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Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to Bt-maize pollen

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SCIENTIFIC OPINION

Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to Bt-maize pollen

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

Using mathematical modelling, the EFSA GMO Panel has previously quantified the risk to non-target (NT) Lepidoptera of conservation concern, potentially occurring within protected habitats, associated with the ingestion of Bt-maize pollen deposited on their host plants. To reduce the estimated larval mortality to a negligible level, an isolation distance of 20 and 30 m was recommended between protected habitats and the nearest fields of maize MON 810/Bt11 and 1507, respectively. Here, the EFSA GMO Panel refines its model predictions, accounting for newly reported information on maize pollen deposition over long distances. For its calculations, the EFSA GMO Panel considered three exposure scenarios at a range of isolation distances, at two protection levels and for a range of lepidopteran species, including hypothetical ones, with a wide spectrum of sensitivities to Bt toxins. An analysis of various sources of uncertainties affecting the exposure of NT Lepidoptera to Bt-maize pollen was conducted, in order to provide quantitative estimates of realistic exposure levels. The EFSA GMO Panel therefore provides risk managers with a tool to estimate and mitigate the risk for NT Lepidoptera of conservation concern. In contrast to its previous outcomes obtained for unrealistically large levels of exposure that would not be expected in practice, the EFSA GMO Panel reports here mortality estimates for a more realistic level of exposure. The EFSA GMO Panel concludes that its previous recommendation for a 20 m isolation distance around protected habitats, within which maize MON810/Bt11 should not be cultivated, remains valid. New calculations show that the previously recommended isolation distance of 30 m from the nearest maize 1507 field would still protect NT Lepidoptera with known levels of sensitivity, including the 'highly-sensitive' *Plutella xylostella*. Should hypothetical species with greater sensitivities exist, larger isolation distances would be needed to ensure the desired level of protection.

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Summary

In this scientific opinion, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) updates its previous risk management recommendations on limiting the exposure of non-target (NT) Lepidoptera of conservation concern, potentially occurring in protected habitats, as defined under Directive 2004/35/EC, to maize MON 810, Bt11 and 1507 pollen. Using the mathematical model developed by Perry et al. (2010, 2011, 2012, 2013), the EFSA GMO Panel previously quantified the risk to NT Lepidoptera associated with the ingestion of Bt-maize pollen deposited on their host plants. To reduce the estimated larval mortality to a negligible level, an isolation distance of 20 and 30 m was recommended between protected habitats and the nearest fields of maize MON 810/Bt11 and 1507, respectively.

New information provided by Hofmann et al. (2014) led the EFSA GMO Panel to reconsider the level of exposure of NT lepidopteran larvae to Bt-maize pollen, in particular over long distances for larvae in protected habitats, and thus to refine the outcomes of the modelling exercise performed by Perry et al. (2010, 2011, 2012, 2013).

The EFSA GMO Panel ran new simulations with exactly the same Bt-related mortality model as used by Perry et al. (2010, 2011, 2012, 2013) but assuming the dose–distance relationship defined by Hofmann et al. (2014). The EFSA GMO Panel considered different exposure scenarios and the uncertainties pertaining to the structure of the Perry et al. model, and/or contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen. In this scientific opinion, the percentage of NT lepidopteran species, for which the predicted mortality is less than a defined threshold protection level (e.g. 0.5 % or 1 %), is given for the three different exposure scenarios and different isolation distances from sources of Bt-maize pollen.

The EFSA GMO Panel emphasises that, in its previous scientific opinions (EFSA GMO Panel, 2011a, b, 2012a–e), larval mortality was estimated with regard to an unrealistically large level of exposure, as no allowance was made for effects that are known to reduce the effective exposure to Bt-maize pollen, such as the proportion of maize pollen that comes from non-Bt-maize varieties. Despite the fact that mortality estimates may have been overestimated for the dose–distance relationship used by EFSA (EFSA GMO Panel, 2011a, b, 2012d, e), no such allowances were necessary in these previous opinions, because the isolation distances commensurate with those mortality estimates were, in any case, relatively small. With the different dose–distance relationships studied here, it is necessary to adopt more realistic levels of exposure and hence isolation distances that are commensurate with appropriate mortality estimates. An uncertainty analysis of several different factors affecting the exposure of NT Lepidoptera to Bt-maize pollen is used to provide quantitative estimates of a more appropriate exposure level.

The EFSA GMO Panel concludes that the new information provided by Hofmann et al. (2014) does not impact greatly on the mortality estimates for NT Lepidoptera of conservation concern, occurring within protected habitats and potentially exposed to maize MON 810/Bt11 pollen. Under the most realistic and even conservative scenarios, the estimated mortality for all species considered is always less than 0.5 % for the previously recommended isolation distance of 20 m. Therefore, the previous EFSA GMO Panel recommendation for isolation distances around protected habitats, within which maize MON 810/Bt11 should not be cultivated, remains valid (EFSA GMO Panel, 2011b).

However, new calculations show that the previously recommended isolation distance of 30 m from the nearest field of maize 1507 would still protect NT Lepidoptera with known levels of sensitivity, including the 'highly-sensitive' *Plutella xylostella* (EFSA GMO Panel, 2011a). Should hypothetical species with greater sensitivities exist, larger isolation distances would be needed to ensure the desired level of protection.

The EFSA GMO Panel provides risk managers with a tool to estimate and mitigate the risk for NT Lepidoptera of conservation concern, considering the above-mentioned three scenarios at a range of isolation distances, at two protection levels and for a range of lepidopteran species with a wide spectrum of sensitivities to Bt toxins, including hypothetical species not yet assessed. This will allow risk managers to select the most appropriate risk management measures (i.e. isolation distances) that are proportionate to the level of risk identified according to appropriate protection goals.

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1. Introduction

Genetically modified (GM) maize events MON 810, Bt11 and 1507 (hereafter referred to as 'Bt-maize') have been developed to be resistant to certain lepidopteran target pests, and in particular the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), and the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefebvre).

The Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) assessed whether or not the insecticidal Cry1Ab and Cry1F proteins expressed in maize MON 810/Bt11[1] and maize 1507 pollen, deposited on the leaves of host plants within or in the surroundings of Bt-maize fields, affect sensitive non-target (NT) lepidopteran larvae, including those of conservation concern found in protected natural habitats, as defined in Directive 2004/35/EC[2] (hereafter referred to as 'protected habitats').

In previous scientific opinions, the EFSA GMO Panel employed a mathematical model (Perry et al., 2011, 2012, 2013) to estimate the larval mortality of known or hypothetical NT lepidopteran species with a range of sensitivities exposed to Bt-maize pollen deposited on the leaves of their host plants (EFSA GMO Panel, 2011a, b, 2012d, e). In its 2011 scientific opinions (EFSA GMO Panel, 2011a, b), the EFSA GMO Panel noted the variability in between-species sensitivity to the insecticidal proteins in Bt-maize pollen and defined five categories of species sensitivity, based on data of Wolt et al. (2005). Whilst the EFSA GMO Panel concluded that certain lepidopteran species (i.e. those in the 'very highly' to 'extremely' sensitive categories) can be at risk when ingesting a certain amount of Bt-maize pollen (EFSA GMO Panel, 2011a, b), it emphasised that no actual species had yet been recorded with that degree of sensitivity and that the species at risk were therefore hypothetical. Consideration of these two hypothetical categories could be disproportionate because in practice there may well be no actual species with that degree of sensitivity. Despite this, the EFSA GMO Panel has taken this worst-case approach to ensure inclusion of all potential species sensitivities within the modelling exercise, in order to study the possible implications for all lepidopteran species of exposure to Bt-maize pollen (EFSA GMO Panel, 2011a, b, 2012d, e).

Based on the model predictions, the EFSA GMO Panel provided risk managers with a set of risk mitigation measures (e.g. non-Bt-maize border rows around Bt-maize field, isolation distances from protected habitats to nearest Bt-maize field) to limit the exposure of known and hypothetical sensitive NT lepidopteran larvae. These mitigation measures vary, depending on the sensitivity of the lepidopteran species and its level of exposure to Bt-maize pollen (EFSA, 2005a, b, 2009; EFSA GMO Panel 2011a, b, 2012d, e).

In this scientific opinion, the EFSA GMO Panel assesses the consequences of the new information reported by Hofmann et al. (2014) on its previous risk assessment conclusions and risk management recommendations for Bt-maize (EFSA, 2005a, b, 2009; EFSA GMO Panel 2011a, b, 2012a–e). Particular attention is paid to the comments by Hofmann et al. (2014) on the isolation distances previously recommended by the EFSA GMO Panel to reduce exposure of sensitive NT lepidopteran larvae of conservation concern potentially occurring in protected habitats. These isolation distances around protected habitats, within which Bt-maize should not be cultivated, are reviewed here by the EFSA GMO Panel.

1.1. Background and Terms of Reference as provided by the requestor

In 2005, the EFSA GMO Panel issued scientific opinions on both maize 1507 and Bt11 for cultivation in the European Union (EU) (EFSA, 2005a, b). In 2009, the EFSA GMO Panel gave its scientific advice on maize MON 810 in the context of the renewal of the authorisation for its continued marketing, including cultivation (EFSA, 2009).

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[1] Data on the biological activity of the Cry1Ab protein variant of maize Bt11 and maize MON 810 against sensitive lepidopteran species confirm that both variants are biologically equivalent. In addition, the reported ranges in the levels of the Cry1Ab protein expressed in maize Bt11 pollen were shown to be similar to those in maize MON 810 pollen. Based on the sensitivity and protein expression data, the EFSA GMO Panel considers that the mortality estimates calculated by Perry et al. (2010) for maize MON 810 apply equally to maize Bt11 (EFSA, 2011b).

