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SCIENTIFIC OPINION

Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

In this Scientific Opinion, the EFSA GMO Panel supplements its previous evaluations of the potential impact of maize 1507 cultivation on a range of non-target lepidopteran species using existing data on species susceptibility and considering various scenarios of exposure which may occur across Europe. The mathematical model, developed for maize MON 810, was recalibrated and extended to estimate the efficacy of certain mitigation measures. In situations where highly sensitive non-target Lepidoptera populations might be at risk, the EFSA GMO Panel recommends that mitigation measures are adopted to reduce exposure. Risk managers are provided with tools to estimate global and, where needed local, mortality of exposed non-target Lepidoptera, both before and after different mitigation measures are put in place, and for different host-plant densities. Mitigation measures are only needed when the proportion of maize and uptake of maize 1507 are sufficiently high, regardless of the other parameters. If maize 1507 cultivation remains below 5% of the Agricultural Unit of Account, then risk mitigation measures are not required. In addition, the EFSA GMO Panel recommends case-specific monitoring to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and scientific uncertainty for (1) the possible resistance evolution to the Cry1F protein in target pests, and (2) the risk to sensitive non-target Lepidoptera from maize 1507 pollen. The EFSA GMO Panel also considers that the plan for general surveillance, and in particular the methodology, needs further details according to the requirements of its 2011 Guidance Document on post-market environmental monitoring of generically modified plants, as well as its Scientific Opinion on the annual 2009 monitoring report on maize MON 810. The EFSA GMO Panel concludes that, subject to appropriate management measures, maize 1507 cultivation is unlikely to raise safety concerns for the environment.

KEY WORDS

GMO, maize (*Zea mays*), 1507, insect resistance, non-target organisms, Lepidoptera, environmental safety, post-market environmental monitoring, mathematical modelling

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SUMMARY

Considering the recurrent requests of the European Commission for reanalysis of the 2005 Scientific Opinion on genetically modified (GM) maize event 1507, the European Food Safety Authority (EFSA) asked the Panel on Genetically Modified Organisms (EFSA GMO Panel) to update the previous environmental risk assessment (ERA) of maize 1507 in light of the scientific data and methodology currently available, and to consider the possible adverse effects that the cultivation of maize 1507 might have on non-target organisms (e.g., Lepidoptera). In addition, the EFSA GMO Panel was asked to reconsider its recommendations to risk managers for methods to reduce exposure and mitigate risks linked to maize 1507 cultivation.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the initial notification C/ES/01/01 for cultivation of maize 1507, including additional data supplied by the applicant and relevant scientific publications.

The EFSA GMO Panel recalibrated its mathematical model, developed by Perry *et al.* (2010) for the ERA of a similar insect resistant maize (event MON 810), in order to simulate and assess potential adverse effects resulting from the exposure of non-target Lepidoptera (butterflies and moths) to pollen from maize 1507 under representative EU cultivation conditions, and extended it to estimate the efficacy of certain mitigation measures. The 2005 EFSA GMO Panel Scientific Opinion on maize 1507 supported '*management recommendations for the cultivation of maize 1507 [with] measures to reduce exposure of non-target Lepidoptera (as well as the target pest), such as the use of non-transgenic border rows as refugia for the target that would also reduce exposure of field margin weeds (and hence non-target Lepidoptera) to pollen from Bt-maize*'. In this Scientific Opinion, the EFSA GMO Panel has used new evidence to explore the complexities of this issue.

The EFSA GMO Panel concludes that the cultivation of maize 1507 could have the following adverse effects on the environment in the context of its intended uses (1) the adoption of altered pest control practices with higher environmental load due to potential evolution of resistance to the Cry1F protein in populations of exposed lepidopteran target pests, and (2) reductions in populations of certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants. In situations where highly sensitive non-target Lepidoptera populations might be at risk, the EFSA GMO Panel recommends that mitigation measures are adopted to reduce exposure.

Considering the wide range and variability of agro-ecosystems and protection goals within the EU, this EFSA GMO Panel Scientific Opinion provides risk managers with tools to estimate global and, where needed local, mortality of exposed non-target Lepidoptera, both before and after different mitigation measures are put in place, and for different host-plant densities. This enables risk managers to choose mitigation measures proportionate to the level of identified risk and to the protection goals pertaining to their region. Special attention should be paid to the degree of large-scale exposure as mitigation measures are only needed when the proportion of maize and uptake of maize 1507 are sufficiently high, regardless of the other parameters. If maize 1507 cultivation remains below 5% of the Agricultural Unit of Account[4,5], the global mortality is predicted to remain below 1%, even for extremely highly sensitive species, and then risk management measures are not required. Whenever mitigation measures are needed, the implementation of non-*Bt*-maize border rows will reduce the mortality of non-target lepidopteran species for both within fields and in field margins.

For protected lepidopteran species in habitats according to Directive 2004/35/EC, it is recommended that maize 1507 is not cultivated within 30 m of their habitat boundary, so that exposure and hence the risks to larvae of lepidopteran populations are minimised in these areas.

[4] For example, an uptake of 20% of maize 1507 in a region where maize represents 25% of the arable land.

[5] i.e., zv = 0.05, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.00625.

In addition to the specific concern on non-target Lepidoptera, the EFSA GMO Panel considered the possible adverse effects of maize 1507 on other non-target organisms, in order to update, where appropriate, its previous evaluations in light of new relevant scientific literature. Having considered all available relevant scientific literature, the EFSA GMO Panel concludes that no new scientific information has been made available that would invalidate the conclusions of its previous Scientific Opinions on maize 1507.

The possible resistance evolution to the Cry1F protein in lepidopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize 1507, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

The EFSA GMO Panel recommends case-specific monitoring (CSM) to assess the efficacy of risk management measures put in place to reduce levels of risk and scientific uncertainty for (1) the possible resistance evolution to the Cry1F protein in lepidopteran target pests, and (2) the risk to sensitive non-target Lepidoptera from maize 1507 pollen. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems.

For (1), the EFSA GMO Panel reiterates its earlier recommendation that appropriate insect resistance management (IRM) strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1F protein in lepidopteran target pests. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be *refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size. In addition, the EFSA GMO Panel makes additional recommendations to the applicant like (a) to focus the sampling of lepidopteran target pests in 'hotspot[6] areas' over time; (b) to include in the samplings surviving lepidopteran target pests within maize 1507 fields in order to detect potentially resistant individuals; (c) to consider regionally important lepidopteran pests (other than corn borers) of maize 1507; and (d) to revise the monitoring protocol aiming at a detecting resistance allele frequency below 5% in 'hotspot areas'. The EFSA GMO Panel recommends caution when predicting future responses of the European and Mediterranean corn borer in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge' strategy, recommends the periodic re-evaluation of the adequacy and efficacy of this IRM strategy.

For (2), the EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera. The purpose of these studies should be to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on host-plants in and adjacent to maize fields at the time of pollen deposition, and if so (a) to estimate the proportions of these populations likely to be affected; and (b) to determine the overall effect on maintaining a favourable status of these populations.

The EFSA GMO Panel agrees with the general surveillance (GS) approach of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize 1507, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize 1507 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize 1507, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize 1507, but requests that its proposals to strengthen GS are implemented. The EFSA GMO Panel considers that the current plan for GS, and in particular the methodology, needs further details according to the requirements laid down in its 2011 Scientific Opinion providing guidance on post-market environmental monitoring (PMEM) of GM plants, as well

[6] In the present document, '*hotspot area*' is defined by an area of high adoption rate of maize 1507 and the presence of multivoltine types of target pests.

as its Scientific Opinion on the annual 2009 PMEM report on maize MON 810. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

In areas where other lepidopteran pests than the European and Mediterranean corn borer are important targets of maize, they might also be subject to resistance evolution due to exposure to the Cry1F protein expressed in maize 1507. Therefore, the EFSA GMO Panel recommends these species are considered by the applicant in the context of the IRM strategy, CSM to monitor resistance evolution to the Cry1F protein in those species, as well as GS through farmer questionnaires.

The EFSA GMO Panel concludes that, subject to appropriate management measures, maize 1507 cultivation is unlikely to raise safety concerns for the environment.

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BACKGROUND AS PROVIDED BY EFSA

On 19 January 2005, the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) issued a Scientific Opinion on the notification for the placing on the market of the insect resistant genetically modified (GM) maize 1507 for feed uses, import, processing and cultivation under Part C of Directive 2001/18/EC (Notification reference C/ES/01/01) (EFSA, 2005). In its 2005 Scientific Opinion, the EFSA GMO Panel recommended that management measures be put in place to delay the possible evolution of resistance to the Cry1F protein in target Lepidoptera. The EFSA GMO Panel was also of the opinion that such measures would reduce the exposure of non-target Lepidoptera to maize 1507 pollen (EFSA, 2005). Based on the evaluation of the environmental risk assessment (ERA), the EFSA GMO Panel concluded that the cultivation of maize 1507 would not pose a significant risk to the environment.

In 2006 and 2008, the European Commission successively requested the EFSA GMO Panel to consider whether new scientific evidence published in the scientific literature required a revision of the conclusions of its 2005 Scientific Opinion on maize 1507. Following these requests, the EFSA GMO Panel evaluated the available new scientific information, and found no new evidence for adverse effects caused by the cultivation of maize 1507 (see Annexes to EFSA, 2005). Therefore, the EFSA GMO Panel concluded that no new scientific information had been made available that would invalidate its previous Scientific Opinion.

In the course of the evaluation of three applications for renewal of authorisation of (1) existing food and food ingredients produced from maize MON 810, (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation, and of (3) food and feed additives, and feed materials produced from maize MON 810 (EFSA, 2009), the EFSA GMO Panel used a new risk assessment methodology, developed by Perry *et al.* (2010), in order to simulate and assess potential adverse effects on non-target Lepidoptera after ingestion of harmful amounts of Cry1Ab-containing maize pollen deposited on their host-plants. On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise, the EFSA GMO Panel concluded that the amounts of maize MON 810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae. The EFSA Panel also noted that all modelling exercises are subject to uncertainties; as with any ecological model, further data would refine the estimates reported. Hence, the EFSA GMO Panel considered it advisable that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by management measures, in order to mitigate the possible exposure of these species to maize MON 810 pollen.

On 14 June 2010, the European Commission requested the EFSA GMO Panel to consider whether new scientific elements might require a revision of the conclusions of its previous Scientific Opinion on maize 1507. On 4 November 2010, the EFSA GMO Panel confirmed that, considering those recent studies and advances in methodology, there was a need to further analyse the potential adverse effects of maize 1507 pollen on non-target Lepidoptera, as well as to clarify its recommendations to risk managers. On 16 December 2010, EFSA endorsed a self-task mandate of the GMO Panel to review its previous safety assessment of maize 1507 in light of recent and relevant methodology and knowledge.

On 20 December 2010, the EFSA GMO Panel requested the applicant to update its application with relevant studies on non-target organisms (NTOs) performed with maize 1507 that would have been generated after the adoption of its 2005 Scientific Opinion. Following this request, the applicant provided new data to support the assessment of direct effects of the Cry1F protein on European species of non-target Lepidoptera on 22 March 2011.

Given the new data currently available on maize 1507 and recent advances in methodology (i.e., Perry *et al.*, 2010, 2011a,b; Perry, 2011a,b), the EFSA GMO Panel decided to supplement its previous conclusions and to clarify its previous recommendations to risk managers. To achieve this goal, the

EFSA GMO Panel considered the most recent relevant data published in the scientific literature, along with the new data submitted by the applicant.

On 5 July 2011, the European Commission asked the EFSA GMO Panel to consider the plan for PMEM of maize 1507 in light of the 2011 Scientific Opinion providing guidance on PMEM of GM plants (EFSA, 2011a).

TERMS OF REFERENCE AS PROVIDED BY EFSA

The EFSA GMO Panel is requested:

- to update the previous evaluation of the ERA of maize 1507 in light of the scientific data and methodology currently available, focusing mainly on possible adverse environmental effects that maize 1507 cultivation may have on non-target organisms (e.g., Lepidoptera);

- to clarify and, where appropriate, to elaborate its previous recommendations to risk managers;

- to investigate whether additional data are needed from the applicant who submitted the application for placing on the market of maize 1507;

- to consider the initial PMEM plan of maize 1507 in light of its 2011 Scientific Opinion providing guidance on PMEM of GM plants.

EVALUATION

1. INTRODUCTION

Maize 1507 has been developed to provide protection against certain lepidopteran target pests (such as the European corn borer, *Ostrinia nubilalis,* and species belonging to the genus *Sesamia*) by the introduction of a part of a *Bacillus thuringiensis* gene encoding the insecticidal Cry1F protein. Maize 1507 also expresses the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein expressed in maize 1507 has been used as selectable marker to facilitate the selection process of transformed plant cells. Since the scope of the application does not cover the use of glufosinate-ammonium-containing herbicides on maize 1507, potential effects due to the use of such herbicides on maize 1507 are not considered by the EFSA GMO Panel.

2. ENVIRONMENTAL RISK ASSESSMENT

2.1. Target specificity of the Cry1F protein[7]

Several lower-tier bioassays with NTOs were performed by the applicant to assess the biological activity, and hence to define the target specificity of the Cry1F protein (see Table 1 for an overview). These studies indicated the lack of toxicity of the Cry1F protein to several arthropod species, representative of different functional groups, such as the predatory ladybird beetle *Hippodamia convergens*, the pollinating honeybee *Apis mellifera*, the parasitic wasp *Nasonia vitripennis*, the predatory green lacewing *Chrysoperla carnea*, and the detrivorous springtail *Folsomia candida*. Most of these non-target arthropod studies were performed with the purified Cry1F protein (*Bt*-plant or *E. coli*-produced). The equivalence of the Cry1F protein produced by *E. coli* and maize 1507 was shown by the applicant[8] and previously evaluated by the EFSA GMO Panel (EFSA, 2005). The toxicity of the Cry1F protein to NTOs that are not arthropods, such as earthworms (*Eisenia fetida*), fish, birds, and mammals, was also analysed by the applicant.

The studies provided by the applicant confirmed that the target specificity of the insecticidal Cry1F protein is limited to arthropod species of the order of Lepidoptera, as no adverse effects on NTOs tested have been reported (see also EPA, 2005; OECD, 2007). Whilst scientific uncertainty has been expressed about the mode of action and specificity of Cry proteins (see publications by Hilbeck and Schmidt , 2006; Then, 2009), the EFSA GMO Panel considers that these are sufficiently understood to inform the ERA of Cry-expressing plants. The general mode of action of Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of susceptible lepidopteran species, leading to death of larvae through pore formation, cell burst and subsequently septicemia (Broderick *et al.*, 2006, 2009; OECD, 2007; Bravo and Soberón, 2008; Raymond *et al.*, 2009; Soberón *et al.*, 2009; Van Frankenhuyzen *et al.*, 2010; Sanahuja *et al.*, 2011). The lepidopteran-active Cry1F protein belongs to the group of three-domain Cry proteins.

[7] Technical dossier / Section 4 / Pages 12-14
[8] Technical dossier / Section 4 / Pages 9 & 12 / Annex 25

Table 1: Overview of laboratory, greenhouse and field studies provided by the applicant that investigate the potential adverse effects of the Cry1F protein or of Cry1F-expressing maize on NTOs

Functional group	Species	Type of study*	Test material	Source of data and additional citations
		Arthropods		
Detrivore / terrestrial / springtail	*Folsomia candida*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007
Herbivore / terrestrial / butterfly larvae	*Danaus plexippus*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007
Predator / terrestrial / ladybird beetle adults	*Hippodamia convergens*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007; [9]
Predator / terrestrial / green lacewing larvae	*Chrysoperla carnea*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007; [10]
Parasitoid / terrestrial / wasp adults	*Nasonia vitripennis*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007; [11]
Pollinator / terrestrial / honeybee larvae	*Apis mellifera*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007; [12]
		Tier 1b	Cry1F-containing pollen	
Filter-feeder / aquatic / water flea	*Daphnia magna*	Tier 1b	Cry1F-containing pollen	EPA, 2005; OECD, 2007
		Organisms that are not arthropods		
Soil decomposer / earthworm adults	*Eisenia fetida*	Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007; [13]
Northern bobwhite quail juveniles	*Colinus virginianus*	Tier 1b	Cry1F-containing grain	EPA, 2005; OECD, 2007
Mice		Tier 1a	Cry1F pure protein	EPA, 2005; OECD, 2007
Broiler chicken		Tier 1b	Cry1F-containing grain	EPA, 2005; OECD, 2007; [14]
Rainbow trout juveniles	*Onchorhynchus mykiss*	Tier 1b	Cry1F-containing grain	EPA, 2005; OECD, 2007; [15]

*In its guidelines for the ERA of GM plants (EFSA, 2010a) and for the assessment of potential impacts of GM plants on NTOs (EFSA, 2010b), the EFSA GMO Panel considered three main tiers, which comprise experimental studies under controlled conditions (e.g., laboratory studies under tier 1a and 1b and semi-field studies under tier 2) and field studies (tier 3). Tier 1a refers to *in vitro* studies carried out with purified metabolites, whereas tier 1b refers to *in planta* testing using bi- or multi-trophic experiments. Semi-field studies are outdoor experiments carried out with some containment that controls for variability, with manipulation treatments on relatively small experimental units (e.g., caged plants, screen houses).

2.2. Interactions of the GM plant with target organisms[16]

The potential of maize 1507 to cause adverse effects through direct or indirect interactions between the GM plant and target organisms was previously evaluated by the EFSA GMO Panel (EFSA, 2005 and its annexes, EFSA, 2010c), and the outcome of these evaluations, which has been updated to consider new relevant scientific literature, is described below.

[9] Technical dossier / Section 4 / Page 12 / Annex 27
[10] Technical dossier / Section 4 / Page 12 / Annex 26
[11] Technical dossier / Section 4 / Page 12 / Annex 28
[12] Technical dossier / Section 4 / Page 13 / Annex 31
[13] Technical dossier / Section 4 / Pages 13 & 14 / Annex 29
[14] Technical dossier / Section 4 / Page 10 / Annex 5
[15] Technical dossier / Section 4 / Page 14 / Annex 24
[16] Technical dossier / Section 4 / Pages 11-12

Because genetic resistance to chemicals, and behavioural resistance to host-plant defenses and cultural practices (Onstad, 2008) such as crop rotation are known to evolve in insect pests, including lepidopteran species (Whalon *et al.*, 2011), the potential evolution of insect resistance to Cry proteins constitutively expressed in *Bt*-crops is considered a relevant environmental and agronomic concern by the scientific community (e.g., Tabashnik *et al.*, 2008a,b, 2009; BEETLE report, 2009). Resistance evolution in target pest(s) to the Cry1F protein is not considered a direct environmental harm, but the consequences of the establishment of lepidopteran target pests with resistance to the Cry1F protein could be that farmers would use other target pest control methods (e.g., insecticides) resulting in higher environmental load or the displacement of biocontrol programmes at a larger scale (Andow, 2008). Other regionally important lepidopteran pests (e.g., *Sesamia cretica*, *Helicoverpa armigera*, *Mythimna unipuncta*) exposed to maize 1507 may also have the potential to evolve resistance to the Cry1F protein.

Instances of field resistance to *Bt*-maize have been reported outside Europe for two lepidopteran target pests in maize that are not present in the European fauna (Tabashnik *et al.*, 2009; Huang *et al*., 2011): *Busseola fusca* (Van Rensburg, 2007; Kruger *et al.*, 2009, 2011b) and *Spodoptera frugiperda* (Matten *et al.*, 2008; Moar *et al.*, 2008; Tabashnik, 2008; Tabashnik *et al.*, 2008a; Storer *et al.*, 2010). Field resistance is defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik, 1994; Andow, 2008).

The first instance of field resistance to *Bt*-maize has been reported in a population of the African stem borer (*B. fusca*) in South Africa, where some larvae were able to survive on Cry1Ab-expressing maize (Van Rensburg, 2007; Kruger *et al.*, 2009, 2011b). It appeared that the field resistance in stem borer in this area has resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting providing a continuous supply of moths (Kruger *et al.*, 2009). The recent survey by Kruger *et al.* (2011a) revealed that compliance with *refugia* requirements in the region was low especially during the initial 5-7 years after release and high number of farmers applied insecticides as preventative sprays on *Bt*-maize and *refugia* irrespective of stem borer infestation levels.

The second instance concerns fall armyworm, *S. frugiperda*. Larvae surviving on Cry1F-expressing maize in some fields on an isolated tropical island in the USA (Puerto Rico) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, where no mortality was observed (Matten *et al.*, 2008; Moar *et al.*, 2008; Tabashnik, 2008; Tabashnik *et al.*, 2008a). Recently, Storer *et al.* (2010) confirmed via laboratory bioassays that *S. frugiperda* collected from the affected area exhibited lower sensitivity to the Cry1F protein compared with typical colonies from other regions, and that the resistance was shown to be autosomally inherited and highly recessive. The unusual combination of biological, geographic, and operational factors (such as high selection pressure for resistance by continuous silage maize production with sequential year-round plantings, high level of overall *S. frugiperda* pest pressure during the year of observing its damage on Cry1F expressing hybrids, drought conditions reducing availability of alternative host plants that encouraged movement of the adult and larval populations into irrigated agricultural maize fields) led to *S. frugiperda* evolving resistance to the Cry1F protein in Puerto Rico. Moreover, no insect resistance management (IRM) measures were put in place at that time in Puerto Rico.

It is considered very unlikely that the levels of selection pressure exerted on *S. frugiperda* by maize 1507 cultivations in Puerto Rico will be experienced in the EU, with the exception of overseas territories. *S. frugiperda* is a migratory seasonal pest across most of the USA, cannot develop at temperatures below 12°C, and displays one or two generations per year in the USA. This reduced selection pressure on *S. frugiperda*, the availability of alternative host-plants and the implementation of IRM plans make the evolution of resistance as observed in Puerto Rico unlikely in other regions.

In other regions where maize 1507 has been cultivated (USA since 2001, Japan and Canada since 2002, Argentina since 2005, Brazil since 2008), no instances of field resistance were reported so far (Tabashnik *et al.*, 2009; Huang *et al.*, 2011). Considering that European climatic and maize cultivation conditions differ from those observed in Puerto Rico and South Africa, it is unlikely that field resistance would develop rapidly in EU target pests such as the European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn borer (*Sesamia nonagrioides*).

However, in line with its previous evaluations of the cultivation of Cry1-expressing maize events, the possible evolution of resistance to the Cry1F protein in lepidopteran target pests is considered by the EFSA GMO Panel as a relevant environmental and agronomic concern associated with the cultivation of maize 1507, as the consequences of resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

2.3. Interactions of the GM plant with non-target organisms[17]

The potential of maize 1507 to have direct or indirect adverse effects on NTOs and the ecological functions they provide in agro-ecosystems was previously evaluated by the EFSA GMO Panel (EFSA, 2005 and its annexes ; EFSA, 2010c). The outcome of these evaluations has been updated in light of new relevant scientific literature, and is described below.

