Appendix 5.2. MON 810 Literature Review – Environment

MON 810 literature review (July 2012)

Appendix 5.2 - Environment

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Review of peer-reviewed publications

Area of the environmental risk assessment: Environmental Safety - Non-Target Organism Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Hendriksma et al., 2011b)	***Objective:*** To evaluate the effects of pollen from MON 810 maize cultivars on the survival and prepupae weight of honeybee (*Apis mellifera*) larvae using an *in vitro* rearing bioassay. ***Experimental Design:*** Pollen of MON 810 maize was grown on an experimental field in a randomized block-design with eight replications. As control, pollen from non-transgenic (near-isogenic, distant related or unrelated) varieties and from the perennial plant *Heliconia rostrata* (false bird of paradise; positive toxicity control) was included. The rearing of larvae upon hatching under laboratory conditions was performed following the protocols by Aupinel *et al.*[1] and Hendriksma *et al.*[2] Three day old larvae were fed the realistic exposure dose of 2 mg pollen in semi-artificial diet. The survival of larvae was noted daily during the 120 h of dietary exposure until the pre-pupal stage where larvae terminate feeding and growing. Potential sub-lethal effects were monitored by weighing pre-pupae after defecation. The data were analysed with mixed models using different packages of the open source statistic software R version 2.11.1. ***Results:*** All larvae fed with MON 810 maize pollen survived the 120 h of dietary exposure of the pre-pupae phase. The survival rate of larvae fed conventional maize pollen did not differ significantly from that of larvae exposed to MON 810 maize pollen. Mean pre-pupae weights of MON 810 maize pollen-fed larvae were almost identical to those of conventional maize pollen-fed larvae. In contrast, significantly fewer larvae survived the larval phase and lower mean pre-pupae weight was observed when they were fed with *H. rostrata* pollen compared to all the other treatments.	MON 810 maize pollen showed no adverse effect on survival and pre-pupal weight of honeybee (*A. mellifera*) larvae. The authors also suggested that "*feeding GM pollen on in vitro reared honey bee larvae is well suited of becoming a standard bioassay in regulatory risk assessments schemes of GM crops.*"	Environment	No adverse effects were determined in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] Aupinel P., Fortini D., Michaud B., Marolleau F., Tasei J.N. *et al.* (2007). Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new *in vitro* standardized feeding method. Pest Manag. Sci. 63:1090–1094;

[2] Hendriksma H.P., Hartel S., Steffan-Dewenter I. (2011a). Honey bee risk assessment: New approaches for in vitro larvae rearing and data analyses. Methods Ecol. Evol. 2:509–517.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Shu *et al*., 2011)	***Objective:*** To evaluate the growth rate and reproduction of earthworm (*Eisenia fetida*) when bred in soil amended with insect resistant *Bacillus thuringiensis* (*Bt*) maize varieties expressing Cry1Ab protein. ***Experimental Design:*** *Bt* maize events MON 810 (variety 5422CBCL) and Bt11 (variety 5422Bt1) expressing Cry1Ab protein, and the near-isogenic non *Bt* maize variety 5422, were cultivated in the greenhouse. Stover including leaves and stalks of maize was cut into small pieces, freeze-dried, ground and sieved. Soil collected from a conventional sweet maize field (South China Agricultural University, China) was air-dried and sieved to remove stones and plant debris. Powdered stover of each maize variety was mixed with 200 g of soil at 2.5, 5 and 7.5% in plastic cups. The water content of the soil−stover mixture was kept at 30% of the water holding capacity. A single approximately 2 month old earthworm with clitellum was transferred to each cup. Thirty replicates per treatment were used and the number of surviving earthworms was recorded on Days 7, 14 and 30. For evaluation of reproductive effects, one pair of earthworms was transferred to a plastic cup (30 replicates). The number of offspring and cocoons was counted, and new offspring were removed from each cup at the interval of 30 days during the 60 days of this experiment. The chemical composition of the maize stover was investigated and Cry1Ab protein concentrations in the mixture of soil and stover, casts and guts of *E. fetida* were analysed by ELISA. Statistical analysis was performed using SPSS software. ***Results:*** More than 90% of *E. fetida* survived over a period of 30 day, irrespective of whether they received *Bt* or non *Bt* maize. There was a significantly higher relative growth rate and more offspring and	No significant differences were observed in survival rate of *E. fetida* between MON 810 and non *Bt* maize treatments. To some extent, *E. fetida* from MON 810 had greater growth and reproduction. The authors suggest these results were unlikely to be directly caused by Cry1Ab protein released from MON 810 but rather by differences in other factors of plants such as plant components (soluble sugar, total organic carbon, total protein and available phosphorus of MON 810 was higher than non *Bt* corn. Of these components however, significant differences were found only in the total protein). The authors state that "Although the presence of Cry1Ab protein in *E. fetida* had no deleterious effects on the survival rate, growth and reproduction, and even a promotion appeared in the MON 810 treatment, the transfer of Cry1Ab protein from	Environment	The authors speculate there is a potential risk for earthworms bred in soils containing Cry proteins from Bt maize varieties. However, these speculations do not constitute an adverse effect [1].
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] The authors used standard test methods for evaluation of the potential effects of transgenic maize on a standard non-target model organism. Their results show no adverse effects to the survival, growth, and reproduction of *E. fetida* with exposure to Cry1Ab protein. The results further reveal decreasing levels of Cry1Ab protein in the soil and stover mixture (substance) throughout the in-life phase of the study, as well as the diminishing Cry1Ab protein levels as the substances pass through the earthworm gut and form castings. Thus the authors state, "No matter which Bt corn stover concentration or which experimental time, Cry1Ab concentrations in substances were always higher than that of casts and guts of *E. fetida"*. Therefore, along with no adverse effects to *E. fetida* from dietary exposure to Cry1Ab protein, this study also illustrates the degradation of Cry1Ab protein.

