efsa
European Food Safety Authority

DEPARTMENT OF SCIENTIFIC EVALUATION OF REGULATED PRODUCTS

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Mr Eric Poudelet
Director
Directorate General for Health and Consumers
European Commission
200, rue de la Loi
B-1049 Brussels – Belgium

Subject: **Request to review the scientific basis of two Testbiotech reports on maize 1507**

Dear Mr Poudelet,

In response to your letter dated 17 February 2014 (with reference Ares (2014) 387725), EFSA assessed the scientific content of the two Testbiotech reports on maize 1507[1] and the scientific publications cited therein (see Annex and its two Appendices for further details).

EFSA did not identify new scientific elements necessitating the revision of the previous conclusions on the safety of maize 1507 made by its Panel on Genetically Modified Organisms (GMO Panel). Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions and risk management recommendations on maize 1507 remain valid and applicable.

Yours sincerely,

Per Bergman
Head of Department

c.c. Mr Vanhoorde, Ms André, Ms Brown, Ms Pelsser
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[1] The report of 11 December 2013 entitled '*high-level-risk-maize 1507 – shortcomings at the European Food Safety Authority (EFSA) and in EU Commission Decision making prompt reassessment of genetically engineered maize 1507*' by Then and Bauer-Panskus and the report of 8 February 2014 entitled '*genetically engineered maize 1507 – industry and EFSA disguise true content of Bt toxins in the plants*' by Then and Bauer-Panskus

ANNEX

1. BACKGROUND

Upon request of the European Commission, EFSA assessed the Then and Bauer-Panskus (2013, 2014) reports and the scientific publications cited therein.

2. ASSESSMENT

During its evaluation of Then and Bauer-Panskus (2013, 2014) reports, EFSA noted that most of the cited scientific publications were addressed previously by EFSA and its GMO Panel in various scientific outputs (e.g., EFSA, 2005, 2009, 2011, 2012a,b). These publications are therefore not considered further here. For the remaining scientific publications, EFSA has focused its assessment on those that are specific to maize 1507 (see Appendices annexed to this letter).

For each area of concern outlined in the Then and Bauer-Panskus (2013, 2014) reports, EFSA assessed whether any of the scientific publications not previously addressed by EFSA and/or its GMO Panel, or any of the arguments put forward by Then and Bauer-Panskus, would invalidate the previous GMO Panel conclusions on the safety of maize 1507.

The EFSA assessment below focuses on Section 2 of the report by Then and Bauer-Panskus (2013) in which the EFSA risk assessment is discussed. The other sections of this report have not been considered, as the topics raised therein are not in EFSA's remit. In addition, the EFSA assessment includes also the Then and Bauer-Panskus (2014) report, as the topic is overlapping.

The EFSA assessment below is structured into the headings used by Then and Bauer-Panskus (2013); where appropriate, it is indicated if the EFSA assessment responds also to the Then and Bauer-Panskus (2014) report.

2.1. EFSA assessment of the Testbiotech Heading 2.1 "*Unintended effects in the plants*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

The scientific publications cited in the Then and Bauer-Panskus (2013) report with relevance to maize 1507 were previously considered by EFSA and/or its GMO Panel (see Appendix A), and therefore are not considered further here.

Points raised

The points raised by Then and Bauer-Panskus (2013) were previously addressed by EFSA and/or its GMO Panel and therefore do not require further assessment.

EFSA conclusion

The arguments put forward by Then and Bauer-Panskus (2013) do not reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.2. EFSA assessment of the Testbiotech Heading 2.2.1 "*Failure to determine the expression rate of Bt toxins*" as referred to in Then and Bauer-Panskus (2013) and the full Then and Bauer-Panskus (2014) report[2]

Cited scientific publications

From the scientific publications cited in the Then and Bauer-Panskus (2013, 2014) reports with relevance to maize 1507, two publications were not previously considered by EFSA and/or its GMO Panel (see Appendices A and B: Pioneer Hi-Bred International, 2001; US EPA, 2010). The possible relevance of these publications for the risk assessment of maize 1507 was scrutinised.

- Pioneer Hi-Bred International (2001): This study report summarises protein expression levels obtained from maize 1507 plants grown in the USA in 1999 at four locations. Different tissues were sampled and the levels of Cry1F and PAT proteins were determined. Comparison of the results of this field trial with other field trials previously assessed by EFSA shows that in stalk, grain and forage the Cry1F levels were lower at other locations/years.

- US EPA (2010): This biopesticides registration action document by the United States Environmental Protection Agency lists expression levels of two Cry1F variants, including the one expressed by maize 1507 (p. 9). Then and Bauer-Panskus (2014) concludes that US EPA only relies on data also used by EFSA (see Appendix B; Pavely, 2002).

Points raised

EFSA considers that it is reasonable to assume that the amount of plant-produced Bt protein will differ owing to differences in plant developmental stages, genetic background of maize varieties and varying environmental conditions (reviewed by CERA, 2013). In addition, the use of various methods for analysis and the application of different work routines between laboratories contribute to the observed variability in measured plant-produced Bt protein concentrations (as noted explicitly in Section 2.3.5.1 of EFSA, 2011). However, as part of the environmental risk assessment, an exposure characterisation is performed to determine how much of the plant-produced Bt protein a particular non-target organism might be exposed to under field conditions. This exposure is estimated mainly from protein expression data, accounting for the variability in expression levels, as well as the respective diets and the feeding behaviour of each non-target organism. Test substance concentrations applied in laboratory studies with non-target organisms are typically selected to be conservative compared with the maximum amount of the plant-produced Bt proteins expected to be available to non-target organisms in the environment. Laboratory studies are often conducted using a single dose, for instance, at the so-called maximum hazard dose (MHD). The MHD is calculated by multiplying the expected environmental concentration (EEC) with a margin of exposure factor. Testing at the MHD level ($\geq 10 \times$ EEC) is considered highly conservative and adds certainty to the assessment (e.g., Romeis et al., 2011). EFSA notes that the EFSA GMO Panel has considered the level and variation of Cry1F protein concentrations in different plant parts of maize 1507 (see e.g., Section 3.2.1 of EFSA (2012a), and in particular pollen in the model used for its environmental risk assessments (EFSA, 2011).

[2] This Section also considers the Then and Bauer-Panskus (2014) report entitled '*genetically engineered maize 1507 – industry and EFSA disguise true content of Bt toxins in the plants*'

EFSA conclusion

Neither the results reported by Pioneer Hi-Bred International (2001) and US EPA (2010), nor the arguments put forward by Then and Bauer-Panskus (2013, 2014) reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.3. EFSA assessment of the Testbiotech Heading 2.2.2 "*Failing to assess the real risk for butterflies*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

From the scientific publications in the Then and Bauer-Panskus (2013) report with relevance to maize 1507, four publications (see Appendix A: Hua et al., 2001; Long et al., 2011a,b; Tan et al., 2013) were not previously considered by EFSA and/or its GMO Panel. The possible relevance of these publications for the risk assessment of maize 1507 was scrutinised.