[2] Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. OJ L 143, 30.4.2004, p. 56–75.

Over the last four years, the EFSA GMO Panel delivered, upon requests of the European Commission and on its own initiative, scientific outputs supplementing the initial environmental risk assessment (ERA) conclusions of maize MON 810, Bt11 and 1507, and, when needed, clarifying the initial set of risk management recommendations (EFSA GMO Panel, 2011a,b, 2012a–e). Overall, EFSA and its GMO Panel were aiming at completing a comprehensive and up-to-date ERA of these maize events in the light of the most recent and relevant scientific literature.

As part of the pre-market ERA of Bt-maize MON 810, Bt11 and 1507, the EFSA GMO Panel assessed the possible adverse effects that these maize events may cause to non-target organisms (NTOs), including NT Lepidoptera, in accordance with its guidance document on the ERA of GM plants (EFSA GMO Panel, 2010). Possible effects on NT Lepidoptera were specifically evaluated due to the known hazard represented by Cry proteins to this NT group. Depending on their sensitivity to the insecticidal Cry proteins expressed in Bt-maize and the amount of ingested pollen, NT Lepidoptera may be at risk following the ingestion of Bt-maize pollen deposited on the leaves of host-plants within and in the surroundings of Bt-maize fields.

The EFSA GMO Panel used the mathematical model developed by Perry et al. (2010, 2011, 2012, 2013) to simulate and predict potential adverse effects resulting from the exposure of NT Lepidoptera to pollen from maize MON 810, Bt11 or 1507 under representative EU cultivation conditions. Based on the outcomes of the modelling exercise, the EFSA GMO Panel recommended mitigation measures (e.g. non-Bt-maize border rows of various strip widths) around the GM maize fields, in order to reduce the exposure of sensitive NT lepidopteran species to Bt-maize pollen deposited on the leaves of host plants in agricultural landscapes (EFSA GMO Panel 2011a, b, 2012d, e).

In the case of lepidopteran species of conservation concern, which potentially occur in protected habitats, an isolation distance of 20 and 30 m was recommended from the nearest fields of maize MON 810/Bt11 and 1507, respectively, so as to reduce their estimated mortality to negligible levels (EFSA GMO Panel 2011a, b, 2012d, e).

The EFSA GMO Panel recently identified a new scientific publication by Hofmann et al. (2014), which provided a comprehensive dataset on the dispersal of maize pollen over long distances. The data led the EFSA GMO Panel to reconsider the level of exposure of NT lepidopteran larvae to Bt-maize pollen, in particular over long distances, and thus to refine the outcomes of the modelling exercise performed by Perry et al. (2010, 2011, 2012, 2013).

The EFSA GMO Panel was therefore requested to update its previous risk management measures for reducing exposure to maize MON 810, Bt11 or 1507 pollen of NT Lepidoptera of conservation concern in protected habitats, in the light of the recent findings by Hofmann et al. (2014).

2.   Data and methodologies

2.1.   Data

In delivering this scientific opinion, the EFSA GMO Panel took into account the new information reported in the scientific publication by Hofmann et al. (2014).

2.2.   Methodologies

According to the EFSA guidance document on the ERA of GM plants (EFSA GMO Panel, 2010), the assessment of possible adverse effects that the cultivation of Bt-maize active against lepidopteran pests might have on NT Lepidoptera constituted a prominent part of the ERA and led to the development of a mathematical model. The model was designed to simulate and predict larval mortality, resulting from the exposure of NT Lepidoptera to pollen from Bt-maize under representative EU cultivation conditions. Details on the structure and parameters of the Bt-related mortality model are given in Perry et al. (2010, 2011, 2012, 2013).

3. Assessment

3.1. Previous environmental risk assessment conclusions and risk management recommendations for Bt-maize events

By using a 14-parameter deterministic mathematical simulation model (Perry et al., 2011, 2012, 2013), the EFSA GMO Panel previously estimated the larval mortality of known and hypothetical NT lepidopteran species with a range of sensitivities[3] exposed to Bt-maize pollen deposited on the leaves of their host plants (EFSA GMO Panel, 2011a, b, 2012d, e). Based on the integration of a mortality–dose relationship from the laboratory with a dose–distance relationship from the field, the model enabled the calculation of larval mortalities at two scales; firstly, spatially within the crop and its immediate margins, and temporally within the period of pollen shed (smaller scales), and secondly, after averaging over a landscape and a growing season (larger scales). The EFSA GMO Panel recalls that, at each stage in the Perry et al. (2011, 2012, 2013) model development, 'worst-case' scenarios have been modelled, in which any assumptions tend towards overestimation instead of underestimation of mortality (EFSA GMO Panel, 2011a, b) (see also Section 3.2). For a full description of the mathematical model, we refer to Section 2.3.5.2 of the EFSA GMO Panel opinion (2011a) and Perry et al. (2010, 2011, 2012, 2013).

The EFSA GMO Panel provided risk managers with recommendations for two categories of NT Lepidoptera (e.g. EFSA GMO Panel 2012d, e): species occurring within maize fields and their margins, and species of conservation concern occurring within protected habitats. For the first category, to reflect a typical agricultural landscape, larger scale mortality was calculated (EFSA GMO Panel, 2011a, b, 2012d, e). For the second category, the EFSA GMO Panel favoured smaller scale estimates to provide thresholds to derive risk management recommendations (EFSA GMO Panel, 2012d), for the following two reasons:

1) In EU farming areas, many protected habitats are isolated and relatively small, contain specific food plants for Lepidoptera and often lack nearby contiguous similar habitat from which colonisation or recovery would allow the replenishment of a population suffering decline through mortality (see Sherratt and Jepson, 1993).

2) Species of conservation concern often have relatively small populations that are not widespread; such vulnerable populations are less able to tolerate mortality and may become extinct in the vicinity. Risk managers might operationalise protection goals for such species by employing lower thresholds for mortality than for more common species occurring in maize fields and margins that are more widespread throughout the maize arable ecosystem.

The EFSA GMO Panel considered the efficacy of a set of mitigation measures to reduce the exposure and thus the mortality of certain sensitive lepidopteran larvae to Bt-maize pollen. The EFSA GMO Panel recommended that risk mitigation measures (e.g. non-Bt-maize border rows) are only required in situations where certain NT Lepidoptera 'extremely sensitive' to Cry1Ab or 'highly sensitive' to Cry1F might be at risk in agricultural landscapes where arable maize fields or their direct field margins contain their host plants (EFSA GMO Panel, 2011a, b). For the protected habitats where lepidopteran species of conservation concern are present, isolation distances[4] of 20 and 30 metres were recommended from the nearest fields of maize MON 810/Bt11 and 1507, respectively, to reduce the estimated mortality of lepidopteran larvae to a negligible level[5] (EFSA GMO Panel, 2011a, b).

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[3] See Table 2 in EFSA GMO Panel, 2011a, reporting the 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 corresponding to five defined categories).

[4] 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 32 ng/mg dry weight of Cry1F protein in pollen of maize 1507 is about 350-fold higher than the Cry1Ab protein content in maize MON 810/Bt11 pollen (see Section 2.3.5.1 of EFSA GMO Panel, 2011a). Since maize 1507 could then pose a greater risk for NT Lepidoptera than maize MON 810/Bt11, different isolation distances are recommended.

[5] In EFSA GMO Panel, 2011a: the EFSA GMO Panel considers that a distance of 30 m is sufficient to reduce the mortality to a negligible level below 0.5 %, even for extremely sensitive species. In EFSA GMO Panel, 2011b: the EFSA GMO Panel considers that a distance of 20 m is sufficient to reduce the mortality to a negligible level below 0.2 % in the margins of the protected areas, even for extremely sensitive species.

The EFSA GMO Panel emphasises that, in its previous scientific opinions (EFSA GMO Panel, 2011a, b, 2012d, e), larval mortality was estimated with regard to an unrealistically large level of exposure, as no allowance was made for effects that are known to reduce the effective exposure to Bt-maize pollen, such as the proportion of maize pollen that comes from non-Bt-maize varieties. Despite the fact that mortality estimates may have been overestimated for the dose–distance relationship used by EFSA in these opinions (EFSA GMO Panel, 2011a, b, 2012d, e), no such allowances were necessary in these previous opinions, because the isolation distances commensurate with those mortality estimates were, in any case, relatively small. With the different dose–distance relationship studied here, it is necessary to adopt more realistic levels of exposure and hence isolation distances that are commensurate with appropriate mortality estimates. An uncertainty analysis of several different factors affecting the exposure of NT Lepidoptera to Bt-maize pollen is presented in Section 3.3 and this helps to provides quantitative estimates of a more appropriate exposure level.

The EFSA GMO Panel is of the opinion that, in contrast to a field that can be looked at as either a whole at a particular time or part of a landscape over a season, a protected habitat is usually considered a spatially 'self-contained' area per se. Since the present scientific opinion addresses only protected habitats where NT lepidopteran species of conservation concern might be found, the EFSA GMO Panel refers here to estimated mortality without further distinction between larger or smaller scale mortality estimates as reported previously (e.g. EFSA GMO Panel, 2011a, b). Furthermore, since the categories of sensitivity are somewhat arbitrary, results are given in this scientific opinion as the percentage of species for which the predicted mortality is less than a defined threshold protection level. The thresholds chosen as examples are 1 % predicted mortality (i.e. a maximum tolerable mortality of 1 in every 100 individual larvae) and 0.5 % predicted mortality (i.e. a maximum tolerable mortality of 1 in 200 individual larvae). As was emphasised explicitly by EFSA (EFSA GMO Panel, 2012e), any specific protection level used here for illustration by the EFSA GMO Panel is intended as an example only. It is reiterated here that the establishing of criteria to define the need for specific mitigation measures is the task of risk managers; any threshold applied must, by necessity, be arbitrary and should be subject to amendment according to the protection goals in operation within the EU (see Section 3.6).