Additionally, though Cry1Ab is detected in castings and guts of *E. fetida*, there is no indication that the protein is biologically active. Hence, any potential risk from exposure to the Cry1Ab in castings or guts is speculation. Any speculation of risk should reside in context of the results of this study which demonstrate that survival, growth and reproduction in *E. fetida* are not adversely effected in the presence and consumption of Cry1Ab.

	cocoons of *E. fetida* in the MON 810 groups. The Cry1Ab protein was detectable in the mixture of soil and stover, casts and guts of *E. fetida* from the MON 810 treatment, of which the highest levels were in the mixture of soil and stover. With the increase of treatment time, a strong decline was observed in Cry1Ab protein from the mixture of soil and stover and casts of *E. fetida*, whereas Cry1Ab in guts increased between 14 and 30 days of MON 810 treatments.	MON 810 to *E. fetida* and the persistence of Cry1Ab in guts and casts could not be ignored, which may constitute a potential hazard to *E. fetida."*		

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Tan *et al*., 2011)	***Objective:*** To evaluate the influence of MON 810 and Bt11 maize on arbuscular mycorrhizal fungi (AMF) *Glomus* communities in maize roots and rhizosphere soils. ***Experimental Design:*** Two Bt maize (MON 810 and Bt11) and their non Bt isolines (5422 and 5422wx) were used in this greenhouse experiment, conducted as a randomized complete block design with three replications. Samples of maize roots and soil adhering to the roots from each block were randomly collected at 36, 50, 64 and 78 days after sowing. AMF colonization in roots was assessed microscopically with the gridline-intersection method followed by one-way ANOVA. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) in combination with sequencing of amplified partial 18S ribosomal DNA was used to detect AMF *Glomus*-specific fragments in root and rhizosphere soil samples. DGGE profiles were classified using Two-Way Indicator Species Analysis. De-trended correspondence analysis (DCA) was used to check the delimitation defined by TWINSPAN and to study interrelationships. ***Results:*** There was no consistent significant difference in AMF colonization between the Bt maize lines (MON 810, Bt 11) and their conventional counterparts over the entire duration of the experiment. DGGE analysis showed differences between Bt and non Bt maize isolines (MON 810 vs. 5422wx and Bt11 vs. 5422wx). Further, differences were also observed between the two non Bt maize cultivars (5422 vs. 5422wx) and between the two Bt maize lines (MON 810 *vs.* Bt 11) in roots.	MON 810 and Bt11 maize showed no adverse effect on the indigenous AMF colonization of roots. However, DGGE analysis showed differences between GM and non-GM maize isolines as well as between the two non-GM maize cultivars and between MON 810 and Bt11. This suggests that Bt toxin is not a unique factor that bears upon the maize genotypes. The maize genotypes may have a greater influence on the AMF community structure of plant roots and rhizosphere soils than other factors, such as the age of the growing plants. The authors conclude that "*Further studies using more sensitive experimental methods are necessary to evaluate the long term influence of Bt maize on the soil microbial communities*."	Environment	No adverse effects were determined in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Environmental Safety – Insect Resistance Management Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Razze *et al*., 2011)	***Objective:*** To explore the feeding behaviour of neonate *Ostrinia nubilalis* (Lepidoptera: crambidae) on MON 810 maize and its potential impact on the development of resistance. ***Experimental Design:*** *O. nubilalis* were collected from maize fields in Iowa. MON 810 maize and near isoline hybrid non-GM plants were grown in pods until the six-leaf stage. One *O. nubilalis* egg mass was placed on the underside of the fifth leaf of each plant with a drop of water. Larvae were exposed to the plants for 6, 12, 24 or 48 h in ten replicates, each consisting of a block with four time treatments and a MON 810 or non-GM plant randomly assigned to each time interval. At the end of each time point, larvae were removed and categorized based on whether they were found on the leaf, in the whorl or on the covering bag. The larvae were mounted onto microscope slides and examined to determine if there was plant material in the gut, which served as evidence they had fed. Material identified included chlorenchyma and tracheary elements. A proportional rating system was used to estimate the amount of plant tissue present in the gut of each larva. Data analysis was performed by analysis of variance. ***Results:*** The mean number of larvae on the covering bag compared with larvae on the leaf was significantly different by treatment and over time. There was a higher percentage of larvae on the leaf that had fed on non-GM maize (ca. 27%) compared with MON 810 maize (ca. 8%) and the percentage of larvae that had fed increased over time. Further, about 50% of the larvae initially left the plant before there was evidence in the gut of feeding regardless of whether the source was MON 810 or non-GM maize. A higher quantity of plant material was found in the gut of larvae recovered from leaves of non-GM compared to MON 810 maize. After 48 h, amongst the larvae that had left the plant, a greater proportion from MON 810 maize had plant material in the gut compared to non-GM maize.	