Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Az. 6790-02-0013 June 2011

Statement of the Central Commission on Biological Safety (ZKBS) on the latest scientific publications on the risk assessment of the maize line MON810

In July 2009 the ZKBS adopted a statement on the risk assessment of MON810 – New studies on the environmental impact of MON810 (Az. 6788-02-13). Here the ZKBS evaluates more recent scientific publications on the risk assessment of the maize line MON810. The publications are those of Álvares-Alfageme *et al.* (2010), Porcar *et al*. (2010) and Perry *et al.* (2010). In the present statement the ZKBS also considers insights drawn from scientific presentations made during a meeting ("Technical Discussion") on “The effects of Cry proteins on the two-spotted ladybird *Adalia bipunctata*” which was hosted by the German Federal Office of Consumer Protection and Food Safety (BVL) on 9 February 2011.

1 Summary

After evaluating the recent studies by Álvares-Alfageme *et al*. (2010) and Porcar *et al.* (2010) and other known data from field trials the ZKBS has come to the conclusion that the *Bt* proteins Cry1Ab and Cry3Bb1 are not expected to have potential adverse affects on ladybirds. So far, only the publication by Schmidt *et al.* (2009), which is based on a laboratory study, has reported adverse effects on two-spotted ladybirds. The critical evaluation of that publication by Meissle and Romeis (2008[1]), Rauschen (2010), Ricroch *et al*. (2010) and the most recent experimental results published by Álvares-Alfageme et *al*. (2010) and Porcar *et al*. (2010) cast considerable doubt on the relevance of the Schmidt et al. (2009) study for the risk assessment.

A further new study (Perry *et al.,* 2010) quantifies the risk to non-target butterflies with the help of a mathematical model. By extrapolating the dose-effect relationships determined in the laboratory for the Cry1Ab protein the authors estimate mortality rates for the larvae of three common species of butterfly (diamondback moth, peacock butterfly and red admiral butterfly) in maize fields and in the biotopes directly adjoining these fields. Their results show that the potential threat posed by the cultivation of MON810 to regional populations of non-target butterflies is only very limited. The calculated mortality rates are so low that at present they would not be technically detectable if mortality rates were monitored during cultivation.

2 Grounds

2.1 New studies on the potential impact of *Bt* proteins on the two-spotted ladybird *Adalia bipunctata*

The ZKBS had already issued a general statement on the risk assessment of new studies on

[1]The publication by Meissle and Romeis (2008) is based on an advance publication (online first) of the work by Schmidt *et al.* (2009) which appeared on the website of the journal Archives of Environmental Contamination and Toxicology on 20 August 2008.

the environmental impact of MON810 on 7 July 2009 (Az. 6788-02-13[2]), in which, among others, the potential effects of Bt proteins on the two-spotted ladybird were evaluated. The ZKBS determined that the study by Schmidt *et al*. (2009) cited at that time exhibited significant shortcomings in terms of the material used, the execution of the experiment and the interpretation of the results, fundamentally calling into question the accuracy of the findings and the statements. For a better understanding of the new studies by Álvares-Alfageme *et al*. (2010) and Porcar *et al*. (2010) under assessment, the study by Schmidt *et al*. 2009 will first be called up again, since the Technical Discussion at the BVL on 9 February 2011 has yielded additional findings.

2.1.1 Current status of the study by Schmidt *et al.* (2009)

In laboratory toxicity tests Schmidt *et al*. (2009) examined the effects of the Bt proteins Cry1Ab and Cry3Bb as well as the expression vector pBD10 on the two-spotted ladybird *Adalia bipunctata* (Coleoptera, Coccinellidae) at different stages of development (L1-L4). The Bt proteins were sprayed at concentrations of 0, 5, 25, and 50 µg/ml and the expression vector pBD10 at concentrations of 0, 10, 50 and 100 µg/ml on eggs of the flour moth (*Ephestia kuehniella*, Lepidoptera, Pyralidae), which constituted the sole food source of the test animals. The trial animals were exposed to the test substances for the entire period of larval development. A significant increase in mortality at different concentrations was detected for Cry1Ab and Cry3Bb compared to the control, whereas the application of the expression vector solution alone did not lead to any increase in mortality compared to the control. Negative effects of Cry proteins on the development time or the weight of the adults were not observed. The authors conclude that the increased mortality rates could be attributable to the direct effects of activated Bt proteins. In the authors' opinion the effect of Cry1Ab on the two-spotted ladybird observed in their experiment calls the postulated host specificity and/or the mode of action of Cry proteins into question, since this class of toxins acts specifically on Lepidoptera. In their interpretation of the environmental significance of their findings, Schmidt *et al*. (2009) come to the conclusion that larvae of the two-spotted ladybird would only be exposed to potentially harmful levels of Bt protein if they feed on Bt maize pollen (direct exposure) or prey (e.g. red spider mites *Tetranychus urticae*) that ingest Bt proteins while feeding on Bt maize (indirect exposure). Feeding on aphids does not represent an exposure pathway for ladybirds in this context since aphids do not ingest any Cry proteins with the phloem liquid of maize plants.