In agro-ecosystem, NTOs provide key ecological functions (including ecosystem services), such as plant pollination, biological control and decomposition, and form important components of farming systems (Sanvido *et al.*, 2009; Arpaia, 2010). Therefore, it is important that the main functional groups mediating these ecological functions as well as their responses to GM plants are considered in the ERA of GM plants (EFSA, 2010c). Because not each of the potentially exposed non-target species can be tested from a practical viewpoint, toxicity of Cry proteins is generally tested on a representative subset of species using a tiered approach (Garcia-Alonso *et al.*, 2006; Rose, 2007; Romeis *et al.*, 2006, 2008a). Lower-tier studies represent a first important step to reach reliable risk assessment conclusions, as they give indications of possible hazards associated with the cultivation of GM plants (see section 2.1). In case a hazard has been identified in lower-tier studies, a detailed exposure characterisation is required to fully characterise the possible risk.

2.3.1. Pollinators

Maize pollen can be collected, stored and consumed by pollinators such as honeybees, especially in regions where there are limited sources of pollen when maize is flowering. Pollen feeding is a route of exposure of honeybees to the Cry1F protein expressed in maize 1507 (e.g., BEETLE report, 2009).

The applicant assessed possible adverse effects of the Cry1F protein on pollinators. In lower-tier dietary bioassays with *Apis mellifera* using either purified Cry1F protein or Cry1F-containing maize pollen incorporated into diet, no adverse effects on larval survival or adult behaviour were reported[18]. In the only peer-reviewed paper assessing the impact of the Cry1F protein on honeybee, Hanley *et al.* (2003) came to similar conclusions as those reported by the applicant (see Table 1, above). Feeding honeybee larvae with the Cry1Ab- or Cry1F-containing maize pollen did not affect larval mortality, pupal mortality, pupal weight or haemolymph protein concentration, compared with larvae fed regular bee-collected pollen or non-transgenic maize pollen.

Malone and Burgess (2009), who reviewed available scientific data on potential adverse effects on honeybees of Cry1 proteins or Cry1-containing maize pollen gathered either under lower- or higher-tier studies, concluded that none of the *Bt*-maize events commercially available at the time of the publication have significant impacts on the health of honeybees. Based on a meta-analysis of 25 independent laboratory studies assessing direct effects on honeybee survival of Cry proteins from currently commercialised *Bt*-crops, Duan *et al.* (2008) noted that the assessed Cry proteins do not negatively affect the survival of either honeybee larvae or adults in laboratory settings. However, Duan *et al.* (2008) considered that in field settings, honeybees might face additional stresses, which could theoretically affect their susceptibility to Cry proteins and generate indirect effects.

[17] Technical dossier / Section 4 / Pages 12-14
[18] Technical dossier / Section 4 / Page 13 / Annex 31

In most cases, the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low (Babendreier *et al*., 2004). The EFSA GMO Panel has no reason to consider that maize 1507 will cause reductions to pollinating insects that are significantly greater from those caused by cultivation of conventional maize.

2.3.2. Natural enemies

Since approximately up to a 1,000 non-target arthropod species can occur in maize fields in the EU (Knecht *et al.*, 2010), several beneficial non-target arthropods such as natural enemies (predators and parasitoids) are likely to be exposed to *Bt*-maize plants and the Cry protein(s) they express when cultivated. These natural enemies can be exposed to Cry proteins when feeding on plant material (including pollen) or honeydew excreted from sap-sucking species, and/or when feeding on prey/host organisms which have previously been feeding on *Bt*-maize (Andow *et al.*, 2006; Romeis *et al.*, 2006, 2008a,b; Lundgren, 2009). These species however are only at risk if the Cry proteins show toxicity at a realistic level of exposure. In some cases, generalist predators show complex feeding habits whereby they feed on plant parts, numerous herbivore prey species and other predators (intra-guild predation) (Arpaia, 2010). Parasitoids can be exposed to metabolites expressed in GM plants via one or more trophic levels (i.e., direct feeding on GM plant material, mainly nectar or exudates, or through their hosts which have previously been feeding on GM plant tissues or their excretions). Data on the susceptibility of natural enemies to Cry1 proteins are available in the scientific literature (reviewed by Romeis *et al.*, 2006, 2008a,b; Lövei *et al.*, 2009), though most data have been generated and analysed for the Cry1Ab and Cry1Ac proteins.

The applicant reported on a number of higher-tier studies[19] (see also EPA, 2005). These studies did not reveal adverse effects on the number and abundance of beneficial and non-target arthropods associated with the cultivation of maize 1507, though in some cases fewer parasitic hymenoptera were observed.

Higgins *et al.* (2009) conducted a 3-year field study with maize 1507 at four locations in the USA, and surveyed a large group of non-target arthropods, including predators, parasitoids, herbivores and detritivores. The range of sampled taxa (including ladybird beetles, lacewings, rove beetles, ground beetles, aphids, thrips, springtails, parasitic wasps, spiders) can be considered sufficiently representative (in functional terms) of maize ecosystems in Europe. Visual counts on maize plants, sticky traps, pitfall traps and litterbags were used to sample specific groups of NTOs. Field data were analysed with a multivariate method to account for general community level responses, whereas an analysis of variance on individual taxa was performed when species abundance was sufficiently high to detect statistically 50% differences. No significant differences in abundance were observed between arthropod assemblages in maize 1507 and its near-isogenic control in any of the field experiments. The first component of the multivariate analysis explained on average the high percentage of 60.3 of the overall variability. All taxa contributed similarly to indices of community abundance and analysis of single taxa always produced differences falling between confidence limits of taxon abundance, thus indicating no significant treatment effects.

On the basis of the data delivered by the applicant and obtained from a literature survey, the likelihood of adverse effects on non-target natural enemies is foreseen to be very low. Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize 1507 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers.

[19] Technical dossier / Section 4 / Page 13 / Annexes 30, 33 & 36

2.3.3. Non-target soil arthropods

2.3.3.1. Fate of Cry1F protein in soil

Proteins are a major nitrogen and carbon source for soil microorganisms. They are readily degradable by widely abundant extracellular microbial proteases (Jan *et al.*, 2009) and there is no indication that Cry proteins would generally behave differently compared to other proteins (reviewed by Icoz and Stotzky, 2008). Even though Cry proteins released into soil from root cells or decaying plant material are degraded within weeks, a small fraction may persist longer under certain environmental conditions. Laboratory studies have shown that, due to their chemical properties (e.g., surface charges), Cry proteins can be sorbed by organo-mineral surfaces, i.e., those provided by clay particles or humic complexes, thereby reducing their accessibility for soil proteases (e.g., Tapp *et al.*, 1994; Tapp and Stotzky, 1995, 1998; Crecchio and Stotzky, 1998, 2001; Pagel-Wieder *et al.*, 2007; Madliger *et al.*, 2011). This sorption slows down degradation rates compared to purely water-dissolved proteins. However, since sorption is an equilibrium process, it does not completely protect Cry proteins from degradation. Depending on the Cry protein and type of soil, sorption characteristics and degradation rates in soil can vary. Due to their relatively strong sorption to soil components, Cry1Ab, the most extensively studied Cry1 protein from GM crops in the literature, exhibits relatively strong sorption characteristics, and was found to be degraded more slowly in soil under similar conditions than e.g. Cry3Bb1 (Baumgarte and Tebbe, 2005; Miethling-Graff *et al.*, 2010). Soil incubation studies with Cry1F under defined laboratory conditions indicated rapid degradation of Cry1F proteins in soil (Herman *et al.*, 2001, 2002). In context of an ERA, the main question is whether the sorption of Cry1F would result in its accumulation in soil up to concentrations that would become toxic to certain non-target soil organisms due to the repeated and large-scale cultivation of maize 1507. Evidence from the cultivation of maize MON810, which expresses the previously mentioned Cry1Ab protein, has never indicated such accumulations under field conditions (Hopkins and Gregorich, 2003, 2005; Baumgarte and Tebbe, 2005; Dubelman *et al.*, 2005; Andersen *et al.*, 2007; Hönemann *et al.*, 2008; Icoz *et al.*, 2008; Gruber *et al.*, 2011) suggesting that despite sorption, degradation rates were sufficiently high. Similarly, field studies with maize 1507 did not detect Cry1F proteins in soils after three subsequent years of their cultivation at three different sites. The threshold of detection was very sensitive with 4.5 ng per g soil dry weight (Shan *et al.*, 2008). Thus, there is no indication that the environmental persistence of the Cry1F protein and the subsequent exposure of non-target soil organisms to this protein would be higher than that for Cry1Ab or similar proteins.

The EFSA GMO Panel concludes that, though the data on the fate of the Cry1F protein and its potential interactions in soil are limited, the relevant scientific publications analysing the Cry1F protein, together with the relatively broad knowledge about the environmental fate of other Cry1 proteins, do not indicate any novel risks that would change its previous conclusion that there are no significant direct effects on the soil environment (EFSA, 2005).

2.3.3.2. Risk to non-target soil arthropods

Springtails and mites are important in the breakdown and recycling of crop residues, and are key indicator species of soil functionality and quality. Since these micro-arthropods can be exposed to the Cry1F protein in the *Bt*-maize field environment, they and the ecological functions they provide could be adversely affected by maize 1507 cultivation.

In general, no negative effects of Cry proteins on springtails and soil mites have been reported in the scientific literature (reviewed by Icoz and Stotzky, 2008). Furthermore, in a lower-tier study performed by the applicant the springtail *Folsomia candida* fed a diet containing the Cry1F protein was not adversely affected (see Table 1, see above).

In addition, Cry1F protein concentrations in decaying plant residues from maize 1507 decrease rapidly and do not accumulate in soil. Therefore, non-target soil organisms will be exposed to relatively low Cry1F protein concentrations within a few months after harvest.

The EFSA GMO Panel considers that there is no evidence to indicate that the cultivation of maize 1507 is likely to cause adverse effects on non-target soil arthropods such as springtails and soil mites due to the expression of the Cry1F protein.

2.3.4. Non-target aquatic arthropods (such as Trichoptera: caddisfly)

Based on findings reported by Rosi-Marshall *et al.* (2007), concerns have been expressed about the transport of *Bt*-maize byproducts (i.e., pollen, detritus) to downstream water bodies and their potential toxic effects on non-target aquatic organisms following consumption. Based on exposure estimates, Carstens *et al*. (2011) identified shredders (Cummins *et al*., 1989) as the functional group most likely to be exposed to Cry proteins.

Rosi-Marshall *et al.* (2007) reported that byproducts of *Bt*-expressing maize entered headwater streams in the USA and claimed on the basis of experimental data obtained under lower-tier conditions that this would reduce growth and increase mortality of some non-target aquatic arthropods, especially trichopteran species (see also Chambers *et al*., 2010). 50% of filtering trichopterans collected by Rosi-Marshall *et al*. (2007) from water streams during peak pollen shed had maize pollen grains in their guts and detritivorous trichopterans were located in accumulations of decomposing maize litter in the streams after harvest.

Rosi-Marshall *et al*. (2007) showed effects on mortality of *Lepidostoma liba* and *Helicospyche borealis* only when they were fed senesced *Bt*-maize leaves or *Bt*-maize pollen at a concentration of 2.75 gm^{-2} (a concentration that is two to three times higher than the maximum observed input rate of pollen in the field), respectively. Since important background information on levels of exposure and sensitivity of caddisflies to *Bt*-proteins are missing in the paper by Rosi-Marshall *et al.* (2007), it is widely concluded by others that the conclusions about risk made by the authors are not supported by the data presented in the paper (ACRE, 2007; EFSA, 2007; Beachy *et al*., 2008; Parrott, 2008). Nonetheless, it could be concluded that a potential hazard for trichopterans has been identified under laboratory conditions when exposed to high doses of *Bt*-proteins (EFSA, 2009).

Under exposure conditions reflecting those reported in the field, recent lower-tier bioassays with four different non-target aquatic leaf-chewing arthropod species (two caddisflies, a crane fly and an isopod) showed no effect on the larvae of caddisflies when fed senesced leaf tissues of Cry1Ab-expressing maize *ad libitum* for 30 days, whereas the negative effects observed on the crane fly and isopod were attributed to tissue-mediated differences among the isogenic line treatments (Jensen *et al*., 2010; Lamp, 2010). The authors attributed the lack of observable toxic effects in their study to the reduction of bioactivity of the Cry1Ab protein, as maize tissues used were previously exposed for two weeks to environmental conditions (terrestrial or aquatic environments). Moreover, no adverse effects on the abundance and biomass of Trichoptera have been reported in natural conditions in Tier 3 studies so far (Chambers *et al*., 2010).

While few data on the fate of Cry1 proteins in senescent and decaying maize detritus in aquatic environments are available, it is important to account for protein degradation from plant debris in an exposure assessment (Wolt and Peterson, 2010; Carstens *et al*., 2011). In their laboratory biosassays based on the European corn borer, Jensen *et al.* (2010) found no bioactivity of the Cry1Ab protein in senesced maize tissue after two weeks of exposure to terrestrial or aquatic environments, suggesting rapid degradation of the protein, although a small fraction can persist longer (Griffiths *et al*., 2009). Even though the occurrence of maize detritus and detectable levels (0.56 ng/mL) of the Cry1Ab protein were reported in water bodies located at less than 500 m from maize fields up to six months after harvest in surveyed water streams in Indiana (USA), the Cry1Ab protein concentrations detected in water bodies were small compared to those measured in fresh maize plants (cf., the mean concentration (± SD) in stream water samples that were positive for the Cry1Ab protein was 14 ± 5 ng/L with a maximum concentration of 32 ng/L) (Tank *et al*., 2010). It was also shown that Cry1Ab-expressing maize tissue does not alter degradation rates, as compared with non-*Bt*-maize (Griffiths *et al*., 2009; Swan *et al*., 2009). Considering the probability of short-term exposure and acute effects to sensitive species, Wolt and Peterson (2010) indicated no concern in 99 % of cases, with limited opportunity for chronic effects, due to the rapid degradation of the Cry1Ab protein. No specific lower-tier studies, assessing the impact of the Cry1F protein on non-target aquatic organisms and the fate of the Cry1F protein in senescent and decaying maize detritus in aquatic environments, have been reported in the scientific literature so far. Although there is indication of a potential hazard for trichopterans under laboratory conditions when exposed to high doses of Cry proteins, no substantial aquatic exposure to the Cry1F protein contained within maize plant tissue is expected. Carstens *et al.* (2011) calculated that, even under worst-case conditions, the exposure of shredders to *Bt*-maize is low. Therefore, the EFSA GMO Panel is of the opinion that it is unlikely that the Cry1F protein in maize 1507 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses.

After consideration of the published literature, the EFSA GMO Panel concludes it is unlikely that the Cry1F protein in maize 1507 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses.

2.3.5. Non-target Lepidoptera

Maize plants are not an important resource of food for indigenous Lepidoptera with the exception of a few pest species. Therefore, the main potential risk to non-target Lepidoptera is expected to be the exposure to potentially harmful amounts of pollen deposited on host-plants in or near maize 1507 fields (EFSA, 2009).

2.3.5.1. Sensitivity of larvae of lepidopteran species to maize 1507 pollen

It is well-documented that larvae of a range of Lepidoptera can be affected by the Cry1F protein with a spectrum of sensitivity which is quantitatively different from the Cry1Ab protein. The content of the Cry1F protein in maize 1507 pollen was estimated to be 32 ng/mg dry weight (EPA, 2001, 2005)[20]. The 32 ng/mg dry weight of Cry1F protein in pollen of maize 1507 is about 350 times the Cry1Ab protein content expressed in maize MON 810 pollen. Estimates of the effect of the Cry1F protein on non-target Lepidoptera is affected by two further sources of variability.

- Firstly, *within-species variability of estimates*. For any particular lepidopteran species, estimates of the sensitivity of larvae to *Bt*-protein vary (Monnerat *et al*., 1999; Saeglitz *et al*., 2006; Schuphan, 2006; Gaspers *et al*., 2010) according to: (1) whether the study utilises activated toxin or protoxins (Monnerat *et al*., 1999), (2) the populations from which the tested larvae were derived (up to 40-fold differences in LD_{50}s; Saeglitz *et al*., 2006, Schuphan, 2006), (3) the batch of toxin used (about 8-fold differences in LD_{50}s; Saeglitz *et al*., 2006), and (4) the methodologies adopted, such as surface application and diet incorporation (minor differences in LD_{50}s, Saeglitz *et al*., 2006). Gaspers *et al*. (2010) found up to 2.25-fold differences in LC_{50}s of *O. nubilalis* for the Cry1F protein in eleven European populations;

- Secondly, *between-species sensitivity*. For example, larvae of *Danaus plexippus* (the Monarch butterfly) are known to be relatively insensitive to the Cry1F protein (Hellmich *et al*., 2001). For this species the LC_{50} for maize MON 810 is considerably less than that for maize 1507; similarly for *S. nonagroides* (the Mediterranean corn borer) (González-Cabrera *et al*., 2006). However, for larvae of *S. frugiperda* (the fall armyworm moth) (Wolt *et al*., 2005), the reverse is the case. There is considerable variability between studies for *O. nubilalis* (the European corn borer) (Siqueira *et al*., 2004; Wolt *et al*., 2005; Gaspers, 2009). Other species for which LC_{50}s have been determined for the Cry1F protein include *Ostrinia furnacalis* (the Asian corn borer) (Xu *et al*., 2010), *Plutella xylostella* (the Diamondback moth), and several other, mainly pest species reported by Wolt *et al.* (2005). There is clearly considerable variability in sensitivity between species and the studies above may also be affected by extra intra-specific variability induced by the factors discussed

[20] Note that this supersedes an earlier determination of 17.5 ng/mg dry weight that was submitted prior to the use by the applicant of an improved protein extraction and quantification system.

above. The sensitivity of the pest species *Galleria mellonella* (see Annexes to EFSA, 2005) could not be quantified by Hanley *et al*. (2003) because, although the Cry1F protein clearly caused mortality, the non-standard bioassay technique involved a diet comprising solely of pollen offered in no-choice feeding trials.

Estimates of the sensitivity of first instars of various lepidopteran species to the Cry1F protein were given in Table 1 of Wolt *et al*. (2005) (and see also Wolt, 2011). However, the great majority of those species reported by Wolt *et al*. (2005) are pest species. Reported species sensitivities of laboratory populations, quoted as the average lethal concentration in units of µg Cry1F g^{-1} diet that kills half of the larvae affected (LC_{50}), ranged widely, from 0.065 to 410. Using a conversion factor in which 825 maize 1507 pollen grains cm^{-2} leaf is equivalent to 1 µg g^{-1} diet, this equates to a range from 54 to 338,352 grains cm^{-2}. The data have a geometric mean LC_{50} approximately equivalent to *ca*. 8,500 maize pollen grains cm^{-2}. This estimate, reported by Wolt *et al*. (2005), might be a slight overestimate, although the EFSA GMO Panel considers it would be unadvisable to attempt a more accurate estimate without further data.

Wolt and Conlan (2001)[21] fitted a normal distribution to these data and then extrapolated the lower value to give an estimate of the 10^{th} percentile of the sensitivity distribution as 553 grains cm^{-2}, an effect level which they regarded as conservative representation of the effect endpoint for the tier 1 risk assessment for a hypothetical sensitive species of concern. Wolt *et al*. (2005) fitted a normal distribution to these data and then extrapolated the lower value to give an estimate of the 5^{th} percentile of the sensitivity distribution as 33 grains cm^{-2}, an effect level which they regarded as representing a worst-case effect endpoint for the tier 1 risk assessment for a hypothetical sensitive species of concern. However, as Wolt *et al*. (2005) stated explicitly concerning the uncertainty of their estimates: "*these data are representative values based only on exploratory assays, and do not necessarily represent definitive values*".

The choice of a particular percentile is arbitrary; a more conservative approach might replace a 5^{th} percentile by a smaller value. In this EFSA GMO Panel Scientific Opinion, a range of assumed sensitivities to the Cry1F protein from maize 1507 is studied that includes, at the lower end, a more pessimistic worst-case effect level than the 5^{th} percentile value of 33 grains cm^{-2} assumed by Wolt *et al*. (2005). In this regard it is relevant to consider the number of non-target Lepidoptera that might be potentially exposed within a maize ecosystem. Austria considers that over 150 butterfly species may be potentially exposed (Dolezel *et al*., 2007). The Rothamsted Insect Survey regularly identifies over 600 macro-lepidopteran adult moths in light-traps across the UK, although many of these traps are not in arable habitats. In a list of 500 species, about 24 are expected to be more sensitive than the lower 5^{th} percentile. By contrast, the most sensitive species would be expected to have an LC_{50} that was of the same order of magnitude as the lower 0.2 percentile of the species sensitivity distribution.

The sensitivity values selected by the EFSA GMO Panel for study in this Scientific Opinion are intended to represent a wider range of hypothetical unspecified lepidopteran species (A-E) that reflect the between-species variability in acute sensitivity to the Cry1F protein from maize 1507. The five LC_{50} values considered form a geometric series with 11.4-fold increments: (A) 1.265; (B) 14.36; (C) 163.2; (D) 1,853; and (E) 21,057 grains cm^{-2}. The smallest value for hypothetical species A, 1.265, may be considered as representing the 'worst-case', where extreme sensitivity to the Cry1F protein from maize 1507 would bring the greatest risk of mortality to a non-target lepidopteran species. This corresponds very closely to the estimated lower 0.2 percentile of the species sensitivity distribution. The next smallest value for hypothetical species B, 14.36, represents a very-highly sensitive species corresponding fairly closely to the 1^{st} percentile. Highly-sensitive species C represents a value, 163.2, between the 5^{th} and 10^{th} percentile of the distribution. Species D represents a value, 1,853, that is highly likely to be below the mean and is termed 'below-average'. The value for species E, 21,057, is highly likely to be above the mean of the distribution and is termed 'above-average'. The ranked LC_{50}

[21] Technical dossier / Section 4 / Page 13 / Annex 35

values reported by Wolt *et al*. (2005) are shown in units of maize 1507 pollen grains cm^{-2}, together with the values studied here, along with information on the species, *Vanessa cardui*, provided by the applicant as additional information[22], in Table 2 below, both on the natural and the logarithmic scale. The experimental methodology in the bioassays used to determine the LC_{50} values for *V. cardui*[16] show that the estimates obtained may be compared with confidence with the other values in Table 2. *V. cardui* is a migrant Nymphalid butterfly, which is widespread and common within Europe. The estimated LC_{50} shows that it is close to average sensitivity.