- Hua et al. (2001): The publication of Hua et al. (2001) ascertained whether Cry1F, Cry9C or Cry9E recognise the Cry1Ab binding sites on *Ostrinia nubilalis* brush border membranes by three approaches: optical sensor chips, surface Plasmon resonance analyses and radioligand binding assays. The authors concluded that there are several Cry toxin binding sites and/or receptors in the midgut epithelia of *O. nubilalis*. Cry1Ac competes for the Cry1Ab binding site, whereas Cry9C and Cry9E appear to compete for a different binding site. Cry1F has multiple binding sites but a low affinity to Cry1Ab binding site. Additionally further investigations on receptor proteins of the different Cry proteins were conducted. The authors speculated that isoforms of aminopeptidates and cadherin in the brush border membrane serve as Cry1Ab, Cry1Ac and Cry1F receptor proteins.

- Long et al. (2011a,b): Long et al. (2011a,b) performed laboratory experiments in which larvae of the lepidopteran species *Vanessa cardui* were exposed to pollen from maize 1507, maize 1507×NK603 or that of the control plant incorporated into an artificial diet at a nominal rate of 1%, 3% and 10 % of the final dry weight. After the seven-day feeding period, larvae were removed from the diet and individual weights and mortality were recorded. The authors noted no statistically significant differences in larval weight between *V. cardui* larvae fed *Bt*- or non-*Bt*-maize for the 1% and 3% maize pollen diets. Statistically significant differences were observed in larval weight for the 10% near-isoline maize pollen diet compared to the corresponding maize 1507 pollen diet, with the larval weights for the near-isoline diet treatment being higher. Mortality rates were comparable in all maize 1507 pollen diet treatments compared to the corresponding near-isoline maize pollen diet treatments.
  Fresh weights of *V. cardui* larvae fed on 1% near-isoline maize pollen diet were comparable to larvae fed on the 1% maize 1507×NK603 pollen diet. Statistically significant differences were observed in larval weight for the 3% and 10% maize 1507×NK603 pollen diets compared to the corresponding near-isoline maize pollen diets. Mortality rates were comparable in all maize 1507×NK603 pollen diet treatments compared to the corresponding near-isoline maize pollen diet treatments.

- Tan et al. (2013): Tan et al. (2013) compared binding patterns of Cry1Ab and Cry1F toxins between the species *O. nubilalis* and *O. furnacalis* as well as the expression of putative cadherin and aminopeptidase-N protein receptors. The authors used brush border membrane vesicles of both corn borer species and conducted ligand blots and immunoblottings. In addition, a comparative cDNA sequence analysis of the Bt protein

putative receptors was performed. The results showed that the Cry1Ab and Cry1F proteins have a high affinity to different protein receptors, thus supporting the observation of low cross-resistance levels between Cry1Ab and Cry1F. Furthermore, these results support the previous experiments indicating the *O. nubilalis* and *O. furnacalis* share similar patterns of susceptibility to Bt proteins.

Points raised

Hanley et al. (2003) is one of the many publications, supporting the non-target risk assessment of maize 1507, previously reviewed by EFSA (e.g., EFSA, 2011). Hanley et al. (2003) observed differences in the sensitivity of a lepidopteran pest species (the greater wax moth, *Galleria mellonella*) to two lepidopteran-active Bt proteins. Similar findings were reported in other publications and previously accounted for by the EFSA GMO Panel. However, Hanley et al. (2003) applied experimental protocols to two different non-target organism species, the honey bee and a lepidopteran pest. While the honey bee testing protocol was judged adequate by the EFSA GMO Panel, the one followed for the testing of the greater wax moth was considered inappropriate to determine the sensitivity of this species to the Cry1Ab and Cry1F proteins with any accuracy, as a non-standard bioassay technique was used relying on a diet comprising solely of pollen, which was offered in no-choice feeding trials.

The Hua et al. (2001) and Tan et al. (2013) publications give useful insights on the binding mechanisms of Bt proteins on receptors in brush border membranes. The results are helpful to better understand the cause of the observed differences in the sensitivity of Lepidoptera towards different lepidopteran-active Bt proteins. However, this information does not change mortality estimates based on pollen exposure of non-target Lepidoptera obtained via the mathematical models developed by Perry et al. (2010, 2012).

In Section 2.3.5 of EFSA (2011), the EFSA GMO Panel assessed the between-species sensitivity of Lepidoptera. It is clearly stated that sensitivity with respect to Cry1Ab and Cry1F is highly variable between species and references to relevant publications were presented. In Section 2.3.5 (EFSA, 2011), the sensitivity was considered in order to estimate mortality following exposure to Bt maize pollen. The EFSA GMO Panel agrees that the observed differences are likely due to differences in binding sites. Yet, this information does not change the risk assessment for non-target Lepidoptera conducted by the EFSA GMO Panel. The EFSA GMO Panel assessment relied on the model by Perry et al. (2011) using worst-case scenarios for a range of Lepidoptera with different sensitivities.

EFSA agrees with Then and Bauer-Panskus that the Long et al. (2011a,b) publications are of limited use owing to limitations in the experimental design of the studies. Therefore, the EFSA GMO Panel did not use these publications as key studies, but only as supportive information in a weight-of-evidence approach. Moreover, instead of focusing its assessment on the sensitivity of *V. cardui* to the Cry1F protein, the EFSA GMO Panel considered the entire range of sensitivities of first instars of various lepidopteran species, together with corresponding values for hypothetical species in its Scientific Opinion. The sensitivity values selected by the EFSA GMO Panel were intended to represent a wide range of hypothetical unspecified lepidopteran species (A-E) that reflect the between-species variability in acute sensitivity (LC_{50}) to the Cry1F protein from maize 1507 (see Table 2 in EFSA, 2011; Wolt et al., 2005). The five LC_{50} values considered form a geometric series with 11.4× increments:

- Hypothetical species (A) with a LC_{50} of 1.265 maize 1507 pollen grains cm^{-2}, representing the 'extreme worst-case' sensitivity Category V, where extreme sensitivity

to the Cry1F protein from maize 1507 would bring the greatest risk of mortality to a non-target lepidopteran species;

- Hypothetical species (B) with a LC_{50} of 14.36 maize 1507 pollen grains cm^{-2}, representing a very-highly sensitive species (Category IV);
- Hypothetical species (C) with a LC_{50} of 163.2 maize 1507 pollen grains cm^{-2}, representing highly-sensitive species (Category III);
- Hypothetical species (D) with a LC_{50} of 1853 maize 1507 pollen grains cm^{-2}, representing 'above-average' sensitive species (Category II);
- Hypothetical species (E) with a LC_{50} of 21057 maize 1507 pollen grains cm^{-2}, representing a species that is highly likely to be above the mean of the distribution and is termed 'below-average' (Category I).

For non-target Lepidoptera, the expression of the Bt protein in pollen is of particular relevance. EFSA (2011) adopted a conservative mean expression level of 32 ng Cry1F/mg pollen for the content of the Cry1F protein in maize 1507 pollen. In the field, it is understood by the EFSA GMO Panel that there will be variability about this mean value. Some plants may shed pollen that has a greater expression level, and this will be compensated for by other plants that will shed pollen with a lower expression level of the Bt protein in pollen. In EFSA (2011), the GMO Panel (Section 2.3.5.2(a)) emphasised that the risk assessment for non-target Lepidoptera and the recommendations for risk management are based on a deterministic mathematical model, i.e. one which depends upon mean values and which performs identically for any given set of initial conditions. The scientific uncertainties associated with the model were discussed explicitly, including the slight effects expected from replacement of assumptions of mean homogenous concentrations of exposure to Cry1F by stochastic heterogeneous fluctuations about that mean value. Therefore, the point made in the Then and Bauer-Panskus (2013, 2014) reports concerning variability in protein expression levels does not represent new scientific knowledge.