The findings of the study by Schmidt *et al.* (2009) have been criticised in a series of scientific publications (Meissle and Romeis, 2008; Rauschen, 2010; Ricroch *et al*., 2010). This criticism has centred on a) doubts about the use of a proper methodological approach and b) doubts about the results presented.

On a) – Methodological approach

- The authors fail to demonstrate that the Cry proteins applied to the eggs of the flour moth were active and that they were actually ingested by the ladybird larvae.
- High mortality rates are exhibited in the controls without toxin. For example, in the experiments with Cry3Bb the mortality rates for the controls do not differ significantly from the mortality rates found at the highest toxin concentration.

On b) – Results

- The authors fail to demonstrate a dose-effect relationship in their tests. There is a decrease in mortality rates at higher concentrations compared with medium concentrations. This is highly unusual for toxicity tests, a fact that the authors

[2]http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/05_plants/zkbs_plants_maize_MON810_2009.pdf?__blob=publicationFile&v=1

refer to but fail to explain in the discussion (compare here e.g. Tabashnik *et al*. 2002; Saeglitz *et al*. 2006a).

- Additional parameters such as development time for the individual larval stages and adult body weight, reveal no differences between the control animals and the animals exposed to the different Cry proteins.

In the view of the ZKBS the senior author (Dr. Angelika Hilbeck) failed to dispel the various doubts about her study during a long exchange in a Technical Discussion of "The effects of Cry proteins on the two-spotted ladybird *Adalia bipunctata*" which took place on 9 February 2011 at the BVL[3].

2.1.2 Assessment of new laboratory studies (Álvares-Alfageme *et al.* 2010; Porcar *et al.* 2010)

Álvares-Alfageme *et al*. (2010) took the study by Schmidt *et al*. (2009) as an opportunity to reproduce the effects of Cry proteins on ladybird larvae observed in the Schmidt *et al*. (2009) study under experimental conditions that on the one hand come closer to a natural exposure of the two-spotted ladybird (tritrophic approach with red spider mites as prey) and on the other hand ensure a quantifiable exposure of the ladybird larvae to high Bt protein doses (worst-case scenario). A further study by Porcar *et al*. (2010) also dealt with the potential toxic effects of Cry proteins on ladybirds in laboratory experiments.

2.1.2.1 Uptake of the Bt protein

In the study by Schmidt *et al.* (2009) insect eggs of the flour moth *E. kuehniella* were sprayed with a Bt protein solution. The larvae of the ladybird (*A. bipunctata*) were to ingest the Bt protein when feeding on the eggs.

Álvares-Alfageme *et al.* (2010) also fed *E. kuehniella* eggs to ladybird larvae of the species *A. bipunctata* and observed their feeding procedure under the microscope. The ladybird larvae were shown to bite open the eggs and to suck out the contents. Ingestion of the eggshells was not observed.

According to the observations of Álvares-Alfageme *et al.* (2010), any noteworthy uptake of the Bt proteins, as assumed in the experimental approach taken by Schmidt *et al.* (2009), is unlikely. Ingestion of the Bt proteins by the ladybird larvae was not checked in the trials conducted by Schmidt *et al.* (2009). Positive controls to determine whether the test organisms actually ingested the administered substances as well as testing for biological activity of the applied Cry proteins are likewise lacking in the study by Schmidt *et al*. (2009). In the studies by Álvares-Alfageme *et al*. (2010) and Porcar *et al*. (2010), by contrast, these important controls were carried out.

