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Statement of the ZKBS on the risk assessment of MON810 – New studies on the environmental impact of MON810

Summary

The German Central Committee on Biological Safety (ZKBS) has taken the cultivation ban on Bt maize MON810 of April 2009 as an opportunity to review its risk assessment of the cultivation of Bt maize MON810 of 2007 and to subject six new studies on the impact of Bt maize on non-target organisms to a detailed assessment. These studies mostly comprise laboratory test and have partially been drawn on as decisive factors for the cultivation ban on MON810 ordered by the BMELV (Federal Ministry of Food, Agriculture and Consumer Protection). The studies have been conducted by Rosi-Marshall et al. (2007), Bøhn et al. (2008), Kramarz et al. (2007), Schmidt et al. (2009), Hofmann (2007) and Hofmann et al. (2009).

A scientific assessment of the study results has revealed that none of them confirm potential adverse effects on non-target organisms by MON810 under cultivation conditions. The assessment is also considering the fact that some of the studies are of scientifically lower quality. The conclusion of the ZKBS is in line with the expert assessment of a French author group (Ricroch et al., 2009) and the opinion of the EFSA (European Food Safety Authority) on the request for renewal MON810 (EFSA, 2009). Both documents regard the German ban as scientifically not justified.

The ZKBS states that the cultivation of MON810 has no adverse effect on the environment.

Introduction

In April 2009 the cultivation of the Bt maize line MON810 was prohibited in Germany at the instigation of the BMELV. At this point, the ZKBS was not consulted on the safety of the cultivation of MON810. Since the last assessment of the potential environmental effects associated with the cultivation of MON810, several scientific studies have been published on this issue, examining potential environmental effects that have previously not been considered but cannot be completely excluded. Some of these studies have been drawn on as decisive factors for the cultivation ban on MON810 (BVL order of 17th April, 2009 - Federal Office of Consumer Protection and Food Safety).

The biological safety of approved Bt plants, including MON810, is supported by the publication of Ricroch et al. (2009) and the opinion for renewal of MON810 application of the EFSA (EFSA, 2009). Ricroch et al. (2009) consider more than 40 publications on the potential environmental effects of Bt plants for 2008-2009 in their statement. The assessment of additional studies published from 1996 to 2008 (376 according to Ricroch et al., 2009) does not question the biological safety of Bt plants either. In its statement, the EFSA (2009) refers to more than 250 environmentally relevant publications. The safety assessment of the ZKBS of 2007 is thus basically confirmed.

This statement of the ZKBS predominantly refers to the studies specifically selected to justify the cultivation ban. These are six publications on five different subject areas:

1. Effects of Bt maize pollen and crop residues on water organisms (Rosi-Marshall et al., 2007)
2. Effects of Bt maize flour on water fleas (Bøhn et al., 2008)
3. Effects of Bt maize plant residues on healthy and nematode-infected snails (Kramarz et al., 2007)
4. Effects of the Bt proteins Cry1Ab and Cry3Bb in feeding tests with larvae of the two-spotted ladybird (Schmidt et al., 2009)
5. Pollen deposition from maize fields to adjacent biotopes (Hofmann et al., 2009)

In view of the significance of these studies for the cultivation ban on MON810, a statement is made to the contents of each of them.

1. Rosi-Marshall et al., 2007

The authors measured the dispersion and transport of pollen and crop residues of Bt and non-Bt maize fields near headwater stream ecosystems. In addition, pollen and maize residues expressing Bt protein were fed in laboratory tests to detritivore larvae of the Trichoptera species *Lepidostoma liba* as well as to filtering larvae of the Trichoptera species *Hydropsyche borealis.* The authors concluded that the intake of Bt maize plant material led to a lower growth rate of the *L. liba* and the intake of Bt maize pollen to an increased mortality rate of the *H. borealis*.

The use of Trichoptera larvae as test organisms for the Lepidoptera-specific Cry1Ab protein is understandable, as Trichopterae are relatively closely related to butterflies. Thus, effects of the Cry1Ab protein on Trichoptera could be possible.

