field trials EFSA recognises that in addition to data on focal species specific data on ecosystem services might be needed (EFSA 2010b).

For the operationalisation of biodiversity protection goals for the ERA of plant protection products EFSA has developed specific guidance using the ecosystem service concept as a conceptual framework (EFSA 2010d). Terrestrial non-target arthropods were identified as key drivers for a range of ecosystem services (e.g. pollination, pest and disease regulation, nutrient cycling, genetic resources and aesthetic values) for which specific protection goals (SPGs) were defined. As EFSA favours a level of protection for agro-ecosystems independent from the stressor and recognizes the need for a harmonized approach when considering biodiversity in the ERA of regulated products (EFSA 2014a), these considerations for the ERA of plant protection products are also relevant for the ERA of GMPs. In addition, in a recent Scientific Opinion on the development of specific protection goal options for ERA schemes, suggestions were made for the ecosystem service concept for ERA purposes also for GMOs (EFSA 2016a). These suggestions specifically addressed non-target Lepidoptera using the case-study of *Bt* maize 1507 (EFSA 2016a). As ecosystem service of major relevance for Lepidoptera the cultural service was identified (for butterflies of conservation concern) but also regulating services, however of minor relevance (EFSA 2016a).

### 11.1.2 **Agricultural protection goals**

Relevant protection goals that need to be considered in the ERA of GMPs also include agricultural protection goals (EFSA 2010a). The relevant legislative documents referred to by EFSA (2010a) comprise e.g. the Biodiversity Action Plan for Agriculture (EC 2001), Directive 2009/128/EC on the sustainable use of pesticides as well as Regulation (EC) No 1107/2009 concerning the placing on the market of PPPs.

The Biodiversity Action Plan for Agriculture (EC 2001) defines priorities for the integration of biodiversity and sustainability considerations into agricultural production practices. Some of these priorities are also relevant for insect resistant GMPs, and in particular *Bt* maize, such as less intensive use of inputs (including plant protection products) and the avoidance of resistance development.

The use of *Bt* maize may lead to changes in the cultivation, management and harvesting techniques of crop plants as a result of resistance development in the target pest (EFSA 2010a) or the occurrence of secondary pests (EFSA 2010a). Such changes of pest control practices may indirectly affect the environment. Such indirect adverse effects have been identified in the ERA of *Bt* maize and led to the adoption of Insect Resistance Management plans as a risk mitigation measure in order to delay the resistance evolution in lepidopteran target organisms (EFSA 2011d, 2011e). Avoidance of resistance development is therefore not only an implicit protection goal in the ERA of GMOs, but also an important goal of European environmental policies. The European Directive 2009/128/EC on the sustainable use of pesticides aims at limiting the levels of pesticide use in order not to increase the risk for resistance development in populations of harmful organisms (Annex III). The promotion of low pesticide input pest management, in particular integrated pest management, is another important aim of this Directive. The potential role of GMOs, in particular *Bt* crops, in integrated pest management has not yet been clarified and is currently being scientifically explored (ARPAIA et al. 2014). Experience gained in the USA with many years of cultivation of *Bt* crops shows that a majority of farmers plant *Bt* maize even if corn rootworm or European corn borer damage is predicted to be low, thereby contradicting general integrated pest management principles (Gray 2010).

An additional relevant protection goal in the context of *Bt* crops is limiting the use of pesticides (see Directive 2009/128/EC). Although it is reported that *Bt* maize reduces pesticide levels compared to conventional maize cultivation (BENBROOK 2012, BENBROOK 2016), assessments during the ERA should evaluate pesticide use of *Bt* crops in accordance with relevant protection goals (e.g. pesticide reduction targets) at EU and Member State level. According to Directive 2009/128/EC, EU Member States need to adopt National Action Plans setting national targets to reduce the risks and impacts of pesticide use on human health and the environment. Aggregated information on objectives and measures set by EU Member States is not available yet, however, the National Action Plans have been criticized for not being ambitious in reducing reliance on pesticides in agriculture (PAN 2014). In this context EFSA specifically refers to “strategic goals for the adoption of certain pest management regimes (e.g. integrated pest management and biological control)” which could serve as the appropriate basis for comparisons of risks (EFSA 2010b).

## 11.2 Limits of Concern for *Bt* maize

### 11.2.1 Introduction

The focus of this report is on LoCs for *Bt* maize and non-target organisms using the example of non-target Lepidoptera. Recommendations for LoCs are based on the suggestions for indicators by KOWARIK et al. (2008) who proposed two indicators for the assessment of adverse effects of toxic substances such as the *Bt* toxin on non-target organisms

- Indicator 1: toxic effects on test and indicator species
- Indicator 2: reduction in the population size of faunal species

The first indicator is situated at the process level of the causal chain of events leading to adverse effects on protection goals (see Methodology). This indicator is usually tested in the laboratory or in semi-field tests. The second indicator assesses effects on NTOs in the field based on the assessment of reduction in the species abundance. According to KOWARIK et al. (2008) the second indicator is of limited value if data from field trials are not available in sufficient detail and accuracy.

The validity of the suggested indicators, i.e. the reliability to indicate a potential hazard for the relevant protection goal, is determined by the following factors:

- The relative effect on the individual versus effects on the population.
- The selection of suitable and sensitive test organisms representing the relevant trophic levels and ecological functions.
- The endpoints (parameters) tested (e.g. mortality, reproduction, growth rate).

### 11.2.2 **Indicator for the assessment of toxic effects on test and indicator species**

This indicator aims at determining toxic effects of the newly expressed *Bt* toxins of *Bt* maize on indicator species. Lethal and sub-lethal effects on individual species can be assessed by the use of laboratory studies. Dose-effect relationships can be calculated from dose-response curves, e.g. by determining the lethal concentration for 50 % of the test species ($LC_{50}$), or in case of sub-lethal effect, the effect concentration causing an effect in 50 % of the test individuals ($EC_{50}$). For Lepidoptera usually larval stages are used for toxicity studies in the laboratory (Tier 1a, 1b studies), feeding either the isolated protein, the relevant GM plant material (LANG & OTTO 2010) or pollen of the respective GM plant applied to host plant leaves (e.g. SCHUPPENER et al. 2012).

KOWARIK et al. (2008) suggest using the PEC/PNEC ratio to determine the potential adverse effect on the selected test species. The ratio between the `predicted environmental concentration´ (the PEC) of a toxic substance and the `predicted no effect concentration´ (the PNEC) on the species tested, derived from the dose-effect studies conducted in the laboratory, is generally used in the risk assessment of other regulated substances. Quotients combining exposure and effect values are calculated to assess the risk of hazardous substances. In the risk assessment of chemicals PEC/PNEC ratios are used (ECB 2003), while for plant protection products hazard quotients (HQ) or toxicity/exposure ratios (TER) are used (Candolfi et al. 2000b, EC 2002, SANCO 2002a).

KOWARIK et al. (2008) suggest calibrating the selected indicator. Calibration in this context is understood as assignment of numerical effect categories to normative classifications of potential adverse effects (e.g. small-medium-high-very high; see Figure 9).

| Indicator | adverse effect categories | | | | |
|---|---|---|---|---|---|
| | none | small | medium | high | very high |
| **toxic effects on test species [PEC/PNEC ratio]** | ≥ 0 to ≤ 1 | > 1 to ≤ 10 | > 10 to ≤ 100 | > 100 to ≤ 1000 | > 1000 |

Figure 9. Example for the calibration of the indicator `toxic effects on test species´ for effects on non-target organisms (KOWARIK et al. 2008).

### 11.2.3 LoCs suggested for toxic effects on test and indicator species

Acceptability thresholds for a toxicity/exposure ratio are common as trigger values for lower tier testing in the ERA of plant protection products, biocides and chemical substances. These trigger values generally indicate an acceptable risk if the trigger value is not exceeded, as the predicted environmental exposure of the species to the substance is considered to be lower than the concentration at which particular effects (e.g. mortality) on the species have been observed. Further refinements (e.g. of the exposure values) of the assessment or risk mitigation measures are required if the trigger value is exceeded. Hence, these trigger values constitute risk-based acceptability thresholds, as they indicate an acceptable risk and not the absence of adverse effects.

For the ERA of GMOs toxicity/exposure ratios are not commonly applied. So far two studies considered the effect/exposure ratio approach for the ERA of GMOs (RAYBOULD et al. 2011, RAYBOULD & VLACHOS 2010). The estimated environmental concentration and the no-observed-adverse-effect concentration (NOAEC) for the non-target species were used to calculate a hazard quotient (HQ). If the HQ was below 1, the risk was considered acceptable (RAYBOULD & VLACHOS 2010). The authors considered the hazard quotient approach as a conservative estimate of risk due to the use of worst-case estimates (RAYBOULD & VLACHOS 2010, RAYBOULD et al. 2011). However, no assessment factors were applied in this approach to account for uncertainties which is generally done in the ERA of plant protection products, biocides or chemical substances.

Suggestions for acceptability thresholds for laboratory toxicity tests have been made by EFSA for the ERA of GMOs (EFSA 2010a). EFSA refers to “a multiplicative effects size of 20 % often taken as a trigger value for further higher tier studies” (EFSA 2010a, 2010b). The scientific rationale behind the values suggested by EFSA remains largely unclear and no further indication is made in which context these trigger values are to be used. It is questionable whether effects sizes of 20 % or less are reliable predictors of harmless effects on a range of non-target organisms in the field when testing the *Bt* toxin in the laboratory.

In contrast to the risk-based thresholds, the trigger value suggested by EFSA is an effect-based acceptability threshold as it refers to the adverse effect of a toxic substance rather than to the risk. There are inconsistencies in the ERA guidelines of EFSA whether the

LoC should be defined at the risk level or at the effect level. According to the proposed LoC concept the observed differences (in effects) between the GMP and its conventional counterpart are compared to the "minimum relevant ecological effect that is deemed biologically significant […]" (EFSA 2010a). This LoC at the effect level needs to be aligned to the effect size that is desired to be detected by a specific statistical test design (PERRY et al. 2009). Therefore the LoC could be considered to be set at the effect level. In contrast, EFSA also outlines that in the risk characterisation the hazard and the exposure assessment are combined and evaluated "whether the risk can be reduced to levels falling within the LoC" (EFSA 2010a). This indicates that the LoC should be based at the risk level.

### 11.2.4 Aspects to consider for LoCs for toxic effects on test and indicator species

*When using risk-based indicators several shortcomings have to be addressed before LoCs can be defined.*

The use of exposure/toxicity ratios is a useful tool for risk characterisation in the ERA of GMOs as it enables a first estimation of the risk of the *Bt* toxin in question, provided that the following methodological shortcomings are addressed:

- Standardized test protocols are needed in order to reliably predict the risk for a particular test species. Currently, no standardized test protocols for a range of test species are available, although efforts have been made to define quality criteria for laboratory toxicity tests for non-target arthropods (BOOIJ & QUI 2015, DE SCHRIJVER et al. 2016, ROMEIS et al. 2011). Laboratory toxicity tests should also account for other than acute toxic effects of the *Bt* toxin on the lepidopteran larvae. In particular for lepidopteran species other effects than those on survival may be of importance, such as effects on fecundity (see references in LANG et al. 2011a), generational effects caused by the transmission of the *Bt* toxins to the F1 generation (PAULA et al. 2014) or population-level effects (CHARLESTON & DICKE 2009). EFSA recommends such measurement endpoints for laboratory tests which take into account sub-lethal effects (EFSA 2010a).
- Laboratory tests for ERA purposes for plant protection products use two standard sensitive indicator species based on sensitivity analyses and associated laboratory test methods (CANDOLFI et al. 2000a). Trigger values used for the two indicator species have been validated by (semi-) field data and a wide range of plant protection products (CAMPBELL et al. 2000, CANDOLFI et al. 2000b). However, for GMOs no such validation has been made for any of the relevant species tested in laboratory.
- For laboratory toxicity studies with GMPs, ERA requirements demand the use of *in-planta* material in addition to purified toxins (EFSA 2010a). It is particularly difficult to use the toxicity-effect ratios based on plant material instead of standardized purified proteins for artificial diets, as dose-response relationships for European non-target Lepidoptera and maize pollen are not known (LANG & OTTO 2010) and might be non-linear, thereby underestimating effects of low concentrations (LANG et al. 2011a).
- In order to account for uncertainties (e.g. using test species versus species occurring in the field, extrapolating results from the lab to the field) assessment factors are generally used when calculating toxicity-exposure ratios. Although these are

somewhat arbitrary, they have been defined for the ERA of plant protection products (EC 2002, SANCO 2002a, 2002b) as well as for chemicals and biocides (ECB 2003). So far assessment factors have not been defined or suggested for the ERA of GMOs.

- Another shortcoming relates to the suggested calibration of the PEC/PNEC for the use in the ERA of GMOs. In ERA frameworks for chemicals or biocides PEC/PNEC ratios of >1 indicate concern and require to refine the assessment (ECB 2003). Also for plant protection products a single trigger value is used, e.g. for the hazard quotient (CANDOLFI et al. 2000a). If determined hazard quotient values exceed or are equal to the trigger value, further testing, refinement of exposure estimations or specific risk mitigation measures are needed (CANDOLFI et al. 2000a). Hence, calibrations suggested for the PEC/PNEC by KOWARIK et al. (2008; see Chapter 11.2.3) are not considered useful for practice as only a single trigger value (one specific PEC/PNEC ratio) should be used to indicate a (unacceptable) risk for the specific organism tested.

### *LoCs for lower tier testing should not decide on the final risk*

The role of the LoC in the tiered testing approach of the ERA of GMOs is still to be clarified. According to the current interpretation of EFSA (2010a), the LoC can be understood as a decision criterion indicating the necessity of further tests at higher tiers in case the LoC is exceeded. Although not explicitly stated by EFSA (2010a), the non-exceedance of a LoC could therefore be interpreted as a stop criterion for the ERA, in the sense that no further testing at higher tiers is considered necessary in order to conclude on the environmental risk of the GMO (DOLEZEL et al. 2017). This approach would comply with the use of trigger values for toxicity/exposure ratios in the ERA of plant protection products, biocides and chemicals, but would need significant improvements when used for toxicity testing of *Bt* crops (see also above).

LoCs for different studies with different containments of a GMO may not necessarily be related to each other as the studies themselves serve different purposes (see also EFSA 2010a, 2010b). For example, lower tier tests in the laboratory may serve to identify hazards (e.g. the sensitivity to a *Bt* toxin), clarify exposure routes or assess the extent of severity of an effect at single-species level under worst case conditions of exposure and best-case environmental conditions; however, they often neglect ecological realism (DOLEZEL et al. 2017). There is insufficient knowledge in how far effects seen in ecotoxicological laboratory studies can predict the likelihood of adverse effects in field experiments (CAIRNS 1983, CAIRNS 1986, KIMBALL & LEVIN 1985).

Consequently, it is questionable what role LoCs should have, when applied to lower tier laboratory studies. In its recommendations to operationalise protection goals for the ERA, EFSA acknowledges that “standard methods and models used in tier 1 levels do not measure specific protection goals directly” (EFSA 2010d). EFSA recommends using a reference tier for each key driver (e.g. a taxonomic group) to allow linking the ERA with the specific protection goal and consequently the maximum tolerable effects. In this context the reference tier is defined as a “sophisticated experimental system or model that is practical for higher tier use”. Consequently, maximum tolerable effects defined for a spe-

cific protection goal should not be directly matched with results from testing at lower tiers such as laboratory toxicity testing.

### *Define LoCs for the relevant measurement endpoints*

The indicator `toxic effect on indicator species', suggested by KOWARIK et al. (2008) was not specified with respect to which endpoint should be used. Various measurement endpoints could be used in order to measure toxic effects of the *Bt* toxin on non-target Lepidoptera (e.g. mortality, reproduction, and development time or intrinsic rate of population increase).