3.2. Summary and relevance of recent scientific data for the environmental risk assessment of Bt-maize events

Recently, the EFSA GMO Panel noted that the scientific publication by Hofmann et al. (2014) reported a comprehensive dataset on maize pollen dispersal and deposition over long distances which might affect the previous ERA conclusions and risk management recommendations for maize MON 810/Bt11 and 1507.

3.2.1. New information reported by Hofmann et al. (2014)

The study by Hofmann et al. (2014) aimed to analyse data on maize pollen deposition in relation to the distance from the nearest maize field. The authors employed a standardised method to record maize pollen grains at 216 sites in Germany, Switzerland and Belgium from 2001 to 2010, using a pollen mass filter (PMF) sampler. The study confirms that the highest pollen deposition is within the nearest maize field and decreases with increasing distance from this field. Maize pollen was sampled up to 4.45 km from the nearest maize field and this made it possible to gather an extended dataset on pollen dispersal. The 95 % confidence interval for a predicted value of pollen deposition spans almost two orders of magnitude.

Hofmann et al. (2014) also discussed the implications of their study on previous risk assessments of Bt-maize and the associated recommendations for mitigation measures. The pollen dose–distance distribution curve used by Perry et al. (2010, 2011, 2012, 2013) differs significantly from that used by Hofmann et al. (2014) for long distances. According to Hofmann et al. (2014), this difference leads to the underestimation of maize pollen deposition over long distances and hence to an underestimation of the exposure of NT lepidopteran species to Bt-maize pollen for distances greater than 10 m. Hofmann et al. (2014) concluded that the isolation distances previously recommended by the EFSA GMO Panel to reduce exposure of certain sensitive NT lepidopteran larvae potentially occurring in protected habitats 'may be inappropriately small'.

3.2.2. Relevance of the new information

The EFSA GMO Panel noted that Hofmann et al. (2014) provided a comprehensive EU dataset on the dispersal and deposition of maize pollen over long distances; few authors have published data on pollen deposition at comparable distances. The EFSA GMO Panel therefore decided to check the validity of its previous risk management recommendations in light of this new information (EFSA GMO Panel, 2011a, b, 2012d, e). In this scientific opinion, the EFSA GMO Panel estimates the mortality of NT lepidopteran larvae using both the Perry et al. (2010, 2011, 2012, 2013) and Hofmann et al. (2014) dose–distance relationships (see Section 3.5). Except for the dose–distance relationship, all of the variables and parameters used by the EFSA GMO Panel (2011a) and/or in the Perry et al. (2010, 2011, 2012, 2013) model were maintained at their same values.

The Perry et al. (2010, 2011, 2012, 2013) and Hofmann et al. (2014) dose–distance relationships differ in several regards. Firstly, Perry et al. (2010, 2011, 2012, 2013) modelled the pollen deposition at varying distances on the basis of data from a single maize field (Perry et al., 2013). In contrast, Hofmann et al. (2014) used data from an unknown number, size and location of fields; the only spatial information concerned the distance to the nearest maize field. Secondly, the period of deposition over which pollen is aggregated in the Perry et al. dose–distance relationship is one week (Perry et al., 2013) and the units are 'number of pollen grains/cm^2'. The period sampled by Hofmann et al. (2014) is an entire flowering period (an average[6] of about four weeks) and the units are 'pollen grains/m^2'. Therefore, throughout this scientific opinion, pollen deposition values recorded by Hofmann et al. (2014) were divided by a factor of 40 000 to ensure an equitable comparison of the two dose–distance relationships. In addition, the pollen sampled by Hofmann et al. (2014) represents a mixture of relatively fresh and relatively old pollen. Thirdly, Perry et al. (2010, 2011, 2012, 2013) assume that pollen deposition occurs over the downwind edge of the nearest field, the edge over which pollen deposition is maximal; no differentiation for wind direction is made by the Hofmann et al. (2014) dose–distance relationship. Fourthly, the Perry et al. (2010, 2011, 2012, 2013) dose–distance relationship is calibrated to relate directly to data from samples of pollen on the leaves of host plants, such as nettles; Hofmann et al. '2014) recorded pollen with a mechanical sampler with no verification at long distances to relate their measurements to pollen on actual leaves. Finally, by integrating the Hofmann et al. (2014) dose–distance relationship over successively distant *annuli* [7] around the nearest field, there appears to be a lack of coherence in the sense that predicted pollen densities increase without limit as the distance of each *annulus* from this field increases; this prevents reliable extrapolation beyond the range of the sampled data. This is not a relevant issue for the Perry et al. (2010, 2011, 2012) dose–distance relationship. Some of the above issues are considered further in Section 3.3.2.

The Perry et al. dose–distance relationship relating dose, 'd' (in pollen grains/cm^2 per week), to distance, 'E' (in metres from edge of field) is:

$$\log_{10}d = 2.346 - 0.145E$$

The Hofmann et al. dose–distance relationship, using the same units, is:

$$\log_{10}d = 1.502 - 0.585 \log_{10}E$$

At the edge of the field exactly (i.e. $E = 0$), the estimated dose under the Perry et al. (2010, 2011, 2012, 2013) dose–distance relationship is 221.8 pollen grains/cm^2 per week. The dose used for the Hofmann et al. (2014) dose–distance relationship cannot be computed under the equation they selected, but can be seen, from Figure 3 of Hofmann et al. (2014), to be between 48 and 89 pollen grains/cm^2 per week.

Within the field (i.e. $E < 0$), the dose used for the Perry et al. (2010, 2011, 2012, 2013) dose-distance relationship is 2.76-fold higher than the dose used at the edge, i.e. 611.6 pollen grains/cm^2 per week. The estimated dose under the Hofmann et al. (2014) dose–distance relationship, from their Figure 3, is about 89 pollen grains/cm^2 per week. As might be expected, within the field and its margins, predicted mortalities based on the Perry et al. (2010, 2011, 2012, 2013) dose–distance relationship

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[6] In Hofmann et al. (2014), 'the exposure time of the PMF samplers covered the main maize pollen flowering period in each particular area (3 to 5 weeks and sometimes longer).'

[7] In mathematics, an *annulus* is a ring-shaped object, especially a region bounded by two concentric circles.

are, on average, more than three-fold higher than those calculated using the Hofmann et al. (2014) model.

Estimated doses for $0 \leq E \leq 9$ are higher based on the Perry et al. (2010, 2011, 2012, 2013) dose-distance relationship than those estimated by the Hofmann et al. (2014) dose-distance relationship; for values of E ≥ 10, the reverse is the case. For values of E > 20, estimated doses under the Perry et al. dose–distance relationship are considerably lower than those under the Hofmann et al. (2014) dose–distance relationship. The effect of this difference is addressed for large values of E in Section 3.5, below.

3.3. Discussion of possible uncertainties pertaining to mathematical models

3.3.1. Introduction

Mathematical models developed to simulate 'reality' are limited by experts' judgement, consequent assumptions and estimates, and hence by the existing data available at that point in time. Since all modelling exercises are subject to uncertainties, as with any ecological model, any new relevant scientific data that become available would therefore automatically lead to the refinement of the model and hence of the reported mortality estimates of NT Lepidoptera of conservation concern potentially occurring in protected habitats. The EFSA GMO Panel previously recognised certain specific weaknesses of the Bt-related mortality model described by Perry et al. (2010, 2011, 2012, 2013).

In its previous outputs on maize MON 810, Bt11 and 1507 (EFSA GMO Panel, 2011a, b, 2012d, e), the EFSA GMO Panel carried out a full exposure assessment building on various assumptions. This took into account many variables and factors, some of which had to be modelled with little available data (EFSA GMO Panel, 2011a). Major sources of variability (e.g. incomplete availability of data concerning the sensitivity of EU Lepidoptera of conversation concern to Cry1 proteins) in the mortality estimates were already discussed in Section 3.1.2.4 of an EFSA GMO Panel scientific opinion (EFSA GMO Panel, 2011a).

In the current scientific opinion, the EFSA GMO Panel discusses additional sources of uncertainties related to the structure of the Perry et al. (2010, 2012) Bt-related mortality model (see Section 3.3.2, hereunder). Appendix A lists 11 sources of uncertainty triggered by biotic and abiotic factors which might affect the actual exposure of Lepidoptera to maize pollen in practice.

Through an elicitation process, these 11 sources of uncertainty were discussed by the experts of the standing working group of the EFSA GMO Panel on ERA; these experts estimated the direction and magnitude of the impacts of these sources of uncertainty on the exposure level. The experts considered that 8 out of the 11 uncertainties were of major relevance and therefore should be accounted for in the calculations of the Perry et al. (2010, 2011, 2012, 2013) Bt-related mortality model in order to reflect a more realistic level of exposure to Bt-maize pollen (see Section 3.3.2). More details on the assessment of the uncertainties are given in Appendix A and in Sections 3.3.2 and 3.3.3, hereunder.

Based on their judgement and the available information, the experts attributed values to the means for each source of uncertainty through a structured discussion process. The value of a multiplicative factor, used to reduce exposure, was then derived from the median values of the individual scores of the uncertainty factors that affect exposure identified during this elicitation process. The estimated exposure was then reduced using this multiplicative factor in order to estimate the larval mortality of Lepidoptera (Section 3.5) under different exposure scenarios (Section 3.4).