The effect of an increase in the expression level in maize 1507 pollen, relative to that previously assumed in the Perry et al. (2011) publication and EFSA (2011), is considered briefly below, as a response to the arguments in the Then and Bauer-Panskus (2013, 2014) reports concerning the issue of variability in protein expression levels in various plant parts of maize 1507. To place this into context, the effects of an increase in mean Cry1F protein expression level of 25% in maize 1507 pollen (i.e., from 32 ng/mg to 40 ng/mg; see Linderblood, 2008 who reported additional expression data for maize 1507 derived from field studies performed in three locations in Spain during the 2005 growing season) on the model predictions were studied.

- An increase in the expression level of 25% would be expected to reduce the LC_{50} values for the species listed in Table 2 of EFSA (2011) by a factor of 1.25. It can be seen from Table 2 of EFSA (2011) that this would have only a minor effect. For example, a species such as *Plutella xylostella*, found at the 8th percentile of the species sensitivity distribution with a LC_{50} of 54 maize 1507 pollen grains cm^{-2} for an expression level of 32 ng Cry1F/mg maize 1507 pollen would have a new LC_{50} of 43.2 maize 1507 pollen grains cm^{-2}, equivalent to the 6.5th percentile of the sensitivity distribution, for an increase in expression level to 40 ng Cry1F/mg maize 1507 pollen. This change in LC_{50} value would not move *P. xylostella* into a higher sensitivity Category. To move a species from the middle of one sensitivity Category to the middle of the next one, a decrease in LC_{50} by 11.4×, which is much larger than the decrease by 1.25× studied here, would be required.

- It is also possible to calculate the effect of this 25% increase in Cry1F expression level in maize 1507 pollen on the estimates of local and global mortality in EFSA (2011). Table 4 of EFSA (2011) gave estimates of local mortality for each Category of

sensitivities studied, at varying distances from the nearest maize 1507 field. The same information has been recalculated, but now under the assumption of a 25% increase in Cry1F protein expression level (S2 in Table 1, below). The average increase in predicted local mortality would be only 1%, and the maximum increase 5.9%. For global mortality (i.e., allowing for large-scale exposure), even for a conservative (worst-case) estimate value of $R = 0.08$ (see Table 3 of EFSA, 2011), the average increase would be 0.08%, and the maximum increase 0.5%.

Table 1: 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, with increasing distances from the nearest maize 1507 field accounting for a conservative mean expression level for the content of the Cry1F protein in maize 1507 pollen of 32 ng Cry1F/mg pollen (S1: scenario 1; based on Table 2 in EFSA, 2011) and 40 ng Cry1F/mg pollen (S2: scenario 2; 25% increase compared to S1)

Distance from field (m)	Categories of non-target Lepidoptera									
	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	
	% local mortality at different Cry1F protein expression levels in maize 1507 pollen (S1: 32 ng/mg; S2: 40 ng/mg)									
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
2	0.3	0.3	3.5	4.4	32.5	37.8	86.2	88.8	98.8	99.1
5	0.1	0.1	1.2	1.6	14.3	17.4	68.4	73.3	96.6	97.3
10	0.0	0.0	0.2	0.3	2.7	3.4	27.2	32.1	82.9	86.0
15	0.0	0.0	0.0	0.0	0.5	0.6	6.0	7.4	45.4	51.3
20	0.0	0.0	0.0	0.0	0.1	0.1	1.0	1.3	12.5	15.3
25	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	2.3	2.9
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.5
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The conclusion from both the abovementioned analyses confirm that moderate fluctuations in expression levels are not expected to have more than a minor effect on estimates of mortality from the mathematical model. Essentially, the recommendations for risk management outlined in EFSA (2011) based on a wide range of different sensitivity of species, are largely unaffected by the degree of change in protein expression level considered here.

EFSA conclusion

Neither the results reported by Hua et al. (2001), Long et al. (2011a,b) and Tan et al. (2013), nor the arguments put forward by Then and Bauer-Panskus (2013) reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.4. EFSA assessment of the Testbiotech Heading 2.2.3 "*Data gaps on non-target organisms besides butterflies*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

From the scientific publications cited in the Then and Bauer-Panskus (2013) report with relevance to maize 1507, one publication (see Appendix A: Dona and Arvanitoyannis, 2009) was not previously considered by EFSA and/or its GMO Panel. The possible relevance of this publication for the risk assessment of maize 1507 was scrutinised.

- Dona and Arvanitoyannis (2009): Dona and Arvanitoyannis (2009) reviewed regulatory progress on GM foods, and discussed possible hazards associated with the consumption of GM foods by human and animals. In particular, the authors mentioned the rat feeding study with maize 1507 performed by Mackenzie et al. (2007) as a publication in which GM-related effects were noted in clinical chemistry (decreased alkaline phosphatase in male rats), haematology (decreased eosinophils in female rats) and organ weight (decreased kidneys weight, relative to body weight). Finally, the authors emphasised the need to perform animal feeding studies in order to test the safety of GM foods, and they called for clinical trials as part of the safety assessment process.

Points raised

Animal feeding studies remain an ongoing topic of debate on the safety assessment of GM foods. In its guidelines for the food and feed safety assessment of GM plants, the EFSA GMO Panel considered that a 90-day feeding study should be carried out as a hypothesis driven exercise. In the case of maize 1507, neither the molecular characterisation, nor the compositional analysis have given indications that would require the performance of the 90-day feeding study. Because the 90-day feeding study had been supplied by the applicant as part of the GM plant market registration application, the EFSA GMO Panel considered these data during its evaluation of maize 1507. The report of the 90-day feeding study provided with the GM plant market registration application refers to the same study as the publication by MacKenzie et al. (2007). The Mackenzie et al. (2007) publication was part of the data package provided to EFSA in the frame of the renewal application for maize 1507 as summarised in the EFSA GMO Panel's Scientific Opinion published in 2009. The study provided in the application and reported by Mackenzie et al. (2007) did not raise safety concerns in the evaluations published in 2005 and 2009 by the EFSA GMO Panel.

EFSA conclusion

Neither the results reported by Dona and Arvanitoyannis (2009), nor the arguments put forward by Then and Bauer-Panskus (2013, 2014) reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.5. EFSA assessment of the Testbiotech Heading 2.2.4 "*True level of complexity ignored by EFSA*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

From the scientific publications cited in the Then and Bauer-Panskus (2013) report with relevance to maize 1507, seven publications (see Appendix A: Thomas and Ellar, 1983; Shimada et al., 2003; Huffmann et al., 2004; Zhang et al., 2006; Dolezel et al., 2011; Mesnage et al., 2012; Bondzio et al., 2013) were not previously considered by EFSA and/or its GMO Panel. The possible relevance of these publications for the risk assessment of maize 1507 was scrutinised.