2.1.2.2 Toxicological studies (comparison of approaches)

Álvares-Alfageme *et al*. (2010) conducted two different types of experiments in which they tested the toxicity of the Bt proteins Cry1Ab and Cry3Bb to larvae of the ladybird *A. bipunctata*:

Experiment type 1: In a tritrophic experiment ladybird larvae in the first and second larval stages were fed for a period of 2 to 5 days with spider mites (*Tetranychus urticae*, Acari, Tetranychidae) which had been raised on either Cry1Ab- or Cry3Bb1-producing maize plants or

[3] During the Technical Discussion the senior authors gave presentations in which Dr. Angelika Hilbeck introduced the study by Schmidt *et al.* (2009) and Dr. Jörg Romeis introduced the study by Álvares-Alfageme *et al*. (2010). Afterwards both authors took part in a detailed discussion with the panel, which comprised representatives of the federal agencies and the ZKBS.

on non-genetically modified isolines. Bt levels in the leaves of the maize plants, the mites and the ladybird larvae were measured. In mites that had been raised on Bt maize plants up to 50% of the Bt content of the leaf material was detectable. Bt proteins were also detected in the ladybird larvae after ingestion of the mites that had been raised on Bt maize plants (<10% of the content present after feeding with leaf material). The Bt protein measurements demonstrated that the ladybird larvae were indeed exposed to the Bt protein during the trials and ingested the protein. It has already been established in biotests that the Bt proteins Cry1Ab and Cry3Bb do not lose their toxicity in red spider mites (Obrist *et al.* 2006; Meissle und Romeis 2009). Despite the fact that protein uptake was demonstrated no increased mortality rates or sub-lethal effects (development time, weight gain) could be detected in the ladybird larvae compared with the negative controls.

Experiment type 2: In direct feeding experiments the Bt protein was offered in a sucrose solution with a protein content that exceeded the protein content in the red spider mites in Experiment 1 by a factor of 10. No lethal or sub-lethal effects were observed in this experiment either.

In the study by Porcar *et al*. (2010) two types of experiments on the potential toxic effects of Cry1Ab and Cry3Bb on ladybird larvae and adults were performed.

Experiment type 1: Ladybird larvae of the species *A. bipunctata* were exposed to non-trypsinised Cry1Ab and Cry3Aa or trypsinised Cry1Ab at a concentration of 50µg/ml for 6 days. Biological activity of the Cry proteins was demonstrated in biotests with the European corn borer (*Ostrinia nubilalis*, Lepidoptera, Crambidae) and the Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera, Chrysomelidae). Water and the buffer solution served as negative controls, the insecticide ZZ Cooper (pyrethroid; piperonyl butoxide) as the positive control. The different substances were presented to the larvae continuously in an artificial diet. On the sixth day there was a sharp increase (>30%) in mortality in the negative control groups and the experiment was abandoned. In the groups with Cry proteins no significant increase in the mortality rates of the ladybird larvae were observed in comparison with the negative control groups. The strong increase in the mortality rate for the positive control group (insecticides) indicated that the experimental design was functional.

Experiment type 2: Adults of the Australian ladybird (*Cryptholaemus montrouzieri*, Coleoptera, Coccinelidae) and the rove beetle (*Atheta coriaria,* Coleoptera, Staphylinidae) were exposed for 15 days to non-trysinised Cry 1Ab and Cry3Aa or trysinised Cry1Ab at a concentration of 50 µg/ml (for Cry1Ab this corresponds to the highest concentration in the study by Schmidt *et al*. (2009)). Water, buffer solution and trypsin-treated buffer solution served as the negative controls, a 5% boric acid solution as a positive control. The substances were offered to the test organisms in an artificial diet (consisting of meat extract, yeast extract, sucrose, agarose gel, honey, vitamins and Nipagin).

Compared with the negative controls no increased mortality was detected in either of the two species in the experimental variants with Cry proteins after 15 days. The clearly observable effects on both species in the positive control confirm the functioning of the experimental design.

Given that the dose-effect relationship in the study by Schmidt *et al*. (2009) was unusual for toxicological tests (see above), it seems very likely that not any effect of Cry proteins is responsible for the increased mortality rates. The studies conducted by Álvares-Alfageme *et al*. (2010) and Porcar *et al.* (2010) reinforce this impression. Neither of the latter studies found significant effects of the proteins on mortality. Consistent with this, the development time and weight development of the trial animals were also unaffected.