Other remaining uncertainties associated with hazards presented to exposed Lepidoptera (e.g. vulnerability of populations and sub-lethal effects) are further discussed in Section 3.3.3, together with the reasoning for not including them in the present modelling exercise.

3.3.2. Identified uncertainties considered in the present modelling exercise

For the new calculations of mortality of NT Lepidoptera of conservation concern (see Section 3.5), the EFSA GMO Panel took into consideration two major types of uncertainties:

Uncertainties pertaining to the structure of the Perry et al. (2012) Bt-related mortality model:

1) As stated in Section 3.1, the Perry et al. (2012) Bt-related mortality model clearly acknowledged that predicted mortality depends on the sensitivity of the species concerned (EFSA GMO Panel, 2011a, b). Sensitivity is estimated through bioassay data which yields an LC_{50} value for the mortality–dose relationship; these data are variable and therefore the determination of LC_{50} values and sensitivities are subject to uncertainty. Furthermore, there are few datasets available for estimating the sensitivity for most assessed Bt-maize events, and for only a very limited number of mainly pest species (EFSA GMO Panel, 2011a). For maize 1507, no species of the 16 studied has been identified as being more sensitive, in practice, than the pest species *Plutella xylostella* (classified as 'highly-sensitive' at just less than the 6th percentile of the estimated sensitivity distribution, see Wolt et al. (2005) and Table 2 in EFSA GMO Panel, 2011a). The EFSA GMO Panel was conservative in considering higher species sensitivity for hypothetical species, and since predictions of mortality for such species extrapolate beyond the range of available sensitivity data, uncertainties are amplified and predictions become less reliable. In particular, for the hypothetical very-highly and extremely sensitive species occurring in the tail of the sensitivity distribution, recommended isolation distances should be regarded with caution. For reasons of transparency, the EFSA GMO Panel gives results here for an extensive range of quantified percentile sensitivities, from the 50th percentile (median average sensitivity) to the 0.1th percentile (extreme sensitivity).

2) The spatial variability of the data from Hofmann et al. (see Figure 3 in Hofmann et al., 2014) was also accounted for in the calculations presented in this scientific opinion. Whereas the data in Hofmann et al. (2014) capture the density of maize pollen over various distances from multiple maize fields, the authors do not report on the location of any of these fields. Also, they only report one distance from field to sampler, that of the nearest one; none of the other distances are reported. For calculations of larval mortality, the EFSA GMO Panel therefore adopted both the expected regression line and the upper 95 % confidence interval from the Hofmann et al. dose–distance relationship (see Figure 3 in Hofmann et al., 2014). The latter reflects between-sample variability in a conservative fashion, since the upper confidence interval represents, on average, a nine-fold higher maize pollen deposition than expected.

3) In developing the present scientific opinion, the EFSA GMO Panel considered the scenario of a NT lepidopteran larva within a 'square-shaped' protected habitat with each side 1 km long (hereafter referred to as '1 km square protected habitat'), separated from the nearest Bt-maize field by 'S' metres. Since there is uncertainty induced by the non-specific spatial information in the data from Hofmann et al. (2014), it is necessary to avoid anomalies due to positional effects as far as possible. Therefore, the average mortality was calculated by integration, for six larvae, in the following two scenarios, each involving three larvae. In the first, one larva was assumed at the near edge, one at the centre, and one at the far edge of a protected habitat 1 km square for which the nearest Bt-maize field, a distance S metres away, was the nearest such field for all three larvae. In the second, one larva was assumed at the near edge, one at the centre, and one at the far edge of a protected habitat for which the next-nearest Bt-maize field was a distance just greater than S metres from the far edge. Effectively, this resulted in computing a weighted average mortality for larvae at the following distances from the nearest Bt-maize field: S, S, S, $S + 500$, $S + 500$ and $S + 1\,000$. Again, this results in a conservative approach (i.e. the assumptions lead to a higher estimated exposure to maize pollen) in which mortality is not underestimated.

Uncertainties contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen:

The eight following sources of uncertainty are further described in Appendix A:

- the proportion of total recorded pollen from Bt-maize, dependent on the mixture of GM and non-GM maize fields in the landscape, as well as on the ratio of non-Bt-maize to Bt-maize plants (as refugia for resistance management of target pests);

- the pollen deposition on the flat surface of pollen samplers used by Hofmann et al. (2014) compared with the three-dimensional structure of a leaf;

- the effect of wind and rain removing pollen from leaves;

- the displacement and accumulation of pollen on areas such as leaf veins and axils, affecting ingestion;

- the competition for resources, resulting in consumption of pollen by non-lepidopteran species unaffected by Bt-protein;

- the degradation of the Bt-protein in pollen;

- the changes in feeding behaviour of NT lepidopteran larvae (e.g. inhibition or repellent effects);

- the lack of temporal coincidence between sensitive larval development stage and maize pollen shed.

All but one of the uncertainties listed above are considered to reduce rather than increase the level of larval exposure to Bt-maize pollen. Furthermore, the effects listed operate independently of one another, and therefore, when considered together, have a multiplicative effect on exposure. This multiplicative effect reduces exposure, sometimes considerably. For example, if each effect in isolation was to lead to a decrease in exposure of one-third, their combined effect would be to reduce exposure to one-twenty-fifth of its original value.

These eight uncertainties were considered of major relevance and therefore accounted for in the calculations with the Perry et al. (2012) Bt-related mortality model (see Sections 3.4 and 3.5) in order to reflect a more realistic level of exposure to Bt-maize pollen.

3.3.3. Identified uncertainties not considered in the present modelling exercise

Some of the uncertainties[8] contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen were not considered of relevance for the new calculations of mortality of NT Lepidoptera of conservation concern. The reasons for not integrating them into the modelling exercise are provided in Appendix A.

The EFSA GMO Panel also considered whether or not the hemizygosity (i.e. one allele for the specific trait) of the newly inserted traits in Bt-maize might be a potential source of variability in exposure. Since the Bt-related mortality model by Perry et al. (2010, 2011, 2012, 2013) integrates a dose-response relationship estimated from available bioassay data (carried out with pollen or purified Bt-proteins), the EFSA GMO Panel is of the opinion that the hemizygous character of the Bt-maize was inherent to the establishment of the sensitivity distribution and does not need to be further accounted for.

The EFSA GMO Panel points out an additional uncertainty pertaining to the Cry toxin dose–response relationship varying across the species considered by Perry et al. (2010, 2011, 2012, 2013). This is acknowledged as an additional limitation of the model as the dose–response was based on an extrapolation of data on Cry1Ab protein in maize Bt 176 to maize MON 810 and Bt11 (see Perry et al., 2011, 2013). Perry et al. (2011, 2012, 2013) explained that this assumption results in highly conservative estimates that tend to overestimate mortality.

Sub-lethal effects, which can result in modified development time, altered fecundity, altered body weight or size and might affect population dynamics of exposed lepidopteran species (Dively et al., 2004; Lang and Vojtech, 2006). Sub-lethal effects are an important issue that can lead to adverse effects on a population over and above those of mortality (Cusham et al., 1994). However, little information exists in the literature with regard to sub-lethal effects of pollen from Bt-maize on NT Lepidoptera and so these effects cannot be factored into the Perry et al. (2010, 2011, 2012, 2013) Bt-related mortality model.

The Perry et al. (2010, 2011, 2012, 2013) Bt-related mortality model was designed to simulate and predict larval mortality, resulting from the exposure of NT Lepidoptera to pollen from Bt-maize under representative EU cultivation conditions. Considering Bt-maize within agro-ecosystems, additional sources of uncertainty linked to mortality (e.g. natural mortality linked to multiple stressors and

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[8] That is, the variability in pollen production from GM and non-GM maize varieties, the spatial distribution of host plants and the nutritional quality of host plants.

vulnerability of endangered species[9] populations to stressors), affecting the overall mortality of NT Lepidoptera, might need further consideration. In the context of the ERA of Bt-maize, the EFSA GMO Panel considered Bt-maize as the sole stressor and did not consider these additional sources of uncertainty linked to mortality and, therefore, they were not included in the new calculations (see Section 3.6).

The shape (i.e. square) and size (i.e. 1 km²) of the protected habitat modelled here are potential sources of uncertainty with regard to estimated larval mortalities. A larva at the centre of a 'square-shaped' protected habitat might be less distant from a neighbouring maize field, and hence more exposed to pollen, than a larva at the centre of a shape with a narrower width. However, this will depend on the orientation of the protected habitat relative to the nearest field and, indeed, the reverse might also be the case. Since the size of most protected habitats under real conditions is likely to exceed 1 km², the exposure of an average larva will probably be less than that modelled here. Despite these uncertainties induced by the non-specific spatial information as regards the Hofmann et al. (2014) data, the EFSA GMO Panel considers that the approach taken is conservative (i.e. the assumptions lead to an overestimation of exposure to maize pollen) and that mortality is not underestimated.

3.3.4. Conclusion of the discussion on uncertainties

The EFSA GMO Panel took into consideration two major types of uncertainties: (1) the uncertainties pertaining to the structure of the Perry et al. (2010, 2011, 2012, 2013) Bt-related mortality model, and (2) the uncertainties contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen.

All but one of the uncertainties are considered to reduce rather than increase the level of larval exposure to Bt-maize pollen.

In Section 3.5, mortality of NT Lepidoptera of conservation concern is estimated for three possible exposure scenarios (see Section 3.4), with different estimations of the aforementioned uncertainties.