The work of Rosi-Marshall et al. (2007), however, exhibits substantial methodological shortcomings. For instance, the source of the Bt maize pollen and the non-Bt maize pollen is not indicated. The type, line and isogenity of the Bt maize inputs remain unclear as well. Dose-effect relations, as usual for toxicological examinations, are not recorded for the Bt protein.

In addition, the authors failed to measure the Bt protein content of pollen and maize residues during the examination of the dispersion into surface water near fields. No clear difference between the decomposition rates of Bt and non-Bt maize waste was observed. Furthermore, information on the possible exposure of the Trichoptera larvae to the Bt protein contained in headwater stream ecosystems is missing. However, from the authors data contained in a figure on the dispersion of Bt pollen into waters and the known contents of Bt proteins in maize pollen one can estimate that the annual dispersion is extremely low (9 - 90 ng/m^2 water surface). With a maximum of 8 g/m^2, the annual amount of dispersed plant material can also be regarded as low. In both cases, the respective Bt protein amounts can be regarded as negligible for Trichoptera larvae even in case of short-term occurrence of the sources of exposure (blooming period) and in view of the protein degradation that starts immediately (Douville et al., 2005, 2007).

Feeding tests with the Trichoptera species *L. liba* also raise questions. The origin of the Bt maize leaves and the non-Bt maize leaves is not indicated either. It is certain, however, that not the leaves of an isogenic maize line have been used as non-Bt variant. This was substantiated by the study of Saxena & Stotzky (2001), who found a higher lignin content of between 33 and 97% in the leaves of the isogenic Bt line. In the authors' opinion, the higher lignin content deteriorates the nutritional quality of the leaves. Thus, Rosi-Marshall et al. (2007) selected another maize line with an alleged lignin content and C/N ratio similar to that of Bt maize. Quantitative data on the constituents (lignin, C/N or others) of the maize plants tested in the laboratory, however, are not specified. Due to the missing data on the origin and the characteristics of the plant material used, it cannot be excluded that the Bt and non-Bt pollen and the Bt and non-Bt leaves did not only differ with respect to the presence of Bt protein. In addition, the concentration of the Bt protein in the leave material has not been determined. The leave amounts administered during the feeding tests have not been indicated either (Quote: “Leaves were added to aquaria as needed.”).

The missing standardisation is a crucial deficit, which above all applies to the laboratory tests conducted by the authors. Irrespective of this fact, the ZKBS attaches little importance to the observed *in vitro* effects on Trichoptera, although possibly large amounts of Bt material might be dispersed into waters. This assessment is based on the following reasons: The natural exposure of Trichoptera larvae to Bt protein in headwater stream ecosystems adjacent to fields is limited both with regard to space (distance to waters and distribution of maize fields in the landscape) and time (short blooming period). Furthermore, the potential exposure of water organisms is substantially limited by the small amount and the low concentration of Bt protein in the plant material as well as its comparatively rapid degradation in waters.

The mortality effects observed in the laboratory have only been found in case of unnaturally high exposure and for one species only. Although the authors verified a significantly higher mortality rate of the Trichoptera species *H. borealis* when fed with Bt maize pollen compared to non-Bt maize pollen, the pollen amount was two to three times higher than the maximum annual pollen input into waters measured. During the experiments with the Trichoptera species *L. liba*, no increased mortality rate could be observed when fed with Bt maize plant material, but a reduced growth rate.

In view of the temporarily limited exposure period in nature (blooming period of maize) in line with the mostly low Bt concentration in natural habitats the reduced growth rate demonstrated in the laboratory can also be classified as non-relevant environmental effect.

<u>Conclusion</u>

The ZKBS states that the causal correlation between the Bt protein or the genetic alteration and the negative effects on Trichoptera larvae has not been sufficiently demonstrated in the publication of Rosi-Marshall et al. (2007)..The study has also been criticised by other authors with regards to its experimental execution and the conclusions drawn (Beachy et al., 2008; Parrott, 2008). The authors themselves admit that they cannot exclude that the difference between the maize varieties used and not the Bt protein may have caused the observed effects (Rosi-Marshall et al., 2008). In addition, the ZKBS states that the results obtained by Rosi-Marshall et al. (2007) within the scope of laboratory experiments cannot be regarded as relevant, when considering the estimated exposure under field conditions. This conclusion has also been considered by the authors in their reply to critical comments (Rosi-Marshall et al., 2008).