The following experimental variations between the studies mentioned above may have led to different results:

a) Duration, continuity and/or reduced exposure of the ladybird larvae as a result of recovery times during the experiment

b) Level of exposure to Cry proteins

c) Origin of the Cry proteins used

On a) In the study by Schmidt *et al.* (2009) the ladybird larvae were exposed to the Cry proteins for the entire period of larval development (10 days) whereas in the experiments performed by Álvares-Alfageme *et al*. (2010) (Experiment 1) and Porcar *et al*. (2010) the larvae were exposed continuously to Cry proteins only for 6 days. However, the long exposure can not have led to the increased mortality in the study by Schmidt *et al*. (2009). In the Schmidt *et al*. (2009) study the largest effects by far were demonstrated in the first larval stage (= 2 to 3 days) (e.g. 24.2% mortality at 5 µg/ml Cry1Ab). In the remaining course of the experiments the mortality rate rose by only 3.2%. For this reason continuous exposure over the entire larval development time is not a determining factor for the observed effects. At 6 days, the initial relevant period of 2-3 days in the study by Schmidt *et al*. (2009) is more than sufficiently covered in the studies by Álvares-Alfageme *et al.* (2010) (Experiment type 1) and Porcar *et al.* (2010).

On b) The study by Schmidt *et al*. (2009) was designed to demonstrate a dose-effect relationship. In contrast, the studies by Álvares-Alfageme *et al*. (2010) and Porcar *et al*. (2010) applied either a natural exposure level of the ladybird larvae to Cry proteins or a level of exposure many times higher than would be expected under natural conditions (worst-case scenario). It is difficult to compare the actual amounts of toxin administered in the studies because exact exposure and uptake values are not given in the study by Schmidt *et al.* (2010). None of the studies by Álvares-Alfageme *et al*. (2010) and Porcar *et al*. (2010) showed rates of mortality that were comparably high to those found in the study by Schmidt *et al*. (2009). Contrary to Schmidt *et al*. (2009), no significant differences were found between the test series with Cry proteins and the negative control groups.

On c) Another difference between the experimental approaches is the application of different, or trypsinised (Schmidt *et al.* 2009) and non-trypsinised Cry3 proteins (Álvares-Alfageme *et al*. 2010; Porcar *et al.* 2010) (see above). At least for Cry3Aa and Cry3Bb, the application of different or differently pre-treated Cry proteins could explain the conflicting effects on ladybird larvae. Nevertheless, the findings of the Schmidt *et al*. 2009 study still contradict the findings from the tritrophic experiments conducted by Álvares-Alfageme *et al*. (2010) with Cry1Ab proteins, which showed no effects on ladybird larvae.

2.1.3 Assessment of additional laboratory and field studies on the impact of Cry proteins on ladybirds

With the help of additional published studies the conflict between results of Schmidt *et al*. (2009) vs. results of Álvares-Alfageme *et al*. (2010) and Porcar *et al*. (2010) can be judged in terms of the ecological relevance: Numerous laboratory and field studies have found no effect of the Bt proteins Cry1Ab and Cry3Bb on *Adalia bipunctata* (Wold *et al.* 2001) or other species of ladybird (Pilcher *et al.* 1997; Jasinski *et al.* 2003; Candolfi *et al.* 2004; Dively & Rose 2004; Bai *et al.* 2005; Lundgren & Wiedenmann 2005; Poza *et al.* 2005; Álvarez-Alfageme *et al.* 2008).

Another argument against a potential threat for ladybird larvae is the low level of exposure of ladybird larvae to Lepidoptera- and Coleoptera-specific Bt proteins under natural conditions. Ladybird larvae feed predominantly on aphids. It has been demonstrated that aphids living on Bt maize plants do not ingest Cry proteins (Head *et al.* 2001; Raps *et al.* 2001; Dutton *et al.*2002; Lundgren & Wiedenmann 2005). The possibility of exposure of ladybirds is only given through the intake of maize pollen. However, with regard to MON810 in particular, an effect on ladybirds is not expected due to the low level of Cry1Ab in pollen. In the same context it should be noted that the concentration of Cry1Ab in MON810 maize pollen given by Schmidt *et al*. (2009) is too high by a factor of 100 compared with the data from the AGBIOS database (AGBIOS 2011) to

which the authors refer. Independent of the experimental results, this contributes to a false perception of the risk of MON810 to non-target organisms.