The use of different measurement endpoints might affect the calibration of the indicator and consequently results in a different acceptability of effects. The same size of an effect could be valued differently depending on the parameters used. For instance a 20 % effect on growth might be tolerated while a 20 % effect on reproduction might be considered inacceptable. The ecological significance of a 20 % effect on reproduction will depend on the reproductive strategy and life table parameters of the particular species (e.g. fecundity, life cycle, dispersal ability). Thus any LoC can only be set specifically for a specific measurement endpoint.

### *Consider a classification system based on the effects of Bt toxins*

Effect data derived from laboratory toxicity studies with non-target lepidopteran species and *Bt* maize could be used for the classification of effects to non-target Lepidoptera and the establishment of distinct categories for which acceptability criteria could be defined. LoCs defined for effect values derived from laboratory tests can have an indicative value as their role is to put the risk posed by a hazard into a broader context (e.g. the toxicity of a specific *Cry* toxin in comparison with other *Cry* toxins).

By Regulation (EC) No 1272/2008 chemical substances are classified into certain hazard categories (e.g. explosives, acute toxicity, carcinogenicity and hazardous to the aquatic environment) based on the nature of their effects. The classification system follows distinct criteria which are based on effect values only (e.g. $LC_{50}$ or $EC_{50}$) and also considers the amount of available data. In general, the lowest of the available toxicity values is used to define the appropriate hazard category.

When applying a similar classification system for GMOs, using standardized test methodologies for laboratory tests is a necessary prerequisite in order to achieve comparable results. In addition to acute toxicity data the following aspects should be taken into account:

- The specificity of the *Cry* toxin for the species tested and alternative mode of actions of toxins to account for effects outside the presumed specificity of the toxin (BØHN et al. 2008, BØHN et al. 2010, VAN FRANKENHUYZEN 2009).
- Delayed or chronic effects with implications for the development of the population (PAULA et al. 2014, ZHANG et al. 2006).
- Adverse effects which are aggravated by additional stressors like predators (BØHN et al. 2008, BØHN et al. 2010).
- Combinatorial effects with other *Cry* toxins (COGEM 2014, HILBECK & OTTO 2015).

### 11.2.5 Indicator for the assessment of the reduction in the population size of faunal species

#### *Indicator suggested by KOWARIK et al. (2008)*

This indicator proposed by KOWARIK et al. (2008) is established at the effect level of the causal chain of events and can be assessed in field trials. Effects on non-target Lepidoptera are dependent on exposure pathways, the concentration of *Bt* toxins ingested and the sensitivity of the individual species. In addition, other environmental stressors (weather conditions, food shortages, parasitism and diseases) as well as the potential for recovery or immigration will modulate the ultimate effect of a *Bt* toxin on the population. Therefore, any indicator that assesses the effect on the population under realistic field conditions will more directly reflect the risk for the relevant protection goal (see also KOWARIK et al. 2008). In this context, the indicator `reduction in population size of faunal species' can be measured by the use of different measurement endpoints, e.g. the reduction in abundance, reduction in site occupancy or via a reduced reproduction rate.

Detecting changes in abundance of non-target Lepidoptera under field conditions is challenging due to the high mobility of the adult life stages and the low abundance of certain species which requires a high number of replications to detect differences (LANG 2004, 2016). Despite the methodological difficulties, non-target Lepidoptera are considered important non-target organisms for *Bt* maize and robust risk assessment methodologies are needed in order to assess adverse effects also in field trials (EFSA 2010a, 2010b). Lepidoptera are also considered suitable bio-indicators for monitoring effects resulting from the cultivation of *Bt* crops and standardized monitoring methodologies have been elaborated (VDI 2010). These include monitoring of adult butterflies and moths but also lepidopteran larvae (LANG et al. 2013, 2016).

Assessing larvae rather than adults on weeds within the crop or in the field margins is a way to avoid variability in the occurrence because they are less mobile than adult butterflies (BOOIJ & QUI 2014). Thus, for field trials it is advantageous to focus on more stationary species and on larval stages. EFSA has based their risk assessments of *Bt* maize on larval mortality estimates predicted by a modelling approach (EFSA 2011d, 2011e, 2012b, 2012d, and 2015b).

| Indicator | adverse effects categories | | | |
|---|---|---|---|---|
| | small | medium | high | very high |
| **reduction in the population size** | > 0 to < 10 % | ≥ 10 to < 30 % | ≥ 30 to < 50 % | ≥ 50 to 100 % |

Figure 10. Example for the calibration of the indicator `reduction in the population size´ for effects on non-target organisms (KOWARIK et al. 2008).

#### *Additional (exposure-based) indicator suggested*

The measurement endpoints for which a LoC is to be defined are most likely effect values (EFSA 2010a, Perry et al. 2009, but see also discussion above). Adverse effects of *Bt* maize on Lepidoptera are mediated via ingestion of *Bt* maize pollen by larval stages. Assessing exposure of lepidopteran larvae e.g. in field margins of *Bt* maize is therefore a suitable indicator for ERA purposes, as it directly links the potential for adverse effects with the relevant protection goal and is the relevant entity on which the potential stressor operates (EFSA 2016a). Although developed for monitoring purposes standardised methodologies for the assessment of lepidopteran larvae have been developed (VDI 2010) and tested for practicality (Lang et al. 2011b).

Using exposure of butterfly larval stages to *Bt* maize pollen also allows to define thresholds for acceptability not on the effect level, but for the exposure to *Bt* maize pollen. Such acceptability thresholds for the exposure to hazardous substances are used in the regulation of ambient air pollutants, either based on emission values or atmospheric input values (Directive 2001/81/EC, Directive 2008/50/EC). Critical loads of air pollutants are set as ecological limits for the input of atmospheric pollutants into a particular ecosystem. No adverse effects on the ecosystem are expected as long as the critical load is not exceeded (see Annex I of Gothenburg-Protocol and Directive 2001/81/EC). However critical levels of exposure are used and defined (such as the concentration of a pollutant in an environmental compartment) above which adverse effects may occur on certain environmental receptors (Directive 2008/50/EC). Importantly, critical load and critical level values for ambient air quality are recommended on well-established scientific knowledge such as physiological and ecological effects of nitrogen-containing pollutants on vegetation (WHO 2000). They are based on the most sensitive type of vegetation or ecosystem (WHO 2000). Using dose-effect relationships for *Bt* maize pollen, considering worst-case pollen deposition scenarios and considering differing sensitivities of non-target lepidopteran larvae would allow to define LoCs at the exposure level for a specific *Bt* maize event.

### 11.2.6 General suggestions for LoCs for non-target species

EFSA suggests an effect size of 30% for semi-field testing and 50 % for field studies as possible LoCs for non-target species (EFSA 2010a, 2010b). The suggested values refer to a study conducted with GM herbicide tolerant crops in the context of the British Farm Scale Evaluations (HEARD et al. 2003a) using a multiplicative difference of R = 1.5 for the study design (PERRY et al. 2003).

Before LoCs can be defined, it is helpful to have a classification scheme for adverse effects. KOWARIK et al. (2008) and EFSA (2015a, Table 11) have proposed classification schemes for effects on populations of non-target faunal species and for non-target arthropods, respectively. KOWARIK et al. (2008) based their scheme on the assessment of the favourable conservation status for protected species (FFH Directive). According to the FFH Directive, a reduction in the population size of a protected species of 6 % within a period of 6 years indicates an unfavourable or bad conservation status of a FFH species. KOWARIK et al. (2008) adapted the classification to a reduction below 10 % within 10 years, thereby considering the maximum authorization period of ten years of a GMP. The authors make a distinction between the spatially and temporally restricted release of the GMP for the purpose of field testing (deliberate release) and cultivation purposes (see Table 11).

In the ERA of plant protection products trigger values for lethal or sub-lethal effects of 50 % is used for semi-field tests with non-target arthropods, due to the assumption that recovery of the species population is not impeded at this effect level but also due to statistical reasons (CANDOLFI et al. 2000a, 2000b). For field tests no fixed threshold for the acceptability of effects are set; expert judgment on a case-by-case is required to assess whether the effects observed are considered acceptable (CANDOLFI et al. 2000a, 2000b).

Recently, EFSA suggested also a classification scheme for adverse effects of plant protection products on (meta-)populations, functional groups and biodiversity of non-target arthropods (EFSA 2015a; Table 11). The effect classes were based on general effect classes in ecotoxicology with no further reference. A definition of negligible effects is provided. Negligible effects should correspond to non-detectable effects and should not tolerate year-on-year declines in abundance or range of occupancy of the population (EFSA 2015a). These suggestions are intended to be used for the local scale. EFSA defines the local scale as the treated field and the immediate surroundings. The ERA for the local scale has to be complemented by a modelling based approach in order to account for effects at landscape level. No strict definition of effect classes for the landscape context has been provided yet, although it is recognized that tolerable effects at landscape scale will have to be lower than effects tolerated at local scale (EFSA 2015a).

Table 11. Classification of adverse effects on non-target organisms according to (EFSA 2015a) and calibration of the indicator `reduction in population size of faunal species' by KOWARIK et al. (2008). n.i. = not indicated

| | | | **Effect size (in %)** | | | | |
|---|---|---|---|---|---|---|---|
| Source | Effects | Scale | Negligible | Small | Medium | Large | Very large |
| EFSA 2015a | reduction of (meta) population or functional group/ biodiversity | Local scale | <10 | 10-35 | 35-65 | > 65 | n.i. |
| Kowarik et al. 2008 | reduction in population size | deliberate release | n.i. | 0-10 | 10-30 | 30-50 | 50-100 |
| Kowarik et al. 2008 | reduction in population size | cultivation | n.i. | 0-5 | 5 -10 | 10-15 | 15-100 |

### 11.2.7 **Specific suggestions for LoCs for non-target Lepidoptera**

Specific suggestions for acceptability thresholds for non-target Lepidoptera for the ERA have been provided by EFSA in the context the risk assessments for three *Bt* maize lines (MON810, Bt11, 1507) since 2011 (EFSA 2011d, 2011e, 2012b, 2012d, and 2015b).

These ERAs used the mathematical model developed by PERRY et al. (2010, 2011, and 2012) to estimate mortality for non-target lepidopteran larvae through the exposure and ingestion of *Bt* maize pollen deposited in their host plants in a typical European maize field. The model included `local´ and `global´ mortality estimates for non-target lepidopteran larvae. Local mortality estimates referred to the crop area and its immediate margins (according to the definition of ROY et al. 2003) and to the short period of maize pollen shed. Global mortality estimates referred to a larger scale (i.e. the entire landscape or region) and to the whole growing period of maize. For non-target lepidopteran larvae occurring within the maize fields and their margins global mortality rates were used, because this population was considered part of a meta-population subject to recolonization and recovery. In contrast, for Lepidopteran species of conservation concern local mortality rates were considered more appropriate, because protected habitats are usually relatively small and isolated.

Maximum tolerable mortalities of 0.5 % (i.e. a mortality of 1 in 200 individual larvae) for non-target lepidopteran species of conservation concern and 1 % (i.e. a mortality of 1 in every 100 individual larvae) for non-target Lepidoptera (species not of conservation concern) were chosen as operational thresholds (Table 12). EFSA (2012d) considered the 0.5 % mortality to be a `negligible effect'. EFSA also stressed that the thresholds proposed are arbitrary and "… should be subject to amendment according to the protection goals…" (EFSA 2015b).

Table 12. Protection levels suggested by EFSA for adverse effects on non-target Lepidoptera by *Bt* maize.

| **Protection threshold levels** | **Protection object** | **Source (EFSA)** |
|---|---|---|
| < 1 % estimated global larval mortality | Non-target Lepidoptera occurring within maize fields and their margins | 2011d, 2011e, 2012b, 2012d |
| < 0.5 % estimated local larval mortality | Non-target Lepidoptera of conservation concern occurring within protected habitats | 2011d, 2011e, 2012b, 2012d |
| < 1 % and 0.5 % estimated larval mortality[1] | Non-target Lepidoptera of conservation concern occurring within protected habitats | 2015b |

[1]two modelling variants; no distinction between global and local mortality

In a recent Scientific Opinion the EFSA Scientific Committee presented the use of the ecosystem service concept for risk assessment purposes for different environmental stressors, such as PPPs and GMOs (EFSA 2016a). In this opinion, the ecosystem service approach was proposed for the assessment of effects of *Bt* maize on non-target Lepidoptera, referring to previous assessments by EFSA on potential adverse effects resulting from the exposure of non-target Lepidoptera to *Bt* maize 1507 pollen. Examples for the concept and a specific protection goal for non-target Lepidoptera including the definition of maximum tolerable effects were outlined (Table 13).

Regarding the suggestions made by EFSA (2016a) several aspects need to be clarified. The attribute to be protected for Lepidoptera refers to the (species) diversity and abundance, the magnitude of tolerable effect is reported to be 1 %, referring to mortality as well as to abundance reduction in the same table (see Table A.5 in EFSA 2016a). Maximum tolerable effects for effects on species diversity are not mentioned. Adult non-target butterflies were defined as the specific entity to be protected, but larval mortality estimates were used to assess effects on the population. Transferring results from larval mortality to the abundance of adult butterflies is not trivial and requires well-grounded knowledge of population dynamics of the specific species as well as population modelling studies. Regarding the temporal scale to which this protection level should refer, a certain effect level on a single generation may me more profound for the butterfly population (e.g. for a multi-voltine species) than the same effect level over the whole growing season. The EFSA opinion considers an effect level of 1 % as `small´ (as compared to the 0.5 % effect level which was considered to be `negligible´, see above).

Table 13. Specific protection goal (SPG) options for non-target Lepidoptera (derived from EFSA 2016a)

| **Dimensions specifying the specific protection goal** | **Suggestions for SPG options for non-target Lepidoptera** |
|---|---|
| Ecological entity to protect | (meta)populations of particular species of Lepidoptera[1] |
| Attribute to protect | within and between species diversity, abundance |
| Maximum tolerable impact/ magnitude of effect | Small effect: 1 % global mortality |
| Spatial scale of protection | Landscape (areas adjacent to fields incl. protected areas) or Region (areas over which agricultural systems may be similar) |
| Temporal scale of protection | Seasons – generations – rotations[2] |

[1] Overall effect on (meta-)populations through estimated percentage larval mortality, because the potential stressor operates on larvae rather than adults; [2] considered the most relevant scale

### 11.2.8 **Suggestions for LoC for exposure-based indicators**

Defining acceptability thresholds for the exposure of non-target lepidopteran larvae to *Bt* maize pollen in a particular environmental compartment (e.g. field margins) requires knowledge of dose-response relationships for *Bt* toxins in pollen consumed by these organisms. Any LoC defined should therefore be related to the amount of pollen present on host plants in field margins at anthesis of the respective *Bt* maize event. Data on pollen deposition of *Bt* maize under realistic field conditions and on larval food plants are available (HOFMANN et al. 2013, 2014, 2016, ZANGERL et al. 2001, LANG et al. 2004, SCHUPPENER et al. 2012). Also dose-response relationships for lepidopteran larvae with different sensitivities to the respective *Bt* toxin from laboratory studies have been reported (e.g. FELKE et al. 2002, FELKE & LANGENBRUCH 2003, HELLMICH et al. 2001, but see also references in EFSA 2011d for maize 1507 and HOLST et al. 2013 for maize MON810). It is

important to notice that these dose-response relationships are based on mortalities ($LC_{50}$ values) observed under laboratory conditions. Assessment factors will therefore be needed in order to cover uncertainties, e.g. due to the extrapolation of results from the lab to the field or between species, or if sub-lethal effects are also to be covered by the LoC derived from the laboratory results.