- Thomas and Ellar (1983): The authors reported *in vivo* studies of delta-endotoxins isolated from *B. israelensis* and from *B. kurstaki* to Balb/c mice by intravenous inoculation via tail vein or by oral feeding. The authors reported that: (1) native crystal delta-endotoxin in phosphate buffered saline from neither host resulted in mortality, independent of the exposure routes and at all doses tested; and (2) alkali-soluble (pH 10.5) delta-endotoxin isolated from *B. israelensis* caused death at the dose of 0.5 mg (60%) and 1 mg per animal (100%), but no death was observed when the delta-endotoxin was isolated from *B. kurstaki*. The characterisation of the delta-endotoxins performed by the authors in this publication does not allow understanding if they share similarities to the newly expressed protein in maize 1507.

- Shimada et al. (2003): Shimada et al. (2003) investigated if the Cry1Ab protein affects primary cultured bovine hepatocytes. The authors concluded that Cry1Ab has little acute toxicity on mammalian cells *in vitro* based on a minor, not significant increase in lactate dehydrogenase activity – a cytotoxicity biomarker – on bovine hepatocytes. However, in this setting neither functional impairment of the Cry1Ab-treated hepatocytes, as demonstrated by the normal albumin secretion, nor morphological changes were noted.

- Huffmann et al. (2004): The authors identified mitogen-activated protein kinase pathways as the defence mechanism in the nematode *Caenorhabditis elegans* to the bacterial pore-forming protein Cry5B.

- Zhang et al. (2006): Zhang et al. (2006) presented a mechanism of cell death induced by the Cry1Ab protein not described before. Cell cultures of transfected ovarian cells of *Trichoplusia ni*-expressing a cadherin receptor relevant to Cry1Ab were exposed to the toxin. The authors observed molecular and cellular changes induced by the Bt protein. The authors showed that binding of the Bt proteins to the receptor induces cell death by activating a previously non-described signalling pathway. Noteworthy, the morphological changes observed are not consistent with apoptotic cell death. Importantly, it is reiterated that toxin-receptor interaction is prerequisite to cytotoxic action, and that no equivalent toxicity has been observed in mammals because of the lack of appropriate receptors and different gastrointestinal tract conditions. The discovery of this new pathway might provide insights on how insects evolve resistance to Bt proteins.

- Dolezel et al. (2011): Dolezel et al. (2011) analysed the environmental risk assessment of GM plant market registration applications for the cultivation of seven maize transformation events (including 1507). The authors mainly focused on the assessment of datasets specific to the GM maize or the relevant protein(s) provided by applicants to support the evaluation of: the agronomic characterisation of GM maize; and possible interactions with target organisms and non-target organisms. The authors

claimed to have identified shortcomings in the datasets. In addition, the authors considered that two principles of Directive 2001/18/EC were largely not fulfilled in such applications: the consideration of the receiving environment and potential indirect effects arising from the application of the complementary herbicide in the case of GM herbicide tolerant maize.

- Mesnage et al. (2012): the authors investigated cytotoxicity on human cells of Cry1Ab and Cry1Ac proteins (expressed in *Escherichia coli*) individually and combined with Roundup at below agricultural dilutions. In summary, the authors reported that Cry1Ab induces low level mortality (via necrosis) on a cellular system at 100 ppm, while Cry1Ac does not. The Roundup formula induces cell mortality via apoptosis at 50 ppm in the same system. The combination of Cry1Ab or Cry1Ac with Roundup reduced cell mortality.

- Bondzio et al. (2013): The authors used an *in vitro* system (IPEC-J2, a non-transformed porcine jejunal epithelial cell line) to test the effects of high concentration of *E. coli*-derived Cry1Ab protein on cellular viability and cytotoxicity by several up to date techniques. The authors did not find any effects on cell viability or cytotoxicity, even at high protein concentration. Proteomic analysis and further ELISA characterisation revealed up to two-fold increase in Hsp70 protein in cells. Bondzio et al. (2013) noted that the Hsp70 protein expression is known to increase in response to various physiological stimuli and it is considered an adaptive response, not adverse *per se*.

Points raised

EFSA considers it is impossible to assess what Then and Bauer-Panskus (2013) meant by the phrase 'true known level of uncertainty'. In its guidelines for the risk assessment of GM plants, the EFSA GMO Panel recommends that uncertainty inherent to the different steps of a risk assessment should be highlighted and quantified as much as possible. The EFSA GMO Panel has explicitly and repeatedly listed the various levels of uncertainty at various stages of the risk assessment of maize 1507 (e.g., see Sections 2.1, 2.3.5, 3.1.1, 3.1.2, 3.1.3, 3.2.2 and 3.2.3 of EFSA, 2011).

The statement that the EFSA GMO Panel considered only investigations carried out with the Cry1Ab protein is incorrect. Reference is made to nine Tier 1a and five Tier 1b studies in which non-target organisms where exposed to the Cry1F protein in EFSA (2011). Moreover, a field study with maize 1507 was assessed in which a large group of non-target organisms was investigated. Based on the information supplied by the applicant and other publications reported in the scientific literature, the EFSA GMO Panel concluded that there are sufficient data to conclude on the spectrum of activity of the Cry1F protein. EFSA acknowledges that the knowledge on the activity spectrum of Bt proteins is growing continuously. Bt proteins are commonly known to be highly target specific, yet evidence suggests that some Bt proteins can affect organisms across orders within phyla and even across phyla (van Frankenhuyzen, 2009, 2013)[3]. Although some Bt proteins are not as specific as initially reported, in most cases toxicity outside a protein's primary target range is orders of magnitude below its toxicity inside that range (EFSA & COGEM, 2013). Therefore, these new findings, which are not specific to Cry1F, do not invalidate previous environmental risk assessment conclusions on maize 1507 made by the EFSA GMO Panel, which took all available data from non-target invertebrates testing into account.

[3] The *Bacillus thuringiensis* toxin specificity database, http://www.glfc.cfs.nrcan.gc.ca/bacillus/

When assessing the findings reported by Shimada et al. (2003), EFSA also considered a more recent publication (Shimada et al., 2006) by the same first author which was published three years later than the Shimada et al. (2003) publication. Shimada et al (2006) reported two findings: (1) the binding of Cry1Ab protein to the brush border membrane vesicles (BBMV) isolated from the small intestine of bovine and porcine, and a much stronger binding in the control - the mid-gut BBMV from silkworm; and (2) the silkworm midgut cells developed severe membrane potential changes within 1 hr of treatment with Cry1Ab, and this change persisted overtime. No such change of membrane potential was detected in the gut cells of bovine or porcine, or in a human intestinal epithelial cell line. Overall Shimada et al. (2003, 2006) indicate that the effects of the Cry1Ab protein on mammalian cells (hepatocytes, intestinal cells) *in vitro* are minor, transient changes, and are not indicative of toxicity, even if associated to a weak binding to some cell components (BBMV in the mammalian intestinal cells; Shimada et al., 2006).