2. Bøhn et al., 2008

Following the OECD directive 211 for toxicological *Daphnia* tests (OECD, 1998), Bøhn et al. (2008) fed Bt and non-Bt maize flour to the test organism “large water flea” (*Daphnia magna*). The goal of the study was to examine whether or not the feeding of Bt maize limits the fitness of water fleas. For this purpose, the survival rate, growth and reproduction were examined. The water fleas reacted to the feeding of Bt maize flour with reduced survival rates, earlier reproduction and reduced egg deposition. The authors interpreted the results as fitness disadvantage and a direct effect of the feeding with Bt maize flour.

The ZKBS notes, that the study of Bøhn et al. (2008) exhibits a series of severe deficits.

The - in particular exclusive - feeding of maize flour in toxicological studies with aquatic non-target organisms is unusual. Although water fleas are capable of surviving with a great variety of food sources, however, the aquaculture with maize flour as the only nutrient constitutes an unbalanced diet and a completely unnatural exposure of water fleas to Bt protein.

This is reflected in the test results. The extremely late maturation period of the water fleas (50% maturation only on the 17. - 18. day) even observed in the reference group (non-Bt maize) as well as their unusually high mortality rate are clear indications for unnatural, species-inappropriate cultural conditions. According to the OECD directive 211, growth and reproduction studies on water fleas should not last longer than 21 days, as the natural mortality rate usually considerably increases after about 28 days. Bøhn et al. (2008), however, doubled the test period (42 days) and obtained different data on the reproduction of the test animals in the second half of the test. Thus, the study in no way complies with the OECD quality requirements on Daphnia tests.

Another key weakness of the study is the plant material used. The used ground maize grains of one genetically modified (hybrid of MON810) and one reference maize line (a local variety of the Philippines) were harvested at the Philippines in 2003. There was no information provided about the conditions under which the material was cultivated (plant phytosanitary measures, pest infestation and the like). The nutritional composition or the equivalence of the used Bt and non-Bt maize flours has not been examined either (e.g. nutrient or mycotoxin contents). The evaluation and interpretation of the observed differences would only have been possible by using clearly characterised maize varieties or by extending the studies (e.g. by feeding studies with further maize varieties of clear origin and treatment). Whether or not the observed effects can be traced back to the presence or absence of Bt protein remains unclear.

The authors recognized the earlier reproduction as a consequence of the feeding with Bt maize flour. They concluded that increased stress caused the premature reproduction in case of the water flea fed with Bt maize flour. According to the OECD directive 211, however, rather a late or reduced reproduction is to be considered as stress indicator in *Daphnia* tests.

The differences in the total number of all eggs produced as observed by Bøhn et al. (2008) are to be classified as minor, when taking the test conditions into account (altogether 80 eggs during Bt treatment and 96 during non-Bt treatment). The reason for the relatively small quantitative difference must be seen in the strongly deviating results of the repeated tests. While two of three repeated tests yielded an increased reproduction rate using the Bt maize variant the third test variant resulted in an extremely low number of eggs per female. This again points to technical problems during experiment.

Furthermore, the authors determined a reduced survival rate of the water fleas fed with Bt maize flour. These data are not convincing in the light of the unnatural and unbalanced nutritional situation. Tests in line with requested conditions for standard tests would presumably have led to reliable results. Also, the inclusion of further reference maize lines would probably have allowed for distinguishing between the effects of the Bt protein and the impacts of the diet as such.

<u>Conclusion</u>

In summary, the ZKBS concludes that the study of Bøhn et al. (2008) provides no reliable and meaningful results due to the improper test conditions (deviation from the OECD directive for tests with water fleas, species-inappropriate nutrition of the test animals, use of uncharacterised maize samples, poor test execution and evaluation). The study is not suitable for questioning the previous risk assessment of MON810. In order to examine the suspected hazardousness of the Bt protein to water fleas, active Bt protein would have to have been used directly or a uniform, species-appropriate feed material, e.g. “spiked” with Bt protein. The publication lacks such clear-cut test approaches.