2.2 New modelling study on exposure and the potential hazard to butterflies through the cultivation of MON810 maize

2.2.1 Perry *et al.* (2010) - original study

The cultivation of Bt maize varieties containing Lepidoptera-specific Cry proteins potentially endangers non-target butterfly species within the Bt maize crop and in the field margin. Given that to date no corresponding studies exist for the EU, Perry *et al*. (2010) applied a mathematical model to estimate the exposure and potential harmful effects of MON810 pollen on the larvae of three species of butterfly (diamondback moth, peacock butterfly, red admiral butterfly) within the maize crop and in directly neighbouring biotopes. With this work the authors[4] introduce an approach in which important influencing factors for quantifying the risk to common species of non-target butterflies are correlated in a relatively simple and transparent way. To do so the authors integrate various individual aspects, such as existing dose-effect studies from the laboratory, abundance and exposure studies from the field, as well as expert evaluations of local exposure parameters for 11 representative maize-growing regions in four EU member states into a comprehensive exposure and effect study.

The model has 11 parameters. For each species a maximum mortality rate (effect probability) within the maize crop and in the field margin was determined. This calculation includes assumptions for the respective species for a mortality-dose relationship (change in mortality in response to change in the concentration of the Cry1Ab protein) derived from laboratory dose-effect trials as well as data for the pollen concentration on the host plants. It is assumed that this is constant within the crop and is distance-dependent in the field margin. First, the maximum mortality rates within the crop and in the field margin are assumed to depend solely on the sensitivity of the larvae to the Cry1Ab protein and are calculated with respect to different exposure characteristics on the basis of maximum values. These rates are then adjusted in the model with respect to species-specific exposure characteristics and applied to the butterfly population of a cultivation region. The information necessary for the adjustment is based on statements and estimations provided by experts who are familiar with the regional particularities of the representative maize-growing areas.

Species-specific adjustments of the exposure are described by means of two parameters: (1) Physical effects derived from expert knowledge, e.g. about the characteristics of the leaves of the host plant (e.g. rough, smooth), the feeding behaviour of the larvae on the host plant (e.g. exposed, hidden) as well as (2) the synchronicity between the emergence of the susceptible larval stage and maize flowering. Both parameters lower the maximum mortality rates. The link from individual butterflies to the population level is created through several region-specific parameters. These are (1) the proportion of the lepidopteran host plant that is found within arable crops and in their field margins, (2) the proportion of maize cultivation area, (3) the proportion of maize fields planted with MON810 maize, (4) the average size of the fields, (5) the average width of a field margin and (6) the density of the host plant within the maize crop and in the field margin. The proportion of the total maize cultivation area used for cultivating MON810 maize could only be given for one region based on actual conditions; a maximum of 80% (100% - 20% refugial area for resistance management) was assumed for all other regions.

[4] As members and experts of the EFSA GMO Panel and various working groups of the GMO Panel dealing with aspects of risk assessment and as EFSA staff, the authors are directly involved in evaluating applications for authorisation to place GMOs on the market in the EU and have access to a range of publicly accessible as well as confidential data sources.

The authors report that whenever they had the option to choose, they supported the worst-case assumption in every aspect of the model so that the model overestimates rather than underestimates mortality rates.

Because as a rule all parameters, with the exception of the species-specific maximum mortality rates within the maize crop and in the field margin, were only estimated by a local expert (no choice), this statement essentially refers to the determination of the species-specific maximum mortality rate within the maize crop and in the field margin. The following decisions were made for the model:

1.) The toxicity of MON810 pollen to the larvae of non-target butterflies is so low that to date no LC_{50} values could be determined for the intake of pollen. For this reason the authors adjusted the published dose-effect relationship for pollen of another GMO, namely Bt176 maize, in such a way that it corresponds to that for the pollen of MON810 maize. Defining the factor by which the concentration of Cry1Ab protein in the pollen of maize MON810 is reduced in comparison to Bt176 maize is decisive here. The authors decide on a factor of approximately 31, which is the mean value of the quotients from the published minimum and maximum concentrations in Bt176 ((1.1 to 7.1 µg/g) and in MON810 maize pollen (0.09 µg/g) (Nguyen & Jehle, 2007; EFSA 2009).