The second type of exposure-based acceptability threshold is the amount of the stressor introduced into the environment, i.e. the cultivation of the GMP. EFSA presented an example of an exposure-based threshold in its Scientific Opinion on *Bt* maize 1507 (EFSA 2011d). In case the cultivation of maize 1507 remains below a value of 5 % of the utilized agricultural area, the global morality even for extremely sensitive lepidopteran species was estimated to remain below 1 % and, consequently, no risk management measures would be required. Risk management strategies were only considered necessary by higher estimates of mortality (EFSA 2011d).

### 11.2.9 Aspects to consider for LoCs for the reduction in the population size of faunal species

In the following some aspects are discussed which are considered crucial for the operationalisation of the LoC concept in the context of the ERA of *Bt* maize with specific regard to the indicator 'reduction in the population size of faunal species'.

#### *Differentiate LoCs between taxa*

The formulation of general LoCs applicable for all groups of non-target organisms will not suffice to address the range and diversity of non-target organisms and the diversity of ecosystem services in agro-ecosystems. Once certain taxa have been selected as relevant for the ERA (see HILBECK et al. 2014) they will require different thresholds of acceptability for adverse effects thereby accounting for differences in the biology of the species (e.g. reproduction, longevity, etc.) or specific environmental aspects of the species (e.g. population status in the respective receiving environment). While e.g. for a particular lepidopteran species with small population sizes a 10 % reduction in abundance may not be considered a small and tolerable effect, this may be the case for more abundant lepidopteran species. Rare, endangered or protected species generally occur in low abundances, and also in agro-ecosystems (AVIRON et al. 2009, LANG 2004). For these species any additional adverse effect on their populations due to GMP cultivation may be considered non-acceptable in order not to deteriorate their conservation status (DOLEZEL et al. 2017). Recommendations for the environmental risk assessment of plant protection products include that decisions on the acceptability of adverse effects observed in field tests are made case-by-case, also accounting for differences of effects on different arthropod taxa, considering the mobility of the species, its reproduction time and affected development stage (CANDOLFI et al. 2000a, 2000b, DOLEZEL et al. 2017).

#### *Thresholds of acceptability should not be determined by statistical constraints under field conditions*

Any LoC needs to be aligned to the effect size that is desired to be detected by a specific statistical test design (PERRY et al. 2009). Arbitrary effect thresholds as used e.g. in testing plant protection products are often due to statistical constraints (e.g. CANDOLFI et al. 2000a, 2000b), but it is questionable whether effect sizes derived from a statistical point

of view can be considered ecologically relevant for a range of different non-target lepidopteran species.

The detection of adverse effects on non-target butterflies is influenced by the respective assessment methodology, such as the endpoint chosen (abundance, species number), transect length or the species biology (e.g. sedentary species, rare species, Lang et al. 2016). Detecting small effects such as 10 - 20 % reduction in species richness or abundance for adult butterflies requires larger sample sizes with a satisfactory statistical power (LANG 2004, LANG et al. 2016). Effects smaller than 30 % can be masked by natural population fluctuations (BÜHLER 2006, LANG & BÜHLER 2012). The use of parameters other than abundance for individual butterfly species (e.g. abundance of mobility classes, overall abundance or absence/presence data) can result in smaller sample sizes required (LANG & BÜHLER 2012). Evaluations of sampling methodology are mostly available for designing monitoring programs for adult lepidopteran species (e.g. LANG et al. 2016). For ERA purposes information is also available from the British Farm Scale Evaluations. Reductions in butterfly counts of approximately 22 % in GM herbicide tolerant oilseed rape fields (HAUGHTON et al. 2003) and 24 % in adjacent field margins (ROY et al. 2003) were observed. Although these data reflect the response of mobile organisms to the availability of weeds as a food source rather than effects due to exposure to *Bt* toxins, they indicate effect sizes to be expected for changes in abundance of adult Lepidoptera when studying effects at field scale (HAUGHTON et al. 2003).

Despite the fact that statistical aspects will influence the detectable effect sizes when designing field tests for ERA purposes and therefore influence the ability to stick to a defined LoC, statistical analysis must clarify beforehand whether defined acceptable effect thresholds can be detected with a given field test design. Specific analysis will be necessary to account for the specificities of non-target Lepidoptera.

### *Decision criteria derived from assessment schemes for species of conservation concern as a starting point for LoCs?*

The estimation of the extinction risk of wild populations of species for the global Red List assessments is based on reductions in population sizes of the taxon in question (IUCN 2012a). The extent of reduction over a time period of 10 years or over three generations, whichever is longer, is used as quantitative decision criterion in order to assign the taxon to a specific extinction risk category. In its ERA guidance for non-target organisms EFSA mentioned the effect size threshold of 30 % over three generations for butterflies based on this classification by IUCN (EFSA 2010b). Reduction of population sizes of larger than 30 % are assigned to the category `vulnerable´ if the causes for the observed reduction may not be reversible and understood and if they may not have ceased (IUCN 2012a); see Table 14).

It has to be emphasized that the reduction in population size is only one of five decision criteria that may lead to the assignment into a particular risk category. Other criteria include the geographic range, the estimated population size together with further subcriteria, very small or restricted populations and a probability of extinction in the wild within a certain time period (IUCN 2012a).

Table 14. Overview on protection levels used in the assessment schemes for the level of endangerment of species according to IUCN (2012a).

| Decision Criteria | Risk Category |
|---|---|
| reduction in population size[10] **≥ 50 %**[1] (≥ 30 %[2]) over the last 10 years or 3 generations | vulnerable |
| reduction in population size[10] **≥ 70 %**[1] (≥ 50 %[2]) over the last 10 years or 3 generations | endangered |
| reduction in population size[10] **≥ 90 %**[1] (≥ 80 %[2]) over the last 10 years or 3 generations | critically endangered |

[1] Observed, estimated inferred or suspected reduction of population size if the causes are reversible and understood and have ceased. [2] where the reduction or its causes may not have ceased or may not be understood or may not be reversible

In the global assessments a population is defined as the total number of mature individuals of a taxon worldwide. The IUCN also provides guidance for regional assessment of Red List categories (IUCN 2012b). The same criteria are used for local, regional and national Red List assessment as for global assessment in order to determine a preliminary estimate of the extinction risk within the region. Then an up- or downlisting of the category may be relevant, considering conspecific populations outside the region which may affect the risk of extinction within the region (IUCN 2012b).

An important aspect is the temporal scale relevant for the IUCN assessment criteria. A 50 % reduction over 10 years would correspond to a detectable reduction in population size of approximately 5 % per year (assuming that the population decline is more or less constant). For GMOs, a reduction in population sizes of non-target Lepidoptera over 10 years can only be assessed in monitoring programmes (LANG & BÜHLER 2012). Ten years is an inappropriate time scale for ERA testing, but reductions in non-target butterfly populations over three generations can be assessed in much shorter time spans as butterflies often have multiple generations per year. Also from a practical point of view a period of one to three years is considered a reasonable time span for semi-field or field trials conducted before market authorization of GMPs. In this context the EFSA Scientific Committee refers to time scales depending on the population dynamics of Lepidoptera: seasons, generations or rotations or a single year (EFSA 2016a). A 50 % reduction of the population size of a non-target butterfly species within three generations would constitute a major population decline of the respective species. In addition, it has to be considered that the values used by the IUCN comprise a range of causes adversely affecting a species. Considering a mono-causal stressor such as *Bt* maize, the respective acceptable thresholds must therefore be much smaller than the values proposed by IUCN.

When defining the magnitude of a tolerable effect, the biological specificities of a species, such as the duration of its life cycle, its growth and reproduction rate, or the number of generations per year should be considered.

### *Define different LoCs for in-crop and off-crop areas*

The spatial scale for which an acceptability threshold is valid has to be defined. In this context different protection goals for different areas within agro-ecosystems may be relevant. A distinction between areas designated for cultivation of crops (in-field or in-crop) and the surrounding areas (off-field or off-crop) is generally applied in the ERA of plant protection products. In order to ensure that acceptable in-field effects do not indirectly affect also off-field non-target populations and thus biodiversity, EFSA suggests landscape level risk assessment in addition to a local scale risk assessment (EFSA 2010d). The local scale assessment comprises two separate assessments, one for in-field areas, which also includes buffer strips, and another for off-field areas (EFSA 2015a). Off-field areas are considered all areas surrounding a field (e.g. hedgerows, grass strips, adjacent field, unmanaged bare land, roads) and can be considered equivalent to field margins as defined by ROY et al. (2003) and referred to by the EFSA for the ERA of GMOs (EFSA 2012d, ROY et al. 2003).

Considering the ecosystem service concept this differentiation between in-field and off-field is explained by the different ecosystem services provided in these different habitats. In the field crop production services have to be weighed against other ecosystem services such as pollination or pest regulation. In off-field habitats there is no crop production and therefore no trade-off between different ecosystem services. Consequently, for plant protection products small to medium effects are tolerable for non-target arthropods in-field, while only negligible effects should be tolerated off-field. Negligible effects are considered reductions of up to 10 % or effects comparable to non-detectable effects (EFSA 2015a).

For non-target Lepidoptera which, according to this concept are representing cultural ecosystem services, this means that a higher protection level is required for species occurring in off-field areas than those occurring in the field. Assessments of *Bt* maize effects on non-target Lepidoptera mostly refer to the field margin and therefore off-field habitats. Generally, few lepidopteran non-target species occur in-field (GATHMANN et al. 2006). Against this background and in order to harmonize protection goals for ERA purposes as required by EFSA, effects by GM crops on non-target lepidopteran species in off-field areas need to be negligible.

### *Consider the status of receiving environments when setting LoCs*

The receiving environment and its specific biological and non-biological features may affect the strengths of possible adverse effects when GMPs are cultivated. According to EFSA the acceptability of effects might be far less in a landscape with low non-target arthropod diversity than in a landscape supporting a high non-target arthropod diversity (EFSA 2015a). It is known that biodiversity levels vary considerably across European agricultural landscapes. Differences in habitat quality and habitat features in agro-environments affect species richness and abundance in butterflies (KUUSSAARI et al. 2007, AVIRON et al. 2009) and species diversity of butterflies is dependent on the level of agricultural intensity (EKROOS et al. 2010) as well as landscape context (AVIRON et al. 2009). This is reflected by the geographic differences in species richness of European butterflies and the patterns of distribution of threatened and endemic butterfly species in Europe (VAN SWAAY et al. 2010).

Therefore also the natural fluctuations in lepidopteran populations need to be considered in a particular environment. Significant declines in European butterfly populations have been recorded during the last 25 years, mainly due to changes in land use including agricultural intensification (EEA 2013). For the definition of a LoC this would require the definition of the minimum environmental quality or the minimum population size to be preserved in a specific receiving environment. In this context it is important that acceptability thresholds for adverse effects due to GMPs may also depend on the individual status of a population in a specific receiving environment. If a population is already in decline and the causes thereof are known and can be prevented, this may influence the decisions on any further pressure on this population due to additional agricultural stressors. If such causes were not reversible and understood and did not cease, a further reduction in population size would possibly not be acceptable (IUCN 2012a). Consequently, defining a specific environmental context, e.g. the level of biodiversity and the causes for its current status in the respective agro-ecosystem, is necessary to put the acceptability levels for additional adverse effects into context (see also (EFSA 2015a). However, tolerating larger reductions in environments with comparably higher biodiversity levels would still contribute to an overall reduction in biodiversity.

## 11.3 Conclusions

EFSA's concept of LoCs triggered a discussion about the relevance and the operationalisation of protection goals for the ERA of GMPs. Protection goals relevant for insect-resistant GMPs such as *Bt* maize relate to biodiversity and ecosystem services. Clearly non-target butterflies are considered as important representatives of biodiversity, but they have also been attributed to cultural services, in particular if they are endangered or aesthetic entities. Although their contribution to other ecosystem services such as regulation of pest species (e.g. through herbivory) has been recognized by EFSA, their role for the provision of other ecosystem services such as pollination services (SETTELE et al. 2009) is not taken into consideration.

In addition the role of endangered species as relevant protection goals in the ERA has been recently recognized by EFSA and suggestions have been made to cover these species by the use of the ecosystem service concept in the ERA of both, GMOs and plant protection products. The use of the ecosystem service concept aims to achieve a harmonized operationalization of general protection goals for use in the ERAs of various regulated products.

In contrast to biodiversity protection goals, agricultural protection goals have so far not been fully addressed in the ERA of GMPs although they are specifically addressed in the relevant guidance documents. For insect-resistant GMPs such as Bt maize the avoidance of resistance development of target organisms and the sustainable use of pesticides are relevant protection goals. For resistance development of target organisms thresholds for acceptability are implicitly set in the ERA of Bt crops and risk management measures required.

With respect to effects on non-target Lepidoptera assessed in laboratory studies discrepancies are evident with regard to the use of effect-based or risk-based acceptability thresholds. While for the environmental risk assessment of plant protection products such risk based thresholds are common, the prerequisites for the use of such thresholds for

the ERA of GMPs are not fulfilled. Any threshold used for lower tier assessments of non-target Lepidoptera should not be used as a stop criterion in the ERA and lead to final conclusions of environmental risks for these organisms. However, thresholds could be used for classification systems, based on effect values of *Bt* toxins. In general, any statistically significant effect observed in laboratory toxicity studies should be considered relevant and followed up by further assessments, e.g. with different methodologies at the same tier or at higher tiers, in order to widen up the ERA approach and reduce uncertainty on possible risks of Bt maize for non-target butterflies.

Acceptability thresholds for the assessment of effects on non-target Lepidoptera under field conditions have been suggested,. The suggested acceptable effect values range from 30 - 50 % and are mostly driven by statistical constraints than by biological necessities. Also decision criteria for the classification of endangered species regarding their extinction risk use similar effect sizes but apply different spatial and temporal limits for the populations in question. LoCs for ERA purposes for non-target Lepidoptera certainly need further differentiation according to individual species, populations as well as their temporal and spatial validity.

The recent advancements in the ERA of plant protection products and the use of the ecosystem service concept lead to the recognition that a differentiation of protection goals is needed between in-field and off-field habitats in agro-environments. This has led to the acknowledgment that it must be ensured that effects outside the crop production area (off-field or off-crop) do not exceed negligible effects, although a specification of this effect class has still to be made for different ecological resources and ecosystem services. Suggested thresholds for butterfly larvae used for current risk management recommendations for *Bt* maize relate to different ecological entities (adults, larvae) and parameters and use different spatial and temporal limits. A harmonization of protection goals is needed between different ERA schemes as well as of the acceptable thresholds for the respective natural resources; this is to guarantee that protection levels are not compromised by different environmental stressors in agro-ecosystems. In particular species of conservation concern will require further discussions regarding the definition of special protection goals to adequately address these particularly vulnerable species.

Strong protection levels and low acceptable effect thresholds are recommended for non-target Lepidoptera considering that current agricultural practices are among the most important pressures on terrestrial biodiversity and ecosystems (EC 2015a, 2015b). Considering that the type of agriculture used as comparator for the evaluation of adverse impacts of GMOs is already leading to farmland biodiversity loss, additional impacts due to GMO cultivation must not be considered acceptable in order not to further deteriorate biodiversity in European agro-ecosystems.

# 12 Stakeholder meeting – feedback on LoC examples

## 12.1 General feedback on the LoC concept

Generally, it was emphasized that there is a need to define the biological relevance of adverse effects for risk assessment purposes. The current definition of LoCs as proposed by EFSA was considered to be very wide and needs specification. It was also emphasized that if effect sizes are proposed they need to be adhered to in the ERA. It was considered important that protection goals and therefore also LoCs are valid for all stressors in agro-ecosystems.