EFSA considers that the publication of Zhang et al. (2006) cited in the Then and Bauer-Panskus (2013) report was misinterpreted. EFSA does not consider that this publication questions the host specificity of Bt proteins, given that a known Cry1Ab protein receptor was introduced into the test system. As outlined in EFSA (2011), the general mode of action and in particular selective binding of Bt proteins to specific receptors are sufficiently understood. The mode of action of Bt proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation and cell burst and subsequently septicaemia. Zhang et al. (2006) and more recent publications do not question the binding of specific Bt proteins to receptors, but rather attempt to elucidate how the binding of the Bt protein to midgut receptors leads to toxic effects, resulting in the death of the exposed target organisms. Understanding such mechanisms is helpful, as this provides insights on how resistance may evolve. However, it is the information on binding sites that is relevant to characterise the host specificity of Bt proteins. EFSA reiterates that the susceptibility of non-target organisms to Bt proteins is assessed through toxicity studies (see e.g., Table 1 of EFSA (2011)) supplied as part of the GM plant registration application, as well as relevant studies published in the scientific literature. In the case of maize 1507, the EFSA GMO Panel considered that sufficient data were supplied by the applicant to conclude on the spectrum of activity of the Cry1F protein.

Regarding the potential safety impact of Bt proteins on mammals, EFSA considers that the publications indicated by Then and Bauer-Panskus (2013) are not providing information impacting on previous conclusions on the safety of maize 1507. In particular, Thomas and Ellar (1983) is considered by EFSA of no value in this context as Bt proteins used in the experiments were not clarified/characterised. The short communication by Mesnage et al. (2012) presents little details on the design of the experiment and reports only some selected findings, preventing the possibility to draw conclusions from this publication. Shimada et al. (2003, 2006) and Bondzio et al. (2013) reported that the Cry1Ab protein is not associated with adverse effects on mammalian cells *in vitro* (hepatocytes, intestinal cells). It should be highlighted that in the rat exposed for 90 days to a diet containing 33% of Bt maize(s) expressing the Cry1F protein (Mackenzie et al., 2007) the intestinal mucosa, exposed to bioactive Cry1F protein (60-200 pg/mg of diets), was extensively explored by macroscopic examination and histopathology, which are well-recognised sensitive tools to detect potential adverse effects on the gastrointestinal tract. This assessment was further corroborated by the assessment of gastrointestinal performance related endpoints (e.g., body weight, body weight gain, food consumption). No safety concerns were identified. Furthermore, it is reported that the administration of the Cry1F protein in a rat model of gastrointestinal toxicity by non steroidal anti-inflammatory drugs (NSAIDs) does not lead to any gastrointestinal morphological change *per se*, and does not worsen the toxicity induced by NSAIDs (Onose et al., 2008). While

providing insights on the mode of action of Bt protein toxicity, Zhang et al. (2006) also reiterates that toxin-receptor interaction is a prerequisite to cytotoxic action in target species. Huffmann et al. (2004) is also considered not relevant by EFSA as it relates to investigations on the Cry5B protein, which is not expressed in maize 1507.

Based on the available information, there is no indication for synergistic and accumulated effects.

In response to the concern related to the appropriate herbicide regimes raised by Dolezel et al. (2011), EFSA notes that the intended uses of maize 1507 exclude treatments with glufosinate-ammonium-based herbicides (EFSA, 2011). In line with EFSA (2010), EFSA concludes that the environmental risk assessment of GM plant market registration applications is to be carried out on a case-by-case basis, starting with a robust problem formulation accounting for all available information and data.

EFSA conclusion

Neither the results reported by Thomas and Ellar (1983), Shimada et al. (2003), Huffmann et al. (2004), Zhang et al. (2006), Dolezel et al. (2011), Mesnage et al. (2012) and Bondzio et al. (2013), nor the arguments put forward by Then and Bauer-Panskus (2013) reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.6. EFSA assessment of the Testbiotech Heading 2.2.5 "*Modelling without reliable data*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

From the scientific publications cited in the Then and Bauer-Panskus (2013) report with relevance to maize 1507, four publications (see Appendix A: Hofmann et al., 2011; Lang et al., 2011; Camastra et al., 2013; Holst et al., 2013a) were not previously considered by EFSA and/or its GMO Panel. The possible relevance of these publications for the risk assessment of maize 1507 was scrutinised.

- Hofmann et al. (2011): Hofmann et al. (2011) presented a new method for recording *in situ* the amount and the distribution of Bt maize pollen deposited on host plant leaves. The method was evaluated during experiments in 2008 to 2010. Pollen deposition and its spatial heterogeneity were recorded on maize and different lepidopteran host plants growing adjacent to maize fields.

- Lang et al. (2011): Lang et al. (2011) commented on uncertainty in the model of Perry et al. (2010) and called for more caution to be exercised regarding predictions of Bt maize effects on non-target Lepidoptera under field conditions. The authors argued that the assessment of such effects is complex, and that modelling approaches are welcomed, as they are especially helpful to better identify and understand complex interactions of key parameters and basic processes. Yet, the environmental risk assessment of GM plants is considered by the authors as a sensitive area, and any quantitative conclusions should be drawn and published with greatest care, as these could have significant policy and regulatory implications. Therefore, the authors advocated a full uncertainty/sensitivity analysis to be performed before making detailed quantitative predictions.

- Camastra et al. (2013): Camastra et al. (2013) assessed some mathematical models used to estimate potential adverse effects on non-target Lepidoptera following

exposure to maize pollen containing the Cry1Ab or Cry1F proteins deposited on their host plants under field conditions. The authors developed an exact formula for the proportion of the population of non-target Lepidoptera suffering mortality and commented on the Perry et al. (2010, 2012) models for the derivation of the species sensitivity parameter.

- Holst et al. (2013a): Holst et al. (2013a) presented an alternative mathematical model and commented on the model of Perry et al. (2010). The authors argued that in Northern Europe, where the protected butterfly *Inachis io* is univoltine, maize MON 810 pollen would not be present on the food plant at the same time as the *I. io* larvae. However, in Central and Southern Europe, where *I. io* is bivoltine, maize MON 810 pollen and the second generation *I. io* larvae would coincide, and an increased mortality of the larvae was predicted.

Points raised

Hofmann et al. (2011) discussed the stochasticity of pollen deposition, and emphasised the heterogeneity these authors previously described in field data (see Hoffman et al. (2010) and earlier publications). The EFSA GMO Panel acknowledged the existence of this heterogeneity (see Section 3.1.2.4 of EFSA, 2010) and its likely effects have previously been studied by Perry et al. (2010). In addition, the deterministic nature of the model and the implications of heterogeneity have been addressed above. Furthermore, Perry et al. (2013) used earlier data of Hofmann and colleagues, as well as data from 13 other studies, in their Appendix A. These data showed that the values adopted in the EFSA mathematical models for mean pollen densities, both within and outside the field, are clearly not underestimated, being at least double the median value and in all cases greater than the 75th percentile of the data from the literature. Perry et al. (2013) concluded that there is no evidence that indicates a requirement to change previous risk assessments.