3. *Kramarz et al., 2007*

Kramarz et al. (2007) fed the snail *Helix aspersa* with plant material of MON810 maize and an isogenic reference maize line. Furthermore, the snails were additionally infected with snail-pathogenic nematodes, as snails are frequently parasitised in the field. The Bt maize material fed had no negative impact on the mortality rate or the growth of the snails; however, the growth of the snails was slightly limited after an additional infection with nematodes during treatment with the largest nematode concentration, compared to the treatment without nematodes. The mortality rate, however, had not changed at all.

The study exhibits a clear experimental design; however, it cannot be distinguished whether the observed effects can be traced back to the Bt protein or the differences of the maize lines. Thus, in future tests several conventional maize varieties should be used as references, so that variety differences can be distinguished from direct Bt effects (comp. the internet page of BMBF on safety research[1]).

<u>Conclusion</u>

Synergetic effects between several, simultaneously effecting stress factors are known in ecotoxicology. This aspect has been a major subject in biological safety research for a long time. Suggestions of the improvement of laboratory test procedures considering such aspects are being discussed (see Duan et al., 2008a; EFSA, 2008).

A solid, environmental risk assessment of MON810 by the ZKBS, however, cannot merely include results from laboratory studies, such as this one. Results of field tests, such as e.g. the ones that have been conducted by the BMBF for many years within the scope of the safety research of genetically modified organisms (GMO), are of special significance. Only in the field effects of GMO can be examined under conditions like in commercial cultivation. Apart from weather and nutritional conditions, a great number of factors with disadvantageous potential, e.g. diseases, the use of pesticides or with advantageous ones, e.g. rapid drop of Bt toxins, avoidance of pathogens and pesticides has an impact on non-target organisms.

Against the background of the minor effect obtained under extreme conditions (high parasite occurrence) and a missing dose-effect relationship, the risk of MON810 for the snail population is regarded by the ZKBS as minor.

4. Schmidt et al., 2009

The authors conducted laboratory toxicity tests with the Bt proteins Cry1Ab and Cry3Bb as well as the expression vector pBD10 at various stages of development (L1-L4) of the two-spotted ladybird *Adalia bipunctata*. The ladybird larvae were fed with the eggs of the flour moth (*Ephestia kuehniella*), which were sprayed with solutions containing the Bt protein or the DNA of the expression vector pBD10. The Bt protein solutions had concentrations of 0, 5, 25 and 50 μg/ml; the pBD10 DNA was applied in the concentrations of 0, 10, 50 and 100 μg/ml. In case of the test variants sprayed with Cry1Ab and Cry3Bb solutions, the mortality rate increased significantly compared to the reference. The expression vector had no effect. According to the au-

[1] http://www.biosicherheit.de/de/mais/oekosystem/652.doku.html

thors, the observed damage of the ladybird larvae could be directly traced back to the activated Bt proteins. The authors called the previous scientific view on the host specificity and the mechanism of action of Cry-proteins into question particularly for the Cry1Ab in view of the effect on the two-spotted ladybird.

The ZKBS states that the study exhibits substantial deficits regarding the material used, the execution of the test and the interpretation of the results, through which the correctness of the results and the statements are principally questioned:

- The description of the test set-up does not indicate how the Bt protein solutions were applied to the *Ephestia* eggs and how it was ensured that the ladybird larvae of the respective stages were exposed to the same Cry-concentrations every time the test was repeated. The level and the homogeneity of the Cry-concentration in the larva feed cannot be estimated. The scientifically sound test execution is thus not given and a test repetition by third parties prevented.
- Neither the concentration by means of quantitative ELISA nor the biological activity by means of biotests were examined in the Bt protein solutions used (see e.g. Duan et al., 2008b). Schmidt et al. (2009) used immunostrips (Agidia Inc.) for the mere qualitative identification of the Bt protein in the solutions. In the view of the ZKBS this is not sufficient for the realistic, scientifically correct estimation of the exposure of the larvae to the active Bt proteins.
- The untypical effects of the Bt proteins on the test animals may be due to some hazardous constituents of the protein preparations. In order to be able to exclude this, inactivated samples of the Bt protein solutions (e.g. by means of chemical or heat treatment) would have to have been used as references during the experiments.
- The ZKBS concludes that the methodical problems lead to the unclear and/or inconsistent results of the study; the effects observed are untypical of scientific laboratory toxicity tests under controlled conditions in several respects.
    - Clear dose-effect relations were obtained neither for Cry1Ab nor for Cry3Bb toxin. For instance, the mortality rate even decreased at the highest concentration stage, compared to the medium concentration stage. The authors cannot explain this effect.
    - The L1-larvae exhibited unusually high mortality rates (up to 21% without the feeding of Bt and 44% with Bt). Occasionally, there was no difference between the mortality rates of the reference and the highest concentration stage (tests with Cry3Bb). In contrast, the L2 to L4 stages were largely unaffected.
    - In contrast to the mortality rate, there was no difference between the references and the variants fed with Cry-protein solutions regarding the development periods of the larval stages, above all of the L1-larvae. At least it would have been expected that the development period of the L1-larvae is prolonged due to the postulated toxic effect.
- The ZKBS notes that the erratic evaluation of results by the authors raises doubts in the careful execution the study. The source AGBIOS 2008[2] quoted by the authors states a Bt concentration of 0.09 µg/g in MON810 pollen ; however, the authors use a value of 7.9-10.3 µg/g for the Bt protein content in MON810 pollen, i.e. a value 100 times too high, and thus overestimate the actual environmental exposure. In the view of the ZKBS,

[2] http://www.agbios.com/dbase.php?action=Submit&evidx=9

the high concentrations used in the study bear no biologically relevant relation to the actual Bt protein content in MON810 pollen (even when considering the fluctuation range in biological systems), to which ladybird larvae could be exposed. It is to be pointed out that the staple food of ladybird larvae and adults is aphids which do not take in Bt proteins from Bt plants, according to Raps et al. (2001).

- As a matter of principle, in a scientific work the authors' own results are related to the current level of knowledge. In this work, however, the results of other laboratory and field tests on the effects of Cry-proteins on ladybirds are not even mentioned, which do not support the results of Schmidt et al. (2009), neither for *Adalia bipunctata* (Wold et al., 2001) nor for other ladybird species (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).

<u>Conclusion</u>

The results of the laboratory study, which supposedly reveal lethal effects of Bt proteins without a clear dose-effect relation at simultaneous lack of impacts on the development and the weight increase of the test animals, are not solid without independent scientific verification considering numerous deficits in the study. Furthermore, several laboratory and field tests do not confirm the results of Schmidt et al. (2009). Even if taking the aspect of precautionary safety assessment into account, the ZKBS sees no reason to change its previous risk assessment of the environmental effects of MON810 due to the study of Schmidt et al. (2009).

5. Hofmann, 2007 and Hofmann et al., 2009

These tests are not available as publications in scientific journals but as reports at the BfN (Federal Agency for Nature Conservation). The study of Hofmann (2007) presents results of the deposition of maize pollen, collected by means of technical pollen samplers. According to the authors, the data (pollen collection starting at the margin of maize fields and extending to different distances) indicates that maize pollen is deposited in much larger amounts than assumed so far.

The ZKBS states that the data of Hoffmann (2007) do not provide any fundamentally new scientific insights. Although the author detected large pollen amounts at first view, this is due to the long measuring periods, reaching over the entire blooming period of maize. As the author himself writes, the results of other works provide similar deposition values, when extrapolated to the entire blooming period (e.g. Wraight et al., 2000; Pleasant et al., 2001; Wolt et al., 2003; Lang et al., 2004; Felke & Langenbruch, 2005; Shirai & Takahashi, 2005).