The LoC concept was considered a useful approach for the ERA of GMPs if certain conditions are met. The results of the project can be useful starting points for discussions on the setting and the elaboration of specific LoCs. However, it has to be considered that the EFSA Guidance Document is relatively "old" (issued in 2010). In between, the Ecosystem Service concept has been developed to be used in the ERA of PPPs translating protection goals in the ERA. In this concept the Protection Goals and therefore also the levels of acceptable effects are set by Risk Managers. A potential for overlap of the two conceptual approaches (Ecosystem Service concept and LoC concept) was identified.

It was generally agreed that LoCs should not be defined exclusively by applicants, although the applicant should be involved in the setting of LoCs as it's the applicants duty to use suggested LoCs in the ERA. Due to its normative element, other relevant stakeholders such as risk assessors, the scientific community, the Member States and the "broader" society also need to be involved for the definition of LoCs. It was emphasized that setting LoCs by applicants means that for each individual GMP application different LoCs may be suggested thereby suggesting different levels of protection. This is in contrast to EFSA`s efforts to harmonize protection goals for ERA purposes.

Regarding the EFSA`s role in the ERA process it was emphasized that EFSA`s role is laid down by Community Law (Regulations (EC) MNO 178/2002 and 1829/2003). As a risk assessing body EFSA's role is to give scientific advice on the safety of GMOs to risk managers, i.e. the European Commission and EU Member States, which have the responsibility as risk managers to decide on the authorisation of GMOs for the European market.

## 12.2 Specific feedback on individual topics

### 12.2.1 LoCs for persistence and invasiveness including plant-to-plant gene flow

For LoCs for GMPs that are able to outcross and persist, the invasive species analogies were emphasized. The role of the indicators proposed was questioned and the use of other indicators, set at lower level, i.e. specific parameters, was suggested. However, these parameters contribute to the indicators suggested by KOWARIK et al. (2008) which were used for this case study.

It was mentioned that LoCs should take into account differences between the conventional plant and the GMP. If e.g. spread and persistence of conventional plants (e.g. oilseed rape) in the environment is acceptable, then the spread and persistence of GM oilseed rape would also have to be accepted, and only potential differences in spread and persistence would have to be assessed regarding their acceptability (e.g. if the GMP

would be more persistent than the conventional plant). Therefore, not only the outcrossing into protected species should be a criterion, but also the comparative level of persistence.

However, for agricultural production systems the comparative approach should not be used, as the occurrence of GM oilseed rape in agricultural habitats poses a problem for agricultural production systems.

It was also emphasized that LoCs for these GMPs have a strong normative dimension (rather than scientific) as the sensitivity of EU Member States regarding hybridisation between GM and non-GM plants differs considerably.

### 12.2.2 **LoCs for effects of the GMP on non-target organisms**

It was mentioned that the exposure-based indicators also include risks and could therefore also be termed risk-based.

Regarding effect thresholds used in the ERA of PPPs it was emphasized that off-field no adverse effects are tolerated (this assessment recognises interactions between in-field and off-field areas) while for in-field the 50 % effect threshold for non-target arthropods is tolerated if recovery within 1 year is guaranteed.

Regarding effects thresholds by IUCN for the extinction risk of wild species it was noticed that these thresholds integrate multiple stressors on a particular species. The question arose if it makes sense to define the LoC for each individual stressor or if the LOC should address several potential stressors (meaning that acceptable effects for individual stressors would have to be lower, e.g. sequential use of pesticides or combined effects by *Bt* and herbicide application).

The ambitious protection levels for non-target Lepidoptera of 0.5 % and 1 % used by EFSA in its modelling approaches were discussed and the normative decision that these levels are considered `negligible ´. It is unclear, on which basis the 0.5 % and 1 % levels were defined and whether they can be detected in practice. These levels were proposed for the modelling, but need to be differentiated from effect sizes for field testing, as it will be difficult to detect such small effects under field conditions. In addition the need for baseline data for the modelling approaches was addressed (e.g. species sensitivities).

It was emphasized that for the ERA of plant protection products small to negligible effects were suggested as specific protection goals.

### 12.2.3 **LoCs for impacts of the cultivation and management techniques**

The use of weed thresholds was considered a useful tool to for setting LoCs.

# 13 Overall Conclusions

### General aim of the concept

The European Food Safety Authority has introduced the concept of Limits of Concern (LoC) for the risk assessment of genetically modified plants. LoCs define the limit where an adverse effect observed in the ERA has the potential to cause harm. The aims of the LoC concept were to open up the ERA for the operationalisation of protection goals when assessing risks to GMPs as well as to conclude on risks in a more quantitative way, and thereby to increase the ERA's conclusiveness. The concept entailed also additional statistical testing (equivalence test in addition to difference test) when assessing differences between the GMP and its non-GM counterpart within the comparative assessment, but also improved guidance for the statistical design of studies. At the same time it became clear that risk assessment requirements for ERA schemes of different environmental stressors in the agro-environment need to consider the same protection goals; therefore, risk assessment specifications have to define common acceptability levels for effects on these protection goals.

### Application of the concept in ERA of GMPs

So far, the LoC concept has not been applied in GMP applications, neither for cultivation purposes nor for import and processing: while the new provisions for statistical testing have been largely followed in the comparative safety assessment, no LoCs have been suggested in GMP applications. Non-equivalent results from the compositional assessment (e.g. for environmentally relevant plant components) are not deemed relevant, e.g. by establishing a link between the substance in question and potentially adverse effects on non-target organisms or effects on pest species. Hence, the requirement of EFSA as well as of Regulation (EC) 503/2013 to assess the biological relevance of statistically significant differences has not been fulfilled by GMP applicants so far. This may be due to a lack of some fundamental prerequisites in order to make the LoC concept operational. In addition, controversies exist regarding the definition, type and size of LoCs as well as the consequences, in case they are exceeded. The LoC concept would substantially improve if LoCs were linked to environmentally relevant parameters assessed in comparative safety assessment. Only when suitable LoCs are used as reference points, the observed differences between the GMO and its conventional counterpart can be related to environmental safety. Here a strict differentiation between the food-feed risk assessment and the ERA is necessary.

### LoCs and protection goals

By introducing the LoC concept in the ERA of GMPs more emphasis is given to environmental protection goals. However, definitions of environmental harm for protection goals are largely lacking. The respective legislative acts for the protection and conservation of biodiversity also lack an indication of specific benchmarks for biodiversity in arable production systems and a definition of a `good ecological status´ for dynamic agro-ecosystems.

Science can support decisions on the relevance of observed adverse effects on biodiversity in agro-environments, but it cannot take any of the normative decisions on what, where and when to protect. Also, it has to be recognized that missing scientific data on

safe ecological limits for agricultural stressors currently impede the definition of LoCs. In addition, specifying what constitutes environmental harm is a highly political issue and is influenced not only by European policies but also by national priorities in conservation efforts. Despite scientific and political limitations to formulate LoCs it will be necessary to define thresholds and criteria for acceptability when assessing adverse effects of GMPs for ERA purposes. In order to make the LoC concept operational, it is important to clarify which protection goals are relevant for a particular GMP in question. For the GMPs discussed in this study it became evident that not only biodiversity protection goals need to be addressed, but that so far agricultural protection goals have been largely neglected in ERA practice.

Agricultural protection goals must not be dismissed in the ERA, in particular as they support agricultural diversity and consequently biodiversity. In this context it has to be recognized that different protection goals may be relevant in and outside agricultural production areas which will affect the definition of LoCs. While in-field a balance has to be found between different ecosystem services no such trade-offs are evident for off-field areas where the conservation of biodiversity must be of highest priority. Experience from current agricultural practice (e.g. weed thresholds, seed production thresholds) may support the definition of LoCs.

If the concept is to be a useful approach for the ERA, LoCs need to be spatially and temporally different from those thresholds that are used for the acceptability of environmental damage defined at the protection goal level. In this context it is important to recognize that various receiving environments exist across Europe which have their own agricultural specificities, diverging biodiversity levels and differing protection goal priorities; these peculiarities must be accounted for when defining what adverse environmental effects are acceptable, for example by using regionally adapted LoCs.

**Exceedance of LoC**

Before the LoC concept is to be made operational, it must be decided what are the consequences and which steps need to be taken in case LoCs are exceeded. Fundamentally different views on the whole ERA process exist among stakeholders involved in the ERA and risk management which have to be resolved before defining LoCs. Controversies remain whether LoCs should represent classical trigger values, comparable to those used in the ERA of plant protection products, or whether they should be considered as decision criteria to inform the risk characterisation and strengthen the ERA by increasing confidence in risk conclusions. Importantly, the introduction of LoCs in the ERA of GMPs needs to be accompanied by improving the data basis and risk assessment approaches in order to derive robust risk conclusions at each step of the ERA.

**LoC and comparative safety assessment**

The LoC concept should also interlink with the comparative safety assessment. Results from the comparative safety assessment, e.g. statistically significant, non-equivalent differences between the GMP and the non-GM counterpart, may be biologically or toxicologically relevant which in turn needs to be scrutinized for their potential to cause environmental harm. Still, results of the comparative assessment are not linked to the LoC concept, which is required when introducing this concept. Such a link implies identifying

possible hazards from the results of the comparative safety assessment, not only in terms of food-feed safety, but also in terms of environmental safety and the lack of harm to related biodiversity protection goals.

**LoCs and uncertainties and long-term effects**

A major challenge for the ERA and the application of LoCs is the need to consider scaling effects. Certain adverse effects at landscape or regional level or certain combinatorial effects (e.g. due to *Bt* toxin effects and herbicidal effects) will become manifest only when cultivating GMPs at large scales. In addition, it has to be recognized that many profound ecological questions still remain unanswered, e.g. what level of biodiversity is needed to sustain certain ecosystem functions or what are the consequences of certain adverse effect sizes of environmental stressors on the biodiversity in arable ecosystems. There are still open questions on how to predict scale-dependent impacts of GMP cultivation across landscapes or regions during the full authorization period of GMPs, which currently can only be addressed via upscaling or modelling approaches in the ERA.

Any decision on LoCs must therefore be aware of the potential consequences of acceptable effect sizes for the relevant ecological entities and ecosystem functions and services if large-scale and long-term GMO cultivation is envisaged. In this context uncertainties have to be made transparent. They are inherently associated with the definition of any acceptability threshold and methodologies have to be found to cover them at least partially, e.g. by using assessment factors. This relates also to the question of how to deal with long-term effects of GMOs which cannot be tested in the ERA. Any LoC defined for such effects needs to be combined with corresponding risk management measures while simultaneously strengthening post-market monitoring.

**Differentiation of LoCs**

EFSA has come up with suggestions for standard values to be used as LoCs. However, these are hardly useful in ERA practice. Standard values for acceptable effect sizes are used in other regulatory areas with more formalised risk assessment procedures and different ERA conditions. For GMPs without standard risk assessment methodologies and case-by-case assessments, standard values are not practicable in the ERA. In this case decisions on LoCs must be made considering the specificities of the crop and the adverse effect scenarios for the protection goals in question. This also implies that LoCs have to discriminate between species, habitats, ecosystem services and ecological functions depending on the biological specificities rather than using standardized threshold values. The definition of LoCs should, however, take into account that – due to prevailing agricultural practices – current pressure on European farmland biodiversity is already high, thereby calling for LoCs for GMOs that do not go beyond current effect levels in conventional agriculture. In this context species and habitats of conservation concern need separate consideration acknowledging their outstanding role for European biodiversity.

**LoCs and areas of risk – the three case studies**

Another important question is, whether LoCs need to be set for all areas of risk in the ERA. However, it is largely unanswered as long as no new applications for cultivation of GMOs are filed in the EU. The prominent role of LoCs in the problem formulation sug-

gests that they are relevant for all areas of risk. This study has shown that the LoC concept has the potential to be applied to several of them.

The case study of GM oilseed rape has shown that the discussion on LoCs can benefit from discussions on environmental harm by the introduction of invasive alien species. In case the definition of thresholds for the acceptability of effects is only based on documented adverse impacts on biodiversity by GM oilseed rape, the precautionary view is missing. It would also ignore that potential effects may be non-reversible and that the novel environmental stressor may be non-retrievable. In the ERA adverse impacts on the relevant protection goals by GM oilseed rape cannot always be assessed *ex-ante*. Instead, biological processes need to be defined which are assessable in the ERA and for which LoCs can be defined, e.g. hybridisation or establishment of the GMP in certain habitats. Whether such biological processes are acceptable depends on the status of the affected entities, e.g. the conservation status of a species or a habitat. In this context also the agricultural biodiversity represented by plant genetic resources is important. Here the LoC concept can be used to support decisions on the acceptability of the presence of GM oilseed rape in receiving environments or in sexually compatible wild or crop plants. However, this requires that legislative texts are amended accordingly and consider the genetic constitution of protected entities. This example also shows that focussing on relevant protection goals helps to make decisions on the acceptability of adverse effects before the ERA is carried out.

Defining LoCs for GM herbicide tolerant plants will be important for adverse effects on in-field weed biodiversity triggered by the use of the complementary, non-selective herbicides. LoCs defined for herbicide tolerant plants must comply with two different authorization regimes, for GMOs and for plant protection products. Relevant protection goals refer to the protection of weed communities, but also to higher trophic levels that are sustained by weeds, as well as different ecosystem services which need to be provided in the field. Further, the conservation of the ecosystem services must be balanced against the productivity of the crop and the use of the non-selective herbicide to suppress crop-competitive weeds. Approaches derived from IPM such as the use of weed thresholds are available to balance different ecosystem services in agro-ecosystems and can be used to establish acceptability thresholds for in-field use. For weed thresholds it is important to widen up the time scale to include crop rotational aspects as well as to consider functional aspects of the impacted species, depending on the specific protection goal to be achieved. LoCs may also be achieved by risk mitigation measures after authorization of the GMP, e.g. by defining spatially distinct areas of the field where the relevant protection goals can be achieved. In addition, for rare and endangered species and weed communities specific habitat protection measures will be necessary. LoCs for agricultural protection goals are also necessary, e.g. low pesticide use, avoidance of resistance development in weeds, preservation of crop rotations.

For the third case study using *Bt* maize and adverse effects on non-target Lepidoptera it is important to spatially differentiate protection goals and related LoCs. Non-target lepidopteran species represent important biodiversity representatives and occur mostly in habitats adjacent to agricultural fields. Existing suggestions by EFSA for non-target Lepidoptera emphasize that strict protection levels tolerating only negligible to small effects are needed, in particular for off-crop areas. This implies serious challenges for the design

of field tests to detect small to negligible effects on these species. In contrast, for laboratory studies decisions have to be made whether to set LoCs at the exposure, effect or risk level. In addition, a classification system based on the characteristics of the *Bt* toxin (e.g. specificity, toxicity, mode of action, chronic effects) is proposed which first allows to define LoCs for each class and second provides a decision making system with clear criteria regarding the acceptability of observed effects. However, establishing such a system requires that test methodologies for *Bt* toxins are standardised. In the meantime any significant difference observed in laboratory tests will have to trigger further examinations on potential consequences of such observed effects.

**Chances of the improved concept**

In the future the LoC concept will be useful as it opens up the ERA for the integration of protection goals and puts pressure on EU Member States to specify their environmental policy objectives. The setting of LoCs will likely be a controversial issue. However, defining LoCs opens the way for discussions on what to protect, where and when in European agro-ecosystems. First steps have to be made and specific LoCs have to be suggested in order to start discussions. This will involve agreeing on priorities with respect to protection goals, but also regarding trade-offs between different ecosystem services provided by the agricultural environments in question. As another attempt to operationalise protection goals, EFSA suggested the ecosystem service concept for the ERA of several environmental stressors. This concept includes the definition of acceptability criteria for adverse effects as well, although in a more structured way than the LoC concept. Both concepts should be carefully compared regarding possible overlaps and differences. At present both concepts are still missing a common understanding of the necessary definitions of effect categories. This requires not only scientific but also political decisions on what is understood by a negligible, low, medium or high effect in a specific biological context. The lack of a scientific justification shall not prevent the formulation of LoCs in the ERA. Decision making on LoCs must therefore involve different stakeholders, such as risk managers, risk assessors, applicants and the scientific community. In any case, political and scientific justifications behind the decisions on LoCs must be made transparent.