Then and Bauer-Panskus (2013) cite the publication of Lang et al. (2011) which commented on uncertainty in the model of Perry et al. (2010). However, Then and Bauer-Panskus (2013) failed to note that this publication referred to the early model developed by Perry et al. (2010) for maize MON 810 expressing Cry1Ab, and did not relate specifically to maize 1507 expressing Cry1F. Moreover, Then and Bauer-Panskus (2013) fail to note that the Lang et al. (2011) publication was answered fully by Perry et al. (2011) who noted that the Perry et al. (2010) publication indeed emphasised precaution; made four separate decisions to model worst-case scenarios; identified six distinct sources of variability to which their results might be sensitive; and emphasised six different bases for the uncertainty of predictions. Perry et al. (2012) demonstrated emphatically that the Perry et al. (2010) publication was not incautious regarding the implications of the results for conclusions regarding regulatory policy. Perry et al. (2012) reaffirmed the conclusions from the Perry et al. (2010) model that concerning the estimated environmental impact of Bt maize pollen on non-target Lepidoptera.

The statement by Then and Bauer-Panskus (2013) that Camastra et al. (2013) "*came to the conclusion that the EFSA data cannot be absolutely considered to fulfil the worst case scenario, recommended in the Directive 2001/18/EC of the European Community*" is wrong. The Camastra et al. (2013) publication contains no such conclusion. Furthermore, the link given by Then and Bauer-Panskus (2013) as part of the supposed citation to the Camastra et al. (2013) publication is not correct; the link points to an apparently unpublished paper in which the authors of Camastra et al. criticise the publication of Holst et al. (2013a). The publication of Camastra et al. (2013) contains one novel scientific advance and addresses a technical issue. The novel advance is that the authors derived an exact formula for a mathematical expression in the Perry et al. (2012) model, which

Perry et al. had solved numerically. This provides an easier way to estimate mortality in the model, but in no way changes the estimates themselves. The technical issue Camastra et al. highlighted was that whilst Perry et al. (2012) clearly in their Figure 2 correctly truncated the values of the in-crop mortality parameter (*h*) to unity, as required for lepidopteran species with high, very-high and extremely-high sensitivity, Perry et al. (2012) did not state this explicitly in their paper. This technical issue has no bearing on uncertainty or safety.

Then and Bauer-Panskus (2013) cited the publication of Holst et al. (2013a) (not 2012 as incorrectly referred to in the Testbiotech text) which presented an alternative model and commented on the model of Perry et al. (2010). However, Then and Bauer-Panskus (2013) failed to note that the Holst et al. (2013a) publication referred to the early model developed by Perry et al. (2010) for maize MON 810 expressing Cry1A, and did not relate specifically to maize 1507 expressing Cry1F. Then and Bauer-Panskus (2013) also failed to cite the (flawed) corrigendum of Holst et al. (2013b) or to note that both these Holst et al. (2013a,b) publications were answered fully by Perry et al. (2013). Perry et al. (2013) noted that Holst et al. (2013a): overestimated the exposure arising from pollen deposition on host plants; underestimated the ratio of the Bt protein concentrations in pollen of the two Bt maize events Bt176 and MON 810; made a gross error in their use of the data of Felke and Langenbruch (2003) and Felke et al. (2010); proposed a methodology for estimation that ran counter to scientific principles; and omitted to account for important extensions to the earlier model of Perry et al. (2010) contained in their later (Perry et al., 2012) work relating to maize 1507. For these reasons, Perry et al. (2013) demonstrated that there was no evidence in the Holst et al. (2013a,b) publications that indicated there is any requirement to change previous risk assessments for non-target Lepidoptera made using the Perry et al. (2010, 2012) models.

EFSA conclusion

Neither the results reported by Hofmann et al. (2011), Lang et al. (2011), Camastra et al. (2013) and Holst et al. (2013a), nor the arguments put forward by Then and Bauer-Panskus (2013) reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

2.7. EFSA assessment of the Testbiotech Heading 2.3 "*Risks of spraying with glufosinate*" as referred to in Then and Bauer-Panskus (2013)

Cited scientific publications

The scientific publications cited in the Then and Bauer-Panskus (2013) report with relevance to maize 1507 were previously considered by EFSA and/or its GMO Panel (see Appendix A), and therefore are not considered further here.

Points raised

In EFSA (2011), it is stated that 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. However, maize 1507 will not be marketed in the European Union (EU) as a herbicide tolerant crop. 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 were not considered by

the EFSA GMO Panel; the GMO Panel had no mandate to consider the potential effects of such uses.

EFSA conclusion

The arguments put forward by Then and Bauer-Panskus (2013) do not reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel.

3. Overall conclusion

Neither the scientific publications cited in the Then and Bauer-Panskus (2013, 2014) reports with relevance to maize 1507, nor the arguments put forward by Then and Bauer-Panskus reveal any new information that would invalidate the previous risk assessment conclusions and risk management recommendations made by the EFSA GMO Panel. Therefore, EFSA considers that the previous GMO Panel risk assessment conclusions and risk management recommendations on maize 1507 remain valid and applicable.

4. References

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Long S, Ostrem J, Kang Q, 2011b. Laboratory characterization of painted lady butterfly (*Vanessa cardui*) larval response to DASØ15Ø71xMONØØ6Ø36 maize pollen in artificial diet. Pioneer Hi-Bred International.

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APPENDICES

European Food Safety Authority

DEPARTMENT OF SCIENTIFIC EVALUATION OF REGULATED PRODUCTS

A. PUBLICATIONS , EXCLUDING EFSA AND EC OUTPUTS, LISTED IN THEN AND BAUER-PANSKUS (2013)

Authors of publication	Title of publication	Publication year	Issue in the remit of the EFSA GMO Panel and relevant to this EC mandate	Publication previously discussed and cited in relevant maize 1507-related applications and/or outputs of EFSA or its GMO Panel
Adamczyk JJ, Perera O, Meredith WR	Production of mRNA from the *cry1Ac* transgene differs among Bollgard lines which correlates to the level of subsequent protein. Transgenic Research, 18:143-149	2008	No	-
Akhtar ZR, Tian JC, Chen Y, Fang Q, Hu C, Chen M, Peng YF, Ye GY	Impacts of six Bt rice lines on nontarget rice feeding thrips under laboratory and field conditions. Environmental Entomology, 39: 715-726	2010	No	-
Bauer-Panskus A, Then C	Testbiotech opinion concerning the application for market approval of genetically modified maize 1507 (DASØ15Ø71)	2010	Yes	Yes[4]

[4] See minutes of the 61st plenary meeting of the Scientific Panel on Genetically Modified Organisms held on 20-21 October 2010 at http://www.efsa.europa.eu/en/events/event/gmo101020.htm

Bondzio A, Lodemann U, Weise C Einspanier R	Cry1Ab treatment has no effects on viability of cultured porcine intestinal cells, but triggers Hsp70 expression. PloS ONE, 8: e67079	2013	Yes	No
Bravo A, Soberón M	How to cope with insect resistance to Bt toxins? Trends in Biotechnology, 26: 573-579	2008	Yes	Yes
Broderick NA, Raffa KF, Handelsman J	Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Sciences of the United States of America, 103: 15196-15199	2006	Yes	Yes
Broderick NA, Robinson CJ, McMahon MD, Holt J, Handelsman J, Raffa KF	Contributions of gut bacteria to *Bacillus thuringiensis* induced mortality vary across a range of Lepidoptera. BMC Biology, 7: 11	2009	Yes	Yes
Camastra F, Ciaramella A, Staiano A	A note on some mathematical models on the effects of *Bt*-maize exposure. Environmental and Ecological Statistics, 19, DOI:10.1007/s10651-013-0264-1	2013	Yes	No
Crickmore N	Using worms to better understand how *Bacillus thuringiensis* kills insects. Trends in Microbiology, 13: 347-350	2005	Yes	Yes
Dona A, Arvanitoyannis IS	Health risks of genetically modified foods. Critical Reviews in Food Science and Nutrition, 49: 164-175	2009	Yes	No
Dolezel M, Miklau M, Hilbeck A, Otto M, Eckerstorfer M, Heissenberger A, Tappeser B, Gaugitsch H	Scrutinizing the current practice of the environmental risk assessment of GM maize applications for cultivation in the EU. Environmental Sciences Europe, 23:33	2011	Yes	No