In the study of Hofmann et al. (2009), the authors developed what is referred to as "worst-case scenarios" for pollen deposition, which are supposed to apply to special weather conditions and unfavourable topographic conditions. The results demonstrate that under certain conditions the deposited pollen amount may theoretically be higher than previously determined by means of the pollen sampling measurements (Hofmann, 2007).

<u>Conclusion</u>

Pollen density data measured by means of pollen samplers are not suggestive of the real exposure of non-target organisms in natural surfaces. In order to determine the exposure of host plants relevant to non-target organisms, climate factors, such as e.g. wind and rain, the surface structure of leaves and host plants as well as the structure (frequency of the specific host plants) and the architecture of the vegetation layer are to be taken into account. In addition, the biology of the non-target organisms has strong influence. Phenology, feeding behaviour, development-dependent exposure and sensitivity to the Bt protein are of central significance for a realistic risk assessment of non-target organisms. As a matter of principle, the ZKBS additionally considers the occasionally occurring higher exposure due to large pollen amounts in its risk assessments. On the other hand, the ZKBS also includes in its assessments the especially low Bt concentration detected in pollen of the maize line MON810 as well as possible impacts of the weather and the vegetation structure on the exposure of non-target organisms (see statement of the ZKBS of July 2007); this point has also especially been taken into account in the EFSA statement on MON810 (EFSA, 2009).

Bearing in mind the influence variables described and insights available at population level, the ZKBS sees no specific hazard posed to non-target organisms, even if in individual cases these may be exposed to higher pollen amounts for a short period of time than expected on the average. The tests of Hofmann (2007) and Hofmann et al. (2009) do not provide any new indications for the potential hazard of non-target organisms but rather confirm the risk assessment approach of the ZKBS.

Final Conclusion

Considering all scientific information available and keeping the precautionary principle in mind, the ZKBS concludes that the cultivation of the maize line MON810 poses no danger to the environment. The risk assessment contained in the statement of the ZKBS of 2007 is thus confirmed, taking the current publications into account.

Literature

Alvarez-Alfageme F., Ferry N., Castanera 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 *Stethoruspunctiltum* Weise (Coleoptera: Coccineliidae). Transgenic Res., DOI: 10.1007/s 11248-008-9177-4.

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

Beachy R.N., Fedoroff N.V., Goldberg R.B., McHughen A. (2008) The burden of proof: A response to Rosi-Marshall et al.. Proc. Nat. Acad. Sci., 105, 7, E9.

Bonn T.$_T$ Primicerio R., Hessen D.O., T. Traavik (2008) Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Arch. Environ. Contam. Toxicol., 55, 584 -592.

Candolfi M.P., Brown K., Grimm C, Reber B., Schmidli H. (2004) A faunistic approach to assesspotential side-effects of genetically modified Bt-corn on non-target arthropods under fieldconditions. Biocon. Sci. Techno!., 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 com. 1st International Symposium on Biological Control of Arthropods, 265-274.

Douville M., Gagne F., Masson L., McKay J., Blaise C. (2005) Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoxin in the environment. Biochem. Syst. Ecol., 33, 219-232.

Douville M., Gagne F., Blaise C, Andre C. (2007) Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt com Cry1Ab gene from an aquatic environment. Ecotox. Environ. Safety, 66, 195 - 203.

Duan J.J., Marvier M., Huesing J., Dively G., Huang Z.Y. (2008a) A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLoS ONE 1, e1415.

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Lebensmittelsicherheit

Duan J.J., Teixeira D., Huesing J.E., Jiang C. (2008b) Assessing the risk to nontarget organisms from Bt com resistant to com rootworms (Coleoptera: ChrysomeJidae): Tier-I testing with *Orius insidiosus* (Heteroptera: Anthocoridae). Environ. Entomol, 37, 838-844.

EFSA (2008) Environmental risk assessment of genetically modified plants - challenges and approaches. EFSA Scientific Colloquium Summary Report 8, 1-159.

EFSA (2009) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMORX-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. The EFSA Journal, 1149, 1-84.

Felke M., Langenbruch G.A. (2005) Auswirkungen des Pollens von transgenem Bt-Mais auf ausge-wählte Schmetterlingslarven. BfN-Skripten 157, 1-143.