2.) The slope of the mortality-dose relationship of non-target butterflies has so far only been experimentally determined for a few species. Felke *et al.* (2010) reported an increase of 5.795 for the European peacock butterfly. Perry *et al.* (2010) emphasize that with the pollen concentrations typically found within the crop or in the margin, the consideration of such a high increase in the model would only lead to very low mortality rates. For all three species the authors decide to apply a lower slope of 1.095, as for instance was determined by Saeglitz *et al.* (2006a, b) and Farinos *et al.* (2004) for lepidopteran pests (European corn borer and pink stalk borer). In doing so they introduce into the model a safety factor that leads to a non-specified overestimation of the mortality rates.

3.) In order to take sublethal effects into account, the maximum mortality rates determined within the crop and in the field margin were multiplied by a factor of four, implying that in addition to the acutely poisoned larvae, three times the number of larvae are so disturbed in their development that they fail to reach the next larval stage.

4.) The concentration of pollen on the host plants in and around the edges of maize fields was based on data from a comprehensive survey of the deposition of maize pollen on microscope slides covered with a coat of petroleum jelly (Wraight *et al.*, 2000). However, a comparison with corresponding counts on leaf surfaces (Pleasants *et al.*, 2001; Lang *et al.*, 2004) suggests that this method overestimates pollen deposition in the model by a factor of three. The decrease in pollen deposition with distance from the field margin was derived from several sets of data (as described in Perry *et al.* 2010).

Considering the relationships and assumptions described, Perry *et al.* (2010) calculate mortality rates that, in relation to the peacock and red admiral butterfly populations, would not exceed 1 in 1572 individuals in any region, and reach a maximum of 1 in 392 individuals for the diamondback moth.

2.2.2 Scientific discussion of the publication by Perry *et al.* (2010)

Lang *et al.* (2011) discussed uncertainties relating to key assumptions in the Perry *et al.* (2010) model. The authors argue that to date there are no data available which would confirm the linearity of the dose-effect relationship for the Cry1Ab protein. They claim that it is theoretically possible that in low concentrations the protein could develop a disproportionately large effect. In this context Lang *et al.* (2011) also criticise the decision taken with regard to the difference in

concentration (factor 31) of the Cry1Ab protein in the pollen of Bt176 maize compared with MON810 maize. They refer to a particularly low measurement value for the level of Cry1Ab protein in pollen from Bt176 maize (Nguyen, 2004), which in this case only exceeds the maximum concentration measured in the pollen of MON810 maize by a factor of four. A further point of criticism cited by the authors is that for one of the three species modelled by Perry *et al.* (2010), the red admiral, there are no data on the sensitivity of the species to Cry1Ab protein available. Given that the sensitivity of individual species can vary greatly, the fact that calculations for the red admiral were based on the assumption of comparable sensitivity for the peacock butterfly may have led to false results. Ultimately, the model would probably underestimate the mortality rates for butterfly larvae because the animals were only exposed for a few days in the laboratory tests on which the estimation is based. The authors also argue that the concept used in the model to consider sublethal effects is not sufficiently substantiated.

The description of pollen dispersal and population dynamics is not addressed by Lang *et al.* (2011).

In a response to the criticism levelled by Lang *et al.* (2011), Perry *et al.* (2011) take up the discussion about variability, uncertainties and the varying sensitivity of the model parameters. Using a graph they illustrate the over-proportional significance attributed to the slope of the dose-effect relationship for the Cry1Ab protein for the calculated mortality rates. According to this the influence of the slope is significantly greater than the assumed difference in the concentration of the Cry1Ab protein in pollen from Bt176 maize in comparison with pollen from MON810 maize. The authors specifically clarify that it was the decision taken (low gradient) that actually made it possible to calculate noteworthy mortality rates for MON810 maize under field conditions.

With regard to the other points of criticism Perry *et al.* (2011) explain that there are no indications whatsoever of a non-linearity of the dose-effect relationship for the Cry1Ab protein. For one of the data sets cited (Lang & Vojtech, 2006) linearity has been established. With regard to the difference in concentration between the pollen of Bt176 maize and MON810 maize Lang *et al.* (2011) compared measurement values from different years. If one compares the values determined by Nguyen (2004) for both maize lines in the respective trial years, then the resulting ratios are 64.8 (2002 trial year) and 30.5 (2003 trial year).