[22] Additional information dated 21/03/2011 / Annex 3

Table 2: Sensitivity of first instars of various lepidopteran species (expressed as LC_{50} values in units of maize 1507 pollen grains cm^{-2}) to the Cry1F protein, together with corresponding values for hypothetical species (A-E, representing a wider range of sensitivities) studied in this Scientific Opinion; after Wolt and Conlan (2001)[23] and Wolt *et al.* (2005). Lower percentiles were estimated from the distribution of species sensitivity

Species	Categorisation	LC_{50}	$Log_{10}(LC_{50})$
Hypothetical species A	**Extremely sensitive**	1.265	0.10
0.2 percentile (estimated from Wolt and Conlan, 2001)		1.27	0.10
1st percentile (estimated from Wolt and Conlan, 2001)		13.8	1.14
Hypothetical species B	**Very highly sensitive**	14.36	1.16
5th percentile (Wolt *et al.*, 2005)		33	1.52
Plutella xylostella		54	1.73
Hypothetical species C	**Highly sensitive**	163.2	2.21
Ostrinia nubilalis		479	2.68
10th percentile (Wolt and Conlan, 2001)		553	2.74
Spodoptera littoralis		817	2.91
Heliothis virescens		1,551	3.19
Trichoplusia ni		1,774	3.25
Hypothetical species D	**Above-average sensitivity**	1,853	3.27
Spodoptera frugiperda		1,980	3.30
Spodoptera exigua		6,435	3.81
Crambus spp.		>8,250	>3.9
Geometric mean of distribution; Wolt *et al.* (2005)		8,497	3.93
Vanessa cardui[24]		8,565	3.93
Hypothetical species E	**Below-average sensitivity**	21,057	4.32
Spodoptera litura		22,275	4.35
Danaus plexippus		>24,750	>4.4
Mamestra configurata		>29,700	>4.5
Diatraea grandiosella		>41,250	>4.6
Agrotis ipsilon		57,090	4.76
Helicoverpa armigera		>82,500	>4.9
Choristoneura fumiferana		115,500	5.07
Lymantria dispar		338,352	5.53

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[24] Additional information dated 21/03/2011 / Annex 3

2.3.5.2. Estimated mortality based on pollen exposure modelling

The EFSA GMO Panel explored a wide range of scenarios, including worst-case assumptions for the exposure of European species of non-target Lepidoptera to the Cry1F protein from maize 1507 pollen to estimate mortality and to provide quantitative risk conclusions for these species.

a) Description of the model

The EFSA GMO Panel estimated mortalities based on an adaption of an existing 11-parameter deterministic mathematical simulation model for the Cry1Ab protein for maize MON 810 (EFSA, 2009; Perry *et al.*, 2010, 2011b) to quantify the risk assessment. Exposure was modelled for combinations of five hypothetical lepidopteran species of differing sensitivities and their host-plants. The model estimates mortality for the most susceptible larval stage of the lepidopteran species concerned directly exposed to pollen of *Bt*-maize. A full exposure assessment was done but it required many factors to be taken into account, some of which had to be modelled with little available data. This approach seeks to identify conditions under which the risk is low and under which it is high, in order to make recommendations on the need for risk management. Different management options are possible to mitigate the risk according to the principle of proportionality; these are detailed in later sections of this EFSA GMO Panel Scientific Opinion.

The model quantifies the potential risk of mortality to the larvae of non-target Lepidoptera from the Cry1F-expressing maize 1507 through the ingestion of harmful amounts of pollen deposited on their host-plants for a typical European maize field of $C = 15$ ha with a $D = 2$ m margin (Perry *et al.*, 2011b). The declining relationship between pollen deposition and distance is modelled using data of Wraight *et al.* (2000, especially Figure 2). Sub-lethal effects are not addressed. Three major factors were studied: (i) a range of five assumed levels of sensitivity of Lepidoptera to the Cry1F protein from maize 1507 representing below-average, above-average, high, very high and extremely high levels (see section 2.3.5.1, above), (ii) two assumed within-crop host-plant densities (parameter e in Perry *et al.*, 2010): zero and moderate (0.01 plants m^{-2}), and (iii) a range of nine levels of mitigation in the form of sown strips of non-*Bt*-maize of different width, w (0 m, 3 m, ..., 24 m) between the main crop and the field margin. In this EFSA GMO Panel Scientific Opinion, the phrase ‘non-*Bt*-maize’ is intended to mean a maize that does not express Cry proteins which are active against Lepidoptera.

Three types of parameters of the model may be identified: (i) parameters concerned with mortality (including the five levels of sensitivity mentioned above), (ii) small-scale parameters (including the within-crop host-plant density, e, and the width of strips for mitigation, w, as mentioned above), and (iii) five large-scale parameters (see below). Mortality is estimated in two phases: firstly locally, using the ‘small-scale’ parameters, and then globally, using the ‘large-scale’ parameters. The term ‘locally’ means here spatially within the crop and its immediate margins, and temporally within the period of pollen shed. The term ‘globally’ means here after averaging over an entire landscape or regional scale and over a whole growing season. The average expected global mortality is always reduced from the local expected mortality because the latter represents an absolute ‘worst-case’ which would never occur in practice since it takes no account of large-scale processes (see Perry *et al.*, 2010). However, in contrast to the previous model for maize MON 810, here the EFSA GMO Panel focuses on providing estimates of mortality at the local, small-scale and giving information that will enable risk managers to translate these to global estimates of mortality appropriate to the region modelled, according to the multiplicative product of the five large-scale parameters.

As for the previous model for maize MON 810, the model for maize 1507 encompasses worst-case scenarios that seek to deliberately avoid the risk of underestimation of larval mortality. For example, within the range of sensitivities adopted here, the very-highly sensitive category is itself over twice as sensitive as the worst-case assumed by Wolt *et al.* (2005) (see section 2.3.5.1, above).

Choice of small-scale parameters

The assumption of a zero density for within-crop host-plants ($e = 0$) models a scenario found typically in certain regions, e.g., in Spain (data from EFSA, 2009 and Perry *et al*., 2010) and Hungary. In the areas around Madrid and Catalonia at the time of maize pollination, there are almost no host-plants for larvae to feed on in the fields or field margins, since weed control and climatic conditions suppress weed and field margin vegetation. In Hungary, Darvas *et al.* (2004) analysed the habitats of 187 protected Lepidoptera species. Of these species, only 30 species had host-plants that might have occurred in maize field margins. Of those 30, only two species, *Vanessa atalanta* and *Inachis io*, actually had any host-plants (in this case *Urtica dioica*) that would be exposed to significant deposition of maize pollen. Despite an intensive survey of ten maize fields and their margins during the first week of August, only one plant species was recorded (*U. dioica*) that was a host-plant of any of the protected species. *U. dioica* was recorded in the margins of four of the ten fields, but was not detected from any position sampled within any of the ten fields. These results are broadly consistent with the data of Novák *et al*. (2009) from a nationwide survey of weeds in arable fields at 193 locations in Hungary over the period 2007-2008, including 3,780 sampling sites within maize fields.

By contrast, evidence for a moderate host-plant density of $e = 0.01$ plants m^{-2} comes from receiving environments to the north and west of those above, including regions within UK (Heard *et al*., 2003a,b), Germany (Schmitz *et al*., 2003), and in Italy, France, the Netherlands and Denmark (Meissle *et al*., 2010). For example, the rare moth *Polia bombycina* is a species of conservation concern, and a priority species in the UK Biodiversity Action Plan. Larvae of *P. bombycina* occur from July onwards on *Sonchus* spp. and other host-plants, close to the time of pollen shed for many maize varieties. *Sonchus* spp. are present in over 80% of all fodder maize fields (Heard *et al*., 2003a,b). Surveys of arable weeds that are host-plants for Lepidoptera were reviewed by Meissle *et al.* (2010) who found that many species showed a gradation from less significant, less widespread and less frequent in central and southern Europe to relatively more significant, more widespread and more frequent in northern and western Europe; their results for northern and western Europe were consistent with those recorded by Heard *et al.* (2003a,b) for the UK. In Germany, near Bonn, Schmitz *et al*. (2003) reported monophagous lepidopteran larvae of *Schiffermuelleria schaefferella* feeding frequently on *Chenopodium album*, a common weed in maize crops (Verschwele, 2011; [25]), and monophagous larvae of *Bedellia somnulentella* and *Emmelina monodactyla* feeding frequently on *Calystegia sepium*, a common bindweed, as reported by Meissle *et al*. (2010).

There is also considerable variability between host-plant densities within regions. For example, in the Po valley in Italy, weed densities varied and did not go below 0.01 plants m^{-2} (see Appendix 2 in Perry *et al*., 2011b). It is not possible to study a wider range of weed densities in this EFSA GMO Panel Scientific Opinion. Furthermore, host-plant density depends on weed management and this varies according to the variety of maize grown and in particular whether the maize is for grain or forage. In addition, larval density (larvae m^{-2}) is a product of host-plant density (plants m^{-2}) and the density of larvae per host-plant. There is no data regarding the density of larvae per host-plant, and in particular how this might differ between the crop and the margin. The model assumes that there is no difference. It should be noted also that, within the model, it is the ratio of host-plant densities between margin and crop that is critical and not their absolute values.

The other small-scale parameter of the model, f, the host-plant density within the field margin, was set at 0.75 m^{-2}, the median value across those regions considered by Perry *et al*. (2010).

[25] http://www.slideshare.net/ARVER/invasive-arten & http://pub.jki.bund.de/index.php/JKA/article/viewFile/212/1421

Choice of the five large-scale parameters for within-crop host-plant density

The five 'large-scale' parameters (assumed independent) (see Perry *et al*., 2010) are:

- *y*, the proportion of the lepidopteran host-plant that is found within arable crops and in their margins (as opposed to other habitats);
- *z*, the proportion of arable fields that are cropped with maize (as opposed to other crops) in any year in the region;
- *v*, the proportion of all maize sown within the defined region that is cropped with maize 1507;
- *x*, the proportion of larvae that remains exposed, after allowance for a set of physical and behavioural effects that tend to reduce exposure;
- *a*, the proportion by which exposure is reduced owing to lack of temporal coincidence between the susceptible larval stage concerned and the period over which pollen from maize 1507 is shed.

There is considerable uncertainty concerning the estimated values of these parameters. However, 'conservative' values (in the sense of values that lead to relatively greater mortality), 'R(MON810)' values (median values from estimates given by experts in Perry *et al.* (2010)), 'typical' values (as judged by the EFSA GMO Panel) and 'non-conservative' values (values leading to relatively smaller mortality) are given in Table 3, below. Estimates of global estimated mortality, after allowance for these effects of large-scale exposure, are calculated by multiplying each estimated local mortality rate by the product of parameters *yzvxa* which is denoted in this Scientific Opinion as *R*. Hence, the four cases highlighted in the graphs below in this EFSA GMO Panel Scientific Opinion are: $R = 0.08$ ('conservative'), $R = 0.02$ ('R(MON810)'), $R = 0.0049$ ('typical'), and $R = 0.00024$ ('Non-conservative'). The values of the five large-scale parameters are subject to considerable regional and between species variability, thus increasing the uncertainty around the value of *R*. Because of this variability, results in this EFSA GMO Panel Scientific Opinion are presented for the full continuum of values of *R* which ranges between zero and unity. In practice, risk managers should calculate the value(s) of the key parameter *R* that pertains to their region(s).

Table 3: Estimates of the five large-scale parameters and of their product *R*

Parameter	Parameter measures proportion of	'Conservative'	R(MON810)	'Typical'	'Non-conservative'
y	Host-plants in arable, rather than non-arable habitat	0.5	0.5	0.35	0.2
z	Maize fields in arable system	0.8	0.2	0.2	0.1
v	Maize that is maize 1507	0.8	0.8	0.5	0.2
x	Larvae exposed after allowance for physical effects	0.5	0.5	0.4	0.3
a	Temporal coincidence	0.5	0.5	0.35	0.2
R = *yzvxa*		**0.08**	**0.02**	**0.0049**	**0.00024**

b) Results from the model

As would be expected, the model predicted that local and global estimated mortality decreased monotonically with the five levels of species sensitivity studied, from 'below-average' to 'extreme'. Results of the quantified risk of mortality, prior to mitigation, are summarised in Figure X(*a*) for a 15 ha field with 2 m margins and a within-crop host-plant density of 0.01 plants m^{-2}; in Figure X(*b*) for a within-crop host-plant density of zero; and in Figure X(*c*) for a 15 ha field with no margins and a within-crop host-plant density of 0.01 plants m^{-2}.

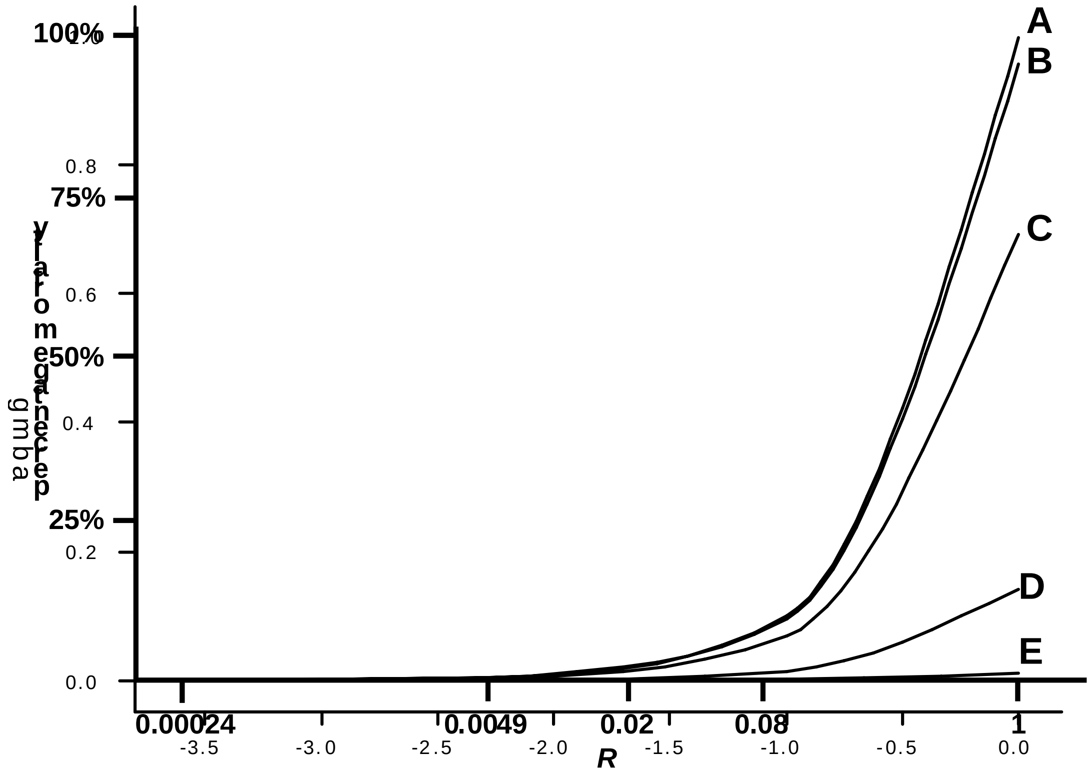

Figure X(*a*) Local estimated percentage mortality (at R = 1) and global estimated percentage mortality (at R < 1) for a 15 ha field of maize 1507 with 2 m margins and a within-crop host-plant density of 0.01 plants m^{-2}. Mortality decreases monotonically with species sensitivity: line A indicates 'extreme' sensitivity; line B indicates 'very-high'; line C 'high'; line D 'above-average'; and line E 'below-average'. Mortality (*y*-axis) is plotted against *R* (*x*-axis), the parameter that measures the degree of large-scale exposure. Local mortality is given by the values corresponding to *R* = 1 (see right hand end of *x*-axis). Global estimated mortality allows for the effects of large-scale exposure and is calculated by multiplying the estimate of local mortality by *R*, where *R* is a proportion between zero and unity. Values shown on the x-axis are: *R* = 0.081 ('conservative'), R = 0.02 ('R(MON810)'), R = 0.0049 ('typical'), and R = 0.00024 ('non-conservative'). A logarithmic scale is used for the *x*-axis to aid visibility.

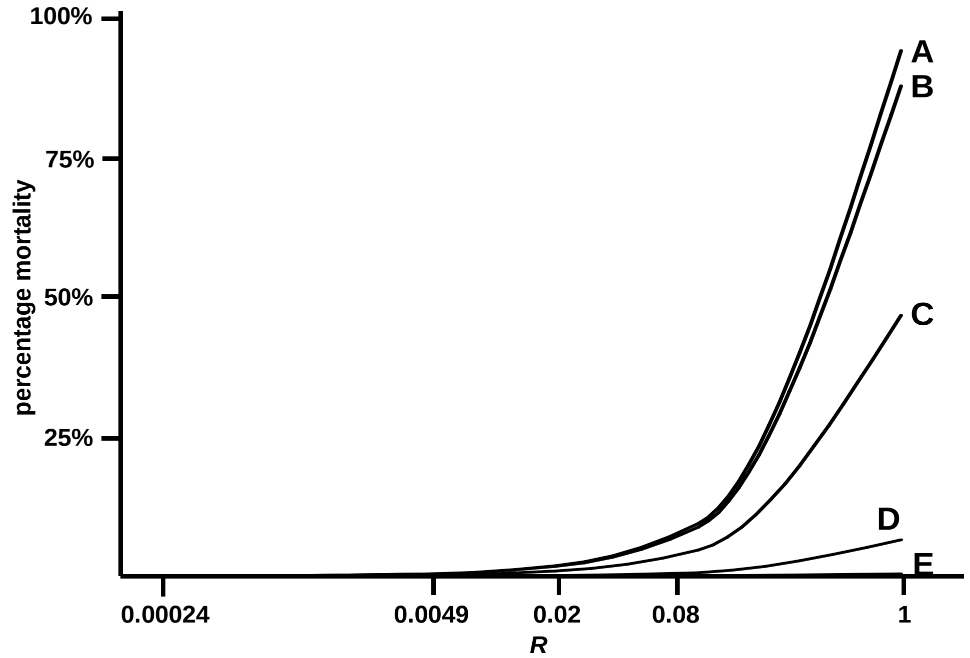

Figure X(*b*) Local and global estimated percentage mortality for a 15 ha field of maize 1507 with 2 m margins and no host-plants within the crop (within-crop host-plant density of 0.00 plants m^{-2}). For other details see the legend to Figure X(*a*), above. A logarithmic scale is used for the *x*-axis to aid visibility.

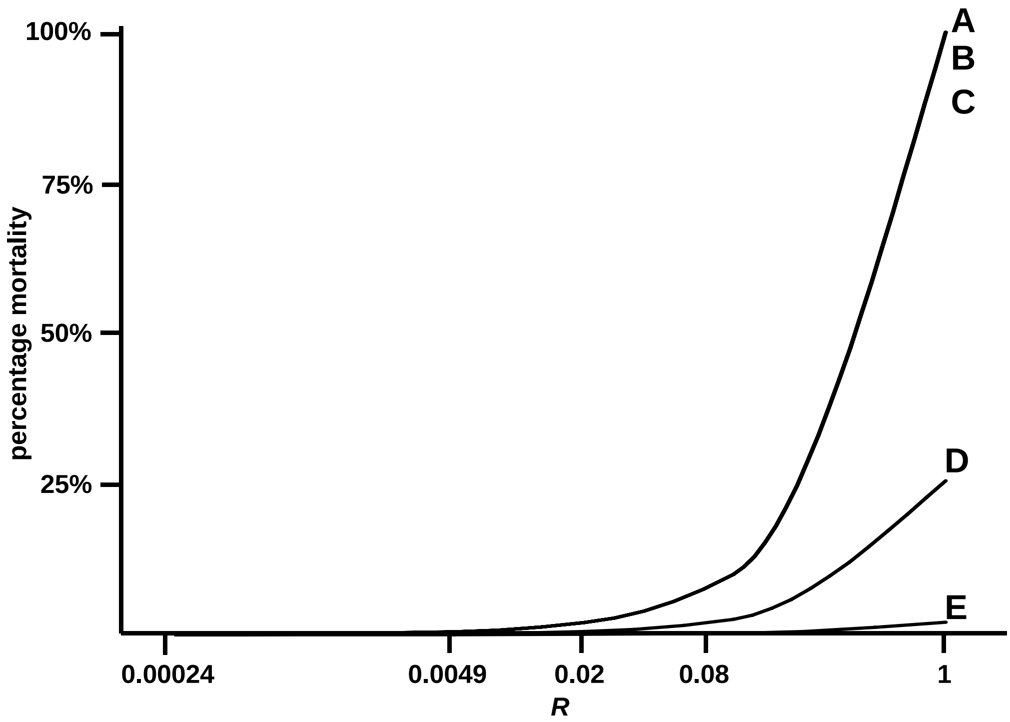

Figure X(*c*) Local and global estimated percentage mortality for a 15 ha field of maize 1507 with no margins and a within-crop host-plant density of

0.01 plants m^{-2}). For other details see the legend to Figure X(*a*), above. A logarithmic scale is used for the *x*-axis to aid visibility.

For any particular environment-species combination, the estimated value of *R* and the sensitivity of the species concerned yield a value of global estimated percentage mortality. Based on this information, the risk management strategies could be determined for each specific case according to protection goals. This EFSA GMO Panel Scientific Scientific Opinion makes no attempt to pre-empt decisions concerning the formulation of such strategies. However, purely to exemplify the implications of the output of this model for risk management decisions, it is useful to illustrate one possible strategy. This is shown graphically in Figure X(*d*), where the results for a 15 ha field with 2 m margins and a within-crop host-plant density of 0.01 plants m^{-2}, shown in Figure X(*a*) above, are repeated but highlighted for the region where global mortalities are less than 5%. Assuming that estimated global percentage mortality has been estimated, a possible strategy might be to impose no explicit management conditions for mitigation if global percentage mortality was less than 1% (green shaded area in Figure X(*d*) below), take definite action to mitigate the risk if global percentage mortality was greater than 4% (red shaded area in figure) and to decide the need for risk management in cases where global percentage mortality was between 1% and 4%, dependent upon pre-defined regional protection goals designed for lepidopteran species in maize ecosystems and other local circumstances (orange shaded area in figure). In this example, for 'typical' values of *R* = 0.0049, no mitigation would be required even for extremely sensitive species.