The behavior of *O. nubilalis* neonates on the plant corresponded with the differences in feeding on the two maize lines. Further research is needed to evaluate these unknown differences in behavior and determine the efficacy of a blended refuge strategy, as well as the associated risk of resistance development in *O. nubilalis* populations. The findings of the present study could be helpful to understand how *O. nubilalis* larvae detect MON 810 maize and how detection affects dispersal behavior over time. Further, these findings may not only be useful for *O. nubilalis* resistance management but also applicable to other species that behave similarly under MON 810 selection pressure.	Environment	No adverse effects were detected in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Pérez-Hedo *et al*., 2011)	***Objective:*** To determine the effect of ingestion of sublethal amounts of Cry1Ab from MON 810 maize on the development and hormonal balance of larvae from the insect pest *Sesamia nonagrioides* (Lepidoptera: noctuidae). ***Experimental Design:*** Larvae of *S. nonagrioides* were reared on a semi-artificial diet. Half were held in a short day (SD) photoperiod, 12:12 (L:D) h inducing diapause and the other half in a long day (LD) photoperiod, 16:8 (L:D) h for continuous development and pupation. Larvae were fed MON 810 maize leaves or sublethal amounts (0, 0.35, 0.9 and 2 mg/kg diet) of Cry1Ab protein. The near-isogenic maize variety PR33P66 (non GM) was used as control. The hemolymph of six larvae of each experimental group was collected for hormonal analysis. The rest of the larvae were observed periodically and mortality, number and duration of the next instars were recorded. Juvenile hormone (JH) was analyzed with UPLC while the level of ecdysteroids (such as 20-hydroxyecdysone) were quantified by competitive ELISA. Two or three-way analysis of variance tests, with a factorial design, were performed using the SAS package. The Chi-square test was used to compare mortality. ***Results:*** Larvae fed MON 810 maize leaves moulted more times than those fed with non-GM maize. Feeding with MON 810 maize leaves was associated with a higher concentration of JH in the hemolymph of larvae developed under the LD photoperiod, but this was not detected under SD or diapausing conditions. In larvae fed non-GM maize, those subjected to LD photoperiod conditions showed a significantly higher concentration of ecdysteroids in the hemolymph than those subjected to SD photoperiod but in the larvae fed with MON 810 maize, this photoperiod effect was not found. LD larvae fed with MON 810 maize leaves did not undergo the increases in moulting hormone necessary for pupation and therefore prolonged their development, reinforcing the above results. Further, all larvae fed with different concentrations of Cry1Ab protein showed higher JH concentrations compared to controls.	The larvae that survived after feeding with MON 810 maize leaves or the higher amount of Cry1Ab protein have higher levels of JH, whereas their level of ecdysteroids did not increase sufficiently to allow pupation, leading to a longer larval development and more larval moults. This response may be considered a defense mechanism that allows some larvae to survive toxin ingestion. Changes in the hormone levels in diapausing larvae were undetectable, probably because these changes were masked by the higher level of JH in the hemolymph of diapausing larvae and because of lack of ecdysteroid titer increase, a phenomenon that is usually observed a few days before pupation in nondiapausing larvae. Authors concluded that *"these results should be taken into account in the establishment of non-Bt refuges to prevent development of Bt-resistance in S. nonagrioides populations."*	Environment	This article shows that the previously published reports documenting a delay in *Sesamia* development, when exposed to Cry1Ab, is due to alterations in JH, and therefore does not report any new adverse effect[1].
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect development	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] Cry1Ab (and Cry1Ac) are known to delay development in various insects including *Sesamia* spp, and this current article shows that this delay in development is due to an increase in Juvenile Hormone (JH). Delayed development (regardless of the cause) could therefore potentially increase Bt resistance risk due to non-random mating and recovery from intoxication leading to viable adults (although not documented for *Sesamia*). However, MON 810 maize provides near complete control of *Sesamia* and appropriate non-Bt refuges are already being promoted in Bt corn growing areas in the EU. Additionally, no change in product performance or Cry1Ab susceptibility has been noted over the period of commercialization, indicating that no significant adaptation in *Sesamia* physiology has