3.4. New calculations

The EFSA GMO Panel compared its previously published results for protected habitats, using the dose–distance relationship of Perry et al. (2010, 2011, 2012, 2013), with those using the dose–distance relationship of Hofmann et al. (2014). Specifically, the mortality of NT lepidopteran larvae was estimated using the relationship between mortality and dose specified by the Perry et al. (2010) and 2012 models for maize MON 810/Bt11 and 1507, respectively, and the dose–distance relationship model of Hofmann et al. (2014). As stated in Section 3.3.2, the estimated mortality is computed for larvae within a 1 km square protected habitat, over a range of isolation distances S metres away from the nearest Bt-maize field.

Three scenarios are considered when estimating mortalities at various distances from the protected habitat:

(1) The first scenario is a direct comparison (hereafter referred to as 'DC' scenario) of the small spatial- and temporal-scale mortality estimates previously published by EFSA (EFSA GMO Panel, 2011a, b, 2012d, e) with all other parameters (other than those in the Hofmann et al. (2014) dose–distance relationship) remaining at the same values. This scenario is unrealistic because it takes no account of the uncertainties associated with exposure, as discussed in Section 3.3.2. The combined effect of these uncertainties will reduce exposure by at least one-half and probably considerably more.

Also, it is clear that, whatever the threshold established for protection (e.g. 1 % predicted mortality) and whatever the isolation distance imposed, it is possible to hypothesise a level of sensitivity for some unknown species for which that threshold would be exceeded. Therefore, rather than specifying the predicted mortality for some arbitrary sensitivity class, it is more informative to state the percentage of species predicted to be protected at a particular protection level. Therefore, the major results reported in this scientific opinion are given in

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[9] Rare and endangered species might be at higher risk because of a higher vulnerability to stressors compared with common species, which may lead to reductions in population size to local extinction levels.

both forms: predicted mortality and percentage of species protected. The protection levels chosen for this study are 0.5 % and 1 %.

For the reasons outlined in Section 3.1, the exposure scenario DC, allowing for a direct comparison of present results with previous Panel outputs (EFSA GMO Panel, 2011a, b, 2012d, e), should also be considered unrealistic.

In contrast to the DC scenario, the EFSA GMO Panel provides, below, the most realistic measure of the likely exposure, under the scenario 'MR'.

(2) The second, 'most realistic' scenario (hereafter referred to as 'MR' scenario) reduces exposure by multiplying the dose calculated from the Hofmann dose–distance relationship by a factor of 0.0376, which is the mean figure of the uncertainties described in Section 3.3.2. This scenario gives the most realistic measure of the likely exposure, in the sense that the EFSA GMO Panel considers it is just as likely to yield an underestimate of true exposure as an overestimate.

(3) The third, 'conservative' scenario (hereafter referred to as 'CO' scenario) is similar to scenario MR, but acknowledges that on 2.5 % of occasions (1 in 40 occasions) a predicted dose from the Hofmann et al. (2014) dose–distance relationship is expected to be as high as the upper 95 % confidence interval, shown in red in Figure 3 of Hofmann et al. (2014). This accounts for between-sample variability. There is no information available from Hofmann et al. (2014) on within-site, within-year variability. Since this upper interval is about nine-fold higher than the expected value from the regression line, the multiplicative factor used is nine-fold higher than that in the second scenario, i.e. 0.338. This third scenario is designed to give a conservative estimate of mortalities, allowing for the worst-case scenario of variability in the doses expressed in the Hofmann et al. (2014) data, but also for the most likely reduction in exposure from those doses (see Appendix A). This scenario might be useful for regions where there are many protected habitats and risk managers regard it as unacceptable that any of these habitats should suffer mortality above a given threshold. The EFSA GMO Panel emphasises that caution is required in the interpretation of this CO scenario, because for every site-occasion for which exposure is nine-fold higher than the expected value, there will be a site-occasion for which exposure is nine-fold lower than expected, and that the overall average exposure remains as in scenario MR, above.

A full list of the parameters used and their values are detailed for maize 1507 in Table 1; these parameters are based on the list given in Table 1 of Perry et al. (2012). Values for maize MON 810/Bt11 are similar, except that the values of parameter[10] 'm' are a multiplicative factor of 67.15 higher, as explained by the EFSA GMO Panel (2011b).

The value of the multiplicative factor 0.0376, used to reduce exposure in scenario MR, was derived from the values of the individual factors that reduce exposure identified in the elicitation process described in Section 3.3.1. These individual values are listed in Table 2. In each case, the quantity given is the proportion by which the Hofmann et al. (2014) predicted dose should be multiplied, to give the effective dose after allowance for the exposure effect. Three values are given for each factor: the median of the eight minimum values over the group of experts that performed the elicitation (i.e. the case in which assumed exposure and mortality are least); the median of the eight maximum values (i.e. the case in which assumed exposure and mortality are greatest); and the median of the eight mid-range values (i.e. (maximum + minimum)/2). The median of the eight mid-range values represent the value considered most realistic, in the sense that the EFSA GMO Panel considers it is just as likely to yield an underestimate of true exposure as an overestimate.

The isolation distances ('S') studied ranged from 5 m to 3 000 m. The EFSA GMO Panel has not considered isolation distances greater than 3 000 m because the uncertainties outlined in Section 3.1, above, are sufficient to caution that extrapolation beyond this distance would yield mortality estimates that could not be relied upon for risk assessment, particularly for relatively large species sensitivities.

The sensitivities studied range from the 50th percentile (i.e. median average sensitivity) up to the 0.1[th] percentile (corresponding to sensitivity exceeded by no more than 1 in every 1 000 species).

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[10] LC50, m (in Table 1)

Table 1: Parameter values used in the Perry et al. (2010, 2011, 2012) Bt-related mortality model to estimate larval mortality for the three scenarios DC, MR and CO

Parameter	Type (units)	Values or derivation
Distance of protected habitat from nearest Bt-maize field, S	Distance (m)	A variable set to various values (see text)
Dose, d	pollen grains/cm^2	The Hofmann power function dose–distance relationship: For DC, $d_{DC} = 31.8 S^{-0.585}$ For MR, $d_{MR} = 0.0376 d_{DC} = 1.20 S^{-0.585}$; For derivation of multiplicative factor 0.0376, see Table 2 below For CO, $d_{CO} = 9 d_{MR} = 0.338 d_{DC} = 10.8 S^{-0.585}$
LC$_{50}$, m	LC$_{50}$ dose (pollen grains/cm^2)	Assumed, for five hypothetical species with sensitivity categorised as: - extreme (0.2th percentile of sensitivity distribution) m = 1.265 - very high (1.1th percentile of sensitivity distribution) m = 14.36 - high (7.5th percentile of sensitivity distribution) 163.2 - above-average (25th percentile) 1853 - below-average (70.7th percentile) 21057
Mortality outside crop, $g(E)$	Percentage (−)	Calculated from mortality–dose relationship
Within-crop mortality, h	Percentage (−)	Not relevant here; protected habitat is remote from crop
Host plant within-crop, e	Density (per m^2)	Not relevant here; protected habitat is remote from crop
Host plant in margin, f	Density (per m^2)	Not relevant here; protected habitat is remote from margin
Size of maize fields, C	Area (ha)	Not relevant here; NTL at risk are remote from field
Width of margin, D	Distance (m)	Not relevant here; NTL at risk are remote from margin
Width of non-Bt strips, w	Distance (m)	Not relevant here; NTL at risk are remote from margin
Host plants in arable, y	Proportion (−)	Not relevant to small spatial and temporal scales
Maize cropping, z	Proportion (−)	Not relevant to small spatial and temporal scales
Utilisation rate, v	Proportion (−)	For DC v=1; for MR and CO, v=0.425, see Table 2 below
Physical effects, x	Proportion (−)	For DC x=1; for MR and CO, x=0.65, see Table 2 below
Temporal coincidence, a	Proportion (−)	For DC a=1; for MR and CO, a=0.5, see Table 2 below
Large-scale exposure, L	Proportion (−)	Not relevant to small spatial and temporal scales

Table 2: The eight sources of uncertainty described in Section 3.3.2 that reduce the Hofmann et al. (2014) estimated pollen doses to more realistic exposure levels, each[(a)] with values identified in the elicitation process of Section 3.3.1.

Source	Parameter name (see Table 1)	Median of minima (least exposure)	Median of maxima (greatest exposure)	Median of mid-range values (most likely exposure)	Product of medians of mid-range values
Proportion of recorded pollen that is from Bt-maize	v	0.05	0.8	0.425	
Three-dimensional structure of host plant leaves		0.32	0.85	0.61	
Effect of wind and rain removing pollen from leaves	x	0.45	0.85	0.65	
Displacement of pollen on leaf		0.55	1.0	0.825	0.0376
Competition for pollen		0.6	1.0	0.8	
Degradation of Bt-protein in pollen		0.625	0.925	0.75	
Changes in feeding behaviour		0.8	1.0	0.9	
Lack of synchrony between larvae and maize pollen shed	a	0.0	1.0	0.5	

(a): Each individual entry in the table gives the value by which the original exposure should be multiplied to yield the effective exposure after allowance for that effect (a value of 0.0 therefore represents the maximum reduction to nil exposure; a value of 1.0 represents the least reduction, implying exposure is unchanged). Individual values are then combined to derive the multiplicative factor 0.0376 used to calculate effective doses for the two scenarios MR and CO.

3.5. Results

This section gives details of the results of the estimation of mortality and required isolation distances for protected habitats, under the three scenarios described in Section 3.4.