Acknowledging the other discussion points, Perry *et al*. (2011) support the critics' wish for a more comprehensive pool of data and explain why, in their view and based on the present state of knowledge, the identified gaps in the data do not lead to a systematic under-estimation of the mortality rates.

Their calculations are based on worst-case assumptions, for which the use of dose-effect relationships for susceptible butterfly species (corn borer and pink stalk borer) is an essential component. In a further publication (Perry *et al*., 2011) the authors clarify the importance of the dose-effect relationship in their model.

Taken together, the ZKBS is of the opinion that, due to the worst-case assumptions applied, the model over-estimates regional mortality rates for common non-target butterfly species. In spite of that, the predicted effects on non-target butterflies are very small and the actual in field effects may be even smaller. Moreover, if the results from the study by Perry *et al.* (2010) are viewed in relation to the conventional control of the pest organism with insecticides and the risk to non-target butterflies associated therewith, the ZKBS assesses the potential risk posed by the cultivation of MON810 maize as negligible.

3 References

AGBIOS (2011) GM Databas. Information on GM approved products. http://cera-gmc.org/index.php?action=gm_crop_database as accessed on 16.03.2011

Álvares-Alfageme, F., Bigler, F. & Romeis, J. (2010) Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipinctata* (Coleoptera:Coccinellidae): the importance of study design. Transgenic Res. DOI: 10.1007/s11248-010-9430-5.

Álvarez-Alfageme F., Ferry N., Castañera P., Ortego F. & Gatehouse A.M.R. (2008) Prey mediated effects of *Bt* maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). Transgenic Res. 17: 943-954.

Bai Y.Y., Jiang M.X. & Cheng J.A. (2005) Effects of transgenic rice pollen on fitness of *Propylea japonica*. J. Pest Sci. 78: 123-128.

Canolfi M.P., Brown K., Grimm C., Reber B. & Schmidli H. (2004). A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. Biocon. Sci. Technol. 14: 129-170.

Dively G.P. & Rose R. (2004) Effects of *Bt* transgenic and conventional insecticide control on the non-target natural enemy community in sweet corn. 1st International Symposium on Biological Control of Arthropods: 265-274.

Dutton A., Klein H., Romeis J. & Bigler F. (2002) Uptake of *Bt*-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. 27: 441–447.

EFSA (2009) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSAGMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA J. 1149, 1–85. See http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej1149_maize-MON810_finalopinion_en_rev.pdf.

Farinos, G. P., de la Poza, M., Herna´ndez-Crespo, P., Ortego, F. & Castanera, P. (2004) Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt-maize cultivation in Spain. Ent. Exp. Appl. 110: 23–30.

Felke, M., Langenbruch, G.-A., Feiertag, S. & Kassa, A. (2010) Effect of Bt-176 maize pollen on first instar larvae of the Peacock butterfly (*Inachis io*) (Lepidoptera; Nymphalidae). Environ. Biosafety Res. DOI: 10.1051/ebr/2010006.

Head G., Brown C.R., Groth M.E., Duan J.J. (2001) Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Appl. 99: 37–45.

Jasinski J.R., Eisley J.B., Young C.E., Kovach J. & Wilson H. (2003) Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environ. Entomol. 32: 407-413.

Lang, A., Brunzel, S., Dolek, M., Otto, M. & Theissen, B. (2011) Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of Bt-maize effects. Proc. R. Soc. B 277. (doi:10.1098/rspb.2010.2085)

Lang, A., Ludy, C. & Vojtech, E. (2004) Dispersion and deposition of Bt-maize pollen in field margins. Z. Pflanzenkr. Pflanzenschutz 111: 417–428.

Lundgren J.G. & Wiedenmann R.N. (2005) Tritrophic interactions among *Bt* (Cry3Bb1) corn, aphid prey, and the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environ. Entomol. 34: 1621-1625.

Meisle, M. & Romeis, J. (2008) Compatibility of biological control with *Bt* maize expressing Cry3Bb1 in controlling corn rootworms. In: Mason P.G., Gillespie, D.R. & Vincent, C. (eds): Proceedings of the third international symposium on biological control of arthropods, Christchurch, New Zealand, 8-13 February 2009, FHTET-2008-06, Morgantown, WV, United states Department of Agriculture, Forest Service, pp 145-160.