occurred in populations exposed to MON 810. Finally, it should be highlighted that there are some ecological factors that have not been accounted for or discussed in Perez-Hedo *et al.* (*e.g.* increased larval development time often results in increased predation or parasitism, overlapping of generations or populations allows for the continued mixing of populations, potential fitness costs associated with increased larval development).

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(George *et al.*, 2012)	***Objective:*** To investigate the effects of MON 810 maize on survival and development of the pest *Busseola fusca* and analyse binding of Cry1Ab protein to the midgut epithelial cells following ingestion. ***Experimental Design:*** Acute toxicity was assessed using artificial diet bioassays performed with 0 (control), 0.5 and 1.0% of purified Cry1Ab protein and *B. fusca* third-instar larvae (n = 20). Larvae were weighed before test start, then at 24 h intervals for 5 days. Mortality was recorded daily. To assess the effects on development and survival, leaf bioassays were carried out with excised leaves from 4 week old plants using both second (L2) and third instar (L3) larvae in two independent experiments conducted in triplicate. Larvae were weighed daily and the developmental stage and mortality were recorded daily for 34 days. As soon as the first mortality was recorded, five larvae from each treatment were fixed for subsequent electron microscopy (EM). The data was analysed with Student's t-test and Kaplan–Meier survival analysis. ***Results:*** Although no acute mortality was observed, 1% Cry1Ab protein reduced larval weight by 60% over the trial period, while larval weight in the control group increased by 25%. Insect survival, developmental rate, pupal and adult weight were significantly reduced ($p < 0.05$) with MON 810 compared to the control line. These differences were more pronounced with L2 than with L3 larvae. Leaf area consumed by MON 810-fed larvae was significantly lower compared to control-fed insects. EM studies revealed that consumption of MON 810 deleteriously affected the gut integrity of the pest.	The findings of the present study confirm that MON 810 maize is deleterious to the pest *B. fusca*, having a significant effect not only on the rate of larval development but also on mortality, with younger larvae being more susceptible. This study confirms the potential for Bt maize, when used as part of an integrated pest management programme, for control of *B. fusca*.	Environment	No adverse effects were detected in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kruger *et al*., 2011)	***Objective:*** To evaluate resistance to MON 810 maize in the moth pest *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts, South Africa. ***Experimental Design:*** One greenhouse and two laboratory studies were conducted in 2008 and 2009, respectively. *B. fusca* populations were collected from MON 810 maize fields and from adjacent refugia in the Vaalharts area, 50 km from the Christiana site where the first incidences of insect resistance have been reported. Control populations were collected from sites where MON 810 was not planted. In greenhouse studies, 720 potted plants were artificially infested with 10 neonate larvae of the F1 generation from field-collected populations reared through to adults. The experiment comprised eight treatments of four stem borer populations (i.e. VAA08Bt, VAA08Ref, CHR08Con and BET08Con) evaluated on MON 810 or non-GM hybrids. In the laboratory, larvae collected from MON 810 maize on six farms at Vaalharts were reared on MON 810 plants for one generation to confirm resistance. One population was also collected from the non-GM maize refugia and the larvae were reared in glass test tubes containing a piece of maize stem with the base of the whorl still intact. The number and mass of live larvae per plant were determined at regular intervals up to 35 day in each experiment. Lethal time (LT50) was calculated. Data analysis was done using analysis of variance. ***Results:*** Larvae of the Christiana (CHR08Con) and Bethal conventional population (BET08Con) did not survive on MON 810 maize for longer than 12 day. The populations collected from both MON 810 maize (VAA08Bt) and refugia (VAA08Ref) at Vaalharts were resistant and the subsequent generation of larvae completed their life cycle on MON 810. Similar results were observed in the laboratory experiments.	This study confirmed resistance of *B. fusca* to Cry1Ab toxin from MON 810 maize, indicating that the geographical distribution of resistant populations in South Africa includes the Vaalharts area. Resistance in larvae collected at Vaalharts showed that the efficacy of the refuge strategy is compromised because refugia did not produce large enough numbers of susceptible individuals to mate with moths of which larvae survived inside MON 810 fields. The authors concluded that "*Further research is needed on possible fitness costs associated with resistance evolution as well as insect resistance management and the high and dose refuge strategy to limit the evolution and spread of Bt-resistant populations to other maize production regions in the country.*"	Environment	This study confirmed *B. fusca* resistance to MON 810 maize in a new location in South Africa[2].
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect resistance	There are no changes to the conclusions of the safety of the initial risk assessment.