# 14 Literature


ADLER W. (Ed.) (1994). Exkursionsflora von Österreich. Verlag Eugen Ulmer. Stuttgart und Wien.

AGES (2004). Die Produktion von Saatgut in abgegrenzten Erzeugungsprozessen zur Vermeidung einer Verunreinigung mit gentechnisch veränderten Organsimen im Kontext mit der Koexistenz von konventioneller Landwirtschaft mit oder ohne GVO und ökologischer Landwirtschaft. Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH.
http://www.ages.at/fileadmin/AGES2015/.../gvo_koexistenz_ages_studie_neu_03

AHMED E. M. (1986). Lectin quantification in peanut and soybean seeds. Peanut Science: January 1986. Vol. 13 (1) 4-7.

ALIX A., BAKKER F., BARRETT K., BRÜHL C. A., COULSON M., HOY S., JANSEN J. P., JEPSON P., LEWIS G., NEUMANN P., SÜßENBACH D., VAN VLIET P. (2010). ESCORT 3: Linking non-target arthropod testing and risk assessment with protection goals. Hotel Zuiderduin, Zeeweg 52, Holland, 8-11th March 2010.

ANDOW D. A., LÖVEI G. L., PAULA D. P. (2016). Equivalence tests to support environmental biosafety decisions: theory and examples. Journal of Biosafety 25 (2): 77-91.

ANDREASEN C., STRYHN H., STREIBIG J. C. (1996). Decline of the flora in Danish arable fields. Journal of Applied Ecology 33: 619-626.

ARPAIA S., MESSEAN A., BIRCH A. N. E., HOKANNEN H., HÄRTEL S., VAN LOON J., LÖVEI G., PARK J., SPREAFICO H., SQUIRE G. R., STEFFAN-DEWENTER I., TEBBE C., VAN DER VOET H. (2014). Assessing and monitoring impacts of genetically modified plants on agroecosystems: the approach of the AMIGA project. Entomologica 2 (154): 79-86.

AVIRON S., SANVIDO O., ROMEIS J., HERZOG F., BIGLER F. (2009). Case-specific monitoring of butterflies to determine potential effects of trasngenic *Bt*-maize in Switzerland. Agriculture, Ecosystems and Environment 131: 137-144.

BAES (2013). Sorten- und Saatgutblatt, Sondernummer 40, Republik Österreich. Bundesamt für Ernährungssicherheit.
www.baes.gv.at/fileadmin/_migrated/.../Feldanerkennung_Kreuzbluetler_01.pdf

BÀRBERI P., BURGIO G., DINELLI G., MOONEN A. C., OTTO S., VAZZANA C., ZANIN G. (2010). Functional biodiversity in the agricultural landscape. Relationships between weeds and arthropod fauna. Weed Research 50: 388-401.

BARZMAN M., BÀRBERI P., BIRCH A. N. E., BOONEKAMP P., DACHBRODT-SAAYDEH S., GRAF B., HOMMEL B., JENSEN J. E., KISS J., KUDSK P., LAMICHHANE J. R., MESSÉAN A., MOONEN A.-C., RATNADASS A., RICCI P., SARAH J.-L. & M. SATTIN (2015). Eight principles of integrated pest management. Agronomy for Sustainable Development 35 (4): 1199–1215.

BAYER CROP SCIENCE (2012): Request for Authorization of genetically modified herbicide tolerant cotton for cultivation in accordance with Regulation (EC) N° 1829/2003 EFSA-GMO-ES 2012-104; Part II - Scientific Information.

BECKER H. C., DAMGAARD C., KARLSSON B. (1992). Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). Theoretical and Applied Genetics 84: 303-306.

BECKER-RITT, A. B., MULINARI, F., VASCONCELOS, I. M., CARLINI, C. R. (2004): Antinutritional and/or toxic factors in soybean (*Glycine max* (L) Merril) seeds: comparison of different cultivars adapted to the southern region of Brazil. Journal of the Science of Food and Agriculture 84 (3) 263-270.

BENBROOK C. M. (2012). Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environmental Sciences Europe 24 (24): 1-13.

BENBROOK C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28: 3.

BILZ M., KELL S. P., MAXTED N., LANSDOWN R. V. (2011). European Red List of Vascular Plants. Luxembourg: Publications Office of the European Union, doi:10.2779/8515.

BLACKBURN T. M., ESSL F., EVANS T., HULME P. E., JESCHKE J. M., KÜHN I., KUMSCHICK S., MARKOVÁ Z., MRUGALA A., PERGL J., PYŠEK P., RABITSCH W., RICCIARDI A., RICHARDSON D. M., SENDEK A., VILÀ M., WILSON J. R. U., WINTER M., GENOVESI P., BACHER S. (2014): A unified classification of alien species based on the magnitude of their environmental impacts. PLOS Biology 12 (5): e1001850.

BØHN T., PRIMICERIO R., HESSEN D. O., TRAAVIK T. (2008). Reduced fitness of *Daphnia magna* fed a *Bt*-transgenic maize variety. Archives of Environmental Contamination and Toxicology 55 (4): 584-592.

BØHN T., TRAAVIK T., PRIMICERIO R. (2010). Demographic responses of *Daphnia magna* fed transgenic *Bt*-maize. Ecotoxicology 19 (2): 419-430.

BOOIJ K. & Y. T. QUI (2014). Ecological and experimental constraints for field trials to study potential effects of transgenic *Bt*-crops on non-target insects and spiders. COGEM CGM 2014-06. Plant Research International, Wageningen. Report Nr. 592.

BOOIJ, K. & Y. T. QUI (2015). Testing impacts of toxic compounds from transgenic crops on non-target arthropods in tier-1 studies: exposure and response. COGEM CGM 2015-08. Report No. 635.

BOMANOWSKA A. (2010). Threat to arable weeds in Poland in the light of national and regional Red Lists. Plant Breeding and Seed Science 61: 55-74.

BRECKLING B. & G. MENZEL (2004). Self-organised pattern in oilseed rape distribution – an issue to be considered in risk analysis. In: Breckling, B. & R. Verhoeven (Eds) Risk Hazard Damage: Specification of Criteria to Assess Environmental Impact of Genetically Modified Organisms. Bonn (Bundesamt für Naturschutz) – Naturschutz und Biologische Vielfalt 1, 73-88.

BRECKLING B. & G. MENZEL (2005). Gentechnisch veränderter, herbizidresistenter Raps. Natur und Landschaft: Heft 7: 316-319.

BRECKLING B., REUTER H., MIDDELHOFF U., GLEMNITZ M., WURBS A., SCHMIDT G., SCHRÖDER W., WINDHORST W. (2011) Risk indication of genetically modified organisms (GMO):

modelling environmental exposure and dispersal across different scales. Oilseed rape in Northern Germany as an integrated case study. Ecological Indicators 11 (4): 936–941.

BROOKS D. R., STORKEY J., CLARK S. J., FIRBANK L. G., PETIT S., WOIWOD I. P. (2012). Trophic links between functional groups of arable plants and beetles are stable at a national scale. Journal of Animal Ecology 81: 4-13.

BROWER L. P., TAYLOR O. R., WILLIAMS E. H., SLAYBACK D. A., ZUBIETA R. R., RAMIREZ M. I. (2012). Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity 5 (2) 95–100.

BUNDESNATURSCHUTZGESETZ (BNatSchG). Gesetz über Naturschutz und Landschaftspflege vom 29. Juli 2009 (BGBl. I S. 2542). http://www.gesetze-im-internet.de/bundesrecht/bnatschg_2009/gesamt.pdf

BÜHLER C. (2006). Biodiversity monitoring in Switzerland: What can we learn for general surveillance of GM crops? Journal für Verbraucherschutz und Lebensmittelsicherheit 1 (Suppl. 1): 37-41.

BYFIELD A. J. & P.J. WILSON (2005). Important arable plant areas: identifying priority sites for arable plant conservation in the United Kingdom. Plantlife International, Salisbury, UK.

CABALLERO-LÓPEZ B., BLANCO-MORENO J. M., PÉREZ N., PUJADE-VILLAR J., VENTURA D., OLIVA F., SANS F. X. (2010). A functional approach to assessing plant–arthropod interaction in winter wheat. Agriculture, Ecosystems and Environment 137: 288-293.

CAIRNS, J. (1983). Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100: 47-57.

CAIRNS, J. (1986). What is meant by validation of predictions based on laboratory tests? Hydrobiologia 137: 271-278.

CALDERON DE LA BARCA A. M., VASQUEZ-MORENO L., ROBLES-BURGUENO M. (1991): Active soybean lectin in foods: isolation and quantification. Food Chemistry 39: 321-327.

CAMPBELL P. J., BROWN K. C., HARRISON E. G., BAKKER F., BARRETT K. L., CANDOLFI M. P., CANEZ V., DINTER A., LEWIS G., MEAD-BRIGGS M., MILES M., NEUMANN P., ROMIJN K., SCHMUCK R., SHIRES S., UFER A., WALTERSDORFER A. (2000). A hazard quotient approach for assessing the risk to non-target arthropods from plant protection products under 91/414/EEC: hazard quotient trigger value proposal and validation. Journal of Pest Science 73, 117-124.

CANDOLFI M. P., BARRETT K. L., CAMPBELL P. J., FORSTER R., GRANDY N., HUET M.-C., LEWIS G., OOMEN P.A., SCHMUCK R., VOGT H. (2000a). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop held at Wageningen International Conference Centre, 21-23 March 2000, Wageningen, The Netherlands. pp 50.

CANDOLFI, M. P., BIGLER F., CAMPBELL P. J., HEIMBACH U., SCHMUCK R., ANGELI G., BAKKER F., BROWN K. C., CARLI G., DINTER A., FORTI D., FORSTER R., GATHMANN A., HASSAN S., MEAD-BRIGGS M., MELANDRI M., NEUMANN P., PASQUALINI E., POWELL W., REBOULET J.-N., ROMIJN K., SECHSER B., THIEME TH., UFER A., VERGNET CH., VOGT H. (2000b). Prin-

ciples for regulatory testing and interpretation of semi-field and field studies with non-target arthropods. Journal of Pest Science 73:141-147.

CARLINI C. R. & M. F. GROSSI-DE-SÁ (2002). Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40 1515-1539.

CBD (1992). Convention on Biological Diversity. https://bch.cbd.int

CBD (2002). 2010 Biodiversity Targets https://www.cbd.int/2010-target

CBD (2011). Updated Global Strategy for Plant Conservation 2011-2020 https://www.cbd.int/gspc/

CBD (2012). Guidance on risk assessment of living modified organisms. Revised on 19 July 2012. Convention on Biological Diversity, Cartagena Protocol on Biosafety. https://bch.cbd.int/protocol/testing_guidance_RA.shtml

CHAMBERLAIN D. E., FREEMAN S. N., VICKERY J. A. (2007). The effects of the GMHT crops on bird abundance in arable fields in the UK. Agriculture, Ecosystems and Environment 118: 350-356.

CHARLESTON D. S. & M. DICKE (2009). Designing experimental protocols to investigate the impacts of GM crops on non-target arthropods. A literature-based study, proposing ecologically relevant experimental protocols to investigate the impact of GM crops on non-target organism Wageningen University and Research Centre. COGEM, Wageningen, the Netherlands. 67pp.

CLAESSEN D., GILLIGAN C. A., LUTMAN P. J. W., VAN DEN BOSCH F. (2005a). Which traits promote persistence of feral GM crops? Part 1: Implications of environmental stochasticity. Oikos 110 (1): 20.

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 (1): 30.

COGEM (2014). International scientific workshop 'Stacked Bt genes: assessment of effects on non-target organisms. COGEM. Amsterdam, the Netherlands. CGM/141217-01.

COMMISSION DIRECTIVE 2008/62/EC of 20 June 2008 providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties. Official Journal of the European Union L 162/13.

COMMISSION DIRECTIVE 2011/78/EU of 20 September 2011 amending Directive 98/8/EC of the European Parliament and of the Council to include Bacillus thuringiensis subsp. israelensis Serotype H14, Strain AM65-52 as an active substance in Annex I thereto.

COMMISSION IMPLEMENTING REGULATION (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. Official Journal of the European Union L 189/4.

CONSTABLE A., JONAS D., COCKBURN A., DAVI A., EDWARDS G., HEPBURN P., HEROUET-GUICHENEY C., KNOWLES M., MOSELEY B., OBERDÖRFER R., SAMUELS F. (2007). History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food and Chemical Toxicology 45: 2513-2525.

COUNCIL DIRECTIVE 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal of the European communities L 230/1.

COUNCIL DIRECTIVE 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities L 206/7.

COUNCIL DIRECTIVE 2005/25/EC of 14 March 2005 amending Annex VI to Directive 91/414/EEC as regards plant protection products containing micro-organisms. Official Journal of the European Union L 90/1.

COUSENS R. (1985). A simple model relating yield loss to weed density. Annals of Applied Biology 107 (2): 239-252.

CRAWLEY M. J. & S. L. BROWN (2004). Spatially structured population dynamics in feral oilseed rape. Proceedings of the Biological Sciences 271 (1551) 1909-1916.

CZAPLA T. H. & B. A. LANG (1990). Effect of plant lectins on the larval development of European Corn Borer (Lepidoptera: Pyralidae) and Southern Corn Rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 83 (6) 2480-2485.

DE LA PAZ CELORIO-MANCERA M., AHN S. J., VOGEL H., HECKEL D. G. (2011). Transcriptional responses underlying the hormetic and detrimental effects of the plant secondary metabolite gossypol on the generalist herbivore *Helicoverpa armigera*. BMC Genomics 12 (1) 575.

DE LA PAZ CELORIO-MANCERA M., HECKEL D. G., VOGEL H. (2012). Transcriptional analysis of physiological pathways in a generalist herbivore: responses to different host plants and plant structures by the cotton bollworm, *Helicoverpa armigera*. Entomologia Experimentalis et Applicata 144: 123-133.

DE SCHRIJVER A., DEVOS Y., DE CLERCQ P., GATHMANN A., ROMEIS J. (2016). Minimal Quality Criteria for Acceptability of in vivo Bioassays with *Bt*-proteins. ISB News Report May/June 2016.

DEVOS Y., REHEUL D., DE SCHRIJVER A., CORS F., MOENS W. (2004). Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. Environmental Biosafety Research 3: 135-148.

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 Research 17: 1059-1077.

DEVOS Y., ROMEIS Y., LUTTIK R., MAGGIORE A., PERRY J. N., SCHOONJANS R., STREISSL F., TARAZONA J. V., BROCK T. C. M. (2015). Optimising environmental risk assessments. EMBO Reports 16 (9): 1060-1063.

DIRECTIVE 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. Official Journal of the European Communities L 123.

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. Official Journal of the European Communities L 106/1.

DIRECTIVE 2001/81/EC of the European Parliament and of the Council of 23 October 2001 on national emission ceilings for certain atmospheric pollutants. Official Journal. L 309.

DIRECTIVE 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethosylate and cement).

DIRECTIVE 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. Official Journal of the European Union. L 143/56.

DIRECTIVE 2004/107/EC of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air.

DIRECTIVE 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. Official Journal of the European Union. L 152/1.