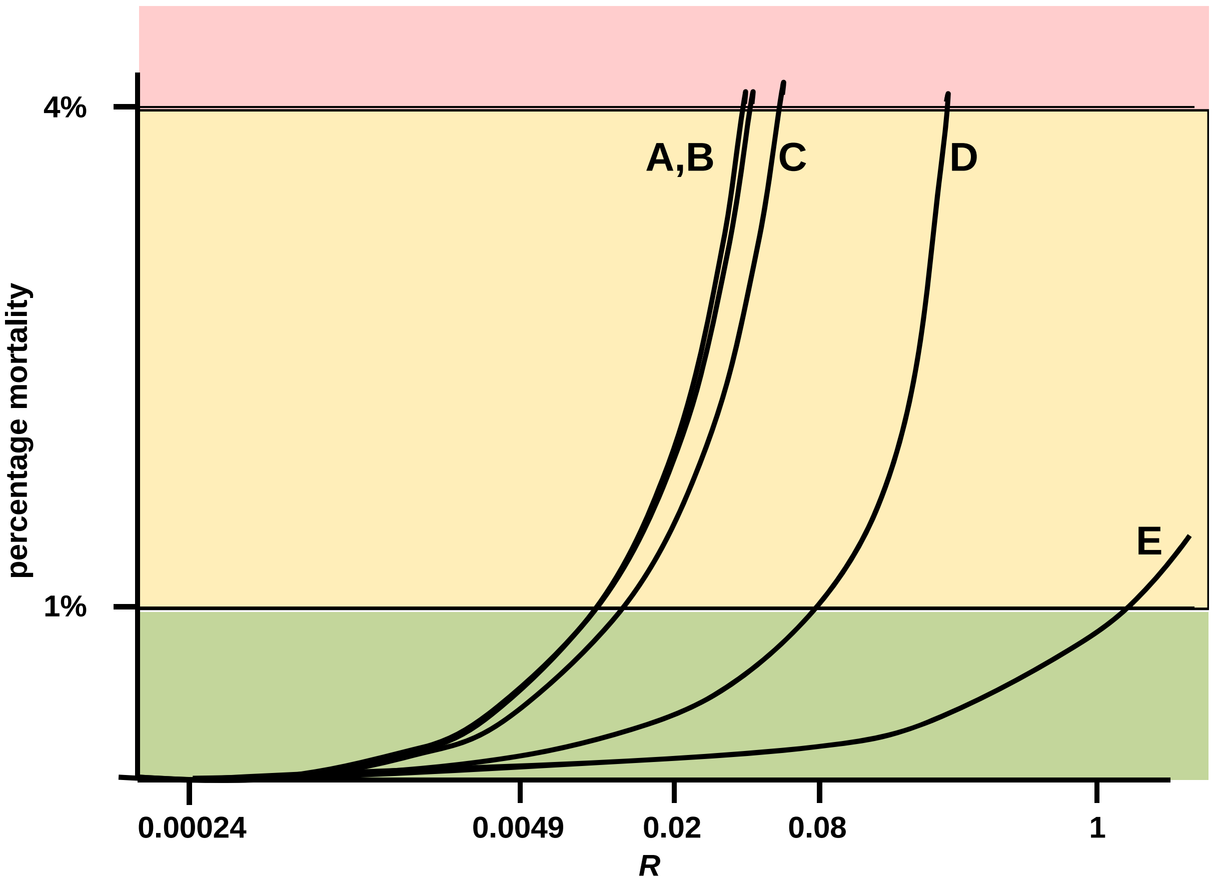

Figure X(*d*) A possible risk management strategy for the situation depicted in Figure X(*a*) is exemplified for illustrative purposes only: estimates of global percentage mortality in the green shaded region (< 1%) might trigger no explicit mitigation measures; estimates in the red shaded region (> 4%) might trigger some form of risk management in the form of mitigation; estimates in the orange region (1% < estimate < 5%) might or might not require risk management, dependant on local protection goals. A logarithmic scale is used for the *x*-axis to aid visibility.

As a summary, global estimated mortality increases monotonically with the five levels of species sensitivity studied, from 'below-average' to 'extreme', as well as decreasing with exposure levels measured by the parameter *R*. If *R* has a typical value (i.e., 0.005), or even if it has a greater value but remains less than 0.01, then negligible adverse effects are foreseen on lepidopteran species, even for those which are extremely sensitive to the Cry1F protein. For example, an uptake of 20% of maize 1507 in a region where maize represents 25% of arable land would lead to negligible adverse effects on lepidopteran species, even if other large scale paremeters (*y*, *x*, *a*) were set to conservative scenario values.

As for Perry *et al*. (2010), results are clearly sensitive to the assumptions regarding the host-plants, both within the crop and in the field margins (and see Appendix 1) (It should be noted that a field with no margins and a within-crop host-plant density of zero would harbour no larvae and therefore none could be at risk of mortality). For maize, weed control to prevent competition with early growth of the crop is crucial for good crop establishment (whether or not it is a *Bt*-maize variety) and this can limit host-plant availability to non-target lepidopteran larvae. If the host-plant population is relatively large within the crop, then there is a complex trade-off for larvae between the advantage of more habitat and a higher population and the disadvantage of a greater mortality within the crop than elsewhere. Only if the overall density of suitable host-plants in the entire arable maize ecosystem was limiting the NTO population size could this disadvantage be ignored, since then there would be no alternative unused habitat for the within-field larval population to exploit.

For the example strategy studied here, for values of *R* of *c*.0.02 as assumed by Perry *et al.* (2010), mitigation might be required for species with high or greater sensitivity. Here, a "highly sensitive species" means a species in one of the three highest sensitivity categories ('high', 'very high' and 'extreme') as defined in Table 2 of this document. To place this into context, note that a species at the lower end of the 'high' sensitivity category would be somewhat less sensitive than the moth pest *Plutella xylostella* and close to the 8th percentile of the species sensitivity distribution. Indeed, in general, the potential risks without mitigation are considerably greater for maize 1507 than was the case for maize MON 810, due to the greater toxicity of the former and assuming the same exposure. Additionally, for fields with margins, some of the predicted outcomes are sensitive to the within-crop host-plant density; there is a greater overall risk at relatively higher densities.

It is possible to base the risk management decisions on global mortality, or on local mortality rates. For example, in case of a 60% local mortality rate and a typical value of *R* = 0.0049, the global mortality rate would then be 0.0049 × 60% = 0.3% and thus categorised as < 1% and 'low'. However, regardless of global, large-scale estimates, this degree (60%) of local mortality may be unacceptable (e.g., if protected non-target Lepidoptera occur directly in maize 1507 fields or in their margins). Risk managers may then require alternative strategies to the mitigation measure modelled, particularly for species of conservation concern (see below).

The results predicted by the model relate to one specific component of mortality at a defined point in the lepidopteran life-cycle and for a limited period of time within the season. For risk managers to place the above results into a population dynamic context, it would be necessary to predict the precise effects of mortality owing to maize 1507 in a particular generation(s) on succeeding generations. This would require the accurate determination of key factors from life-table data (Varley *et al*., 1973), which is beyond the scope of this analysis. Appropriate calculations should account for the number of generations per year of the species concerned and the number of these that are likely to be temporally coincident with maize 1507 pollen shed. Small declines in lepidopteran populations are difficult to detect in practice (Aviron *et al*., 2009) because of the natural fluctuations and trends in lepidopteran populations (Conrad *et al*., 2006). Regarding local mortality rates, it should be noted that, by comparison, abiotic mortality factors analysed in field studies for some lepidopteran species can reduce the larval population by more than 50% (Annamalai *et al*., 1988) in one season. Also, biotic mortality factors such as the impact of larval and pupal parasitoids can be high, since parasitisation rates as high as 80% are often found in field conditions (e.g., Telekar and Shelton, 1993; Liu *et al.*, 2000).

The applicant estimated for *V. cardui*, a median mortality rate for all regions included in their modelling exercise of 1 out of 926 individuals (approximately 0.11%)[26]. The applicant concluded that these low levels compare to those reported previously by Perry *et al.* (2010), and are expected to be representative of other Nymphalidae in EU agroecosystems (e.g,. *Inachis io* and *Vanessa atalanta*). However, the EFSA GMO Panel does not agree with the applicant's conclusion that this study provided adequate evidence that there is a negligible risk of maize 1507 to non-target Lepidoptera in the EU. Firstly, as stated above, there is no evidence that the average sensitivity found for the single species *V. cardui* will be typical of other species of Lepidoptera or even of other Nymphalids. Secondly, the exposure assessment used by the applicant assumes that the host-plant is exclusively the nettle *Urtica dioica* when it is known that some populations of *V. cardui* are known to prefer thistles (*Cirsium* spp. and *Carduus* spp.) (Janz, 2005). Finally, the use of the data of Gathmann *et al.* (2006) on densities of *Urtica* in maize fields in the exposure assessment was incorrect.

2.3.5.3. Conclusion

From existing data on species susceptibility and considering various scenarios of exposure which may occur across Europe, the EFSA GMO Panel has assessed the potential impact of maize 1507 cultivation on a range of non-target lepidopteran species.

Local and global estimated mortality increase monotonically with the five levels of species sensitivity studied, from 'below-average' to 'extreme' and with the level of exposure. Maize 1507 pollen grains found in and up to 30 m distance (for justification, see Table 4, in section 3.1.2.3, below) from maize 1507 fields could locally adversely affect differing proportions of non-target lepidopteran larvae, the proportion depending upon the sensitivity spectrum of the lepidopteran species under consideration, and other factors. However, global estimated mortality decreases monotonically with exposure level (measured by the parameter *R*) determined by factors such as the proportion of the land cropping maize 1507. For typical maize production conditions where maize represents 25% or less of arable land and as long as the proportion of maize 1507 is only moderate (uptake below 20%), the global mortality is likely to be less than 1%, even for extremely sensitive non-target lepidopteran species.

Nevertheless, the EFSA GMO Panel concludes that there is a risk to certain highly[27] sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants.

2.4. Impacts of the specific cultivation, management and harvesting techniques

The PAT protein expressed in maize 1507 has been used as a selectable marker during the transformation process. The scope of the application for maize 1507 cultivation does not cover the use of glufosinate-ammonium-containing herbicides on maize 1507. Therefore, potential environmental adverse effects due to the applications of glufosinate-ammonium-containing herbicides and possible changes in weed management are not considered by the EFSA GMO Panel in this Scientific Opinion.

Bt-crops, such as maize 1507, may reduce the use of insecticides and may cause changes in crop rotations in response to reduced pest pressure (Gómez-Barbero *et al.*, 2008a; Brookes and Barfoot, 2010). However, this reduction in pesticide use and the narrow spectrum of activity of Cry proteins may provide an opportunity for secondary pests, previously controlled by insecticides used against key target pests, to reach damaging levels (Wang *et al.*, 2008, Lu *et al.*, 2010). Natural enemies failing to

[26] Additional information dated 21/03/11 / Annex 3

[27] Here, a "highly sensitive species" means a species in one of the three highest sensitivity categories ('high', 'very high' and 'extreme') as defined in Table 2 of this document. To place this into context, note that a species at the lower end of the 'high' sensitivity category would be somewhat less sensitive than the moth pest *Plutella xylostella* and close to the 8th percentile of the species sensitivity distribution.

fully control secondary pests, and reduced competition with target pests might also play a role in secondary pest outbreaks (Catangui and Berg, 2006; Sanvido *et al.*, 2007; Eichenseer *et al.* 2008; Romeis *et al.*, 2008; Fitt, 2008; Kennedy, 2008; Naranjo *et al.*, 2008; Dorhout and Rice, 2010; Lu *et al.*, 2010; Virla *et al.*, 2010). During the last decade *Striacosta albicosta* (the western bean cutworm) expanded across the cornbelt in the USA due to the decrease of competition from other lepidopteran target pests as a consequence of *Bt*-maize cultivation (Michel *et al.*, 2010). The western bean cutworm is not affected by the Cry1Ab protein expressed in *Bt*-maize, and was therefore able to occupy the ecological niche of the more susceptible *Helicoverpa zea* (corn earworm) and European corn borer (Catangui and Berg, 2006; Dorhout and Rice, 2010). However, *S. albicosta* is not present in European maize cultivation.

Where secondary pests remain uncontrolled, they can build up higher populations, affecting other crops in the agricultural landscape (Meissle *et al.*, 2011). Such a situation has been recently reported for mirid bugs in *Bt*-cotton in China: mirid bug infestation levels increased in alternative host crops (Chinese date, grapes, apple, peach and pear), and were significantly correlated with regional proportion of *Bt*-cotton planted (Lu et *al.*, 2010). However, it is considered unlikely that a similar situation occurs in *Bt*-maize in Europe, as it has a smaller pest spectrum than cotton, and the insecticide input in conventional maize is generally lower than in conventional cotton (Meissle *et al.*, 2011). It should also be noted that the emergence of secondary pests is not specific to *Bt*-crop cultivations only, or maize 1507 in particular. Arthropod assemblages in agricultural fields are in a continuous fluctuation in terms of their species number, composition and individual densities over time and space. Human interventions, including pest control, influence these parameters. Whenever pest management of crops changes, the abundance of some pest species may decline and other pest species may increase.

If secondary pests reached damaging levels, additional pest control measures might be necessary and some changes in management could result in adverse environmental effects. In general, it is recommended to adhere to integrated pest management (IPM) principles to manage secondary pests and minimise environmental impacts (Meissle et *al.*, 2011). Predicting the incidence of secondary pests and the environmental consequences of changes in management measures is highly dependent upon cultivation practices, farming systems and regional environmental factors.

The EFSA GMO Panel concludes that, apart from changes in insecticide regimes, there are no anticipated changes in management that will occur with the cultivation of maize 1507. The EFSA GMO Panel notes that the incidence of secondary pests and the environmental consequences of changes in management measures is highly dependent upon farming systems and regional environmental factors, and is therefore difficult to predict. Risk managers should be aware that, whenever pest management measures change, species assemblages will change accordingly and the environmental consequences should be considered in the framework of IPM in National Action Plans according to Directive 2009/128/EC.

2.5. Conclusions on the environmental risk assessment

The potential of maize 1507 to have direct or indirect adverse effects on NTOs and the ecological functions they provide in agro-ecosystems has been updated in light of new relevant scientific literature.

From existing data on species susceptibility and considering various scenarios of exposure which may occur across Europe, the EFSA GMO Panel has assessed the potential impact of maize 1507 cultivation on a range of non-target lepidopteran species. Local and global estimated mortality increase monotonically with the five levels of species sensitivity studied, from ‘below-average’ to ‘extreme’ and with the level of exposure. Maize 1507 pollen grains found in and up to 30 m distance (see Table 4) from maize 1507 fields could locally adversely affect differing proportions of non-target lepidopteran larvae, the proportion depending upon the sensitivity spectrum of the lepidopteran species under consideration. However, global estimated mortality decreases monotonically with exposure level (measured by the parameter *R*) determined by factors such as the proportion of the land cropping maize 1507. For typical maize production conditions where maize represents 25% or less of arable land and as long as the proportion of maize 1507 is only moderate (uptake below 20%), the global mortality is likely to be less than 1%, even for extremely sensitive non-target lepidopteran species. However, certain highly sensitive[28] non-target lepidopteran species are at risk where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants.

In addition to the specific concern on non-target Lepidoptera, the EFSA GMO Panel considered the possible adverse effects of maize 1507 on other NTOs (such as pollinators, aquatic NTOs), in order to update, where appropriate, its previous evaluation in light of new relevant scientific literature. The EFSA GMO Panel concludes that no new scientific scientific information has been made available that would invalidate its previous conclusions on the environmental safety of maize 1507.

- Several beneficial non-target arthropods such as natural enemies (predators and parasitoids) can occur in maize fields in the EU and are therefore likely to be exposed to maize 1507 plants and the Cry1F protein they express when cultivated. Existing studies did not reveal adverse effects on the number and abundance of beneficial and non-target arthropods associated with the cultivation of maize 1507. On the basis of the data delivered by the applicant and obtained from a literature survey, the likelihood of adverse effects on non-target natural enemies is foreseen to be very low. Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize 1507 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers.

- No specific lower-tier studies, assessing the impact of the Cry1F protein on non-target aquatic arthropods and the fate of the Cry1F protein in senescent and decaying maize detritus in aquatic environments, have been reported in the scientific literature so far. Although there is indication of a potential hazard for trichopterans under laboratory conditions when exposed to high doses of Cry proteins, no substantial aquatic exposure to the Cry1F protein contained within maize plant tissue is expected, except for possibly small amounts of pollen during pollen shed. Therefore, the EFSA GMO Panel is of the opinion that it is unlikely that the Cry1F protein in maize 1507 products would cause adverse effects on non-target aquatic arthropods in the context of its proposed uses.

- The Cry1F protein does not negatively affect honeybee larvae and adults in laboratory settings. Considering that the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low, the EFSA GMO Panel does not consider that maize 1507 will cause reductions to pollinating insects that are significantly greater from those caused by cultivation of conventional maize.

- Whilst data on the fate of the Cry1F protein and its potential interactions in soil are limited, the relevant scientific publications analysing the Cry1F protein, together with the relatively broad knowledge about the environmental fate of other Cry1 proteins, do not indicate any novel risks that would change previous EFSA GMO Panel conclusion that there are no significant direct effects on the soil environment (EFSA, 2005). The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the cultivation of maize 1507 is likely to cause adverse effects on non-target soil arthropods such as springtails and soil mites due to the expression of the Cry1F protein.

The possible resistance evolution to the Cry1F protein in lepidopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize 1507, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. In addition to

[28] Here, a "highly sensitive species" means a species in one of the three highest sensitivity categories ('high', 'very high' and 'extreme') as defined in Table 2 of this document. To place this into context, note that a species at the lower end of the 'high' sensitivity category would be somewhat less sensitive than the moth pest *Plutella xylostella* and close to the 8th percentile of the species sensitivity distribution.

the European and Mediterranean corn borers, which are the major target pests, other regionally important lepidopteran pests exposed to maize 1507 may also have the potential to evolve resistance to Cry1F protein.

The EFSA GMO Panel also concludes that, apart from changes in insecticide regimes, no other changes in management are anticipated with the cultivation of maize 1507. The reduction in pesticide use and the narrow spectrum of activity of Cry proteins may permit populations of herbivore arthropods to develop that are no longer controlled by insecticides previously applied. Thus, reduced or no insecticide applications in maize 1507 may provide an opportunity for secondary pests, previously controlled by insecticides used against key target pests, to reach damaging levels. The incidence of such dynamics will depend upon a series of factors, including cultivation management applied at the farm level, the crop rotation and the receiving environments.

The EFSA GMO Panel concludes that the cultivation of maize 1507 could have the following adverse effects on the environment in the context of its intended uses (1) the adoption of altered pest control practices with higher environmental load due to potential evolution of resistance to the Cry1F protein in populations of exposed lepidopteran target pests, and (2) reductions in populations of certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants.

3. Risk management strategies (including post-market environmental monitoring)

3.1. Risk mitigation measures[29]

3.1.1. General aspects of risk mitigation

According to the EFSA GMO Panel Guidance Document on the ERA of GM plants (EFSA, 2010a) and in line with Annex II of Directive 2001/18/EC (EC, 2001), the risk assessment can identify risks that require management and propose mitigation measures to reduce the levels of risk. In order to reduce the identified risks associated with the GM plant deployment to a level of no concern, the EFSA GMO Panel evaluated the scientific quality of the management and mitigation measures proposed by the applicant, as well as their adequacy and efficacy. Risk mitigation should be proportionate to the results of the different risk scenarios studied, the specific protection goals in the receiving environments, and to the levels of scientific uncertainty and risk identified in the ERA (EFSA, 2011a).

3.1.2. Interplay between environmental risk assessment and risk mitigation

The ERA of maize 1507 concluded that:

(1) the potential consequences of resistance evolution to the Cry1F protein in populations of exposed lepidopteran target pests may cause adverse environmental effects. Resistance to the Cry1F protein is likely to evolve in exposed populations of target lepidopteran pest species, particularly those subjected to the highest selection pressures, such as in areas of continuous or very extensive maize 1507 cultivation or in rotation with other crops expressing the Cry1F protein (if any). While this is not considered a direct environmental harm, the consequences of resistance evolution may require altered pest control practices with higher environmental load. Considering that lepidopteran target pests may evolve resistance to Cry1F-expressing maize under conditions of continuous exposure, the applicant proposed to put in place risk management measures to delay the possible evolution of resistance;

(2) exposed non-target Lepidoptera that are highly sensitive to the Cry1F protein may be at risk if exposed to harmful amounts of maize 1507 pollen.

[29] Technical dossier / Section 4 / Pages 28-31 /Annex 37

The EFSA GMO Panel considers that the risks identified during the ERA may require management and, in the following sections, makes recommendations for appropriate management and mitigation measures, wherever it is necessary. The suggested management measures take into consideration the level of scientific uncertainty associated with the conclusions of the ERA (e.g., by considering hypothetical very high levels of sensitivity and exposure of non-target Lepidoptera). In order to reduce the identified risks and remaining scientific uncertainty associated with the cultivation of maize 1507 to a level of no concern, the scientific quality of several management measures, as well as their reliability and efficacy were evaluated by the EFSA GMO Panel. These aspects are described below.

3.1.2.1. Risk mitigation measures to delay resistance evolution to the Cry1F protein in lepidopteran target pests

Insect resistance management plan proposed by the applicant

In line with the applicants' EU working group on IRM (as referred to by Alcalde *et al.* (2007)), the applicant proposed to put risk management measures in place to delay the possible resistance evolution in the target insect pests[30]. According to the IRM plan proposed by the applicant, farmers growing more than 5 ha of Cry1F-expressing maize in the EU shall establish refuge areas with non-*Bt*-maize, corresponding to at least 20 % of the surface planted with *Bt*-maize. The applicant's reasoning for implementing the *refugia* only on farms where the total area of *Bt*-maize is greater than 5 ha is based on (1) the high fragmentation of the European agricultural landscape, (2) the lack of economic feasibility for providing *refugia* on farms with less than 5 ha *Bt*-maize, and on (3) the negligible risk of resistance evolution in areas with *Bt*-maize fields smaller than 5 ha (Alcalde *et al.*, 2007).

In addition to maintaining an adequate level of refuge areas with non-*Bt*-maize, the IRM plan proposed by the applicant covers the following elements[31]: (1) monitoring target pests for any potential evolution of resistance to maize 1507, (2) the implementation of a comprehensive education programme to aid farmers in understanding the importance of IRM to delay the resistance evolution by planting refuge areas, and (3) the application of a remedial action plan addressing any contingency if resistance should occur.

High dose/refuge strategy

The EFSA GMO Panel agrees with the applicant that appropriate IRM strategies are capable of delaying possible evolution of resistance under field conditions (Alstad and Andow, 1995; Andow, 2008; Tabashnik *et al.*, 2008a, 2009; Huang *et al.*, 2011). Resistance management strategies, relying on a 'high dose/refuge' strategy, have been endorsed for several Cry-expressing crops in several countries (Bates *et al.*, 2005; Andow, 2008; MacIntosh, 2010; Gaspers *et al.*, 2010; Huang *et al.*, 2011). The 'high dose/refuge' strategy proscribes planting *Bt*-maize that produces a very high concentration of the insecticidal Cry protein (25 times the amount needed to kill > 99 % of susceptible individuals), so that nearly all target insects that are heterozygous for resistance do not survive on it. In addition, a nearby refuge of non-*Bt*-maize is required where the target insect pests do not encounter the Cry protein (Ives and Andow, 2002). (Note that in this Scientific Opinion, a refuge is intended to mean a refuge area with maize that does not express Cry proteins which are active against Lepidoptera). Under these conditions, most of the rare resistant individuals surviving on *Bt*-maize will mate with abundant susceptible individuals emerging from nearby refuges to produce heterozygous progeny that is phenotypically susceptible. If inheritance of resistance is recessive, the hybrid progeny from such matings will die on *Bt*-maize.

The underlying assumptions contributing to the success of the 'high dose/refuge' strategy in delaying resistance evolution are that: (1) the Cry protein is expressed in relevant plant tissues at a high dose, (2) initial resistance alleles are rare in the target insect population, so that nearly all resistance alleles will be in heterozygote individuals that cannot survive on the *Bt*-crop, (3) random mating occurs

[30] Technical dossier / Section 4 / Pages 28-31 / Annex 37

[31] Technical dossier / Section 4 / Pages 28-31 / Annex 37

between resistant insects emerging in *Bt*-crops and susceptible insects preserved on non-*Bt*-crops (refuge) at sufficient levels, (4) resistance alleles are partially or fully recessive, and that (5) fitness costs are associated with the resistance.