Duan JJ, Marvier M, Huesing J, Dively G, Huang ZY	A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PloS ONE, 3: e1415	2008	Yes	Yes
Gathamm A, Wirooks L, Hothhorn LA, Bartsch D, Schuphan I	Impact of Bt-maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. Molecular Ecology, 15: 2677-2685	2006	Yes	Yes
Hanley AV, Huang ZY, Pett WL	Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. Journal of Apicultural Research, 42:77-81	2003	Yes	Yes
Hilbeck A, Schmidt JEU	Another view on Bt proteins – How specific are they and what else might they do? Biopesticides International, 2: 1-50	2006	Yes	Yes
Holst N, Lang A, Lövei G	Increased mortality is predicted of *Inachis io* larvae caused by Btmaize pollen in European farmland. Ecological Modelling, 250: 126-133	2013	Yes	No
Hofmann F, Epp R, Kruse L, Kalchschmied A, Maisch B, Müller E, Kuhn U, Kratz W, Ober S, Radtke J, Schlechtriemen U, Schmidt G, Schröder W, van den Ohe W, Vögel R, Wedl N, Wosniok W	Monitoring of Bt maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008. Umweltwissenschaften und SchadstoffForschung, 22: 229-251	2010	Yes	Yes

Hofmann F, Otto M, Kuhn U, Ober S, Schlechtriemen U, Vögel R	A new method for *in situ* measurement of Bt-maize pollen deposition on host-plant leaves. Insects, 2: 12-21	2011	Yes	No
Hua G, Masson L, JuratFuentes JL, Schwab G, Adang MJ	Binding analyses of *Bacillus thuringiensis* Cry endotoxins using brush border membrane vesicles of *Ostrinia nubilalis*. Applied and Environmental Microbiology, 67: 872-879	2001	Yes	No
Huffmann DL, Abrami L, Sasik R, Corbeil J, van der Goot G, Aroian RV	Mitogenactivated protein kinase pathways defend against bacterial poreforming toxins. Proceedings of the National Academy of Sciences of the United States of America, 101: 10995-11000	2004	Yes	No
Ito A, Sasaguri Y, Kitada S, Kusaka Y, Kuwano K, Masutomi K, Mizuki E, Akao T, Ohba M	*Bacillus thuringiensis* crystal protein with selective cytocidal action on human cells. Journal of Biological Chemistry, 279: 21282-21286	2004	No	-
Janz N	The relationship between habitat selection and preference for adult and larval food resources in the polyphagous butterfly *Vanessa cardui* (Lepidoptera: Nymphalidae). Insect Behaviour, 18: 767-780	2005	Yes	Yes
Kramarz PE, Vaufleury A, Zygmunt PMS, Verdun C	Increased response to cadmium and *Bacillus thuringiensis* maize toxicity in the snail *Helix aspersa* infected by the nematode *Phasmarhabditis hermaphrodita*. Environmental Toxicology and Chemistry, 26: 73-79	2007	Yes	Yes

Kramarz P, de Vaufleury A, Gimbert F, Cortet J, Tabone E, Andersen M, Krogh P	Effects of *Bt*-maize material on the life cycle of the land snail *Cantareus aspersus*. Applied Soil Ecology, 42: 236-242	2009	Yes	Yes
Lang A, Brunzel S, Dolek M, Otto M, Theißen B	Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of *Bt*-maize effects. Proceedings of the Royal Society B: Biological Sciences, 278: 980-981	2011	Yes	No
Long S, Ostrem J, Kang Q	Laboratory characterization of painted lady butterfly (*Vanessa cardui*) larval response to DASØ15Ø71 maize pollen in artificial diet. Pioneer Hi-Bred International	2011	Yes	No
Long S, Ostrem J, Kang Q	Laboratory characterization of painted lady butterfly (*Vanessa cardui*) larval response to DASØ15Ø71xMONØØ6Ø36 maize pollen in artificial diet. Pioneer Hi-Bred International	2011	Yes	No
Lövei GL, Andow DA, Arpaia S	Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environmental Entomology, 38: 293-306.	2009	Yes	Yes
Matthews D, Jones H, Gans P, Coates S, Smith LMJ	Toxic secondary metabolite production in genetically modified potatoes in response to stress. Journal of Agricultural and Food Chemistry, 53: 7766-7776	2005	No	-
Mesnage R, Clair E, Gress S, Then C, Székács A, Séralini GE	Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology, 33: 695-699	2012	Yes	No
Nguyen HT, Jehle JA	Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810: Journal of Plant Diseases and Protection, 114: 82-87	2007	Yes	Yes

Obrist LB, Dutton A, Albajes R, Bigler F	Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecological Entomology, 31: 143-154	2006	Yes	Yes
OECD	Consensus Document on the safety information on transgenic plants expressing *Bacillus thuringiensis*-derived insect control proteins/ Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2007)14), No 42:1-10	2007	Yes	Yes
Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB	A mathematical model of exposure of non-target Lepidoptera to *Bt*-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society B: Biological Sciences, 277: 1417-1425	2010	Yes	Yes
Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Leureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB	The usefulness of a mathematical model of exposure for environmental risk assessment. Proceedings of the Royal Society B: Biological Sciences, 278: 982-984	2011	Yes	Yes

Perry JN, Devos Y, Arpaia S, Bartsch D, Ehlert C, Gathmann A, Hails RS, Hendriksen NB, Kiss J, Messéan A, Mestdagh S, Neemann G, Nuti M, Sweet JB, Tebbe CC	Estimating the effects of Cry1F *Bt*-maize pollen on non-target Lepidoptera using a mathematical model of exposure. Journal of Applied Ecology, 49: 29-37	2012	Yes	Yes
Peterson JA, Obrycki JJ, Harwood JD	Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events. Biocontrol Science & Technology, 19: 613-625.	2009	Yes	Yes
Pigott CR, Ellar DJ	Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiology & Molecular Biology Reviews, 71: 255-281	2007	Yes	Yes[5]
Raymond B, Johnston PR, Wright DJ, Ellis RJ, Crickmore N, Bonsall MB	A mid-gut microbiota is not required for the pathogenicity of *Bacillus thuringiensis* to diamondback moth larvae. Environmental Microbiology, 11: 2556-2563	2009	Yes	Yes
Saeglitz C, Bartsch D, Eber A, Gathmann A, Priesnitz KU, Schuphan I	Monitoring the Cry1Ab susceptibility of European corn borer in Germany. Journal of Economic Entomology, 99: 1768-1773	2006	Yes	Yes
Sanahuja G, Banakar R, Twyman RM, Capelle T, Christou P	*Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnology Journal, 9: 283-300	2011	Yes	Yes