DIRECTIVE 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. Official Journal of the European Union. L 309/71-86.

DIRECTIVE (EU) 2015/412 of the European Parliament and of the Council of 11 March 2015 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of genetically modified organisms (GMOs) in their territory. Official Journal of the European Union L 68/1.

DOLEZEL M., MIKLAU M., HILBECK A., OTTO M., ECKERSTORFER M., HEISSENBERGER A., TAPPESER B., GAUGITSCH H. (2011). Scrutinizing the current practice of the Environmental Risk Assessment of GM maize applications for cultivation in the EU. Environmental Sciences Europe 23: 33.

DOLEZEL M., MIKLAU M., HEISSENBERGER A., REICHENBECHER W. (2017). Are Limits of Concern a useful concept to improve the environmental risk assessment of GM pants? Environmental Sciences Europe 29: 7.

DONALD P. F., GREEN R. E., HEATH M. F. (2001). Agricultural intensification and the collapse of Europe's farmland bird populations. Proceedings of the Royal Society London B Biological Sciences 268: 25–29.

EC (2000). Guidance Document on Persistence in Soil. Working Document. DG Agri 9188/VI/97rev.8 12 July 2000.

EC (2001). Biodiversity Action Plan for Agriculture. Communication from the Commission to the Council and the European Parliament. COM(2001)162 final

EC (2002). Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final. Draft Working Document. 17 Oct 2002.

EC (2015a). Report from the Commission to the European Parliament and the Council. The mid-term review of the EU Biodiversity Strategy to 2020. European Commission. COM (2015) 187 final

EC (2015b). The State of Nature in the EU. Reporting under the EU Habitats and Birds Directives 2007–2012. European Commission. Luxembourg: Office for Official Publications of the European Union.

ECB (2003). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment of existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II, Chapter 3: Environmental Risk Assessment. European Chemical Bureau.

ECHA (2008). Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose[concentration]-response for environment. www.echa.europa.eu

ECHA (2010). How to report weight of evidence. Practical guide 2. European Chemicals Agency. www.echa.europa.eu

ECHA (2012). Guidance on information requirements and chemical safety assessment. Part E: risk characterisation. www.echa.europa.eu

ECHA (2013). Guidance on information requirements. Guidance on Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (BPR). Version 1.0. www.echa.europa.eu

EEA (2013). The European Grassland Butterfly Indicator: 1990-2011. European Environment Agency. Luxembourg, Publication Office of the European Union.

EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA Journal 99, 1-100.

EFSA (2008): Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on gossypol as undesirable substance in animal feed. EFSA Journal 908, 1-56.

EFSA (2010a). Guidance on the environmental risk assessment of genetically modified plants. Scientific Opinion. EFSA Journal 8 (11): 1879.

EFSA (2010b). Scientific Opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. EFSA Journal 8 (11): 1877.

EFSA (2010c). Scientific Opinion on statistical considerations for the safety evaluation of GMOs. EFSA Journal 8 (1): 1250.

EFSA (2010d). Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal 8 (10): 1821.

EFSA (2011a). Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. Scientific Opinion. EFSA Journal 9 (5): 2149.

EFSA (2011b): Guidance for risk assessment of food and feed from GM plants. Scientific Opinion. EFSA Journal 9 (5): 2150.

EFSA (2011c). Statistical significance and biological relevance. Scientific Opinion. Scientific Committee. EFSA Journal 9 (9): 2372.

EFSA (2011d). Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation. EFSA journal 9 (11): 2429.

EFSA (2011e). Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize Bt11 for cultivation. EFSA journal 9 (12): 2478.

EFSA (2012a). Scientific Opinion on herbicide tolerant genetically modified soybean 40-3-2 for cultivation. EFSA Journal 10 (6):2753.

EFSA (2012b). Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize 1507 for cultivation. EFSA Journal 10 (11): 2934.

EFSA (2012c). Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize 1507. EFSA Journal 10 (10):2933.

EFSA (2012d). Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810. EFSA Journal 10 (12): 3016.

EFSA (2012e). Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA Journal 10 (12): 3017.

EFSA (2012f): Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize Bt11. EFSA Journal 10 (12): 3018.

EFSA (2013). Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 11 (7): 3295.

EFSA (2014a): Editorial: Specifying biodiversity-related protection goals for environmental risk assessment. EFSA Journal 12 (6): e14062.

EFSA (2014b). Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 12 (7): 3800.

EFSA (2015a). Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 13 (2): 3996.

EFSA (2015b). Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to *Bt*-maize pollen. EFSA Journal 13 (7): 4127.

EFSA (2016a). Guidance to develop specific protection goal options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 14 (6): 4499.

EFSA (2016b). Recovery in Environmental Risk Assessments at EFSA. Scientific Committee. EFSA Journal 14 (2): 4313.

EFSA (2016c). Coverage of endangered species in environmental risk assessments at EFSA. EFSA Journal 14 (2): 4312.

EFSA (2017). Guidance on the Use of the Weight of Evidence Approach in Scientific Assessments. Draft document.

EKROOS J., HELIÖLÄ J., KUUSSAARI M. (2010). Homogenization of lepidopteran communities in intensively cultivated agricultural landscapes. Journal of Applied Ecology 47: 459-467.

ELIAS P., ELIAS P., BARANEC T. (2005). The New red list of Slovak endangered weeds. International Scientific Conferences on Threatened Weedy Plant Species, Sept 20-21, 2005, Slovak University of Agriculture, Nitra, Slovakia.

ELMQUIST T., FOLKE C., NYSTRÖM M., PETERSON G., BENGTSSON J., WALKER B., NORBERG J. (2003). Response diversity, ecosystem change and resilience. Frontiers in Ecology and the Environment 1 (9): 488-494.

EPPO (2003a). Environmental risk assessment scheme for plant protection products. Chapter 4: Soil. OEPP/EPPO Bulletin 33: 147-149.

EPPO (2003b): Environmental risk assessment scheme for plant protection products. Chapter 8: Soil organisms and functions. OEPP/EPPO Bulletin 33: 195-209.

EPPO (2003c): Environmental risk assessment scheme for plant protection products. Chapter 12: non-target terrestrial higher plants. OEPP/EPPO Bulletin 33: 239-244.

EPPO (2010a). Environmental risk assessment scheme for plant protection products. Chapter 10: honeybees. OEPP/EPPO Bulletin 40: 323-331.

EPPO (2010b). Efficacy evaluation of plant protection products. Side-effects on honeybees. EPPO Standards PP1/170(4). Bulletin OEPP/EPPO Bulletin 31, 323-330.

EPPO (2015). Efficacy evaluation of plant protection products. PP 1/213 (4) Resistance risk analysis. European and Mediterranean Plant Protection Organization. EPPO Bulletin 45 (3), 371-387.

ESSL F., NEHRING S., KLINGENSTEIN F., MILASOWSZKY N., NOWACK C., RABITSCH W. (2011). Review of risk assessment schemes of IAS in Europe and introducing the German-Austrian Black List Information System (GABLIS). Journal of Nature Conservation 19: 339-350.

FAHRIG L. (2003). Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution and Systematics 34: 487–515.

FANGÚNDEZ J. (2014). The paradox of arable weeds: diversity, conservation, and ecosystem services of the unwanted. In: Benkeblia N. (Ed). Agroecology, Ecosystems, and Sustainability, CRC Press 2014, pp. 139–150.

FAO (2001). International Treaty on Plant Genetic Resources for Food and Agriculture. http://www.fao.org/plant-treaty/en/

FELKE M., LORENZ N., LANGENBRUCH G. A. (2002). Laboratory studies on the effects of pollen from *Bt*-maize on larvae of some butterfly species. Journal of Applied Entomology 126 (6): 320-325.

FELKE M. & G. A. LANGENBRUCH (2003). Wirkung von *Bt*-Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch. Gesunde Pflanzen 55 (1): 1-7.

FIRBANK L. G., ROTHERY P., MAY M. J., CLARK S. J., SCOTT R. J., STUART R. C., BOFFEY C. W. H., BROOKS D. R., CHAMPION G. T., HAUGHTON A. J., HAWES C., HEARD M. S., DEWAR A. M., PERRY J. N., SQUIRE G. R. (2006). Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. Biology Letters 2: 140-143.

FREUDLING C. (2004). The circumstances surrounding glyphosate resistant horseweed in more than nine US states. In: Breckling B. & R. Verhoeven (eds) Risk Hazard Damage, Naturschutz und Biologische Vielfalt, Heft 1. Federal Agency for Nature Conservation, Bonn, Germany.

FRIESEN L. F., NELSON A. G., VAN ACKER R. C. (2003). Evidence of contamination of pedigreed Canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. Agronomy Journal 95 (5) 1342-1347.

GADHELA I. C., FONSECA N. B., OLORIS S. C., MELO M. M., SOTO-BLANCO B. (2014). Gossypol toxicity from cottonseed products. The Scientific World Journal 2014 (Article ID 231635).

GATHMANN A., WIROOKS L., HOTHORN L. A., BARTSCH D., SCHUPHAN I. (2006). Impact of *Bt* maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. Molecular Ecology 15: 2677–2685.

GEROWITT B. & R. HEITEFUSS (1990). Weed economic thresholds in cereals in the Federal Republic of Germany. Crop Protection 9: 323-331.

GIBSON D. M., STACK S., KRELL K., HOUSE J. (1982). A comparison of soybean agglutinin in cultivars resistant and susceptible to *Phytohpthora megasperma* var. *sojae* (Race1). Plant Physiology 70: 560-566.

GOEDHART P. W., VAN DER VOET H., BALDACCHINO F., ARPAIA S. (2014). A statistical simulation model for field testing of non-target organisms in environmental risk assessment of genetically modified plants. Ecology & Evolution 4 (8) 1267–1283.

GONZALEZ DE MEJIA E., BRADFORD T., HASLER C. (2003). The anticarcinogenic potential of soybean lectin and lunasin. Nutrition Reviews 61 (7): 239-246.

GOVE B., POWER S. A., BUCKLEY G. P., GHAZOUL J. (2007). Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long term impact assessments and field surveys. Journal of Applied Ecology 44 (2): 374-384.

GRAY M. E. (2010). Relevance of traditional integrated pest management (IPM) strategies for commercial corn producers in a transgenic agroecosystem: A bygone era? Agricultural and Food Chemistry 59 (11): 5852–5858.

GRUTTKE H. & G. LUDWIG (2004). Konzept zur Ermittlung der Verantwortlichkeit für die weltweite Erhaltung von Arten mit Vorkommen in Mitteleuropa: Neuerungen, Präzisierungen und Anwendungen. – Natur und Landschaft 79 (6): 271 – 275.

GRUTTKE H., LUDWIG G., SCHNITTLER M., BINOT-HAFKE M., FRITZLAR F., KUHN J., ASSMANN T., BRUNKEN H., DENZ O., DETZEL P., HENLE K., KUHLMANN M., LAUFER H., MATERN A., MEINIG H., MÜLLER-MOTZFELD G., SCHÜTZ P., VOITH J. & WELK E. (2004). Memorandum: Verantwortlichkeit Deutschlands für die weltweite Erhaltung von Arten. – In Gruttke H. [Red.]: Ermittlung der Verantwortlichkeit für die Erhaltung mitteleuropäischer Arten. – Naturschutz und Biologische Vielfalt 8: 273-280.

GUO P., WANG Y., ZHOU X., XIE Y., WU H., GAO X. (2013). Expression of soybean lectin in transgenic tobacco results in enhanced resistance to pathogens and pests. Plant Science 211: 17-22.

HAGENBUCHER S., OLSON D. M., RUBERSON J. R., WÄCKERS F. L., ROMEIS J. (2013). Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. Critical Reviews in Plant Sciences 32 (6) 458-482.

HAGENBUCHER S., WÄCKERS F. L., ROMEIS J. (2014). Indirect multi-trophic interactions mediated by induced plant resistance: impact of caterpillar feeding on aphid parasitoids. Biology Letters 10 (2): 20130795.

HAMMER K. & K. KHOSHBAKHT (2005). Towards a `red list´for crop plant species. Genetic Resources and Crop Evolution 52: 249-265.

HARTZLER R.G. (2010). Reduction in common milkweed (*Asclepias syriaca*) occurrence in Iowa cropland from 1999 to 2009. Crop Protection 29: 1542–1544.

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. G., GARNER B. H., HAYLOCK L. A., HORNE S. L., MASON N. S., SANDS R. J. N., WALKER M. J. (2003). Invertebrate responses to the management of genetically modified herbicide -tolerant and conventional spring crops. II. Within-field epige-

al and aerial arthropods. Philosophical Transactions of the Royal Society London B 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. Philosophical Transactions of the Royal Society London B 358: 1899-1913.

HAWES C., HAUGHTON A. J., BOHAN D. A., SQUIRE G. R. (2009). Functional approaches for assessing plant and invertebrate abundance patterns in arable systems. Basic and Applied Ecology 10: 34-42.

HAWES C., SQUIRE G. R., HALLETT P. D., WATSON C. A., YOUNG M. (2010). Arable plant communities as indicators of farming practice. Agriculture, Ecosystems and Environment 138: 17-26.

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. (2003a). Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. Philosophical Transactions of the Royal Society London B 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. (2003b). Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. II. Effects on individual species. Philosophical Transactions of the Royal Society London B 358: 1833-1846.

HELLMICH R. L., SIEGFRIED B. D., SEARS M. K., STANLEY-HORN D. E., DANIELS M. J., MATTILA H. R., SPENCER T., BIDNE K. G., LEWIS L. C. (2001). Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. Proceedings of the National Academy of Sciences of the United States of America 98 (21): 11925-11930.

HILBECK A., MEIER M., RÖMBKE J., JÄNSCH S., TEICHMANN H., TAPPESER B. (2011). Environmental risk assessment of genetically modified plants - concepts and controversies. Environmental Sciences Europe 23:13.

HILBECK A., WEISS G., OEHEN B., RÖMBKE J., JÄNSCH S., TEICHMANN H., LANG A., OTTO M., TAPPESER B. (2014). Ranking matrices as operational tools for the environmental risk assessment of genetically modified crops on non-target organisms. Ecological Indicators 36: 367–38.

HILBECK A. & M. OTTO (2015). Specificity and combinatorial effects of *Bacillus thuringiensis Cry*-toxins in the context of GMO Environmental Risk Assessment. Frontiers in Environmental Science 3 (71).

HOFMANN F., SCHLECHTRIEMEN U., KUHN U., WITTICH K.-P., KOCH W., OBER S., VÖGEL R., OTTO, M. (2013). Variation of maize pollen shedding in North Germany and its relevance for GMO-monitoring. In: Breckling B. & R. Verhoeven (Eds): GM-Crop Cultivation - Ecological Effects on a Landscape Scale. Theorie in der Ökologie 17.

HOFMANN F., OTTO M., WOSNIOK W. (2014). Maize pollen deposition in relation to the distance from the nearest pollen source under common cultivation - Results of 10 years of monitoring (2001-2010). Environmental Sciences Europe 26: 24.

HOFMANN F., KRUSE-PLASS M., KUHN U., OTTO M., SCHLECHTRIEMEN U., SCHRÖDER B., VÖGEL R., WOSNIOK W. (2016). Accumulation and variability of maize pollen deposition on leaves of European Lepidoptera host plants and relation to release rates and deposition determined by standardised technical sampling. Environmental Sciences Europe 28: 14.

HOLST N., LANG A., LÖVEI G., OTTO M. (2013). Increased mortality is predicted of *Inachis io* larvae caused by *Bt*-maize pollen in European farmland. Ecological Modelling 250: 126-133.

HRON R. J., KUK M. S., ABRAHAM G. (1990) Determination of free and total gossypol by high performance liquid chromatography. Journal of the American Oil Chemists` Society 67 (3): 182-187.