Whether the underlying assumptions of the 'high dose/refuge' strategy are met for the European and Mediterranean corn borer and maize 1507-expressing maize is considered below.

(1) *The Cry protein is expressed in relevant plant tissues at a high dose:* The predicted duration of susceptibility of the target insect pests to the insecticidal protein is dependent upon many factors (e.g., Tyutyunov *et al.*, 2008), including the dose of the protein in the *Bt*-crop. It is generally assumed that the expression level in plant tissues must be sufficiently high to kill a high proportion of heterozygous resistant genotypes, so that any resistance allele in the target insect pest population remains functionally recessive (Andow, 2008). Instances of field resistance, reported so far (reviewed by Tabashnik *et al.*, 2009; Huang *et al.*, 2011), support model predictions that target insect pests are at greater risk of evolving resistance if managed by *Bt*-crops that are not high dose (Tabashnik *et al.*, 2004). Maize 1507 was shown to provide a high dose against the European corn borer (EPA, 2005).

(2) *Resistance alleles are rare*: Studies, in which the frequency of resistance alleles to the Cry1F protein in populations of corn borer populations are directly estimated, have not been published in the scientific literature.

It has been shown that alleles conferring resistance to the Cry1Ab protein are sufficiently rare in European populations of corn borers for the 'high dose/refuge' strategy to successfully delay resistance development (Bourguet *et al.*, 2003; Schuphan, 2006; Andreadis *et al.*, 2007). In field populations of the European corn borer, no resistant homozygotes were found for major resistance genes, and the estimated frequency of Cry1Ab resistance alleles were low (Andow *et al.*, 1998, 2000; Bourguet *et al.*, 2003; Stodola *et al.*, 2006; Andreadis *et al.*, 2007). In European populations of corn borers, the frequency of resistance alleles was estimated as <0.0009 for European corn borer populations from France (Bourguet *et al.*, 2003) and as <0.0097 for Mediterranean corn borer populations from Greece and Spain (Andreadis *et al.*, 2007).

(3) *Mating occurs between resistant insects emerging in Bt-crops and susceptible insects preserved on non-Bt-crops (refuge) at sufficient levels*: The EU research project ProBenBt, in which various aspects of European and Mediterranean corn borer genetics and *Bt*-resistance in lepidopteran target pest species were studied, revealed that gene flow among European populations of both pest species is likely to be high enough to delay resistance development to *Bt*-toxins in maize (Schuphan, 2006). Likewise, Bourguet *et al.* (2000a,b) reported a high level of gene flow within and between European corn borer populations feeding on maize in France. In contrast, host-plants other than maize have been shown to constitute an ineffective refuge to support sufficient numbers of susceptible European corn borers that would mate freely with adults emerging from maize (Bourguet *et al.*, 2000b; Losey *et al.*, 2001, 2002; Leniaud *et al.*, 2006).

The fact that some adults of the European corn borer mate at a more restricted spatial scale (Hunt *et al.*, 2001; Qureshi *et al.*, 2005; Dalecky *et al.*, 2006; Bailey *et al.*, 2007; Engels *et al.*, 2008) than previously assumed in the high-dose/refuge strategy might under certain circumstances (e.g., crop rotated landscape) decrease its efficiency (Dalecky *et al.*, 2006; Schuphan, 2006). However, predictions generated by a recently developed demo-genetic dynamic model confirm that applying the high-dose/refuge strategy is likely to maintain the sensitivity to *Bt*-toxins in the European corn borer (Tyutyunov *et al.*, 2008). Similar modelling work has not been performed on the Mediterranean corn borer so far. It remains thus difficult to extrapolate the predictions obtained for the European corn borer to the Mediterranean corn borer, especially due to its less polyphagous and more sedentary behaviour (Eizaguirre *et al.*, 2004, 2006). Based on field data from Spain (Eizaguirre *et al.*, 2004, 2006), the authors concluded that the numbers of males and females caught in directional light traps were not different in traps oriented towards *Bt* or non-*Bt*-

maize fields, thus males from adjacent *Bt* and non-*Bt*-maize fields mate indiscriminately with females emerging in any of the two kinds of maize fields. According to field mark/recapture data from aforementioned studies, there are important inter field dispersal flights by Mediterranean corn borer adults. Specifically male Mediterranean corn borer may fly at least up to 400 m from the place of origin.

(4) *Resistance alleles are recessive*: Because no resistant corn borer strains are known from the field, it has not been possible to define if the resistance in corn borer populations to *Bt*-maize would be recessive. However, it is considered a valid assumption that resistance alleles are recessive. Despite intensive *Bt*-maize cropping in various areas, no resistant corn borers have been found in areas with proper resistance management, and resistance against *Bt*-crops known from other insects has been shown to be recessive (Andow, 2008; Perreira *et al.*, 2008a,b).

(5) *Fitness costs are associated with the resistance:* Modeling results have shown that fitness costs can delay or reverse resistance by selecting against Cry-resistant genotypes in refuges where resistant insects are not exposed to the Cry protein (Carrière and Tabashnik, 2001). Few studies analysed fitness costs associated with resistance to Cry1F-expressing maize in corn borers (reviewed by Gassmann *et al.*, 2009), but a weak fitness and recessive cost associated with Cry1F resistance in European corn borer has been reported by Perreira *et al.* (2011).

The evidence discussed above suggests that most of the underlying assumptions contributing to the success of the 'high dose/refuge' strategy in delaying resistance evolution are fulfilled for maize 1507 and corn borers.

Conclusion

According to the IRM plan proposed by the applicant, only farmers growing more than a total area of 5 ha of *Bt*-maize in the EU shall establish refuge areas with non-*Bt*-maize, corresponding to at least 20 % of the surface planted with *Bt*-maize. In practice, this would mean that non-*Bt*-maize *refugia* would not be implemented on a considerable proportion of farms in certain EU countries, as the area planted to *Bt*-maize on these farms would cover less than 5 ha. Considering experiences in Spain and other EU countries, this would not pose a risk, as *Bt*-maize would not be widely adopted in a given region. The Spanish experience illustrates that only in regions where pest infestation is high (e.g., Cataluña), does the adoption rate of *Bt*-maize reach approximately 60% (Gómez-Barbero *et al.*, 2008b). Therefore, it is likely that sufficiently large areas of non-*Bt*-maize will remain providing widely distributed mosaics of both non-*Bt* and *Bt*-maize at regional scales. However, if *Bt*-maize was adopted on a larger scale in a region, the risk of resistance evolution is likely to increase requiring more specific refuge management measures. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be *refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size.

In its 2005 Scientific Opinion on maize 1507, the EFSA GMO Panel concluded that the intended expression of the Cry1F protein was demonstrated and the expression levels were in the same range for different locations and growing seasons (see EFSA, 2005). In addition, the stability of the expression of the *cry1F* gene in maize 1507 was demonstrated. The EFSA GMO Panel is not aware of new information that would invalidate its previous evaluation and therefore agrees with the applicant to implement an IRM plan that relies on the 'high dose/refuge' strategy, in order to delay resistance evolution in lepidopteran target pests, namely *O. nubilalis* and *S. nonagrioides*. Based on field data on interfield dispersal flight characteristics of Mediterranean corn borer adults (Eizaguirre *et al*., 2004, 2006), the implementation of appropriate IRM (i.e., size and distance of *refugia*) will enable successful management of the potential resistance evolvement for this species as well.

Considering that other regionally important lepidopteran pests exposed to maize 1507 may also have the potential to evolve resistance to the Cry1F protein (e.g., *Sesamia cretica*, *Helicoverpa armigera*, *Mythimna unipuncta*), the EFSA GMO Panel advises the applicant to consider regionally important lepidopteran pests (other than the European and Mediterranean corn borers) of maize 1507 in the context of the IRM strategy. However, the Cry1F protein might not be expressed in relevant plant tissues at high toxicity dose for some of these lepidopteran pest species, meaning that one of the underlying assumptions contributing to the success of the 'high dose/refuge' strategy in delaying resistance evolution would not be fulfilled for maize 1507 for those species.

Appropriate adaptation of the IRM plan to local and/or regional conditions (e.g., IPM, farming system) is a key element of successful IRM (Tyutyunov *et al.*, 2008, MacIntosh, 2010). Therefore, the EFSA GMO Panel recommends that stewardship agreements pertaining to IRM, as proposed by the applicant, consider the following factors:

- the biology and ecology of target pest(s) (e.g., number of generations, alternative host-plants, dispersal behavior, pest density level);

- the management of maize 1507 fields (e.g., cultivation practices and IPM measures, configuration of non-Cry1F-expressing maize refugia);

- the local characteristics (e.g., adoption rate of maize 1507, farming systems, landscape structure and heterogeneity);

- the stakeholders/growers (e.g., communication, socio-economic background, education/training).

In addition, PMEM will provide important feedback on the status of resistance evolution, as well as on status of maize production systems (see section 3.2).

3.1.2.2. Risk mitigation measures to reduce the exposure of non-target lepidopteran species occurring within maize fields and their margins to maize 1507 pollen

An important step in risk management is to apply mitigation measures to decrease exposure of concerned species at risk, considering their most sensitive life stages, host-plants and their location within the maize arable ecosystem. In addition, any estimates of mortality due to maize 1507 need to be placed into the context of natural fluctuations in populations caused by other sources of mortality for particular lepidopteran species (see section 2.3.5.2(b)). For example, for host-plants and larvae occurring predominantly in field margins, the focus for mitigation must be to reduce the exposure in field margins. Since the outcome of mitigation measures depends on several factors, many of which are local factors, the relationship between mortality before and after the implementation of mitigation measures is too complex to be translated into simple guidance to risk managers. Nonetheless, based on data retrieved from the scientific literature, and those generated by the extended Perry *et al.* (2010) model, general principles can be given, which can be followed in most situations.

Mitigation may take two forms: (1) direct mitigation measures, usually at small spatial scales, which seek to reduce the identified risk, and (2) indirect mitigation measures that seek to increase the overall population of particular non-target lepidopteran species, often at a spatial scale larger than single fields for which the risk has been identified.

(1) An example of a direct mitigation measure is the planting of buffer strips, consisting of rows of non-*Bt*-maize (not expressing a lepidopteran-active Cry protein) at the edges of the maize 1507 fields (i.e., between the maize 1507 plants and field margins). In its 2005 Scientific Opinion, the EFSA GMO Panel noted that *refugia*, meant to delay resistance evolution in target pests, may also reduce levels of exposure of non-target Lepidoptera to maize 1507 pollen. However, no quantification of the appropriate size or location of such strips could be given at that time. For larvae within field margins, mitigation works by increasing the effective distance of these larvae from the source of *Bt*-maize pollen. Similarly, larvae in the non-*Bt*-maize strips at the edge of the field suffer correspondingly less mortality the further they are located from the *Bt*-maize field interior. Mortality of Lepidoptera within the *Bt*-maize field interior is assumed to be unaffected by the presence or absence of the non-*Bt*-maize strips. For a typical field size of 15 ha, strip widths of

21 m and 24 m would result in a percentage of non-*Bt*-maize in the assumed field of, respectively, 20.5% and 23%, both close to the 20% recommended by many authorities (e.g., US EPA) as non-*Bt*-maize *refugia* to delay the possible evolution of resistance to Cry proteins amongst target pest species (e.g., Bates *et al*., 2005; MacIntosh *et al*., 2010). It should be noted also that mitigation with a single block of 4.48 ha of non-*Bt*-maize represents 23% of the total of 19.48 ha of maize, made up of 4.48 ha of non-*Bt*-maize and 15 ha of *Bt*-maize. Further details on this matter are provided in Appendices 2 and 3 and see also Perry *et al.* (2011b).

To reduce exposure within field margins, it is not necessary for headlands to be sown with non-*Bt*-maize or indeed sown with any crop; any increase in the effective distance between maize 1507 and non-target Lepidoptera within field margins or headlands will act to reduce the identified risk.

Another example of a direct mitigation measure would be crop management in which herbicide was applied to reduce host-plant densities within maize 1507 field. However, this could also reduce both floristic and faunistic biodiversity (e.g., Heard *et al.*, 2003a,b), and there could be trade-offs between maize 1507 measures and general maize crop management. It is also important to consider that the implementation of the provisions of Directive 2009/128/EC (EC, 2009b) establishing a framework for Community action to achieve the sustainable use of pesticides will most likely change weed control programmes in EU farming systems. Integrated weed management would aim at reducing the use of herbicides, while still maintaining weeds at levels that do not compete with the maize crop. A higher presence of weeds in maize fields would therefore be tolerated as long as they do not reduce crop productivity over time. The presence of weed host-plants of non-target Lepidoptera may, in turn, have implications on the level of exposure of these species to maize 1507 pollen. An alternative direct strategy would be to integrate herbicide use with field margin management according to approved practices consistent with Directive 2009/128/EC (EC, 2009b). This would typically entail a reduction of herbicide usage in the field margins and/or protection of field boundaries against spray drift to create 'conservation headlands' (Sotherton, 1991). Another direct mitigation measure that might be considered is the detasseling of maize 1507 plants in border rows in order to limit maize 1507 pollen dispersal outside of the maize field.

(2) An example of an indirect measure would be the establishment and maintenance of additional habitats for non-target Lepidoptera (Candolfi *et al.*, 2000). This might be achieved by, for example, the planting of additional host-plant seeds in areas greater than 30 m from maize 1507, so as to increase the host-plant density and provide a food source in an area where the risk of mortality from maize 1507 pollen is negligible. Such areas might include road or footpath verges and on-farm ecological compensation areas (Boller *et al*., 2004). Further examples of indirect mitigation measures include changes in cropping systems to reduce the frequency of maize in a rotation and/or the total area of maize planted and compensation to growers for electing not to grow maize 1507. It should be noted that the implementation of these further measures operate at large spatial scales and alter the values of the parameters z and v in the model described in section 2.3.5.2(a), above. Implementation of mitigation measures may present problems at the landscape or regional scale, because of the added complexity due to the need for coordination between many growers. Similarly, for this general case, where species of conservation concern (see section 3.1.2.3, below) are not present, it seems unnecessary to impose measures for each field separately. The farm seems the most appropriate scale for practicable mitigation measures.

Here, examples of alternative strategies are presented for risk management to illustrate and compare possible risk mitigation strategies. It is important to maintain the flexibility of the mitigation measures, since what may be a good option for one region or Member State may not be sensible in another. Hence, the strategies outlined in this section should be seen as examples of a semi-quantitative approach that might utilise estimates from mathematical models in order to reduce the risk to non-target Lepidoptera. Because some estimates of local mortality are available from the modelling exercise (described in section 2.3.5.2(a), above), the sections 3.1.2.2-3.1.2.3, below, focus on direct mitigation measures.

Spatial aspects of field margins

In practice, mitigation measures are likely to be implemented differently by farmers depending on: field management; field size and shape; as well as the habitat and extent of adjacent field margins. The presence and extent of field margins as well as of roads, paths, ditches and all other forms of field edges play an important role in risk assessment and risk management.

In general, less-intensive maize ecosystems with fragmented small fields (e.g. Berkatal, Germany, see Appendix 1) tend to have a ratio of marginal area to crop area which exceeds that of more-intensive ecosystems where fields are larger (e.g., Grebbin, Germany, see Appendix 1). The implications for risk management are explored more fully in Appendix 1.

Whilst it is not possible to give specific recommendations, in general, the greater the area of non-*Bt*-maize habitat in which host-plants are found, the less the risk to non-target Lepidoptera; and the greater the distance of a larva from a source of maize 1507 pollen, the less is the risk. This applies whether the habitat is a field margin or an area of non-*Bt*-maize.

Spatial aspects of mitigation using sown areas of non-Bt-maize

If it is assumed that for mitigation to manage insect resistance, 20% of the total cropped area should be non-*Bt*-maize, then general recommendations can be given concerning where and how to place the non-*Bt*-maize in relation to fields cropped with maize 1507. The arrangement of non-*Bt*-maize should seek to maximise the average distance of larvae from the nearest source of maize 1507 pollen. Detailed implications for risk management are given in Appendix 2. These depend on whether the maize field has margins and/or host-plants within the maize crop.

Outcomes of mitigation using sown areas of non-Bt-maize

For illustrative purposes, the efficacy of various mitigation scenarios for fields with margins, are compared in Appendix 3, for mortalities estimated from the model as described in section 2.3.5.2, above. Clearly, when there are within-crop host-plants, then there is considerable variability in the efficacy of mitigation, according to the strategy chosen and dependent on other factors. Efficacy is defined by P, the proportional reduction in mortality due to mitigation.

As a summary of the results, for a field with margins, when it can be assumed that there are no host-plants within the crop the model predicts that it is possible to mitigate using buffer strips so as to almost completely eliminate mortality. In this case, the use of the mitigation strategy in the form of strips of width 24 m reduces estimated global mortality, for all factor combinations, to below 1%. By contrast, when the host-plant density is 0.01 or greater and the estimated sensitivity is high or greater, mitigation of the type considered here, even for buffer strips of width 24 m, cannot eliminate more than two-thirds of the local estimated mortality. However, it should be stressed, as stated above, that for typical exposure levels ($R = 0.005$) that global mortality would be negligible.

In conclusion, wherever non-target Lepidoptera populations could be at risk (see above), mitigation may take two forms:

(1) direct mitigation measures, such as non-*Bt*-maize strips, which seek to reduce the identified risk;

(2) indirect mitigation measures, such as the establishment and maintenance of additional habitats for non-target Lepidoptera, that seek to increase the overall population of particular non-target lepidopteran species, often at a spatial scale larger than single fields for which the risk has been identified.

It is important to maintain the flexibility of mitigation measures, since what may be an appropriate option for one region or Member State may not be sensible in another. Estimated local or global mortality, both before and after mitigation, depends in a complex manner on different combinations of a number of factors that include: the sensitivity of individual lepidopteran species, the degree of large-scale exposure effects, and host-plant density. The first of these factors may be assumed constant over Europe, but the other two factors vary according to regional agronomy and may be estimated locally using official agricultural statistics and other sources of information. Risk managers should select the appropriate values for the other two factors according to protection goals and management needs applying within their jurisdiction.

Special attention should be paid to the degree of large-scale exposure as mitigation measures are only needed when the proportion of maize and uptake of maize 1507 are sufficiently high, regardless of the other parameters. Indeed, maize 1507 cultivation remains below 5% of the Agricultural Unit of Account[32] (i.e., zv = 0.05, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.00625), the global mortality is predicted to remain below 1%, even for extremely highly sensitive species. In such situations, risk mitigation measures are not required as the potential adverse effect on the overall population would be virtually undetectable. Whenever mitigation measures are needed, the implementation of non-Bt-maize border rows will reduce the mortality of non-target lepidopteran species for both within fields and in field margins. The width of such border rows should be adapted to the size of maize 1507 fields to keep them proportional to the actual risk . Thus 12 m non-GM borders would be sufficient for a 5 ha field, 21 m borders for 15 ha field and 36 m for a 50 ha field of maize 1507 where exposure in both margins and within fields needs to be reduced.

3.1.2.3. Risk mitigation measures to reduce the exposure of non-target lepidopteran species of conservation concern and protected habitats to maize 1507 pollen

Bt-maize pollen might be hazardous to a range of lepidopteran species of conservation concern (Darvas *et al.*, 2004; Lang, 2004; Traxler *et al.*, 2005; Lang and Otto, 2010), and should therefore be the focus of specific risk management (Hofmann *et al.*, 2010).

The purpose of mitigation measures described here is to avoid harm to non-target lepidopteran species of conservation concern and occurring in protected habitats, as defined under Directive 2004/35/EC (EC, 2004). Emphasis is taken of any harm that has significant adverse effects on reaching or maintaining the favourable conservation status of such species in their protected habitats. The significance of such effects is to be assessed locally with reference to the baseline condition, taking account of the criteria set out in Annex I of Directive 2004/35/EC. The major mitigation tool to avoid environmental harm to these species is the establishment of isolation distances to all relevant habitats as defined in Directive 2004/35/EC. Table 4 gives estimates of distances from the nearest maize 1507 field that would be necessary to decrease the estimated local mortality below a certain level. Hence, as an illustration, the procedure might be (1) to decide what category(-ies) of sensitivity the species of conservation concern falls into, subsequently (2) to select the column of the table referring to the most sensitive of these categories, (3) in this column, to chose the row representing the maximum mortality that could be tolerated, and finally (4) to read the distance corresponding to this entry.

The EFSA GMO Panel considers that a distance of 30 m is sufficient to reduce the local mortality to a negligible level below 0.5%, even for extremely sensitive species.

[32] For example, an uptake of 20% of maize 1507 in a region where maize represents 25% of the arable land.

Table 4: Estimated local mortality (%) of five categories of non-target Lepidoptera, whose first instars are defined to have different levels of sensitivity to the Cry1F protein (based on Table 2, above), with increasing distances from the nearest maize 1507 field

Distance from field (m)	Category I: below average sensitivity	Category II: above average sensitivity	Category III: high sensitivity	Category IV: very high sensitivity	Category V: extreme, worst-case sensitivity
2	0.3	3.5	32.5	86.2	98.8
5	0.1	1.2	14.3	68.4	96.6
10	0.0	0.2	2.7	27.2	82.9
15	0.0	0.0	0.5	6.0	45.4
20	0.0	0.0	0.1	1.0	12.5
25	0.0	0.0	0.0	0.2	2.3
30	0.0	0.0	0.0	0.0	0.4
40	0.0	0.0	0.0	0.0	0.0

3.1.2.4. Scientific uncertainties associated with effects of maize 1507 on non-target lepidopteran populations

All modelling exercises are subject to scientific uncertainty (Perry *et al.*, 2009, 2010, 2011a,b; Perry, 2011a,b). The major sources of variability in the estimates reported in this EFSA GMO Panel Scientific Opinion arise from (i) assumptions made in the model structure; (ii) variation in small-scale parameters: field size, margin width, and host-plant densities *e* and *f*; (iii) variation in large-scale parameters: *y*, *z*, *v*, *x* and *a*; (iv) incomplete availability of data; and (v) variability in data induced by lack of standardised methodology.

- Examples of (i) include possible non-linearity of the relationship between logit-transformed mortality and logarithmically-transformed dose. This possibility was raised by Lang *et al.* (2010); see the response by Perry *et al*. (2011). Another source of variability occurs when pollen deposition varies spatially, especially in weather conditions (Hofmann, 2009) where turbulence is largely due to vertical wind movements or gusts in thundery conditions on summer afternoons. Then, a particular small area may experience a larger than average concentration of pollen, although such larger than average pollen concentrations are balanced by smaller than average values elsewhere, where effects are diluted. This effect was quantified in Figure 4 of Hofmann (2007). For maize MON 810 pollen, Perry *et al.* (2010) found that allowance for such a stochastic effect may result in mortalities between 1.5 and 1.7 times greater than those predicted by a purely deterministic approach.