[2] However, the level appears to be low, with appreciable fitness costs such as reduced growth rate. This could be quite important as insects collected from the Christiana region showed no detectable resistance, suggesting that fitness costs or other measures have reduced resistance in this area. Additionally, there is no data on what has happened to resistance levels in 2009 - 2011. Furthermore, the authors do acknowledge that low initial levels of compliance to refuge requirements most likely played an important role in resistance evolution. Reporting of *Busseola fusca* resistance is not new and was already reported by van Rensburg in 2007 (van Rensburg JBJ, 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to *Bt*-transgenic maize. South African Journal of Plant and Soil, 24, 147-151.

Area of the environmental risk assessment: Environmental Safety - Protein / DNA Fate in Soil Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Gruber *et al*., 2011)	***Objective:*** To explore the fate of recombinant Cry1Ab protein in a field receiving liquid manure from dairy cows fed with MON 810 maize. ***Experimental Design:*** Two groups of 5 cows were fed a partial total mixed ration (PTMR) containing 63% MON 810 maize or its near-isogenic line. Liquid manure from the MON 810-fed or control group was applied to grassland and maize fields. A validated ELISA method was used for quantification of Cry1Ab in various samples collected along the agricultural chain, i.e. MON 810 plants, feed, liquid manure, soil and grass crops grown on manured fields. Student's t test was used to compare Cry1Ab mean concentrations in maize plants, PTMR and liquid manure of MON 810 and non-GM origin, considering p <0.05 as significant. ***Results:*** Cry1Ab concentrations in MON 810 maize were equivalent to 23.7 µg/g dry weight. Subsequently, there was a rapid decline, with 2.6 and 0.9% of the Cry1Ab from the MON 810 maize detected in feed and liquid manure, respectively. Half of this residual Cry1Ab persisted during slurry storage for 25 weeks. After application to experimental fields, Cry1Ab was degraded to below detectable levels in soil. Cry1Ab exhibited a higher rate of degradation compared to total protein in the agricultural processes. Immunoblotting revealed a degradation of the 65 kDa Cry1Ab protein into immunoreactive fragments of lower size in all analysed materials.	The authors concluded that "*The field trial shows the extensive and, compared to other proteins, rapid degradation of recombinant Cry1Ab protein during the course of liquid manure management of a dairy farm feeding GM maize (event MON 810), leading to non-detectable levels in soil and the following crop.*"	Environment	No adverse effects were detected in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Protein persistence	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Gruber *et al*., 2012)	***Objective:*** To analyse the amount of Cry1Ab protein in the agricultural soils from four different experimental field sites in a nine-year field trial with MON 810 maize. ***Experimental Design:*** Four experimental field sites in Southern Germany were cultivated with the Bt maize variety Kuratus (event MON 810) and its near isogenic line (Gavott) in four replicates for 9 continuous maize growing seasons (2000–2008). Each year, whole plants were harvested for grain maize according to Good Agricultural Practices. Chopped plant residues, maize stubble and root material remained on the field plots. Soil samples were collected from experimental plots at depths of 0–30 and 30–60 cm at ten sampling points in 2007, 2008 and 2009. Cry1Ab protein was quantified using an in-house validated ELISA method with a decision limit (CCα) of 2.0 ng Cry1Ab protein/g soil. Soil texture analysis was performed on aliquots of the eight replicate field site soil samples. Student's t-test was used to compare the means of Cry1Ab concentrations in the samples. ***Results:*** In general, high clay content was associated with low Cry1Ab protein recovery. Cry1Ab protein was not detected in any of the soil samples collected from the control plots. Cry1Ab protein was also not detected in the soil samples collected in the spring before the next farming season at any of the four experimental sites planted with MON 810 maize except for the one field site, with 2.91 and 2.57 ng Cry1Ab protein/g soil (in the top and lower soil samples, respectively) collected 6 weeks after the eighth growing season.	