3.5.1. Direct comparison scenario

The newly obtained results for the Hofmann et al. (2014) dose–distance relationship with unrealistic (i.e. unadjusted for any of the eight exposure effects in Section 3.3.2) exposure estimates, but directly comparable to the small spatial- and temporal-scale percentage mortality estimates published by the EFSA GMO Panel (2011a, b, 2012d, e), are given below in Table 3 for maize 1507 and in Table 4 for maize MON 810/Bt11. In addition to the four sensitivity categories given previously, results are also given for the most sensitive species of the 16 species for which data exists, i.e. the pest *Plutella xylostella* (see Table 2 in EFSA GMO Panel, 2011a).

As mentioned above, the results for species in the 'very high' and 'extreme' sensitivity categories are, therefore, hypothetical. In addition, since predictions of mortality for such species extrapolate beyond the range of available sensitivity data, uncertainties are amplified and predicted mortalities should be regarded with caution. Within the tables, the type indicates whether or not the percentage mortality is expected to be below protection levels of 0.5 % and/or 1 %.

Table 3: Estimated percentage larval mortality in a 1 km square protected habitat in the DC scenario where NT lepidopteran larvae are exposed during the four-week period of Bt-maize pollen production. Results given for four categories of species sensitivity and for the pest species *Plutella xylostella*, at increasing distances[(b)] from the nearest field of Bt-maize 1507, with no margins.

Distance from field (m)	Dose–distance relationship	'Above-average' sensitivity (25th percentile)	'High' sensitivity (7.5th percentile)	*Plutella xylostella* (6th percentile)	'Very high', hypothetical sensitivity (1.1th percentile)	'Extreme', hypothetical sensitivity (0.2th percentile)
5	Perry et al.	1.7	18.8	37.8	75.9	97.7
5	Hofmann et al.	*0.2*	3.1	4.3	24.9	63.9
20	Perry et al.	*< 0.1*	*0.1*	*0.4*	1.4	16.5
20	Hofmann et al.	*0.1*	1.4	2.0	15.0	59.2
30	Perry et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	0.5
30	Hofmann et al.	*0.1*	1.2	1.6	12.7	57.1
50	Hofmann et al.	*< 0.1*	**0.9**	1.2	10.2	54.0
100	Hofmann et al.	*< 0.1*	**0.6**	**0.9**	7.5	48.8
200	Hofmann et al.	*< 0.1*	*0.4*	*0.6*	5.5	42.6
500	Hofmann et al.	*< 0.1*	*0.3*	*0.4*	3.6	33.4
1 000	Hofmann et al.	*< 0.1*	*0.2*	*0.3*	2.6	26.3
2 000	Hofmann et al.	*< 0.1*	*0.1*	*0.2*	1.8	19.8
3 000	Hofmann et al.	*< 0.1*	*0.1*	*0.2*	1.4	16.4

(b): Distances of 5 m, 20 m and 30 m allow a direct comparison between the Hoffman et al. (2014) dose–distance relationship considered in this scientific opinion and the Perry et al. dose–distance relationship described by the EFSA GMO Panel (2011a, 2012d). Values for which estimated percentage mortality is less than 0.5 % shown in bold italic type; values less than 1 % shown in bold type.

For all events, Tables 3 and 4 show that, for the small isolation distance of 5 m, the model using the Perry et al. dose–distance relationship predicts greater mortality than the Hofmann et al. dose–distance relationship, and vice-versa for isolation distances of 20 m and greater. For the Hofmann et al. dose–distance relationship, predicted mortality declines only slowly with increasing isolation distance, especially for maize 1507 and species with the hypothetical 'very-high' or 'extreme' sensitivity. For example, for maize 1507 (Table 3), if the protection level was set at 1 % (i.e. the maximum mortality that was tolerable was 1 in every 100 individual larvae), then the results indicate that at an isolation distance of 100 m, *Plutella xylostella* and all species up to and including those categorised as highly sensitive (in fact, an estimated 94.5 % of all species) would be expected to be protected at that level. This represents all the species that have so far been tested, so the only species that would not be protected at that level and at that isolation distance are hypothetical.

Table 4: Estimated percentage larval mortality in a 1 km square protected habitat in the DC scenario where NT lepidopteran larvae are exposed during the four-week period of Bt-maize pollen production. Results given for four categories of species sensitivity and for the pest species *Plutella xylostella*, at increasing distances[(c)] from the nearest field of Bt-maize MON 810/Bt11, with no margins.

Distance from field (m)	Dose–distance relationship	'Above-average' sensitivity (25th percentile)	'High' sensitivity (7.5th percentile)	*Plutella xylostella* (6th percentile)	'Very high', hypothetical sensitivity (1.1th percentile)	'Extreme', hypothetical sensitivity (0.2th percentile)
5	Perry et al.	*< 0.1*	*0.3*	**0.8**	3.3	31.8
5	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	**0.5**	5.9
20	Perry et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.2*
20	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*0.2*	2.8
30	Perry et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*
30	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*0.2*	2.3
50	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*0.1*	1.7
100	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	1.2
200	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.9*
500	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.6*
1 000	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.4*
2 000	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.3*
3 000	Hofmann et al.	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*0.2*

(c): Distances of 5 m, 20 m and 30 m allow a direct comparison between the Hoffman et al. (2014) dose–distance relationship considered in this scientific opinion and the Perry et al. dose–distance relationship described by the EFSA GMO Panel (2011a, b, 2012d, e). Values for which estimated percentage mortality is less than 0.5 % shown in bold italic type; values less than 1 % shown in bold type.

As expected, since maize MON 810/Bt11 has a lower Bt-protein content than maize 1507, expected mortalities in Table 4 are lower than those in Table 3. As an example, if the protection level was set at 1 %, then the results indicate that at an isolation distance of 100 m, all species up to those categorised as extremely sensitive (in fact, an estimated 99.7 % of all species) would be expected to be protected at that level. Again, this includes all species that have so far been tested.

3.5.2. Most realistic and conservative scenarios

Newly obtained results for the Hofmann et al. (2014) dose–distance relationship under scenarios MR and CO, i.e. with more realistic exposure estimates, are given below in Tables 5 and 6, for maize 1507 and maize MON 810/Bt11. Again, results are provided for the two protection levels of 0.5 % and 1 % mortality.

In Table 5, results are given first for one 'real' and then for three hypothetical species. The first species is the most sensitive species for which data exists, the pest *Plutella xylostella* (see Table 2 in EFSA GMO Panel, 2011a). The other three hypothetical species, 'Px5', 'Px10' and 'Px25', represent increasing levels of sensitivity compared with *Plutella xylostella*, in the sense that the dose of pollen for which their assumed mortality is 50 % (i.e. their LC_{50} values) are, respectively, 5-, 10- and 25-fold lower than that of *Plutella xylostella*. This provides a 'safety factor' as commonly adopted for the risk assessment of insecticides; a 10-fold increase in sensitivity relative to the most sensitive species yet recorded is thought to provide risk managers with a sufficient margin of error for the determination of adequate separation distances.

For maize MON 810/Bt11, Tables 5 and 6 show that, for both scenarios, the previous recommendation of a 20 m isolation distance would be expected to protect the vast majority of species. The difference between the two scenarios is minimal, because for all species tested thus far there is low mortality, even in the more conservative scenario 'CO'.

For maize 1507, Table 5 shows that for hypothetical species Px10 with its 10-fold safety factor, an isolation distance of 200 m would be expected to give adequate protection at the 0.5 % level under the MR scenario. For the CO scenario (for 1 in every 40 site-occasions), this isolation distance would result in no more than an estimated 2.3 % mortality, even for such a hypothetically sensitive species.

Table 6 shows that for maize 1507, under the MR scenario, the expectation is that the great majority of species (> 99 % of all species) are protected to a 1 % mortality level, even with the previously

recommended isolation distance of 30 m. This represents all the species that have so far been tested, so the only species that would not be protected at that level and at that isolation distance are hypothetical.

Under the CO scenario, an isolation distance of 200 m would protect over 98 % of all species at that level, but an isolation distance of 1 000 m would be required to protect 99 % of species.

As expected, the results in Tables 3, 4, 5 and 6 indicate that any increase in an isolation distance increases the percentage of species protected. However, the results indicate consistently that increasing an isolation distance by an order of magnitude, or even two orders of magnitude, makes relatively little difference to the percentage of species protected at any given level. At any given isolation distance, even a large one, there will always remain a theoretical small percentage of exceptionally sensitive species that could suffer mortality above this threshold. To emphasise the hypothetical nature of such species in Table 6, certain values are given in bold type. These represent the situation where, for a particular isolation distance, at a particular protection level, the species expected to be protected include all those tested thus far plus some hypothetical species not yet identified. It is clear that only a few values, and for very small isolation distances, are not of this kind.

Table 5: Estimated percentage[d] larval mortalities using the Hofmann et al. (2014) dose–distance relationship for NT lepidopteran larvae exposed to Bt-maize pollen in a 1 km square protected habitat when exposure is reduced by an isolation distance of S metres from the nearest Bt-maize field, under two scenarios (see text).