- Examples of (ii) include natural variation between areas, reflecting expected agronomic and environmental heterogeneity, such as those relating to host-plant densities. Risk managers should certainly allow for local variation in host-plant densities in recommending management options.

- For (iii), one example relating to parameter *x* is the degree to which larval behaviour may lead to a reduction in exposure, for example by species that create barriers such as leaf bags or webs, although there is no data concerning the extent to which this protects larvae from pollen deposition. Another example relates to local variability in the regional or national statistics relating to parameter *v*, the uptake of maize 1507 by growers. Furthermore, parameter *a*, that reflects temporal coincidence between maize 1507 pollen deposition and sensitive lepidopteran larval stages may be more variable after maize 1507 commercialisation because it will be integrated into several different commercial maize varieties, resulting in a range of flowering dates, and thus increasing temporal variability in exposure to the Cry1F protein (Van Hout *et al.*, 2008). However, the EFSA GMO Panel has allowed for these and other sources of large-scale parameter variability by giving recommendations for a range of values of *R*, the parameter that is the product of all five large-scale parameters. It is recommended that risk managers should estimate the particular value of *R* that pertains to their region or Member State prior to developing policy for appropriate management.

- Regarding (iv), the incomplete availability of Cry1 sensitivity data concerning EU Lepidoptera of conservation concern has been remarked upon by many authors; recently Perry *et al.* (2010, 2011a) and Lang *et al.* (2010) have both emphasized that further information is required. Gaspers *et al.* (2010) emphasised that lack of standardised experimental methodology may induce variability and/or bias into estimates of LC_{50}s from laboratory bioassays; future international standardisation of methodology might be desirable to ensure consistency between studies.

One of Lang *et al.*'s (2010) main objections to quantitative conclusions from such models for the ERA of GM plants was the need to allow for the fact that sensitivity to a Cry protein can vary greatly among lepidopteran species, even within genera, making a prediction of the sensitivity to a Cry protein for any given species difficult. As Perry *et al.* (2010) confirmed, extrapolating observations made on one non-target lepidopteran species to another is problematic because of between-species variability in acute sensitivity to Cry1 proteins (see also Schmitz *et al.*, 2003). In this Scientific Opinion, the EFSA GMO Panel has addressed this problem by providing output from the model for a range of non-target lepidopteran species with different sensitivities. Risk managers will need to assess which species may be at risk, and modulate management options according to any known or assumed sensitivities of these species.

The parameter to which estimates of mortality are most sensitive is undoubtedly the variable measuring the rate of change of mortality with concentration/dose of the Cry1F protein (i.e., the slope of the logit regression on logarithmically-transformed dose of the bioassays performed to estimate LC_{50}s). The value assumed here for the coefficient in the logit regression was 2.473, identical to the 'worst-case' value assumed by Perry *et al.* (2010) (and see discussion in Perry *et al.*, 2011a). In their reply to questions raised by the EFSA GMO Panel on 20 December 2010, the applicant provided important new data concerning bioassays of the common European lepidopteran species of conservation concern, *V. cardui*, and of the pest species *O. nubilalis*[33]. The regression slope for neonate larvae of the former species was estimated as 1.91 and for the latter as 3.00. These values lend strong support to the value assumed in this EFSA GMO Panel Scientific Opinion and by Perry *et al.* (2010).

The predictions made here are relatively robust because at each stage in the model development, where there was a choice, 'worst-case' scenarios have been modelled, in which any assumptions would tend towards overestimation rather than underestimation of mortality.

It should be emphasised that the above results relate to mortality from one hazard, that of exposure to maize 1507 pollen. There are many other sources of mortality that are suffered by lepidopterans in egg, larval, pupal and adult stages. Examples include predation, parasitism, weather, viral and entomofungal pathogens, etc. To place the mortality considered here into a quantified population dynamic context, it would be necessary to predict the precise effects of mortality owing to maize 1507 in a particular generation(s) on succeeding generations of a defined species, which would require the accurate determination of key factors from life-table data. This is beyond the scope of this EFSA GMO Panel Scientific Opinion (see section 2.3.5.2(b), above).

Sublethal effects are an important issue that can lead to adverse effects on a population over and above those of mortality. Little information exists in the literature concerning sublethal effects other than those on larval weight. Sublethality is also beyond the scope of this EFSA GMO Panel Scientific Opinion.

[33] Additional information dated 21/03/2011 / Annex 3

The value of the model is that it provides a transparent, structured and simple approach to exposure analysis that may be followed for several non-target lepidopteran species and other taxa with alternative hazards, if sufficient data become available. Further, in its derivation of an integrated mortality-distance relationship, it offers the opportunity for relatively accurate laboratory-based estimation of mortality-dose relationships to supplement relatively inaccurate determinations of mortality in the field.

3.1.3. Conclusion on risk mitigation measures

The EFSA GMO Panel considered that the risks identified during the ERA may require management and made recommendations for appropriate management and mitigation measures, wherever it is necessary. The suggested management measures take into consideration the level of scientific uncertainty associated with the conclusions of the ERA (e.g., by considering hypothetical very high levels of sensitivity and exposure of non-target Lepidoptera). In order to reduce the identified risks and remaining scientific uncertainty associated with the cultivation of maize 1507 to a level of no concern, the scientific quality of several mitigation measures, as well as their reliability and efficacy, were evaluated by the EFSA GMO Panel.

The EFSA GMO Panel reiterates its earlier recommendation that appropriate IRM strategies (i.e., ‘high dose/refuge’ strategy) should be employed, in order to delay the potential evolution of resistance to the Cry1F protein in target pests. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be *refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size.

Possible resistance evolution by other regionally important lepidopteran pests should also be considered. Therefore, the EFSA GMO Panel advises the applicant to consider regionally important lepidopteran pests (other than the European and Mediterranean corn borers) of maize 1507 in the context of the IRM strategy. However, the Cry1F protein might not be expressed in relevant plant tissues at high toxicity dose for some of these lepidopteran pest species, meaning that one of the underlying assumptions contributing to the success of the ‘high dose/refuge’ strategy in delaying resistance evolution would not be fulfilled for maize 1507 for those species.

The EFSA GMO Panel recommends caution when predicting future responses of the European and Mediterranean corn borer in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Therefore, the EFSA GMO Panel, while agreeing with the ‘high dose/refuge’ strategy, recommends the periodic re-evaluation of the adequacy and efficacy of this IRM strategy.

Effects on non-target Lepidoptera populations occurring in maize 1507 cultivation areas will be very location, season and species specific, as they depend on the interaction of maize pollen deposition on host-plants at a time when sensitive larvae are present. The EFSA GMO Panel acknowledges that predicting the effects of maize 1507 pollen on populations of non-target Lepidoptera in any location is problematic particularly when information on the sensitivity of many Lepidoptera species is not available and there will be fluxes in populations of both exposed larvae and host-plants. Therefore, the EFSA GMO Panel used a model to generate different scenarios, in order to reach conclusions for more sensitive Lepidoptera species. Risk management strategies have been developed by considering a range of different scenarios, including worst-case scenarios for both sensitivity of Lepidoptera and exposure of highly sensitive species.

The EFSA GMO Panel recommends that, in situations where highly sensitive non-target Lepidoptera populations might be at risk, mitigation measures are adopted to reduce exposure. In agricultural landscapes where arable maize fields or their direct field margins contain host-plants of highly sensitive larvae and where maize 1507 cultivation is greater than 5% of the Agricultural Unit of Account, mitigation measures are required to reduce exposure of these plants to maize 1507 pollen. Such mitigation may take two forms: (1) direct mitigation measures (such as non-*Bt*-maize border rows), usually at small spatial scales, which seek to reduce the identified risk, and (2) indirect mitigation measures that seek to increase the overall population of particular non-target lepidopteran species, often at a spatial scale larger than single fields for which the risk has been identified. For (1), the width of such border rows should be adapted to the size of maize 1507 fields to keep them proportional to the actual risk. Thus 12m non-GM borders would be sufficient for a 5 ha field, 21 m borders for 15 ha field and 36 m for a 50 ha field of 1507 maize where exposure in both margins and within fields needs to be reduced.

The EFSA GMO Panel recognises that the proximity of areas that (1) contain non-target lepidopteran species of conservation concern, and that (2) maintain important sources of food host-plants for local populations of lepidopteran species should also be considered, as these areas may have high significance for the protection of these populations. For protected lepidopteran species in habitats according to Directive 2004/35/EC (EC, 2004), the EFSA GMO Panel considers that a distance of 30 m is sufficient to reduce the local mortality to a negligible level below 0.5%, even for extremely sensitive species (see Table 4).

It is important to maintain the flexibility of the mitigation measures, since what may be a good option for one region or Member State may not be sensible in another. Estimated local or global mortality, both before and after mitigation, depends in a complex manner on different combinations of a number of factors that include: the sensitivity of individual lepidopteran species, the degree of large-scale exposure effects, and host-plant density. The first of these factors may be assumed constant over Europe, but the other two factors vary according to regional agronomy and may be estimated locally using official agricultural statistics and other sources of information. Risk managers should select the appropriate values for the other two factors according to protection goals and management needs applying within their jurisdiction.

In conclusion, the EFSA GMO Panel considers that, subject to the implementation of appropriate management measures, the identified risks of maize 1507 cultivation on non-target Lepidoptera could be reduced to a level of no concern.

The EFSA GMO Panel concludes that risk mitigation measures are only required in situations where highly sensitive non-target Lepidoptera populations might be at risk. For example, highly sensitive non-target Lepidoptera and their host plants are present in *Bt*-maize fields and margins in areas where there is a high proportion of maize in arable fields and a high rate of adoption of maize 1507. Similarly, resistance evolution to target species is only expected when the selection pressure is high due to high adoption of maize 1507 in a region.

3.2. Post-market environmental monitoring[34]

3.2.1. General aspects of post-market environmental monitoring

Directive 2001/18/EC (EC, 2001) introduces an obligation for applicants to implement monitoring plans in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the aforementioned Directive. According to Annex VII, the objectives of (an environmental) monitoring plan are (1) Case-Specific Monitoring (CSM) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct, and (2) General Surveillance (GS) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA (EFSA, 2011a).

[34] Technical dossier / Section 5 / Pages 1-10 / Annexes 37 & 38

3.2.2. Interplay between environmental risk assessment, risk mitigation and post-market environmental monitoring

With the consideration of risk mitigation measures, the ERA of maize 1507 concluded that:

(1) the potential consequences of resistance evolution to the Cry1F protein in populations of exposed lepidopteran target pests may cause adverse environmental effects. Resistance to the Cry1F protein is likely to evolve in exposed populations of lepidopteran target pest species, particularly those subjected to the highest selection pressures, such as in areas of continuous or very extensive maize 1507 cultivation or in rotation with other crops expressing the Cry1F protein (if any). While this is not considered a direct environmental harm, the consequences of resistance evolution may require altered pest control practices with higher environmental load. Considering that lepidopteran target pests may evolve resistance to Cry1F-expressing maize under conditions of continuous exposure, the applicant proposed to put in place risk mnagement measures to delay the possible evolution of resistance. Therefore, the EFSA GMO Panel reiterated its earlier recommendation that appropriate IRM strategies (i.e., ‘high dose/refuge’ strategy) should be employed, in order to delay the potential evolution of resistance to the Cry1F protein in target pests;

(2) exposed non-target Lepidoptera that are highly sensitive to the Cry1F protein may be at risk if exposed to harmful amounts of maize 1507 pollen. The EFSA GMO Panel recommended that, in situations where highly sensitive non-target Lepidoptera populations might be at risk, mitigation measures are adopted to reduce exposure of sensitive populations, and provided risk managers with tools to estimate global and, where needed local mortality of exposed non-target Lepidoptera, both before and after different mitigation measures are put in place, and for different host-plant densities. Such mitigation measures may take two forms: (1) direct mitigation measures, such as non-*Bt*-maize strips, which seek to reduce the identified risk; the width of such strips should be adapted to the size of maize 1507 fields to keep them proportional to the actual risk (the larger the field, the larger the width), and (2) indirect mitigation measures, such as the establishment and maintenance of additional habitats for non-target Lepidoptera, that seek to increase the overall population of particular non-target lepidopteran species, often at a spatial scale larger than single fields for which the risk has been identified.

Since EFSA GMO Panel concluded that risk management measures should be undertaken for both of the identified risks, CSM is recommended in both cases to confirm the assumptions made in the ERA and in the development of appropriate risk management measures.

The EFSA GMO Panel recommends caution when predicting future responses of the European and Mediterranean corn borer in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Therefore, the EFSA GMO Panel, while agreeing with the ‘high dose/refuge’ strategy, recommends the periodic re-evaluation of the adequacy and efficacy of this IRM strategy.

The suggested mitigation measures to reduce exposure of highly sensitive non-target Lepidoptera under the aforementioned conditions took into consideration the level of uncertainty associated with the conclusions of the ERA (e.g., by considering a wide range of levels of sensitivity and exposure of non-target Lepidoptera). Therefore, the risk mitigation measures that might be adopted are more conservative than necessary to protect these species. Hence, the proposed risk mitigation measures may be disproportionate to the level of risk or scientific uncertainty in some cases, and put unnecessary burden on farmers. Therefore, if applicants, in agreement with risk managers, wish to reduce the mitigation measures because they are considered too conservative, then CSM should be conducted to reduce the levels of uncertainty associated with the revised management levels. Therefore, the EFSA GMO Panel recommends that monitoring studies are conducted to confirm the risk conclusions on the sensitive non-target Lepidoptera and thus to reduce uncertainty in representative receiving environments where maize 1507 will be cultivated.

3.2.3. Case-specific monitoring

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the ERA, then CSM should be carried out after placing on the market, in order to confirm assumptions made in the ERA and to further inform the ERA. CSM should be targeted at assessment endpoints and environmental protection goals identified as being at risk during the ERA, or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant. CSM should be put in place, in order (1) to confirm that any assumption in the ERA regarding the occurrence and impact of potential adverse effects is correct, and (2) to determine the efficacy of risk mitigation measures and/or ultimately to allow the modification of risk mitigation measures, so that their efficacy and proportionality can be improved (see EFSA, 2011a).

To assess the efficacy of risk mitigation measures put in place to reduce levels of risk and scientific uncertainty, the EFSA GMO Panel recommends CSM to address (1) resistance evolution to the Cry1F protein in lepidopteran target pests, and (2) the risk to sensitive non-target Lepidoptera to maize 1507 pollen.

3.2.3.1. Monitoring resistance evolution to the Cry1F protein in lepidopteran target pests

The applicant proposed to measure the baseline susceptibility of corn borer populations to the Cry1F protein and changes in that susceptibility in the EU. Resistance monitoring, through targeted field sampling in areas where maize 1507 adoption is the highest and selection pressure is greatest, should reveal changes in susceptibility of these populations. In this way, changes relative to the baseline susceptibility could be detected in time to enable proactive management before control failures occur (Siegfried *et al*., 2007; Tabashnik *et al*., 2008a,b, 2009). The EFSA GMO Panel agrees this approach and considers that susceptibility data can reveal potential changes in resistance levels in corn borer populations. Such data will also indicate the efficacy of the implemented ‘high dose/refuge’ strategy in delaying resistance evolution in the target pest species, and reduce the remaining scientific uncertainty related to the adequacy of the IRM plan proposed by the applicant.

The EFSA GMO Panel considers that the overall framework to monitor resistance evolution proposed by the applicant is consistent with those described in the scientific literature (reviewed by Tabashnik *et al*., 2009).

With regard to the monitoring of resistance evolution, the EFSA GMO Panel expects that the use of standard procedures will allow baseline susceptibility testing on small numbers of European populations for an efficient monitoring of resistance evolution to the Cry1F protein. Therefore, the EFSA GMO Panel recommends:

- utilising appropriate sampling strategies of larvae of corn borers to set the most adequate and precise susceptibility baselines through random sampling, and to measure changes in susceptibility of populations at greatest risk of resistance evolution through targeted sampling in areas of high adoption rate of maize 1507. The sampling strategy should include fields cropped to this *Bt*-maize and adjacent fields cropped to non-lepidopteran-active-*Bt*-maize or conventional maize, annual sampling during each maize growing season, follow up sampling of the same populations in subsequent seasons and sampling at appropriate times;

- accounting for relevant factors when designing an appropriate sampling strategy (e.g., the abundance, distribution and dispersal behaviour of corn borers, local variability in susceptibility levels).

In addition to the monitoring of baseline susceptibility and changes in susceptibility, the EFSA GMO Panel considers it relevant that unexpected field damage resulting from corn borer control failures is monitored and reported. Such observations may reveal the occurrence of localised resistance before it spreads, and may serve as a trigger for further investigations to detect emerging resistance at an early stage (i.e., maize 1507 fields may be followed closely to check if adult corn borers are found in these fields). The EFSA GMO Panel considers that farmer questionnaires provide a relevant early alert system to report unexpected field damage caused by corn borer larvae (see section 3.2.4.1).

The EFSA GMO Panel makes the following additional recommendations to the applicant:

- to focus the sampling of lepidopteran target pests in 'hotspot[35] areas' over time to increase the likelihood of detecting resistance evolution. Sampling in areas with lower adoption rate of maize 1507 is also required but at a lower frequency in order to establish susceptibility baselines;

- to include in the samplings surviving lepidopteran target pests within maize 1507 fields in order to detect potentially resistant individuals. The sampling should be mainly done as late as possible within the growing season in order to increase the likelihood of detecting surviving individuals;

- to consider regionally important lepidopteran pests (other than corn borers, see section 3.1.2.1) of maize 1507 in the context of CSM for IRM strategy and, where appropriate, adjust the design and implementation of the IRM plan accordingly;

- to revise the monitoring protocol aiming at a detecting resistance allele frequency below 5% (between 1% and 3%) in 'hotspot areas'. The EFSA GMO Panel recommends to increase the number of larvae collected or to use a F2 screening (see EFSA, 2011b for further details).

3.2.3.2. Monitoring the risk to non-target Lepidoptera

The EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera and considers that the purpose of these studies should be:

- to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on plants in and adjacent to maize fields at the time of pollen deposition (see point (a) below), and if so:
    - to estimate the proportions of these populations likely to be affected (see point (b) and in specific cases (c) below);
    - to determine the overall effect on maintaining a favourable status of these populations (see point (c) below).

Various approaches for such confirmatory studies are indicated below which could be adopted according to assessments of local need, the cost-effectiveness and considering whether the studies are likely to produce results that inform both risk managers and risk assessors.

The EFSA GMO Panel recommends monitoring approaches focus on:

a) *Determining the sensitivity and risk to exposed non-target lepidopteran species*

In the model (see section 2.3.5.2), conservative assumptions based on worst-case assumptions were made on the sensitivity of non-target lepidopteran species to the Cry1F protein. The recommended mitigation measures described in section 3.1.2.2 were made in line with these worst-case assumptions. It is likely that, in many maize growing regions, the exposed non-target Lepidoptera are not highly sensitive to the Cry1F protein, so that the mitigation measures could be reduced to make them proportional to the risk to non-target Lepidoptera. Conversely, some exposed non-target Lepidoptera may be found that have high levels of sensitivity to the toxin, so that they fully justify the

[35] In the present document, '*hotspot area*' is defined by an area of high adoption rate of maize 1507 and the presence of multivoltine types of target pests

recommended measures and may require other measures and monitoring to ensure populations are not being adversely affected.

In this respect, data are required on the sensitivity of non-target Lepidoptera which are estimated to have a high or greater sensitivity for the Cry1F protein, and where such species are present in different regions of Europe where maize 1507 will be grown. It is therefore recommended that applicants and Member States cooperate in determining which focal non-target Lepidopteran species should be examined, and where such lepidopteran larvae and their host-plants might be found during flowering of maize in maize 1507 growing regions. Such observations could trigger further investigation on the sensitivity of these non-target lepidopteran species to the Cry1F protein. Subsequently, using the model developed by Perry *et al.* (2010, 2011a,b), levels of local and global mortality likely to be found in and around maize 1507 fields and the effects on local and overall populations could be assessed.

b) Confirming the level of exposure of non-target Lepidoptera

In areas where it is known that highly sensitive lepidopteran species are present, the level of exposure of the larvae can be assessed by recording the populations of their host-plants within fields of maize 1507 and in the field margins, as well as in the adjacent fields and margins of other non-*Bt*-crops. The proportion of exposed and non-exposed host-plants can be related to that of the highly sensitive non-target lepidopteran population that is exposed and, assuming mortality is known, to the effect on populations.

c) Monitoring the effects on non-target Lepidoptera in the field and field margins of maize 1507 compared with conventional fields

The effects on numbers of larvae found on host-plants in and around maize 1507 fields could be assessed by counting the numbers of larvae on their host-plants in both maize 1507 fields and in nearby non-*Bt*-maize fields during and the flowering period. However, for many non-target lepidopteran species, it is likely that populations will be low or very variable, so that such studies will not be able to generate sufficient data to show differences between treatments. Therefore, the EFSA GMO Panel recommends to focus such monitoring on a limited number of ‘hotspot’ situations where the likelihood of adverse effects is high (see figure X(a)), i.e., where there are measurable numbers of highly sensitive species and a high uptake of maize 1507 in intensive areas of maize cultivation. In these cases, it is recommended that monitoring is accompanied by the monitoring described in (b) (above) since this is likely to give a better indication of exposure levels of the population of a non-target lepidopteran species in an area and this can be related to any treatment differences that might be observed in populations.

d) Monitoring the efficacy of risk management strategies (e.g., the pollen deposition from maize 1507)

Risk management strategies are designed to limit the amount of pollen deposition on larval host-plants. Monitoring could be conducted to determine the amounts of pollen deposited on larval host-plants within and at set distances from maize crops, in order to determine exposure levels of larvae and to determine whether isolation distances are sufficient to reduce exposure levels. In addition, the potential mortality of non-target Lepidoptera feeding on these host-plants after pollen deposition can be modelled in more detail if their sensitivity is known (see a)).

The EFSA GMO Panel considers that monitoring and additional studies are only required in situations where there is a potential risk to populations of sensitive non-target Lepidoptera due to high adoption of maize 1507 (i.e., above adoption rate of 20%).

3.2.4. General surveillance

According to Directive 2001/18/EC, the objective of GS is to detect any unanticipated adverse effects on protected and valued entities of the environment that may be due to the cultivation of GM plants, including biodiversity and ecosystem services (EFSA, 2011a).

The applicant proposed to conduct GS for maize 1507 throughout the period of validity of the authorisation. The GS will take into consideration and be proportionate to the extent of cultivation of maize 1507 in the EU Member States. The applicant proposed to build its GS on four approaches (1) the use of annual farmer questionnaires, (2) the review of scientific information provided by existing monitoring networks, (3) the monitoring and review of ongoing research and development, as well as scientific literature, and (4) the implementation of industry stewardship programs, in order to identify potential adverse effects associated with the intended uses of maize 1507.