Findings of the present study indicate that there was no accumulation or persistence of Cry1Ab protein in different soils after nine-year continuous cultivation with MON 810 maize.	Environment	No adverse effects were determined in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Protein persistence	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Environmental Safety – Ecology Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Bell *et al*., 2012)	*Objective:* To analyse the population dynamics of *Ostrinia nubilalis* between the states of Wisconsin and Minnesota and evaluate if landscape-level manipulations can be used to restrict the cycle amplitude of this pest. *Experimental Design:* Long-term larval time series were analysed from Minnesota (1963–2009; site-years n = 282) and Wisconsin (1964–2009; site-years n = 276). Larval populations were sampled routinely during September and October from about 500 commercial maize fields per state. In Minnesota, larvae were assumed to be absent in insect resistant *Bacillus thuringiensis* (Bt) maize, so only non-GM fields were sampled from 1996 onward. All fields were randomly sampled in proportion to their presence on the landscape in Wisconsin. Generalized additive models (GAMs), Fisher's *g*-test and a wavelet multi-resolution analysis (MRA) were used to demonstrate the differences in population dynamics of *O. nubilalis* between the states. The effect of Bt maize was evaluated using a mechanistic model. *Results:* In Minnesota, the dominant peak in the periodogram was 6.57 years except for one site. Averaged over sites, the GAM showed a strong cycle of 5–7 years between peaks. The peaks were 1970, 1977, 1984, 1989 and 1995 and 2001. For Wisconsin, the dominant peak in the periodograms had a periodicity of 5.75 years for all except one site. When averaged over sites, the GAM showed that there was strong cycle of 5–7 years, although the 95% confidence intervals were wider and the amplitude post-Bt were more emphasised than in Minnesota. Although periodicity was evident in both states, the amplitude was lower in Wisconsin. In Minnesota there was an immediate damping effect after Bt introduction. However, in Wisconsin, the cycles continued to grow in amplitude until they damped in the second post-Bt oscillation. Regarding spatial synchrony, the autocorrelations decayed with distance. At 275 km, *O. nubilalis* was spatially random. Further, the pattern of peaks as resolved by spectral estimators indicated a degree of spatial dependence.	The introduction of Bt maize explained cycle damping when the adoption of the crop was high (Minnesota); oscillations were damped but continued to persist when Bt maize was used less intensely (Wisconsin). The host plant quality is the key to understanding both epidemic persistence and the success of intervention strategies. In particular, different maize management regimes (i.e. Bt or non-GM) within and between states is thought to limit the potential spatial autocorrelation of *O. nubilalis* either directly or indirectly via its regulators such as pathogens, parasitoids and predators etc.	Environment	No adverse effects were detected in this study[3].
			Observed parameter	**Feedback on initial environmental risk assessment**
			Population dynamics	There are no changes to the conclusions of the safety of the initial risk assessment.

[3] This paper does not show any adverse effects for Bt maize with activity against *Ostrinia nubilalis*. Note that this paper discusses Cry1Ab generically, but it is unclear if only Cry1Ab acreage is used for this study. The only effects discussed in this paper are that the high adoption of Bt maize changes epizootic periodicity due to *Nosema pyrausta*, a natural enemy of *O. nubilalis*. This effect should not be considered an adverse effect as it is widely known, and expected, that by using Bt maize, fewer of the target pests (e.g. *O. nubilalis*) will be available for predation or parasitism by specific natural enemies of *O. nubilalis.*

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