Event	Scenario	Species/sensitivity	S, m									
			5	20	30	50	100	200	500	1 000	2 000	3 000
1507	MR	*Plutella xylostella*	*0.14*	*0.06*	*0.05*	*0.04*	*0.03*	*0.02*	*0.01*	*0.01*	*0.01*	*< 0.01*
		Px5	**0.8**	*0.36*	*0.29*	*0.22*	*0.15*	*0.11*	*0.07*	*0.05*	*0.03*	*0.03*
		Px10	1.7	**0.75**	*0.60*	*0.45*	*0.32*	*0.22*	*0.14*	*0.10*	*0.07*	*0.05*
		Px25	5.0	2.3	1.9	1.4	1.0	**0.72**	*0.46*	*0.32*	*0.22*	*0.18*
	CO	*Plutella xylostella*	1.4	**0.65**	**0.52**	*0.39*	*0.27*	*0.19*	*0.12*	*0.09*	*0.06*	*0.05*
		Px5	7.4	3.6	2.9	2.2	1.6	1.1	**0.72**	**0.50**	*0.35*	*0.28*
		Px10	13.6	7.1	5.8	4.5	3.2	2.3	1.5	1.1	**0.72**	*0.58*
		Px25	28.7	18.3	15.6	12.7	9.5	7.0	4.6	3.3	2.3	1.8
MON 810/Bt11	MR	*Plutella xylostella*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
		Px5	*0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
		Px10	*0.02*	*0.01*	*0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
		Px25	*0.06*	*0.03*	*0.02*	*0.02*	*0.01*	*0.01*	*0.01*	*< 0.01*	*< 0.01*	*< 0.01*
	CO	*Plutella xylostella*	*0.02*	*0.01*	*0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
		Px5	*0.09*	*0.04*	*0.03*	*0.03*	*0.02*	*0.01*	*0.01*	*0.01*	*< 0.01*	*< 0.01*
		Px10	*0.20*	*0.09*	*0.07*	*0.05*	*0.04*	*0.03*	*0.02*	*0.01*	*0.01*	*0.01*
		Px25	**0.62**	*0.28*	*0.22*	*0.17*	*0.12*	*0.08*	*0.05*	*0.04*	*0.03*	*0.02*

(d): Bold italic type indicates species are protected to the degree that their estimated percentage mortality is less than or equal to 0.5 % (i.e. 1 in 200 individuals); bold type indicates protection to level of 1 % (i.e. 1 in 100 individuals).

Table 6: Results using the Hofmann et al. (2014) dose–distance relationship for NT lepidopteran larvae exposed to Bt-maize pollen in a 1 km square protected habitat. Values given are the percentage of those species where larvae are protected[e] to the degree that their estimated percentage mortality is less than or equal to 0.5 % (i.e. 1 in 200 individuals) or less than or equal to 1 % (i.e. 1 in 100 individuals), by an isolation distance of *S* metres from the nearest Bt-maize field, under two scenarios (see text).

Scenario	Event	Protection level	S, m									
			5	20	30	50	100	200	500	1 000	2 000	3 000
MR	1507	0.5%	**97.5**	**98.7**	**98.9**	**99.1**	**99.3**	**99.4**	**99.6**	**99.6**	**99.7**	**99.7**
		1 %	**98.6**	**99.2**	**99.3**	**99.4**	**99.5**	**99.6**	**99.7**	**99.7**	**99.82**	**99.84**
	MON810/Bt11	0.5%	**99.86**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**
		1 %	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**
CO	1507	0.5%	88.0	92.5	93.5	**94.5**	**96.0**	**96.5**	**97.5**	**98.3**	**98.7**	**98.9**
		1 %	92.0	**95.5**	**96.0**	**96.5**	**97.5**	**98.2**	**98.7**	**99.0**	**99.2**	**99.3**
	MON810/Bt11	0.5%	**99.5**	**99.7**	**99.7**	**99.7**	**99.82**	**99.84**	**99.88**	**99.88**	**> 99.9**	**> 99.9**
		1 %	**99.7**	**99.8**	**99.82**	**99.84**	**99.86**	**99.88**	**> 99.9**	**> 99.9**	**> 99.9**	**> 99.9**

(e): Bold values indicate that for this isolation distance the species protected at the particular level includes all species tested thus far plus some hypothetical species not yet identified.

To aid in the interpretation of these results, the EFSA GMO Panel points out the absence of correlation between the level of sensitivity and the rarity of NT lepidopteran species. There is no reason to expect a species of conservation concern to be any more or any less sensitive to the insecticidal Cry proteins than a pest species. However, in line with its conservative approach, the EFSA GMO Panel has assumed that some rare species have extreme sensitivity.

3.6.   Discussion

The mathematical model described in the present scientific opinion focuses on exposure and estimated mortality caused by Bt-maize pollen to larvae of lepidopteran species of conservation concern, with different sensitivities to the Bt-protein expressed. This single stressor (Bt-maize pollen) is one of several potential stressors that can impact Lepidoptera, at one or more stages in their development (eggs, larvae, pupae, adults). Mortality at specific stages may or may not affect the mortality of the population as a whole, and does not necessarily affect it linearly. Recognised techniques, such as key factor analysis (Varley and Gradwell, 1960), use population dynamics data to place mortality at specific stages into the context of the whole population. Currently, there are insufficient data available to allow Bt-related larval mortality to be put into the context of overall mortality.

The EFSA GMO Panel therefore recommends that the results of the mathematical model to estimate the Bt-related mortality of NT lepidopteran larvae are put into a broader ecological context by risk managers.

4.   Conclusions

New information provided by Hofmann et al. (2014) led the EFSA GMO Panel to reconsider the level of exposure of NT lepidopteran larvae to Bt-maize pollen, in particular over long distances for larvae in protected habitats, and thus to refine the outcomes of the modelling exercise performed by Perry et al. (2010, 2011, 2012, 2013).

The EFSA GMO Panel ran new simulations with exactly the same Bt-related mortality model as used by Perry et al. (2010, 2011, 2012, 2013) but assuming the dose–distance relationship defined by Hofmann et al. (2014). The EFSA GMO Panel considered different exposure scenarios and the uncertainties pertaining to the structure of the Perry et al. model, and/or contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen. In this scientific opinion, the percentage of NT lepidopteran species, for which the predicted mortality is less than a defined threshold protection level (e.g. 0.5 % or 1 %), is given for the three different exposure scenarios and different isolation distances from sources of Bt-maize pollen.

The EFSA GMO Panel emphasises that, in its previous scientific opinions (EFSA GMO Panel, 2011a, b, 2012d, e), larval mortality was estimated with regard to an unrealistically large level of exposure, as no allowance was made for effects that are known to reduce the effective exposure to Bt-maize pollen, such as the proportion of maize pollen that comes from non-Bt-maize varieties. Despite the fact that mortality estimates may have been overestimated for the dose–distance relationship used by EFSA (EFSA GMO Panel, 2011a, b, 2012d, e), no such allowances were necessary in these previous opinions, because the isolation distances commensurate with those mortality estimates were, in any case, relatively small. With the different dose–distance relationships studied here, it is necessary to adopt more realistic levels of exposure and hence isolation distances that are commensurate with appropriate mortality estimates. An uncertainty analysis of several different factors affecting the exposure of NT Lepidoptera to Bt-maize pollen is used to provide quantitative estimates of a more appropriate exposure level.

The EFSA GMO Panel concludes that the new information provided by Hofmann et al. (2014) does not impact greatly on the mortality estimates for NT Lepidoptera of conservation concern, occurring within protected habitats and potentially exposed to maize MON 810/Bt11 pollen. Under the most realistic and even conservative scenarios, the estimated mortality for all species considered is always less than 0.5 % for the previously recommended isolation distance of 20 m. Therefore, the previous EFSA GMO Panel recommendation for isolation distances around protected habitats, within which maize MON 810/Bt11 should not be cultivated, remains valid (EFSA GMO Panel, 2011b).

However, new calculations show that the previously recommended isolation distance of 30 m from the nearest field of maize 1507 would still protect NT Lepidoptera with known levels of sensitivity, including the 'highly-sensitive' *Plutella xylostella* (EFSA GMO Panel, 2011a). Should hypothetical species with greater sensitivities exist, larger isolation distances would be needed to ensure the desired level of protection.

The EFSA GMO Panel provides risk managers with a tool to estimate and mitigate the risk for NT Lepidoptera of conservation concern, considering the above-mentioned three scenarios at a range of isolation distances, at two protection levels and for lepidopteran species with a wide spectrum of sensitivities to Bt toxins, including hypothetical species not yet assessed. This will allow risk managers to select the most appropriate risk management measures (i.e. isolation distances) that are proportionate to the level of risk identified according to appropriate protection goals.

5.   Recommendations

In Section 3.3.2, the EFSA GMO Panel acknowledges the uncertainty in the Perry et al. (2010, 2011, 2012, 2013) model caused by the lack of data from bioassays estimating the sensitivity of a wider range of 'real' NT Lepidoptera for most assessed Bt-maize events. Furthermore, in sections 3.2.2 and 3.3.2, the EFSA GMO Panel indicates unresolved uncertainties inherent in the data of Hofmann et al. (2014).

Given the fact that these uncertainties mainly affect results on hypothetical species, the EFSA GMO Panel reinforces its previous recommendations for further studies on the effective exposure and level of sensitivity of NT Lepidoptera to Bt-maize pollen; for example, it recommends that studies are performed to estimate the range of NT Lepidoptera, with high sensitivity levels, effectively exposed to and likely to be affected by maize 1507 pollen (EFSA GMO Panel, 2011a), and to confirm the estimated sensitivity of NT Lepidoptera and whether or not NT lepidopteran larvae, with an 'extremely high' sensitivity to the Cry1Ab protein, are present and feeding on host plants occurring in and adjacent to maize fields at the time of pollen shed (EFSA GMO Panel, 2011b).

Documentation provided to EFSA

1.   Note, dated 16 December 2014, from the Chair of the EFSA GMO Panel to the EFSA Executive Director to request a self-task mandate to revise its previous risk mitigation measures reducing exposure of sensitive non-target Lepidoptera, which potentially occur in protected natural habitats, as defined in Directive 2004/35/EC, to maize MON 810, Bt11 or 1507 pollen.