ILN (1996). LANA Gutachten: Methodik der Eingriffsreglung. Gutachten zur Methodik der Ermittlung, Beschreibung und Bewertung von Eingriffen in Natur und Landschaft, zur Bemessung von Ausgleichs- und Ersatzmaßnahmen sowie von Ausgleichszahlungen. Teil III. Vorschläge zur bundeseinheitlichen Anwendung der Eingriffsregelung nach § 8 BNatSchG. Universität Hannover. Institut für Landschaftspflege und Naturschutz. Stuttgart, Juni 1996. http://www.fachdokumente.lubw.baden-wuerttemberg.de/servlet/is/50038/

IUCN (2010a). European Red List.
http://ec.europa.eu/environment/nature/conservation/species/redlist

IUCN (2010b). IUCN Red List of Threatened Species. Version 2010.1.
www.iucnredlist.org

IUCN (2012a). IUCN Red List Categories and Criteria: Version 3.1. Second Edition. IUCN. Gland, Switzerland and Cambridge, UK. iv+32pp.

IUCN (2012b). Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. IUCN. Gland, Switzerland and Cambridge, UK. iii+ 41pp.

JÄNSCH S., RÖMBKE J., HILBECK A., WEIß G., TEICHMANN H., TAPPESER B. (2011). Assessing the potential risks of transgenic plants for non-target invertebrates in Europe: a review of classification approaches of the receiving environment. BioRisk 6: 19–40.

KAKADE M. L., SIMONS N. R., LIENER I. E., LAMBERT J. W. (1972). Biochemical and nutritional assessment of different varieties of soybeans. Journal of Agricultural and Food Chemistry 20 (1) 87-90.

KAUR M., SINGH K., RUP P. J., KAMBOJ S. S., SINGH J. (2009). Anti-insect potential of lectins from *Arisaema* species towards *Bactrocera cucurbitae*. Journal of Environmental Biology 30 (6).

KEMPENAAR C., LOTZ L. A. P., VAN DER HORST C. L. M., BELTMAN W. H. J., LEEMANS K. J. M., BANNINK A. D. (2007). Trade-off between costs and environmental effects of weed control on pavements. Crop Protection 26: 430–435.

KIMBALL K. & S. A. LEVIN (1985). Limitations of laboratory bioassays: need for ecosystem-level testing. Bioscience 35 (3): 165-171.

KOWARIK I., BARTZ R., HEINK U. (2008). Bewertung „ökologischer Schäden“ infolge des Anbaus gentechnisch veränderter Organismen (GVO) in der Landwirtschaft. Naturschutz und Biologische Vielfalt 56: pp 248. Bundesamt für Naturschutz, Bonn-Bad Godesberg.

KREMEN C. & A. MILES (2012). Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecology and Society 17(4): 40.

KUUSSAARI M., HELIÖLÄ J., LUOTO M., PÖYRY J. (2007). Determinants of local species richness of diurnal Lepidoptera in boreal agricultural landscapes. Agriculture, Ecosystems and Environment 122: 366-376.

LANG A. (2004). Monitoring the impact of Bt maize on butterflies in the field: estimation of required sample sizes. Environmental Biosafety Research 3 (1): 55-66.

LANG A., LAUBER E., DARVAS B. (2007) Early-tier tests insufficient for GMO risk assessment. Nature Biotechnology 25(1):35–36.

LANG A. & M. OTTO (2010). A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (*Bt*) maize on non-target Lepidoptera. Entomologia Experimentalis et Applicata 135 (2): 121-134.

LANG A., BRUNZEL S., DOLEK M., OTTO M., THEIßEN B. (2011a). Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of *Bt*-maize effects. Proceedings of the National Academy of Sciences of the United States of America 278: 980-981.

LANG A., DOLEK M., THEIßEN B., ZAPP A. (2011b). Are adult crambid snout moths (Crambinae) and larval stages of Lepidoptera suitable tools for an environmental monitoring of transgenic crops? Insects 2: 400-411.

LANG A. & C. BÜHLER (2012). Estimation of required sampling effort for monitoring the possible effects of transgenic crops on butterflies: Lessons from long-term monitoring schemes in Switzerland. Ecological Indicators 13 (1): 29-36.

LANG, A., THEIßEN, B., DOLEK, M. (2013). Standardised methods for the GMO monitoring of butterflies and moths: the whys and hows. BioRisk 8.

LANG A., BÜHLER C., DOLEK M., ROTH T. (2016). Estimating sampling efficiency of diurnal Lepidoptera in farmland. Journal of Insect Conservation 20: 35-48.

LEPPING M. D. (2011). Nutrient composition of a transformed soybean cultivar containing aryloxyalkanoate dioxygenase-12 (AAD-12), double mutant maize EPSPS gene (2mEPSPS), and phosphinothricin acetyltransferase (PAT) - event DAS-44406-6. Study ID 101104.03. Dow AgroSciences LLC.

LIENER I. E. (1955). The photometric determination of the haemagglutinating activity of soyin and crude soybean extracts. Archives of Biochemistry and Biophysics 54 (1): 223-231.

LORENZETTI F. & V. NEGRI (2009). The European Seed Legislation on Conservation Varieties. In: Veteläinen M., Negri V., Maxted N. (Eds.). European landraces on farm conservation, management and use. Bioversity Technical Bulletin No. 15. Biodiversity International, Rome, Italy. pp 287-295.

LUDWIG G., MAY R. & CH. OTTO (2007). Verantwortlichkeit Deutschlands für die weltweite Erhaltung der Farn- und Blütenpflanzen. Vorläufige Liste. BfN Skripten 220: 1-102.BfN Bonn-Bad Godesberg.

LUDWIG G. & M. SCHNITTLER (2009). Rote Liste der Pflanzen Deutschlands. http://www.bfn.de/fileadmin/MDB/documents/RoteListePflanzen.pdf

LUNA T. & R. K. DUMROESE (2013). Monarchs (*Danaus plexippus*) and milkweeds (*Asclepias* species). Native Plants 14 (1): 5-14.

LUTMAN P. J. W., BERRY K., PAYNE R. W., SIMPSON E.; SWEET J. B., CHAMPION G. T., MAY M. J., WIGHTMAN P., WALKER K., LAINSBURY M. (2005). An update on the persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). In: Proceedings of the Second International Conference on Co-Existence between GM and non-GM based agricultural supply chains; Messean, A. (Ed), 14-15 November 2005, Montpellier, pp 93-96.

LUTMAN P. J. W., BOWERMAN P., PALMER G. M., WHYTOCK G. P. (2000). Prediction of competition between oilseed rape and *Stellaria media*. Weed Research 40 (3): 255-269.

MAO Y. B., CAI W. J., WANG J. W., HONG G. J., TAO X. Y., WANG L. J., HUANG Y. P., CHEN X. Y. (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnology 25 (11): 1307-1313.

MARSHALL E. J. P., BROWN V. K., BOATMAN N. D., LUTMAN P. J. W., SQUIRE G. R., WARD L. K. (2003). The role of weeds in supporting biological diversity within crop fields. Weed Research 43: 77–89.

MARVIER M. & R. C. VAN ACKER (2005). Can crop transgenes be kept on a leash? Front in Ecology and the Environment 3 (2): 93-100.

MATEOS-APARICIO I., REDONDO CUENCA A., VILLANUEVA-SUÁREZ M. J., ZAPATA-REVILLA M. A. (2008). Soybean, a promising health source. Nutrición Hospitalaria 23 (4): 305-312.

MAURO I. J., MCLACHLAN S. M., VAN ACKER R. C. (2009). Farmer knowledge and a priori risk analysis: pre-release evaluation of genetically modified Roundup Ready wheat across the Canadian prairies. Environmental Science and Pollution Research International 16 (6): 689-701.

MEISSLE M., MOURON P., MUSA T., BIGLER F., PONS X., VASILEIADIS V. P., OTTO S., ANTICHI D., KISS J., PALINKAS Z., DORNER Z., VAN DER WEIDE R., GROTEN J., CZEMBOR E., ADAMCZYK J., THIBORD J.-B., MELANDER B., CORDSEN NIELSEN G., POULSEN R. T., ZIMMERMANN O., VERSCHWELE A. & E. OLDENBURG (2010). Pests, pesticide use and alternative options in European maize production: current status and future prospects. Journal of Applied Entomology 134: 357–375.

MEISSLE M., ÁLVAREZ-ALFAGEME F., MALONE L. A., ROMEIS J. (2012). Establishing a database of bio-ecological information on non-target arthropod species to support the envi-

ronmental risk assessment of genetically modified crops in the EU. EFSA Supplementing Publication: EN-334. https://www.efsa.europa.eu/en/supporting/pub/en-334

MEYER S. & CH. LEUSCHNER (2015). 100 Äcker für die Vielfalt. Initiativen zur Förderung der Ackerwildkrautflora in Deutschland. Universitätsverlag Göttingen. ISBN-978-3-86395-184-9. 351 S.

MEYER S., HILBIG W., STEFFEN K. & S. SCHUCH (2013). Ackerwildkrautschutz – Eine Bibliographie. BfN Skripten 351, BfN, Bad Godesberg. www.bfn.de

MICHIELS K., VAN DAMME E. J. M., SMAGGHE G. (2010). Plant-insect interactions: What can we learn from plant lectins? Archives of Insect Biochemistry and Physiology 73 (4): 193-212.

MEA (2005). Ecosystems and Human Well-being: Synthesis. Millennium Ecosystem Assessment. Island Press, Washington, DC, 160 pp.

NEGRI V., MAXTED N., VETELÄINEN M. (2009). European Landrace Conservation: An introduction. In Veteläinen M., Negri V., Maxted N. (Eds.). European landraces on farm conservation, management and use. Biodiversity Technical Bulletin No. 15. Biodiversity International, Rome, Italy. 1-22.

NIENSTEDT K. M., BOCK T.C.M., VAN WENSEM J., MONFORTS M., HART A., AAGAARD A., ALIX A., BOESTON J., BOPP ST.K., BROWN C., CAPRI E., FORBES V., KÖPP H., LIESS M., LUTTIK R., MALTBY L., SOUSA J.P., STREISSL F., HARDY A.R. (2012). Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Science of the Total Environment 415 (1): 31-38.

NORRIS R. F. & M. KOGAN (2000). Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. Weed Science 48 (1): 94-158.

NORRIS R. F. & M. KOGAN (2005). Ecology of interactions between weeds and arthropods. Annual Review of Entomology 50: 479-503.

O`DONOVAN J. T. (1996). Weed economic thresholds: useful agronomic tool or pipe dream? Phytoprotection 77 (1): 13-28.

OECD (2001). Consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and anti-nutrients. www.oecd.org

OECD (2009). Consensus document on compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): key food and feed nutrients and anti-nutrients, OECD Environmental Directorate, Paris, France, 32pp. ENV/JM/MONO(2004)16, www.oecd.org

OERKE E.-C. (2006). Crop losses to pests. Journal of Agricultural Science 144: 31–43.

OLIVER L. (1988). Principles of weed threshold research. Weed Technology 2 (4): 398-403.

PAN (2014). Reducing pesticide use across the EU. Implementation if the EU directive on sustainable use of pesticides. Pesticide Action Network Europe.

PASCHER K. & M. DOLEZEL (2005). Koexistenz von gentechnisch veränderten, konventionellen und biologisch angebauten Kulturpflanzen in der österreichischen Landwirtschaft. Bundesministerium für Gesundheit und Frauen. Forschungsbericht der Sektion IV. Band 2/2005.

PASCHER K. & G. GOLLMANN (1997). Ökologische Risikoabschätzung von Freisetzungen gentechnisch veränderter Organismen für die spezielle Situation in Österreich. Studie im Auftrag des Bundesministeriums für Gesundheit und Konsumentenschutz. Wien.

PASCHER K., NARENDJA F., RAU D. (2006). Feral oilseed rape – investigations on its potential for hybridisation. Research Report in commission of the Federal Ministry of Health and Women, Section IV, January 2006.

PATIL C. D., BORASE H. P., SALUNKHE R. B., SURYAWANSHI R. K., NARKHADE C. P., SALUNKE B. K., PATIL S. V. (2013). Mosquito larvicidal potential of *Gossypium hirsutum* (*Bt* cotton) leaves extracts against *Aedes aegypti* and *Anopheles stephensi* larvae. Journal of Arthropod-Borne Diseases 8 (1): 91-101.

PAULA D. P., ANDOW D. A., TIMBO R. V., SUJII E. R., PIRES C. S. S. (2014). Uptake and transfer of a *Bt* toxin by a Lepidoptera to its eggs and effects on its offspring. PLoS One 9 (4): e95422.

PERRY J. N., ROTHERY P., CLARK S. J., HEARD M. S., HAWES C. (2003). Design, analysis and statistical power of the farm-scale evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40: 17–31.

PERRY J. N., TER BRAAK C. J. F., DIXON P. M., DUAN J. J., HAILS R. S., HUESKEN A., LAVIELLE M., MARVIER M., SCARDI M., SCHMIDT K., TOTHMERESZ B., SCHAARSCHMIDT F., VAN DER VOET H. (2009): Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. Environmental Biosafety Research 8: 65-78.

PERRY J. N., DEVOS Y., ARPAIA S., BARTSCH D., GATHMANN A., HAILS R. S., KISS J., LHEUREUX K., MANACHINI B., MESTDAGH S., NEEMANN G., ORTEGO F., SCHIEMANN J., SWEET J. B. (2010). A mathematical model of exposure of nontarget Lepidoptera to *Bt*-maize pollen expressing *Cry*1Ab within Europe. Proceedings of the Royal Society B 277 (1686): 1417-1425.

PERRY J. N., DEVOS Y., ARPAIA S., BARTSCH D., GATHMANN A., HAILS R. S., KISS J., LHEUREUX K., MANACHINI B., MESTDAGH S. (2011). The usefulness of a mathematical model of exposure for environmental risk assessment. Proceedings of the Royal Society B 278 (1708): 982-984.

PERRY J. N., DEVOS Y., ARPAIA S., BARTSCH D., EHLERT C., GATHMANN A., HAILS R. S., HENDRIKSEN N. B., KISS J., MESSÉAN A. (2012). Estimating the effects of *Cry*1F *Bt*-maize pollen on non-target Lepidoptera using a mathematical model of exposure. Journal of Applied Ecology 49 (1): 29-37.

PLEASANTS J. M. & K. S. OBERHAUSER (2012). Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity. doi: 10.1111/j.1752-4598.2012.00196.x

POWER A. G. (2010). Ecosystem services and agriculture: trade-offs and synergies. Philosophical Transactions of the Royal Society B 365: 2959-2971.

POWLES S. B. (2008). Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64 (360): 365.

RAYBOULD, A. & D. VLACHOS (2010). Non-target organism effects tests on Vip3A and their application to the ecological risk assessment for cultivation of MIR162 maize. Transgenic Research 20 (3): 599-611.

RAYBOULD A., CARON-LORNIER G., BOHAN D. A. (2011). Derivation and interpretation of hazard quotients to assess ecological risks from the cultivation of insect-resistant transgenic crops. Journal of Agriculture and Food Chemistry 59: 5877-5885.

REGULATION (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Union L 396.

REGULATION (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union L 353.

REGULATION (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union L 309.

REGULATION (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union. L 309.

REGULATION (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Official Journal of the European Union L 176.

REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93.

REGULATION (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93/85.

REGULATION (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union L 317/35.

RIECKEN U., FINCK P., RATHS U., SCHRÖDER E., SSYMANK A. (2006). Rote Liste der gefährdeten Biotoptypen Deutschlands. Naturschutz & Biologische Vielfalt 34, pp 318.