Meisle M. & Romeis J. (2009) Insecticidal activity of Cry3Bb1 expressed in Bt-maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) Ent. Exp. Appl. 131: 308-319.

Nguyen, H.T. & Jehle, J.A. (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810. Z. Pflanzenkr. Pflanzenschutz 114: 82–87.

Nguyen, H.T. (2004) Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais: Expression, Nachweis und Wirkung von rekombinantem Cry1Ab in heterologen Expressionssystemen. PhD thesis, Georg-August Universität Göttingen, Germany.

Obrist L., Dutton A., Romeis J. & Bigler F. (2006) Biological activity for Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. Biocontrol 51: 31-48.

Perry J.N., Devos Y., Arpaia, S., Bartsch D., Gathmann A., Hails R.S., Kiss J., Lheureux K., Manachini B., Mestdagh S., Neemann G., Ortego F., Schiemann J. & Sweet J.B. (2010) A mathematical model of exposure of nontarget Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proc. R. Soc. B 277: 1417– 1425.

Perry J.N., Devos Y., Arpaia S., Bartsch D., Gathmann A., Hails R.S., Kiss J., Lheureux K., Manachini B., Mestdagh S., Neemann G., Ortego F., Schiemann J. & Sweet J. B. (2011) The usefulness of a mathematical model of exposure for environmental risk assessment. Proc. R. Soc. B DOI: 10.1098/rspb.2010.2667.

Pilcher C.P., Obrycki J.J., Rice M.E. & Lewis L.C. (1997) Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26: 446-454.

Pleasants J.M., Hellmich R.L., Dively G.P., Sears M.K., Stanley-Horn D.E., Mattila H.R., Foster J.E., Clark T.L. & Jones G.D. (2001) Corn pollen deposition on milkweeds in or near corn field. Proc. Natl. Acad. Sci. USA 98: 11 919–11 924.

Porcar M., García-Robles I., Domínguez-Escribà L., Latorre A. (2010) Effects of Bacillus thuringiensis Cry1Ab and Cry3Aa endotoxins on predatory Coleoptera tested through artificial diet-incorporation bioassays. Bull Entomol Res. 100: 297-302.

Poza de la M., Pons X. Farinós G.P., López C., Ortego F., Eizaguirre M., Castanera P. & Albajes R. (2005) Impact of farm-scale *Bt* maize on abundance of predatory arthropods in Spain. Crop Protection 24: 677-684.

Raps A., Kehr J., Gugerli P., Moar W.J., Bigler F. & Hilbeck A. (2001) Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Mol. Ecol. 10: 525–533.

Rauschen, S. (2010) A case of „pseudo science“? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is a reminiscent of the case of the green lacewing. Transgenic Res 19: 13-16.

Ricroch A., Bergé J.B. & Kuntz M. (2010) Is the German suspension of MON810 maize cultivation scientifically justified? Transgenic Res. 19: 1-12.

Saeglitz C., Bartsch D., Eber S., Gathmann A., Priesnitz K. U. & Schuphan I. (2006a) Monitoring the Cry1Ab susceptibility of European corn borer in Germany. J. Econ. Entomol. 99: 1768–1773.

Saeglitz C., Engels H. & Schuphan I. (2006b) Final Report of ProBenBt, Workpackage 1, Task 4. See http://www.bio5.rwth-aachen.de/german/downloads/EU-Review.pdf.

Schmidt J.E.U., Braun C.U., Whitehouse L.P. & Hilbeck A. (2009) Effects of activated *Bt* transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in laboratory ecotoxicity testing. Arch. Environ. Contam. Toxicol. 56: 221-228.

Tabashnik B.E., Liu Y.-B., Dennehy T.J., Sims M.A., Sisterson M.S., Biggs R.W., Carrière Y. (2002) Inheritance of resistance to Bt toxin Cry 1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelechiidae) Journal of Economic Entomology, 95: 1018-11026.

Wold S.J., Burkness E.C., Hutchison W.D. & Venette R.C (2001) In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177-187.

Wraight, C. L., Zangerl, A. R., Carroll, M. J. & Berenbaum, M. R. (2000) Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proc. Natl. Acad. Sci. USA 97: 7700–7703.