3.2.4.1. Farmer questionnaires

The EFSA GMO Panel agrees with the approach of the applicant to establish farmer questionnaires as a reporting format that provides relevant information. The questionnaires to farmers exposed to or using GM plants are regarded by the EFSA GMO Panel as an adequate tool for addressing several aspects of GS (EFSA, 2011a). The EFSA GMO Panel is of the opinion that farmer questionnaires enable the reporting of any on-farm observations of effects associated with the cultivation of maize 1507, as this approach uses first-hand observations and rely on farmers' knowledge and experience of their local agricultural environments, comparative crop performance and other factors that may influence events on their land (Schmidt *et al.*, 2008; Wilhelm *et al.*, 2010). Some of the questions link directly to assessment endpoints or give indirect indications of effects on assessment endpoints (EFSA, 2011a).

Farmer questionnaires should be designed to determine whether the farmer/manager/worker has noticed any differences between the GM plant and its management and that of similar non-GM plants growing on the farm, nearby or previously (EFSA, 2011a). The applicant and risk managers are advised to consider the new EFSA GMO Panel guidance document on PMEM (EFSA, 2011a) and the specific recommendations on the annual PMEM report of maize MON 810 cultivation in 2009 (EFSA, 2011b) when finalising their or evaluating monitoring plans.

While the EFSA GMO Panel considers the format and contents of the farmer questionnaire, as provided by the applicant, comprehensive, it proposes the following modifications:

- to add questions on the possible occurrence and observation of (GM) volunteer maize from previous crops (whenever relevant) and feral maize plants in field margins for the consideration of unanticipated effects on the persistence and invasiveness potential of maize 1507;

- to consider the occurrence of regionally important lepidopteran pests other than corn borers in maize 1507 fields and surrounding areas;

- in addition to the questions on pest and disease incidences on maize 1507, the farmer questionnaire should specifically request information on the occurrence of possible unexpected field damaged maize 1507 plants which might be associated with corn borer control failures, as this information will complement the CSM of resistance evolution to the Cry1F protein in target pests;

- to add questions on the proportion of non-Cry1F-expressing maize compared with maize 1507 on the farm, the distance between the refuge area and the monitored maize 1507 field in case the refuge is planted as a separate field adjacent to the *Bt*-maize field, the differences in pest management practices of the refuge.

In line with the general recommendations on the farmer questionnaire set in its 2011 Scientific Opinion on PMEM (EFSA, 2011a), the EFSA GMO Panel advises farmer questionnaires:

- are designed to ensure the appropriate statistical validity and representativeness of the collected data, including the proportion of fields growing maize 1507 in a region and a minimum percentage or number of questionnaires required to achieve statistical power in the data collected;
- are designed to generate data on the agronomic management of maize 1507, as well as data on impacts on farming systems and the farm environment;
- use a field or group of fields growing maize 1507 as the basic unit for monitoring in representative farming regions and for representative cropping systems within the country. The precise fields should be identified, so that their locations can be subsequently retrieved from registers of GM plant sites;
- clearly identify the comparator (e.g., variety, location) and whether it is being grown adjacent to maize 1507, on the same farm or in another location. If no comparators are being grown spatially or temporally close to maize 1507, then the rationale for selecting another comparator (e.g., historical data) should be fully described;
- where appropriate, observe the field/fields in subsequent years for any unusual residual effects;
- provide information on other GM plant events being grown at the same sites and farms;
- are adapted, where needed, to each GM plant monitoring on a case-by-case basis by considering additional data requirements relevant for each species/event, its management and its receiving environments;
- are user friendly but also information rich;
- are constructed to encourage independent and objective responses from farmers, land managers and others involved with maize 1507 or its transgene products;
- are audited to ensure the independence and integrity of all monitoring data.

In addition to the general recommendations on the farmer questionnaire (EFSA, 2011a) and in line with its 2011 Scientific Opinion on the annual PMEM report on maize MON810 cultivation in 2009 (EFSA, 2011b), the EFSA GMO Panel advises the applicant to take into account the following points:

- the sampling frame should be comprehensive and a stratification should be applied consistently in each country. Adequate sampling should be carried out from the previous stratification exercise;
- the cultivation areas, with high uptake of maize 1507 and where maize 1507 has been continuously grown in previous years, should be over-represented in the sampling scheme;
- the number of farmers not participating in the survey and the reasons thereof should be documented;
- impartial and standardised interviews should be carried out by independent parties and effective quality and auditing procedures should be considered;
- additional questions in the farmer questionnaire should be considered to describe in more detail the cultivation of *Bt*-maize in the local area and/or the previous years, the receiving environments and the management systems in which maize 1507 is being grown;

- relevant data as from other sources of information (e.g., official statistics on crop management practices) should/could be considered for validity check of the questionnaires (e.g., consistency, representativeness);

- the raw data, programmes, logs and output files related to the statistical analysis of the farmer questionnaires should be provided. Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report;

- appropriate statistical procedures should be used based on using a distribution for appropriate outcomes;

- the use of a standard default effect size of 5 % is not relevant for all assessment endpoints and, where scientifically justified, different default effect sizes should be considered for some assessment endpoints;

- data should be pooled and statistically analysed over years. At the end of the ten years of GS, the applicant should conduct a statistical analysis with all pooled data;

- a codification for farmers repeatedly surveyed over years should be set up. These farmers should be particularly monitored;

- the number of years the surveyed farmer has grown maize 1507 and other GM plants should be indicated.

3.2.4.2. Existing monitoring networks

Since farmer questionnaires focus mainly on the cultivation area of the GM plant and its surroundings, the EFSA GMO Panel supports the consideration of additional information sources for GS (EFSA, 2011a). In this respect, Directive 2001/18/EC proposed to make use of established routine surveillance networks, in order to obtain data on environmental impacts in the landscape where GMOs are cultivated from a range of existing monitoring networks which observe changes in biota and production practices from farm up to regional level. EU Member States have various networks in place – some of which have a long history of data collection – that may be helpful in the context of GS of GM plant cultivations. Existing monitoring networks involved in routine surveillance offer recognised expertise in a specific domain and have the tools to capture information on important environmental aspects over a large geographical area. However, the EFSA GMO Panel recognises that existing monitoring networks fully meeting all the needs of the monitoring of GM plant cultivations can be limited (Bühler, 2006; Mönkemeyer *et al*., 2006; Schmidtke and Schmidt, 2007; Graef *et al*., 2008). The development of harmonised criteria for the systematic identification, specification and analysis of existing surveillance networks across the EU is therefore considered important (EFSA, 2011a).

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using existing monitoring networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in the Directive 2004/35/EC on environmental liability (EC, 2004) in farming regions where maize 1507 will be cultivated and intends to investigate their suitability for providing data on potential changes in biota.

Knowing the limitations of existing monitoring networks, it is important to describe the processes and criteria that will be used for selecting and evaluating existing monitoring networks for supplying data related to the unanticipated adverse effects of GM plants in GS. Therefore, the applicant, in consultation with Member States, should:

- consider the protection goals, the assessment endpoints and their indicators that could be monitored through existing monitoring programmes;

- identify the type of existing monitoring networks that would be appropriate to survey the protection goals considered to be at risk in the countries where maize 1507 will be grown;
- describe the generic approach and develop more detailed criteria to evaluate existing monitoring networks and how appropriate networks will be selected (considering the hereunder list of points);
- identify what changes need to be made to these monitoring networks and describe how these might be implemented, and identify gaps in information that could be filled by additional surveys;
- encourage these networks to adopt the proposed modifications and describe how data from these networks will be integrated and assessed.

In addition, when selecting existing monitoring networks to be part of GS, the applicant is recommended to consider the following points for assessing the suitability of these existing networks to supply relevant GS data:

- the relevance of protection goals and their indicators monitored through existing monitoring networks;
- the type (e.g., raw data) and quality of the data recorded;
- the statistical power and the effect sizes detected by monitoring networks, where appropriate;
- the ease of access to the data collected by existing monitoring networks (e.g., availability of data via Internet, free access to data or access subject to a fee, protected data of ongoing research projects);
- the track record and past performance of existing monitoring networks;
- the methodology used by existing monitoring networks (e.g., sampling and statistical approach) including: (1) the spatial scale of data collection (e.g., local, regional, national, zonal): existing monitoring networks focusing on agricultural areas cultivated with GM plants or with conventional plants like maize, potato (for which GM are also available and grown) should be preferred; (2) temporal scale of data collection: appropriate frequency of data collection and reporting (e.g., short-term vs. long-term data sets, regularity of data collection); and (3) other parameters such as the language of the reports, impartiality.

Furthermore, the EFSA GMO Panel recommends that the applicant describes arrangements with any third parties participating in its GS plan. It is recommended to consider how arrangements for collecting, collating and analysing data will be made, and to describe how formal agreements, procedures and communication will be established with the European Commission and Member States or other third parties, although detailed arrangements may not have been agreed at the time of the application.

The EFSA GMO Panel also recommends to include in the sources of information that support GS of maize 1507, existing monitoring networks that monitor herbicide usage, botanical diversity on farms and weed resistance evolution, so that the scientific requirements for the detection of any unforeseen environmental effects due to altered farm management practices associated with maize 1507 cultivation are met.

3.2.4.3. Monitoring and review of ongoing research and development, as well as scientific literature

An additional approach to support GS is to review all new scientific, technical and other information pertaining to maize 1507, including information on GM plants with similar traits or characteristics, which has emerged during the reporting period. This will include reviewing of results from ongoing research and development studies (e.g., variety registration trials) and all publications including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted (EFSA, 2011a).

The EFSA GMO Panel recommends that the applicant:

- to cover all relevant peer-reviewed publications, including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted;

- to describe the criteria for selecting and evaluating the scientific reliability of publications;

- to adhere to systematic literature review methodology to select relevant papers (EFSA, 2010d).

3.2.4.4. Industry stewardship programs

The EFSA GMO Panel welcomes the applicant's proposal to develop stewardship programs for the introduction, marketing and management of maize 1507, but advises that these programmes should be made available well in advance of the time of commercialisation so as to allow risk managers to validate the implementation of risk management measures and detailed monitoring plans.

3.2.5. Reporting results of post-market environmental monitoring

The applicant will submit a report on an annual basis covering CSM and GS. In case of adverse effects altering the conclusions of the ERA, the applicant will immediately inform the European Commission and Member States. The EFSA GMO Panel agrees with the proposal made by the applicant on reporting intervals. The EFSA GMO Panel recommends that effective reporting procedures are established with the Competent Authorities of Member States and the European Commission as required under the Council Decision 2002/811/EC on monitoring.

The results of PMEM should be presented in accordance with the standard reporting formats established by the 2009/770/EC Commission Decision on standard reporting formats (EC, 2009a). In addition, the applicant is recommended to provide raw data, in order to allow different analyses and interrogation of the data and to allow scientific exchange and co-operation between Member States, the European Commission and EFSA. The EFSA GMO Panel recommends that the applicant describes whether the PMEM reports contain cumulative analyses of data with previous years' results.

3.2.6. Conclusions and recommendations on PMEM

The EFSA GMO Panel gave its opinion and made recommendations on the scientific quality of the PMEM plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the ERA, the EFSA GMO Panel recommends CSM to address (1) the possible resistance evolution to the Cry1F protein in lepidopteran target pests, and (2) the risk to sensitive non-target Lepidoptera to maize 1507 pollen. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems.

The EFSA GMO Panel agrees with the GS approach of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize 1507, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize 1507 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize 1507, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize 1507, but requests that its proposals to strengthen GS are implemented. The EFSA GMO Panel considers that the current plan for GS, and in particular the methodology, needs further details according to the requirements laid down in its 2011 Scientific Opinion providing guidance on PMEM of GM plants, as well as its Scientific Opinion on the annual

2009 PMEM report on maize MON 810 (EFSA, 2011b). The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

CONCLUSIONS AND RECOMMENDATIONS

Considering the recurrent requests of the European Commission for reanalysis of the 2005 Scientific Opinion on GM maize event 1507, the EFSA GMO Panel updated the previous ERA of maize 1507 in light of the scientific data and methodology currently available, and to consider the possible adverse effects that the cultivation of maize 1507 might have on NTOs (e.g., Lepidoptera). In addition, the EFSA GMO Panel was asked to reconsider its recommendations to risk managers for methods to reduce exposure and mitigate risks linked to maize 1507 cultivation.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the initial notification C/ES/01/01 for cultivation of maize 1507, including additional data supplied by the applicant and relevant scientific publications.

The EFSA GMO Panel recalibrated its mathematical model, developed by Perry *et al.* (2010) for the ERA of a similar insect resistant maize (event MON 810), in order to simulate and assess potential adverse effects resulting from the exposure of non-target Lepidoptera (butterflies and moths) to pollen from maize 1507 under representative EU cultivation conditions, and extended it to estimate the efficacy of certain mitigation measures. The 2005 EFSA GMO Panel Scientific Opinion on maize 1507 supported '*management recommendations for the cultivation of maize 1507 [with] measures to reduce exposure of non-target Lepidoptera (as well as the target pest), such as the use of non-transgenic border rows as refugia for the target that would also reduce exposure of field margin weeds (and hence non-target Lepidoptera) to pollen from Bt-maize*'. In this Scientific Opinion, the EFSA GMO Panel has used new evidence to explore the complexities of this issue.

The EFSA GMO Panel concludes that the cultivation of maize 1507 could have the following adverse effects on the environment in the context of its intended uses (1) the adoption of altered pest control practices with higher environmental load due to potential evolution of resistance to the Cry1F protein in populations of exposed lepidopteran target pests, and (2) reductions in populations of certain highly sensitive non-target lepidopteran species where high proportions of their populations are exposed over successive years to high levels of maize 1507 pollen deposited on their host-plants. In situations where highly sensitive non-target Lepidoptera populations might be at risk, the EFSA GMO Panel recommends that mitigation measures are adopted to reduce exposure.

Considering the wide range and variability of agro-ecosystems and protection goals within the EU, this EFSA GMO Panel Scientific Opinion provides risk managers with tools to estimate global and, where needed local, mortality of exposed non-target Lepidoptera, both before and after different mitigation measures are put in place, and for different host-plant densities. This enables risk managers to choose mitigation measures proportionate to the level of identified risk and to the protection goals pertaining to their region. Special attention should be paid to the degree of large-scale exposure as mitigation measures are only needed when the proportion of maize and uptake of maize 1507 are sufficiently high, regardless of the other parameters. If maize 1507 cultivation remains below 5% of the Agricultural Unit of Account[36] (i.e., zv = 0.05, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.00625), the global mortality is predicted to remain below 1%, even for extremely highly sensitive species, and then risk mitigation measures are not required. Whenever mitigation measures are needed, the implementation of non-*Bt*-maize border rows will reduce the mortality of non-target lepidopteran species for both within fields and in field margins.

For protected lepidopteran species in habitats according to Directive 2004/35/EC, it is recommended that maize 1507 is not cultivated within 30 m of their habitat boundary, so that exposure and hence the risks to larvae of lepidopteran populations are minimised in these areas.

[36] For example, an uptake of 20% of maize 1507 in a region where maize represents 25% of the arable land.

In addition to the specific concern on non-target Lepidoptera, the EFSA GMO Panel considered the possible adverse effects of maize 1507 on other NTOs, in order to update, where appropriate, its previous evaluations in light of new relevant scientific literature. Having considered all available relevant scientific literature, the EFSA GMO Panel concludes that no new scientific information has been made available that would invalidate the conclusions of its previous Scientific Opinions on maize 1507.

The possible resistance evolution to the Cry1F protein in lepidopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize 1507, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects.

The EFSA GMO Panel recommends CSM to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and scientific uncertainty for (1) the possible resistance evolution to the Cry1F protein in lepidopteran target pests, and (2) the risk to sensitive non-target Lepidoptera from maize 1507 pollen. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems.

For (1), the EFSA GMO Panel reiterates its earlier recommendation that appropriate IRM strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1F protein in lepidopteran target pests. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be *refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size. In addition, the EFSA GMO Panel makes additional recommendations to the applicant like (a) to focus the sampling of lepidopteran target pests in 'hotspot[37] areas' over time; (b) to include in the samplings surviving lepidopteran target pests within maize 1507 fields in order to detect potentially resistant individuals; (c) to consider regionally important lepidopteran pests (other than corn borers) of maize 1507; and (d) to revise the monitoring protocol aiming at a detecting resistance allele frequency below 5% in 'hotspot areas'. The EFSA GMO Panel recommends caution when predicting future responses of the European and Mediterranean corn borer in the EU based on experiences elsewhere, as resistance evolution in target insect pests is dependent upon many factors. Therefore, the EFSA GMO Panel, while agreeing with the 'high dose/refuge' strategy, recommends the periodic re-evaluation of the adequacy and efficacy of this IRM strategy.

For (2), the EFSA GMO Panel recommends to carry out further field studies on non-target Lepidoptera. The purpose of these studies should be to estimate whether non-target Lepidoptera larvae, with a high sensitivity to the Cry1F protein, are in reality feeding on host-plants in and adjacent to maize fields at the time of pollen deposition, and if so (a) to estimate the proportions of these populations likely to be affected; and (b) to determine the overall effect on maintaining a favourable status of these populations.

The EFSA GMO Panel agrees with the GS approach of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize 1507, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize 1507 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize 1507, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize 1507, but requests that its proposals to strengthen GS are implemented. The EFSA GMO Panel considers that the current plan for GS, and in particular the methodology, needs further details according to the requirements laid down in its 2011 Scientific Opinion providing guidance on PMEM of GM plants, as well as its Scientific Opinion on the annual 2009 PMEM report on maize MON 810. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

[37] In the present document, '*hotspot area*' is defined by an area of high adoption rate of maize 1507 and the presence of multivoltine types of target pests

In areas where other lepidopteran pests than the European and Mediterranean corn borer are important targets of maize, they might also be subject to resistance evolution due to exposure to the Cry1F protein expressed in maize 1507. Therefore, the EFSA GMO Panel recommends these species are considered by the applicant in the context of the IRM strategy, CSM to monitor resistance evolution to the Cry1F protein in those species, as well as GS through farmer questionnaires.

The EFSA GMO Panel concludes that, subject to appropriate management measures, maize 1507 cultivation is unlikely to raise safety concerns for the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Note, dated 9 November 2010, from the Chair of the EFSA GMO Panel to the EFSA Executive Director to request a self-task mandate to update the Environmental Risk Assessment of GM maize 1507 for cultivation.
2. Acknowledgement letter, dated 16 December 2010, from the EFSA Executive Director to the Chair of the EFSA GMO Panel.
3. Letter from EFSA to the applicant, dated 20 December 2010, requesting additional information.
4. Note, dated 8 February 2011, from the Chair of the EFSA GMO Panel to the EFSA Executive Director to request an extension of deadline.
5. Acknowledgement letter, dated 17 February 2011, from the EFSA Executive Director to the Chair of the EFSA GMO Panel.
6. Letter from the applicant to EFSA, dated 21 March 2011, providing the additional information requested by EFSA.
7. Letter, dated 5 July 2011, from the Director General of DG SANCO to the EFSA Executive Director asking EFSA to consider the PMEM of maize 1507.
8. Note, dated 31 August 2011, from the Chair of the EFSA GMO Panel to the EFSA Executive Director to request a modification of the terms of reference and an extension of deadline.
9. Acknowledgement letter, dated 14 September 2011, from the EFSA Executive Director to the Chair of the EFSA GMO Panel.
10. Letter, dated 20 September 2011, from the EFSA Executive Director to the Director General of DG SANCO in response to the European Commission's request to consider the PMEM of maize 1507.

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APPENDICES

Appendix 1. Some spatial aspects of non-cropped areas in farmland

In practice, mitigation measures are likely to be implemented differently by farmers depending on field management, field size and shape, as well as the habitat and extent of adjacent field margins. The presence and extent of field margins, as well as of roads, paths, ditches and all other forms of field edges play an important role in risk assessment and risk management. Field margins and edges are typical non-cultivated habitats of agricultural landscapes that are habitat for wild annual or perennial plant species (Neemann *et al.*, 2006). Neemann *et al.* (2007) revealed that the frequency of these (often linear) habitats is a key variable associated with the fragmentation of agricultural landscapes. Fragmentation is a useful indicator to rank agricultural landscapes for the likelihood of region-specific potentially adverse environmental effects of the cultivation of genetically modified plants. For example, in agricultural landscapes where *Bt*-maize is grown, the local abundance of fields having host-plants for non-target Lepidoptera is related closely to the frequency of these linear non-cultivated habitats (Neemann, unpublished). Regions of less-intensive maize cultivation often have fragmented small fields (e.g., Berkatal, Germany; see Figure A1_1, below).

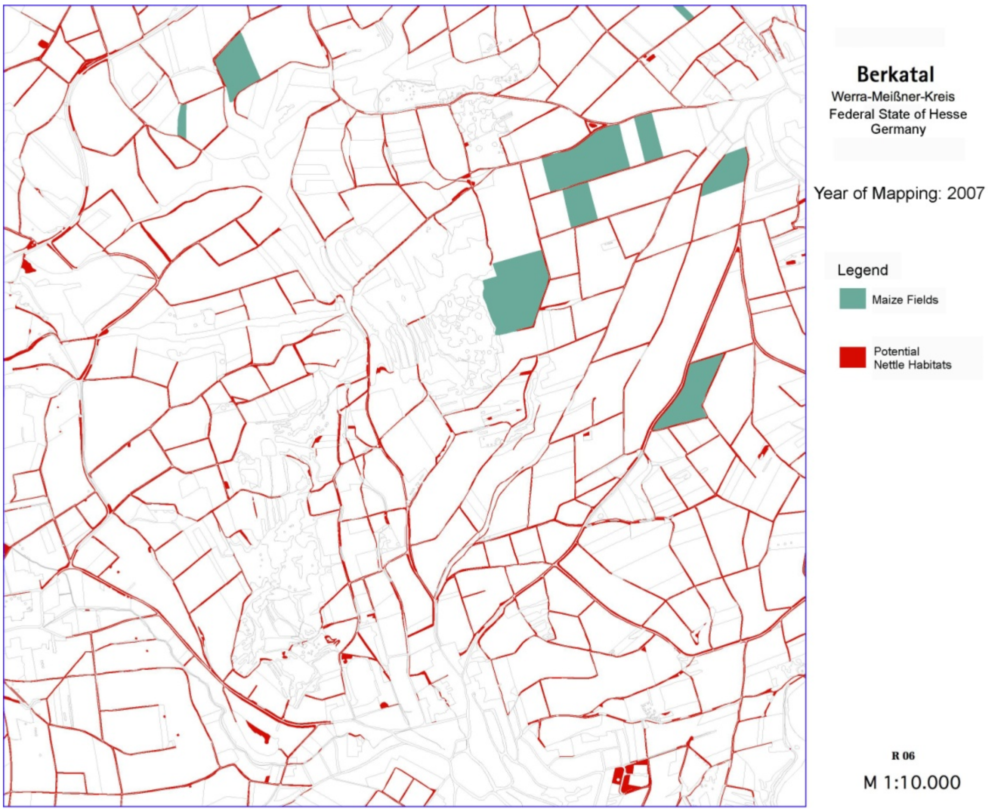

Figure A1_1 Distribution of maize fields (grey) and marginal and edge habitats for the host-plant genus *Urtica* (red) in a 9 km^2 sector around the village of Berkatal (Federal State of Hesse, northern part of German Highlands).