2.   Acknowledgement letter, dated 3 February 2015, from the EFSA Executive Director to the Chair of the EFSA GMO Panel.

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Appendix A – Sources of uncertainty affecting the level of exposure of non-target Lepidoptera occurring in protected habitats to Bt-maize pollen

Sources of uncertainty[a]	Reasoning and related publications[b]	Corresponding parameter in Perry et al. model[c]	Uncertainty accounted for in the present modelling exercise[d]	Direction and magnitude of the multiplicative effect on the level of exposure following experts elicitation[e]		
				Median of maximum exposure [f]	Median of minimum exposure[g]	Median of mid-range exposure[h]
Proportion of GM/non-GM and Bt/non-Bt-maize leading to a dilution effect	Hofmann et al. (2014) consider that all maize pollen collected by the samplers is derived from GM maize. In practice, this is unlikely since a minimum of 20 % of the maize area should be cropped with non-Bt-maize as refuge for the insect resistance management. Moreover, it is not expected that most of the maize fields are GM or GM of the same event. Such a 'dilution effect' was observed by Hofmann et al. (2008). The authors report that the proportion of Bt-maize pollen collected by pollen samplers ranges between 7 and 44 % at distances of between 5 m and 120 m from a single Bt-maize field. Therefore, the ratio of GM to non-GM pollen emitted in an agricultural area might have an impact on exposure of lepidopteran species (Eastham and Sweet, 2002)	v	Yes	0.8	0.05	0.425

Sources of uncertainty[a]	Reasoning and related publications[b]	Corresponding parameter in Perry et al. model[c]	Uncertainty accounted for in the present modelling exercise[d]	Direction and magnitude of the multiplicative effect on the level of exposure following experts elicitation[e]		
				Median of maximum exposure[f]	Median of minimum exposure[g]	Median of mid-range exposure[h]
Pollen deposition on the flat surface of pollen sampler compared with leaf surface leading to a dilution effect	The Hofmann et al. (2014) model estimates the pollen deposition on a flat/horizontal surface. However, vegetation is characterised by a three-dimensional structure. This structure can be described by the leaf area index (LAI). The LAI is defined as 'the one-sided green leaf area per unit ground surface area'. The LAI varies between vegetation types. In the literature, values of between 2.5 and 8 were measured for crops, 1.6 to 13 for grasslands, 0.5 and 0.8 for rural areas and up to 19 for forests (Geyger, 1977; Hough, 1990; Schulla, 1997; Bronstert et al., 2001; Baldocchi, 2012). Technical pollen samplers might therefore under- or overestimate the real exposure of lepidopteran species to Bt-maize pollen	x	Yes	0.85	0.32	0.61
Translocation/removal of pollen from leaf surfaces by wind and rain	Pollen on leaf surfaces can be removed by wind and rain. This can lead to a reduction of exposure of larvae (Zangerl et al., 2001)	x	Yes	0.85	0.45	0.65
Displacement and accumulation of pollen from leaf surfaces by wind and rain	Pollen on leaf surfaces can be displaced because of rain and wind. Pollen can accumulate on lower leaves (Gathmann et al., 2006b; Hofmann et al., 2013), or on leaf veins and leaf axils (Hofmann et al., 2013). This could lead to a higher exposure of larvae of those species that feed on lower leaves. In most cases, larvae do not prefer to feed on leaf veins and leaf axils; this could lead to a lower exposure of larvae of species showing such inhibition	x	Yes	1.0	0.55	0.825
Competition for resources, resulting in consumption of pollen by non-lepidopteran species unaffected by Bt-protein	Pollen is a nutritionally rich food resource for many species. It can be expected that exposure of lepidopteran species might be reduced because of food competition with other species which consume pollen, e.g. ladybird beetles or hoverflies. In addition, other herbivores which also consume host plants of lepidopteran species will consume pollen together with leaf material (Wäckers et al., 2007)	d	Yes	1.0	0.6	0.8

Sources of uncertainty[a]	Reasoning and related publications[b]	Corresponding parameter in Perry et al. model[c]	Uncertainty accounted for in the present modelling exercise[d]	Direction and magnitude of the multiplicative effect on the level of exposure following experts elicitation[e]		
				Median of maximum exposure[f]	Median of minimum exposure[g]	Median of mid-range exposure[h]
Degradation of the Bt-protein in pollen	The data on degradation of Bt-protein in pollen is scarce. Ohlfest et al. (2002) observed that UV radiation did not affect the Bt-protein concentration after continuous radiation over 240 hours. However, the Bt-protein content and the biological activity of the protein were reduced by a factor of around 45, regardless of some flaws of the publication by Ohlfest et al. (2002). Pollen expression data compared with other studies are few and the mortality of the control is unacceptably high. In a further study, Xing et al. (2008) observed that Bt-proteins in maize pollen were not detectable after 15 or 18 days, depending on the event	*d*	Yes	0.925	0.625	0.75
Changes in feeding behaviour of lepidopteran larvae	Scientific publications report examples of feeding inhibition, repellent effects and changes in feeding behaviour, particularly for Cry1F protein (Prasifka et al., 2007, 2009; Goldstein et al., 2010; Gaspers et al., 2011). In contrast, Halcomb et al. (2000) observed no repellent effect on two lepidopteran species feeding on GM cotton expressing Cry1Ac protein		Yes	1.0	0.80	0.90
Lack of temporal coincidence between sensitive larval development stage and maize pollen shed	Only a limited number of lepidopteran species might be at risk. Reasons are synchronicity between larval development and pollen shed, hidden lifestyle of larvae (e.g. feeding inside stalks) or feeding behaviour. In different studies, the proportion of affected species was estimated. The study by Schmitz et al. (2010) estimated that seven butterfly species in Germany might be exposed to maize pollen. In specific field studies, the number of exposed lepidopteran species was limited. Gathmann et al. (2006a) identified 27 potentially exposed species. A similar number (33) was identified by Lang (2004). Furthermore, the exposure of lepidopteran species differs between years because of climatic factors (Gathmann et al., 2006b)	*a*	Yes	1.0	0.0	0.50

Sources of uncertainty[a]	Reasoning and related publications[b]	Corresponding parameter in Perry et al. model[c]	Uncertainty accounted for in the present modelling exercise[d]	Direction and magnitude of the multiplicative effect on the level of exposure following experts elicitation[e]		
				Median of maximum exposure [f]	Median of minimum exposure[g]	Median of mid-range exposure[h]
Mixture of different maize varieties leading to a dilution effect	The number of pollen grains varies when produced by different maize varieties and from distinct maize fields. It is influenced by factors such as maturity class, morphological characteristics, flowering time, and nutritional and climatic conditions. Furthermore, the amount of emitted pollen varies temporally, both throughout the day and the flowering time		No	–	–	–
Spatial distribution of host plants	Spatial distribution of host plants of lepidopteran species is very variable. Host plants in protected habitats normally have lower relative densities than field margins, and oviposition is not restricted to host plants in the protected area (e.g. *Brassicaceae* in Italian Community Interest Sites). Therefore, a limited portion of the population is feeding at a certain patch and only parts of a population might be affected. Furthermore, sunny sites are preferred for egg laying. Development time and mortality of small tortoiseshell larvae is dependent on microclimate (Bryant et al., 1997). This might lead to a higher exposure of larvae because the preferred host plants might be located in field margins (Gathmann et al., 2006a)	*e, f*	No. This is considered an effect on density of larvae per unit area, rather than on exposure. Given a particular protected habitat, and given the larval density within it, the percentage mortality, which is unaffected by the density, is estimated here	–	–	–

Sources of uncertainty[a]	Reasoning and related publications[b]	Corresponding parameter in Perry et al. model[c]	Uncertainty accounted for in the present modelling exercise[d]	Direction and magnitude of the multiplicative effect on the level of exposure following experts elicitation[e]		
				Median of maximum exposure [f]	Median of minimum exposure[g]	Median of mid-range exposure[h]
Nutritional quality of host plants	The nutritional quality influencing the choice of host plants for egg laying. Pullin (1987) reports that females of *Aglais urticae* prefer to lay their eggs on freshly sprouted, shorter nettles because of their better nutritional quality compared with older nettles		No. This is considered an effect on density of larvae per unit area, rather than on exposure. Given a particular protected habitat, and given the larval density within it, the percentage mortality, which is unaffected by the density, is estimated here	–	–	–

(a): Biotic and abiotic factors constituting sources of uncertainty on the level of exposure of NT Lepidoptera to Bt-maize pollen.

(b): The relevance of the identified sources of uncertainty is supported by a scientific reasoning and related scientific publications.

(c): Corresponding parameters were accounted for in the modelling exercise by Perry et al. (2010, 2011, 2012, 2013). For further details, see Table 3 of EFSA GMO Panel, 2011a.

(d): Some uncertainties were accounted for in the present scientific opinion to re-run the Perry et al. Bt-related mortality model using the Hofmann et al. (2014) dose–distance relationship data.

(e): A total of eight experts were asked to quantify the direction and magnitude of the impact of the uncertainties on the overall exposure level of NT Lepidoptera to Bt-maize pollen. Each individual entry gives the value by which the original exposure should be multiplied to yield the effective exposure after allowing for that effect (a value of 0.0 therefore represents the maximum reduction to nil exposure; a value of 1.0 represents the least reduction, implying exposure is unchanged).

(f): Median of the maximum end of the range over all the experts, expressed as a percentage (highest exposure).

(g): Median of the minimum end of the range over all the experts, expressed as a percentage (lowest exposure).

(h): Median of the middle of the range over all the experts, expressed as a percentage (most likely exposure).