[5] See http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?unit=GMO (with Question Number EFSA-Q-2011-01067)

Saxena D, Flores S, Stotzky G	Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology & Biochemistry, 34: 133-137	2002	Yes	Yes
Shimada N, Kim YS, Miyamoto K, Yoshioka M, Murata H	Effects of *Bacillus thuringiensis* Cry1Ab toxin on mammalian cells. Journal of Veterinary Medical Science, 65: 187-191	2003	Yes	No
Soberón M, Gill SS, Bravo A	Signaling versus punching hole: how do *Bacillus thuringiensis* toxins kill insect midgut cells? Cellular and Molecular Life Science, 66: 1337-1359	2009	Yes	Yes
Székács A, Weiss G, Quist D, Takács E, Darvas B, Meier M, Swain T, Hilbeck A	Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay. Food and Agricultural Immunology, 23: 99-121	2011	Yes	Yes
Tan SY, Cayabyab BF, Alcantara EP, Huang F, He K, Nickerson KW, Siegfried BD	Comparative binding of Cry1Ab and Cry1F *Bacillus thuringiensis* toxins to brush border membrane proteins from *Ostrinia nubilalis*,*Ostrinia furnacalis* and *Diatraea saccharalis* (Lepidoptera: Crambidae) midgut tissue. Journal of Invertebrate Pathology, 114: 234-240	2013	Yes	No
Then C, Lorch A	A simple question in a complex environment: How much Bt toxin do genetically engineered MON 810 maize plants actually produce? In: Breckling B, Reuter H and Verhoeven R (eds), 2008, Implications of GM Crop Cultivation at Large Spatial Scales, Theorie in der Ökologie 14. Frankfurt, Peter Lang	2008	Yes	Yes
Then C	Risk assessment of toxins derived from *Bacillus thuringiensis* synergism, efficacy, and selectivity. Environmental Science and Pollution Research, 17: 791-797	2009	Yes	Yes

Thomas WE, Ellar DJ	*Bacillus thuringiensis* var *israelensis* crystal delta-endotoxin: effects on insect and mammalian cells *in vitro* and *in vivo*. Journal of Cell Science, 60: 181-197	1983	Yes	No
van Frankenhuyzen K	Insecticidal activity of *Bacillus thuringiensis* crystal proteins. Journal of Invertebrate Pathology, 101: 1-16	2009	Yes	Yes
van Frankenhuyzen K, Liua Y, Tonona A	Interactions between *Bacillus thuringiensis* subsp. *kurstaki* HD-1 and midgut bacteria in larvae of gypsy moth and spruce budworm. Journal of Invertebrate Pathology, 103: 124-131	2010	Yes	Yes
Wraight CL, Zangerl AR, Carroll MJ, Berenbaum MR	Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proceedings of the National Academy of Sciences, 97: 7700-7703	2000	Yes	Yes
Zeilinger AR, Andow DA, Zwahlen C, Stotzky G	Earthworm populations in a northern US cornbelt soil are not affected by long-term cultivation of Bt maize expressing Cry1Ab and Cry3Bb1 proteins. Soil Biology & Biochemistry, 42: 1284-1292	2010	Yes	Yes
Zhang X, Candas M, Griko NB, RoseYoung L, Bulla LA	A mechanism of cell death involving an adenylyl cyclase/PKA signaling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. Proceedings of the National Academy of Sciences of the United States of America, 103: 9897-9902	2006	Yes	No

Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, Yin Y, Wang C, Zhang T, Zhu D, Zhang D, Xu J, Chen Q, Ba Y, Liu J, Wang Q, Chen J, Wang J, Wang M, Zhang Q, Zhang J, Zen K, Zhang CY	Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of crosskingdom regulation by microRNA. Cell Research, 22: 107-126	2011	Yes	Yes[6]
Zurbrügg C, Nentwig W	Ingestion and excretion of two transgenic Bt corn varieties by slugs. Transgenic Research, 18 (2): 21525.	2009	Yes	Yes

[6] See http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?unit=GMO (with Question Number EFSA-Q-2012-00283)

B. PUBLICATIONS , EXCLUDING EFSA AND EC OUTPUTS, LISTED IN THEN AND BAUER-PANSKUS (2014)

Authors of publication	Title of publication	Publication year	Issue in the remit of the EFSA GMO Panel and relevant to this EC mandate	Publication previously discussed and cited in relevant maize 1507-related applications and/or outputs of EFSA or its GMO Panel
Pavely C	Quantitative ELISA analysis of Cry1F and PAT protein expression levels, and composition of the hybrid maize line containing event TC1507. Part of application EFSA-GMO-NL-2004-02 by Pioneer Overseas Corporation	2002	Yes	Yes[7]
Phillips AM	Cry34Ab1, Cry35Ab1, Cry1F and PAT protein levels in hybrid maize TC1507, DAS-59122-7, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a conventional control from the Monsanto 2006 Production Plan 06-01-52-04. Dow AgroSciences LLC	2008	Yes	Yes[8]
Pioneer Hi-Bred International	Quantitative ELISA analysis of poCry1F and PAT protein expression levels in hybrid and inbred lines of TC1507 and the inbred line of TC1360. Part of application A446 submitted to Food Standards Australia New Zealand (FSANZ)	2001	Yes	No

[7] GM plant market registration application with reference EFSA-GMO-NL-2004-02 / Annex 7D (import)
[8] GM plant market registration applications with reference EFSA-GMO-CZ-2008-62 and EFSA-GMO-NL-2009-65

Stauffer C	Quantitative ELISA analysis of poCry1F and PAT protein expression levels, composition and efficacy of hybrid lines 1360 and 1507 – EU field sites. Part of application EFSA-GMO-NL-2004-02 by Pioneer Overseas Corporation	2000	Yes	Yes[9]
Stauffer C, Rivas J	Quantitative ELISA analysis of Cry1F and PAT expression levels in and compositional analysis of maize inbred and hybrid lines 1362 and 1507. Part of application EFSA-GMO-NL-2004-02 by Pioneer Overseas Corporation	1999	Yes	Yes[10]
Székács A, Weiss G, Quist D, Takács E, Darvas B, Meier M, Swain T, Hilbeck A	Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay. Food and Agricultural Immunology, 23: 99-121	2011	Yes	Yes
Then C, Bauer-Panskus A	High-level-risk-maize 1507: Shortcomings at the European Food Safety Authority (EFSA) and in EU Commission decision making should prompt reassessment of genetically engineered maize 1507. Testbiotech Background Report, 11-12 – 2013	2013	Yes	No
US EPA	Biopesticides Registration Action Document - *Bacillus thuringiensis* plant-incorporated protectants	2001	Yes	Yes
US EPA	Biopesticides Registration Action Document - *Bacillus thuringiensis* Cry1F corn	2005	Yes	Yes
US EPA	Biopesticides Registration Action Document - Cry1Ab and Cry1F Bt plant-incorporated protectants	2010	Yes	No

[9] GM plant market registration application with reference EFSA-GMO-NL-2004-02 / Annex 4 (import)
[10] GM plant market registration application with reference EFSA-GMO-NL-2004-02 / Annex 2 (import)