By contrast, more-intensive landscapes tend to feature larger fields; in such landscapes margins and edge habitats are clearly less extensive, e.g., Grebbin, Germany; see Figure A1_2, below).

Figure A1_2 Distribution of maize fields (grey) and marginal and edge habitats for the host-plant genus *Urtica* (red) in a 9 km^2 sector around the village of Grebbin (Federal State of Mecklenburg-Vorpommern, northeastern part of German Flatlands).

In general, agricultural landscapes with less-intensive maize cultivation and with fragmented small fields (e.g. Berkatal, Germany, see Figure A1_1, above) tend to have a ratio of marginal area to crop area which exceeds that of more-intensive landscapes (e.g., Grebbin, Germany, see Figure A1_2, above).

It is possible to take some initial steps towards the quantification of these aspects of maize ecosystems insofar as they may impact the ERA of non-target Lepidoptera. It is clear that, for several fragmented small fields, if each have margins around all sides, the risk to non-target Lepidoptera is less than in a single field with the same width of margin (compare arrangements (*a*) and (*b*) in Figure X below), because the ratio of margin area to crop area is twice as great for the smaller fields (see Perry *et al.*, 2010 and section 2.3.5.2 of main document).

By contrast, if instead the width of the field margins is proportional to the length of the side of the field, i.e., if the larger field in Figure X had instead a margin of width $2D$ as in arrangement (*c*), then its total marginal area would be $16LD$, equal to that of the total of the smaller fields in (*a*). Also, note that the average distance of a lepidopteran larva in a margin of the arrangements in (*a*) and (*b*) is $D/2$ metres from the nearest crop, less than the corresponding average distance of D for the larger field in (*c*). Under those assumptions, the relative risk to non-target Lepidoptera from pollen deposition from crop to margin would therefore be *less* for arrangement (*c*).

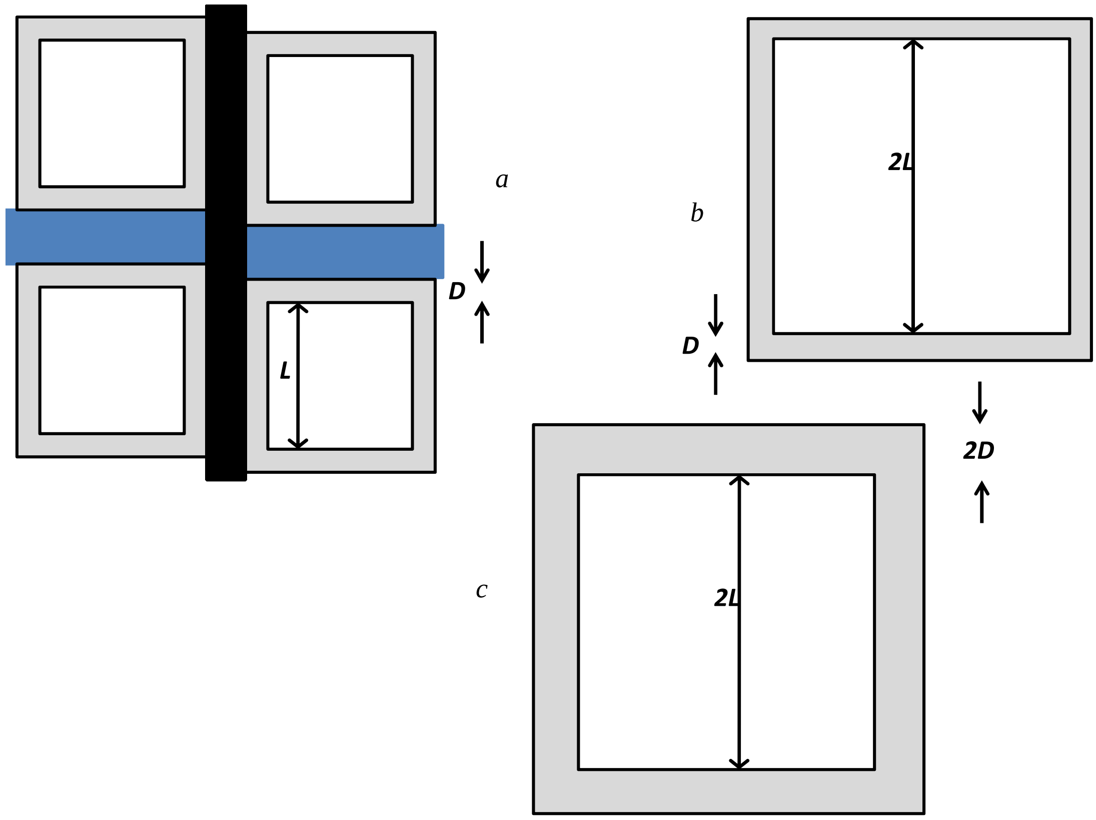

Figure X Simplified representation of three hypothetical arrangements of *Bt*-maize fields; white areas indicate crop, grey areas field margins, black rectangle a road and blue areas a river. It is assumed that the *Bt*-maize fields shown border, as neighbours, fields in which *Bt*-maize is not grown. (*a*) Four fragmented square fields with sides each of length *L* and margins each of width *D*; each field has a crop area of L^2 and total margin area of approximately 4*LD*, so total crop area over the four fields is $4L^2$ and total margin area is approximately 16*LD*. Average distance between margin and nearest crop is D/2. (*b*) A single square field with side of length 2*L* and margin of width *D* also has crop area of $4L^2$, but total margin area is approximately 8*LD*, about half that for the fragmented fields in (*a*). Average distance between margin and nearest crop is D/2. (*c*) A single square field with side of length 2*L* and margin of width 2*D* also has crop area of $4L^2$, but total margin area is approximately 16*LD*, the same as that for the fragmented fields in (*a*). Average distance between margin and nearest crop is D, greater than that for (*a*) or (*b*).

It is easy to change the parameters *C* (where $C = L^2$) and *D* in the model of Perry *et al*. (2010) in order to calibrate risk management for particular regions and thus to apply local characteristics to estimate the risk of mortality. It is not possible to give more specific recommendations concerning the effects of the degree of fragmentation of the EU agricultural landscape and field and margin sizes, because of lack of data and complexity of the possible scenarios. However, in general, *the greater the area of non-Bt habitat in which host-plants are found, the less the risk to non-target Lepidoptera*; and *the greater the distance of a larva from a source of Bt-maize pollen the less is the risk*. This applies whether the habitat is a field margin or an area of non-*Bt*-maize.

Appendix 2. Spatial aspects of mitigation using sown areas of non-*Bt*-maize

If it is assumed that, to manage insect resistance, 20% of the total cropped area should be non-*Bt*-maize, then recommendations can be given concerning where and how to place the non-*Bt*-maize in relation to the fields cropped with maize 1507, in order to minimise effects on non-target Lepidoptera. In general, it must be reiterated that for non-target Lepidoptera, arrangements should seek to maximise the average distance of larvae from the nearest source of maize 1507 pollen, since these minimise the risk.

Consider the four hypothetical arrangements of non-*Bt*-maize in and around the maize 1507 fields depicted in Figure W, below. Clearly, arrangement (*a*) cannot be recommended under any circumstances; the average distance of a larva to the crop, $L/40$, is relatively small and no larva is at a greater distance than $L/20$ from the *Bt*-crop. Furthermore, growers would have the greatest difficulty in sowing this arrangement. For the other arrangements, the recommendations will vary as to whether the field depicted has margins or not.

For fields without margins, the arrangement that maximises the average distance of larvae from the nearest source of maize 1507 pollen is clearly (*d*). It should be noted that for this arrangement, the block of non-*Bt*-maize is sufficiently distant (> 50 m) from the crop to make the risk from pollen deposition negligible, but is sufficiently close to it (< 500 m) to qualify as a refuge under the insect resistance management strategy (see relevant sections of main document).

For fields with a margin on each side, there is no optimal arrangement. Arrangement (*b*) is *usually* superior to (*c*) and (*d*), since it maintains a minimum distance of $L/20$ between crop and margin; the minimum distance is zero for (*c*) and (*d*). For a margin of width *D,* the average distance of a larva in the margin from the crop is: ($D/2 + L/20$) for arrangement (*b*), approximately the same ($D/2 + L/20$) for arrangement (*c*), and $D/2$ for arrangement (*d*). However, these calculations take no account of the mortality of larvae within the crop; the efficacy of mitigation for an entire field plus margins depends in a complex fashion on the balance between within-crop host-plant density (and therefore within-crop larval density), on potential larval mortality in the margin and on other factors.

Table 5, below, summarises the outcomes of different mitigation strategies for these different factors through the estimated mortality from the modelled maize field of 15 ha, in terms of proportional reduction in mortality. Then, for illustrative purposes, *P*, the proportional reduction in mortality achieved through mitigation under the best strategy(ies), is presented both in Table 5 and in Appendix 3, below. The efficacy of mitigation for non-*Bt*-maize buffer strips is calculated for increments of 3m width up to a width of 24 m; blocks are assumed to be 4.48 ha. For buffer strips of and for blocks the proportion of the total cropped area that is non-*Bt*-maize is in both cases 23%.

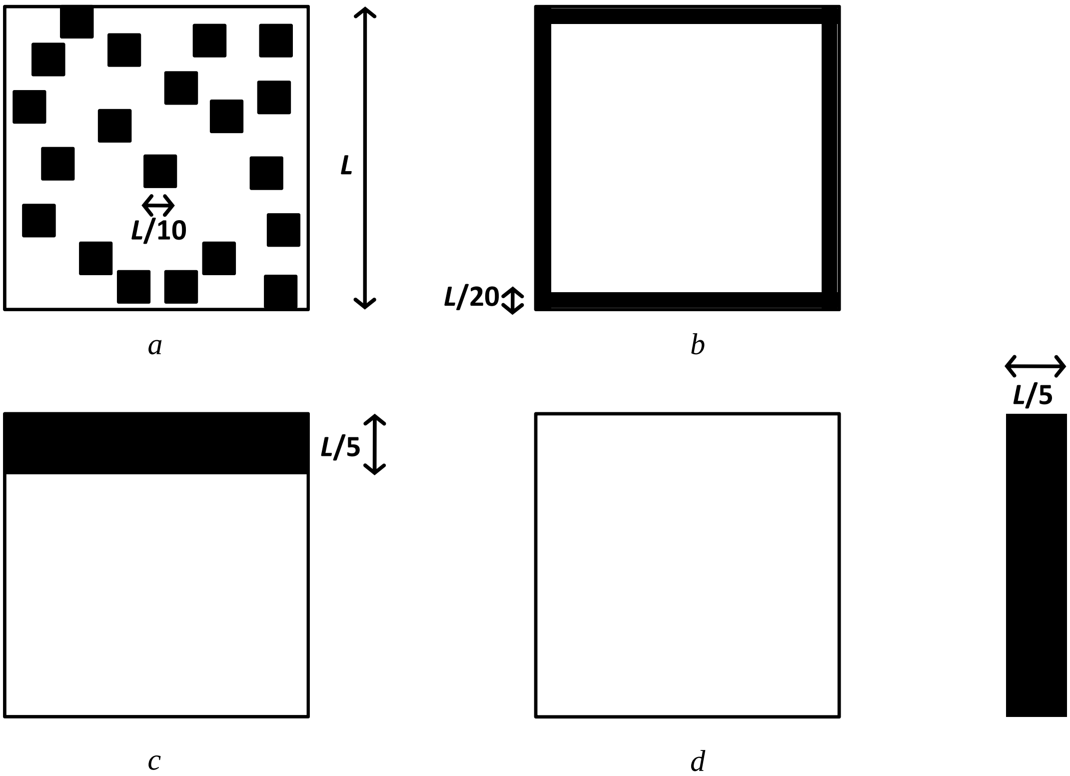

Figure W Four hypothetical arrangements of blocks of non-*Bt*-maize (black shading) placed within and around fields for mitigation purposes; white areas represent *Bt*-maize. In each case, 20% of the total cropped area comprises non-*Bt*-maize. (*a*) Twenty blocks of non-*Bt*-maize placed randomly; (*b*) four buffer strips of non-*Bt*-maize placed along each edge of field; (*c*) a single block of non-*Bt*-maize placed along one side of the field; and (*d*) a single block of non-*Bt*-maize placed remotely at a distance between 50 m and 500 m from the *Bt*-maize field.

Table 5: Mitigation strategies for different within-crop host-plant densities and for fields with and without margins. The proportional reduction in mortality, *P*, measures efficacy of mitigation. Mitigation strategies follow either arrangement (*b*) or (*d*) in Figure W, above.

Field type	With margins		Without margins	
Within-crop host-plant density	Zero	Non-zero (0.01 plants m^{-2})	Zero	Non-zero (0.01 plants m^{-2})
Mitigation strategy	The best strategy is arrangement (*b*), buffer strips of varying widths, *P* is given in Figure V(*a*), Appendix 3, below. The poorest outcome is given by arrangement (*d*), in a single block, *P* = 0	There is no best strategy. For arrangements (*b*), buffer strips of varying widths, and for arrangement (*d*), a single block, *P* is given in Figure V(b), Appendix 3, below	No need for mitigation, since there are no host-plants in field, therefore no larvae and no risk	The best strategy is arrangement (*d*), a single block, for which *P* = 0.23. For the less good arrangement (*b*), buffer strips, *P* depends on the strip width, but can never be as large as 0.23

Under mitigation, larvae in the margin would be exposed to a mixture of pollen from both *Bt*- and non-*Bt*-maize. It is possible that there might therefore be a slight reduction in the effective dose of the Cry1F protein through dilution by the non-harmful non-*Bt*-maize pollen, to some value less than that of the *Bt*-maize, depending on larval ingestion and the strength of any dilution effect. This effect has been ignored here, and the results quoted in this Scientific Opinion therefore represent a worst-case scenario, where mortality estimates are not underestimated (Perry *et al*., 2011b).

Appendix 3. Outcomes of mitigation using sown areas of non-*Bt*-maize

For illustrative purposes, the scenarios for fields with margins, leading to mortalities estimated from the model as described in Appendix 2, above, are used to demonstrate the variability in the efficacy of mitigation, defined by *P*, the proportional reduction in mortality due to mitigation. Specifically, Figure V(*a*) relates to the scenario for which host-plants are assumed absent from within the crop and the mitigation strategy is given by Figure W(*b*) in Appendix 2, above. Values of *P* are plotted for each of the five sensitivities studied and for each of the eight buffer strip widths considered. Clearly, the estimated efficacy of mitigation depends in a non-linear fashion on the sensitivity of the species concerned (and therefore its estimated mortality) and on the width of the buffer strips employed.

By contrast, Figure V(*b*) relates to the scenario for which the within-crop host-plant density is assumed to be 0.01 plant m^{-2} and the mitigation strategies considered are those given by Figure W(b) and W(d), in Appendix 2, above. Here, for the buffer strips, the efficacy of mitigation increases monotonically with *w*, as expected, but depends on species sensitivity in a complex manner. For most, but not all combinations of *w* and sensitivity, mitigation using buffer strips is more efficacious than a single block. By comparing Figures V(*a*) and (*b*), note how the efficacy of mitigation depends very sensitively on the assumed host-plant density.

When it can be assumed that there are no host-plants within the crop, it is possible to mitigate using buffer strips so as to almost completely eliminate mortality. In this case, use of the mitigation strategy in the form of strips of width 24 m reduces estimated global mortality, for all factor combinations, to below 1%. In fact, for all cases where the host-plant density within-crop may be assumed to be zero, the local estimated mortality never exceeds 4%, even for the extreme sensitivity level.

By contrast, when the host-plant density is 0.01 or greater and the sensitivity is high or greater, mitigation of the type considered here, even for buffer strips of width 24 m, cannot eliminate more than two-thirds of the estimated mortality. As an example, for a moderate value of parameter *R* = 0.4 (see section 2.3.5.2), estimated global mortality is around 5%, and this would only be reduced to 2% after mitigation. Equally clearly, mitigation with strips of width of 12 m instead of 24 m could not be recommended for scenarios other than those with non-conservative, large-scale effects or for species of less than high sensitivity.

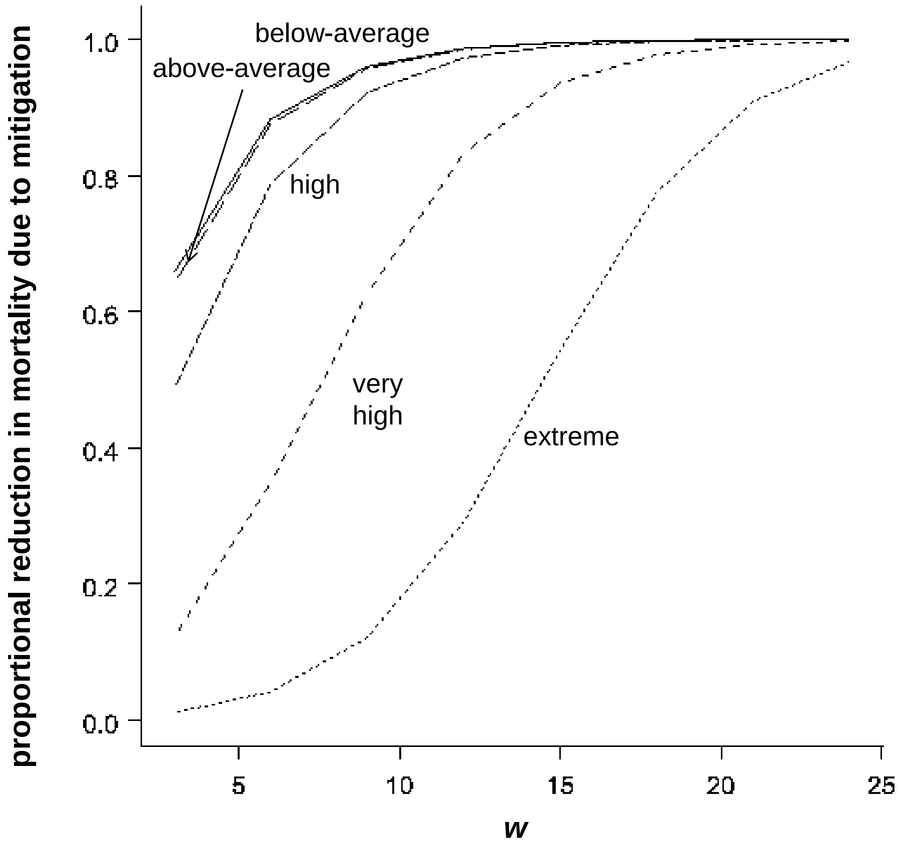

Figure V(*a*) Efficacy of mitigation, measured by proportional reduction in mortality, *P*, for a 15 ha maize 1507 field with a 2 m margin on each side, assuming no within-crop host-plants are present. Mitigation is effected by sowing non-*Bt*-maize buffer strips of width *w*, as in arrangement (*b*) of Figure W, above. The efficacy of mitigation increases monotonically with *w* and decreases monotonically with species sensitivity: solid line indicates 'extreme' sensitivity; long-dashed line indicates 'very-high'; medium dashed line 'high'; short dashed line 'above-average'; and dotted line 'below-average'.

In conclusion, estimated local or global mortality, both before and after mitigation, depends in a complex manner on different combinations of a number of factors that include: the sensitivity of individual lepidopteran species; the degree of large-scale exposure effects; and host-plant density. The first of these may be assumed constant over Europe, but the other two factors vary according to regional agronomy and may be estimated locally using official agricultural statistics and other sources of information. Risk managers should therefore select the appropriate values for the other two factors according to protection goals and management needs applying within their jurisdiction.

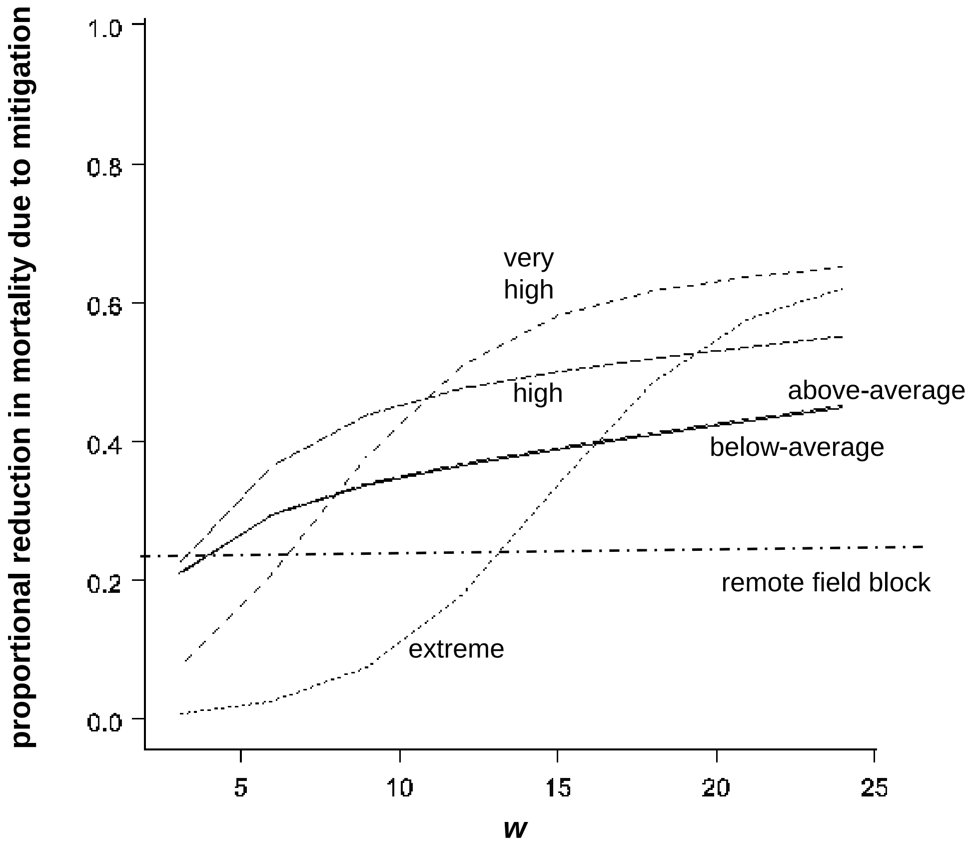

Figure V(*b*) Efficacy of mitigation, measured by proportional reduction in mortality, *P*, for a 15 ha maize 1507 field with a 2 m margin on each side, assuming a within-crop host-plant density of 0.01 plants m^{-2}. Mitigation is effected by sowing non-*Bt*-maize buffer strips of width *w*, as in arrangement (*b*) of Figure W, above, or a single block of non-*Bt*-maize as in arrangement (*d*) of Figure W. The efficacy of mitigation increases monotonically with *w*, but depends on species sensitivity in a complex manner: solid line indicates 'extreme' sensitivity; long-dashed line indicates 'very-high'; medium dashed line 'high'; short dashed line 'above-average'; and dotted line 'below-average'. Efficacy, *P*, is shown for these five sensitivities for the eight buffer strip widths considered in arrangement (a). Also shown is the value of *P* for the single block, which has a constant value of 0.23 for all sensitivities studied.