ROMEIS J., HELLMICH R. L., CANDOLFI M. P., CARSTENS K., DE SCHRIJVER A., GATEHOUSE A. M. R., HERMAN R. A., HUESING J. E., MCLEAN M. A., RAYBOULD A., SHELTON A. M., WAGGONER A. (2011). Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Research 20 (1): 1-22.

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. Philosophical Transactions of the Royal Society London B 358: 1879-1898.

RÜDELSHEIM P. L. J. & G. SMETS (2012). Baseline information on agricultural practices in the EU. Cotton (*Gossypium hirsutum* L.). Study performed for EuropaBio, Belgium. http://www.europabio.org (accessed 14 Dec 2015).

RÜDIGER H. & H.-R. GABIUS (2001). Plant Lectins: occurrence, biochemistry functions and applications. Glykoconjugate Journal 18: 589-631.

RYAN M. R., SMITH R. G., MORTENSEN D. A., TEASDALE J. R., CURRAN W. S., SEIDEL R., SHUMWAY D. L. (2009). Weed-crop competition relationships differ between organic and conventional cropping systems. Weed Research 49: 572-580.

SAATGUT-GENTECHNIK VERORDNUNG (2001). Verordnung des Bundesministers für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft über die Verunreinigung von Saatgut mit gentechnisch veränderten Organismen und die Kennzeichnung von GVO-Sorten und Saatgut von GVO-Sorten (Saatgut-Gentechnik-Verordnung) StF: BGBl. II Nr. 478/2001.

SANCO (2002a). Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC. Draft Working Document. SANCO/10329/2002rev2final, 17 October 2002.

SANCO (2002b). Guidance document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC. Working Document. SANCO/3268/2001rev.4(final), 17 October 2002.

SANVIDO O., ROMEIS J., GATHMANN A., GIELKENS M., RAYBOULD A., BIGLER F. (2012). Evaluating environmental risks of genetically modified crops: ecological harm criteria for regulatory decision-making. Environmental Science & Policy 15: 82-91.

SCHUPPENER M., MÜHLHAUSE J., MÜLLER A.-K., RAUSCHEN S. (2012). Environmental risk assessment for the small tortoiseshell *Aglais urticae* and a stacked *Bt*-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes. Molecular Ecology. doi: 10.1111/j.1365-294X.2012.05716.x

SETTELE, J., SHREEVE, T., KONVIČKA, M., VAN DYCK, H. (2009). Ecology of Butterflies in Europe. Cambridge University Press. The Edinburgh Building, Cambridge CB2 8RU, UK.

SHUKLA S., ARORA R., SHARMA H. C. (2005). Biological activity of soybean trypsin inhibitor and plant lectins against cotton bollworm/legume pod borer, *Helicoverpa armigera*. Plant Biotechnology 22 (1): 1-6.

SHUKLE R. H. & L. L. MURDOCK (1983). Lipoxygenase trypsin inhibitor, and lectin from soy-beans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). Environmental Entomology 12 (3): 787-791.

SINGH K., KAUR M., RUP P. J., SINGH J. (2006). Exploration for anti-insect properties of lectin from seeds of soybean (*Glycine max*) using *Bactrocera cucurbitae* as a model. Phytoparasitica 34 (5): 463-473.

SPILATRO S. R., COCHRAN G. R., WALKER R. E., CABLISH K. L., BITTNER C. C. (1996). Characterization of a new lectin of soybean vegetative tissues. Plant Physiology 110 (3): 825-834.

SQUIRE G. R., HAWES C., BOHAN D. A., BROOKS D. R., CHAMPION G. T., FIRBANK L. G., HAUGHTON A. J., HEARD M. S., MAY M. J., PERRY J. N., YOUNG M. W. (2005). Biodiversity effects of the management associated with GM cropping systems in the UK. Final report of Defra Project EPG 1/5/198. Defra, UK.

SQUIRE G. R., HAWES C., BEGG G. S., YOUNG M. W. (2009). Cumulative impact of GM herbicide-tolerant cropping on arable plants assessed through species-based and functional taxonomies. Environmental Science and Pollution Research 16: 85–94.

STORKEY J., MEYER S., STILL K. S., LEUSCHNER C. (2012). The impact of agricultural intensification and land-use change on the European arable flora. Proceedings of the Royal Society B 279: 1421–1429.

SUKOPP U. & H. SUKOPP (1993). Das Modell der Einführung und Einbürgerung nicht einheimischer Arten. Ein Beitrag zur Diskussion über die Freisetzung gentechnisch veränderter Kulturpflanzen. GAIA 2 (5): 267-288.

SUNILKUMAR G., CAMPBELL L. M., PUCKHABER L., STIPANOVIC R. D., RATHORE K. S. (2006). Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proceedings of the National Academy of Sciences 103 (48): 18054-18059.

SWANTON C. J., WEAVER S., COWAN P., VAN ACKER R., DEEN W., SHRESHTA A. (1999). Weed thresholds: theory and applicability. Journal of Crop Production 2 (1): 9-29.

SWEDISH CHEMICALS AGENCY (2013). Restriction report. Proposal for a restriction Nonylphenol and Nonylphenolethoxylates in textiles. http://echa.europa.eu

SWIFT M. J., IZAC A.-M. N., VAN NOORDWIJK M. (2004). Biodiversity and ecosystem services in agricultural landscapes – are we asking the right questions? Agriculture, Ecosystems and Environment 104: 113-134.

THAKUR K., KAUR M., KAUR S., KAUR A., KAMBOJ S. S., SINGH J. (2013): Purification of *Colocasia esculenta* lectin and determination of its anti-insect potential towards *Bactrocera cucurbitae*. Journal of Environmental Biology 34 (1): 31-36.

TRAXLER A., MINARZ E., ENGLISCH T., FINK B., ZECHMEISTER H. & ESSL F. (2005a) 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), 286 pp. www.umweltbundesamt.at

TRAXLER A., MINARZ E., HÖTTINGER H., PENNERSTORFER J., SCHMATZBERGER A., BANKO G., PLACER K., HADROBOLEC M. & H. GAUGITSCH (2005b). Biodiversitäts-Hotspots der Agrarlandschaft als Eckpfeler für Risikoabschätzung und Monitoring von GVO. Forschungsbericht der Sektion IV, Band 5/2005. Bundesministerium für Gesundheit und Frauen. Wien. www.bmgf.gv.at

UMWELTBUNDESAMT (2015). Protection of Biodiversity in the Risk Assessment and Risk Management of Pesticides (Plant Protection Products & Biocides) with a focus on arthropods, soil organisms and amphibian. Texte 76/2015, Umweltbundesamt Dessau-Roßlau, Germany. www.umweltbundesamt.de

UMWELTBUNDESAMT (2016). 5-Punkte-Programm für einen nachhaltigen Pflanzenschutz. Umweltbundesamt Dessau-Roßlau, Germany. www.umweltbundesamt.de

VACHER C., WEIS A. E., HERMANN D., KOSSLER T., YOUNG C., HOCHBERG M. E. (2004). Impact of ecological factors on the initial invasion of *Bt* transgenes into wild populations of birdseed rape (*Brassica rapa*). Theoretical and Applied Genetics 109: 806-814.

VAN DAMME E. J., BARRE A., ROUGÉ P., PEUMANS W. J. (2004). Cytoplasmic/nuclear plant lectins: a new story. Trends in Plant Science 9 (10): 484-489.

VAN DER VOET H., PERRY J. N., AMZAL B., PAOLETTI C. (2011). A statistical assessment of differences and equivalences between genetically modified and reference plant varieties. BMC Biotechnology 11:15.

VAN ELSEN T. & H. LORITZ. (2013). Vielfalt aus der Samentüte? Naturschutz und Landschaftsplanung 45 (5): 155-160. ISSN 0940-6808, Stuttgart.

VAN ELSEN T., BERG M., DRENCKHAHN D., DUNKEL F.-G., EGGERS T., GARVE E., KAISER B., MARQUART H., PILOTEK D., RODI D., WICKE G. (2006). Karlstadter Positionspapier zum Schutz der Ackerwildkräuter. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Sonderheft XX: 527-533, Stuttgart.

VAN FRANKENHUYZEN K. (2009). Insecticidal activity of *Bacillus thuringiensis* crystal proteins. Journal of Invertebrate Pathology 101 (1): 1-16.

VAN SWAAY C., CUTTELOD A., COLLINS S., MAES D., LOPZE MUNGUIRA M., SASIC M., SETTELE R., VERSTRAEL T., WARREN M., WIEMERS M., WYNHOFF I. (2010). European Red List of Butterflies. Publications Office of the European Union. Luxembourg.

VANDENBORRE G., VAN DAMME E. J., SMAGGHE G. (2009). Natural products: plant lectins as important tools in controlling pest insects. In: Ishaaya I. & A. Rami Horowitz (Eds.): Biorational Control of Arthropod Pests. Springer. pp 163-187.

VDI (2010). Monitoring the effects of genetically modified organisms (GMO). Standardised monitoring of butterflies and moths (Lepidoptera). Transect method, light trap and larval survey Beuth Verlag. Verein Deutscher Ingenieure, Berlin, Germany. VDI 4330.

VETELÄINEN M., NEGRI V., MAXTED N. (Eds) (2009). European landraces on farm conservation, management and use. Biodiversity Technical Bulletin No. 15. Bioversity International, Rome, Italy.

VODKIN L.O. & N.V. RAIKHEL (1986). Soybean Lectins and realted proteins in seeds and roots of Le+ and Le- soybean varieties. Plant Physiology 81: 558-565.

VON KRIES C. & G. WINTER (2011) Legal implications of the step-by-step principle. Environmental Sciences Europe 23 (1): 32.

WATKINSON A. R., FRECKLETON R. P., ROBINSON R. A., SUTHERLAND W. J. (2000). Predictions of biodiversity response to genetically modified herbicide-tolerant crops. Science 289: 1554.

WHO (2000). Air Quality Guidelines for Europe. Second Edition. WHO Regional Publications, European Series, No. 91.

WILLIAMSON M. (2001). Can the impacts of invasive plants be predicted? In: Brundu G., Brock J., Camarda I. et al. (Eds.): Plant Invasions. Species Ecology and Ecosystem Management. Backhuys Publishers, Leiden. pp 11-19.

WULFERT K. (2016). FFH-Abweichungsverfahren und artenschutzrechtliches Ausnahmeverfahren. Schriftenreihe des Fachgebietes Landschaftsentwicklung / Umwelt- und Planungsrecht 2. Kassel University Press. pp 386.

XU B., WANG Y., LIU X., YUAN F., SU N., CHEN Y., WU Y., ZHANG Q. (2006). Effects of Cry-IAc and secondary metabolites in Bt transgenic cottonseed on *Lycoriella pleuroti* (Diptera: Sciaridae). Environmental Entomology 35 (3): 807-810.

YOUNG J. E. B., GRIFFIN M. J., ALFORD D. V., OGILVY S. E. [Eds] (2001). Reducing agrochemical use on the arable Farm: The TALISMAN and SCARAB Projects. London: DEFRA.

ZANGERL A. R., MCKENNA D., WRAIGHT C. L., CARROLL M., FICARELLO P., WARNER R., BERENBAUM M. R. (2001). Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. Proceedings of the National Academy of Sciences 98 (21): 11908-11912.

ZANIN G. & M. SATTIN (1988). Threshold level and seed production of velvetleaf (*Abutilon theophrasti* Medicus) in maize. Weed Research 28 (5): 347–352.

ZHANG G. F., WAN F.-H., LÖVEI G. L., LIU W.-X., GUO J.-Y. (2006). Transmission of *Bt* toxin to the predator *Prophylaea japonica* (Coleoptera: Coccinellidae) through its aphid prey feeding on transgenic *Bt* cotton. Environmental Entomology 35 (1): 143-150.

ZHANG W., RICKETTS T. H., KREMEN C., CARNEY K., SWINTON S. M (2007). Ecosystem services and dis-services to agriculture. Ecological Economics 64 (2): 253-260.

ANNEX I

Dates and participants of three workshops for the development of suggestions for LoCs for different types of GMOs and risk areas.

Workshop 1: Limits of Concern for the risk area `Persistence and invasiveness including plant-to-plant gene-flow´, 11th-12th May 2015

Participants:

- Ulrich Heink, Heimholtz Zentrum für Umweltforschung, Leipzig, DE
- Beate Koller, ARCHE NOAH Verein zur Erhaltung der Kulturpflanzenvielfalt, Schiltern, AT
- Alexandra Ribarits, Institut für Saat- und Pflanzgut, Pflanzenschutzdienst und Bienen, Agentur Gesundheit und Ernährungssicherheit, Wien, AT
- Christina Topitschnig, Institut für Saat- und Pflanzgut, Pflanzenschutzdienst und Bienen, Agentur Gesundheit und Ernährungssicherheit, Wien, AT
- Iris Kröger, Bundesamt für Naturschutz, Bonn, DE
- Wolfram Reichenbecher, Bundesamt für Naturschutz, Bonn, DE
- Wolfgang Rabitsch, Abt. Naturschutz, Umweltbundesamt Wien, AT
- Marianne Miklau, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Helmut Gaugitsch, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Marion Dolezel, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT

Workshop 2: Limits of Concern for the risk area `impacts of the specific cultivation and management techniques´, 1st-2nd June 2015

Participants:

- Matthew Heard, Natural Environmental Research Council, Centre for Ecology and Hydrology, UK
- Wolfgang Krämer, Institut für Pflanzenschutzmittel, Agentur Gesundheit und Ernährungssicherheit, Wien, AT
- Martha Mertens, ibn – Institut für Biodiversität, München, DE
- Geoffrey Squire, John Hutton Institute, Dundee, UK
- Wolfram Reichenbecher, Bundesamt für Naturschutz, Bonn, DE
- Gerhard Zethner, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Helmut Gaugitsch, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Marianne Miklau, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Marion Dolezel, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT

Workshop 3: Limits of Concern for the risk area `LoCs for effects of the GMP on non-target organisms´, 21st -22nd September 2015

Participants:

- Thomas Bøhn, GenØk – Centre for Biosafety, Tromso, NO
- Angelika Hilbeck, ETH Zürich, Zürich, CH
- Andreas Lang, Universität Basel, Basel, CH
- Patrik Schröttle, Institut für Pflanzenschutzmittel, Agentur Gesundheit und Ernährungssicherheit, Wien, AT
- Klaus Peter Zulka, Abt. Naturschutz, Umweltbundesamt Wien, AT
- Simone Mühlegger, Abt. Chemikalien, Umweltbundesamt Wien, AT
- Wolfram Reichenbecher, Bundesamt für Naturschutz, Bonn, DE
- Andreas Heissenberger, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Marianne Miklau, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT
- Marion Dolezel, Abt. Landnutzung & Biologische Sicherheit, Umweltbundesamt Wien, AT

ANNEX II

List of participants of feedback workshop on LoCs

- Martin Batic, Ministry of the Environment and Spatial Planning (SI)
- Ulrike Middelhoff, Federal Office of Consumer Protection and Food Safety (DE)
- Klaus Swarowsky, Umweltbundesamt (DE)
- Anne-Gabrielle Wust-Saucy, Federal Office for the Environment (FOEN) (CH)
- Wolfram Reichenbecher, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Margret Engelhard, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Iris Kröger, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Hanka Teichmann, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Birgit Winkel, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Mathias Otto, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Friedrich Wassmann, Federal Agency for Nature Conservation (DE); Division II.3.3; GMO-Regulation, Biosafety
- Samson Simon, Federal Agency for Nature Conservation; Division II.3.3; GMO-Regulation, Biosafety (DE)
- Marion Dolezel, Environment Agency Austria, Landuse & Biosafety (AT)
- Andreas Heissenberger, Environment Agency Austria, Landuse & Biosafety (AT)