Hofmann F. (2007) Kurzgutachten zur Abschätzung der Maispollendeposition in Relation zur Entfer-nung von Maispollenqueilen mittels technischem Pollensammler PMF. BfN, Bonn. www.bfn.de/fileadmin/MDB/documents/themen/agrogentechnik/07-05-31_Gutachten_Pollendeposition_end.pdf

Hofmann F. Janicke L., Janicke U., Wächter R_M Kuhn U. (2009) Modellrechnung zur Ausbreitung von Maispollen unter Worst-Case-Annahmen mit Vergleich von Freilandmessdaten. BfN Bonn, http://www.bfn.de/fileadmin/MDB/documents/service/Hofmann_et_al_2009_Maispollen_Worst Case_Modell.pdf

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

Kramarz P.E., De Vaufleury A., Zygmunt P.M.S., Verdun C. (2007) Increased response to cadmium and *Bacillus thuringiensis* maize toxicity in the snail *Helix aspersa* infected by the nematode *Phas-marhabditis hermaphrodita.* Environ. Tox. Chem., 26, 73-79.

Lang, A., Ludy C, Vojtech E. (2004). Dispersion and deposition of Bt maize pollen in field margins. J. Plant Dis. Prot, 111,417-428.

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

OECD (1998) *Daphnia magna* reproduction test. OECD Guidelines for Testing of Chemicals, 211, 1-21.

Parrott W. (2008) Study of Bt impact on caddisflies overstates its conclusions: response to Rosi-Marshall et al.
Proc. Nat. Acad. Page 105, 7, E10.

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* com. Environ. Entomol., 26, 446-454.

Pleasants J.M., Hellmich R.L., Diveiy G.P., Sears M.K., Stanely-Horn D.E., Mattila H.R., Foster J.E., Clark T.L., Jones G.D. (2001) Com pollen deposition on milkweeds in and near cornfields. Proc. Nat. Acad. Page 98, 11919-11924.

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

Raps A., Kehr J., Gugerli P., MoarW.J., Bägler F., Hilbeck A. (2001) Immunological analysis of phloem sap of *Bacillus thuringiensis* com and of the nontarget herbivore *Rhopaiosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Mol. Ecol., 10, 525-533.

Ricroch A., Berge J.B., Kuntz M. (2009) Is the German Suspension of MON810 maize cultivation scientifically justified? Transgenic Res., http://www.springerlink.corn/content/r6052757667ng364/fulltext.pdf.

Rosi-Marshall E.J., Tank J.L., Royer T.V., Whiles M.R., Evans-White M., Chambers C, Griffiths N.A. (2007). Toxins in transgenic crop by-products may affect headwater stream ecosystems. Proc. Nat. Acad. Page 104, 204-208.

Rosi-Marshall E.J., Tank J.L., Royer T.V., Whiles M.R. (2008) Reply to Beachy et al. and Parrott: Study indicates Bt com may affect caddisflies. Proc. Nat Acad. Page 105, E11.

Saxena D., Stotzky G. (2001) Bt com has a higher lignin content than non-Bt com. Amer. J. Bot, 88, 1704-1706.

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 bipunetata* in laboratory ecotoxicity testing. Arch. Environ. Contam. Toxicol., 56, 221-228.

Shirai Y., Takahashi M. (2005) Effects of transgenic Bt com pollen on a non-target lycaenid butterfly, *Pseudozizeeria maha.* Appl. Entomol. Zool., 40, 151-159.

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

Wolt J.D., Peterson R.K.D, Bystrak P., Meade T. (2003) A screening level approach for nontarget insect risk assessment: Transgenic Bt com pollen and the monarch butterfly (Lepidoptera: Danaidae). Environ. Entomol., 32, 237-246.

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. Nat. Acad. Page 97, 7700-7703.

ZKBS (2007) Stellungnahme der ZKBS zum Bescheid des BVL (Teilweises Ruhen der Inverkehrbringens-Genehmigung des gentechnisch veränderten Maises MON810) vom 27. April 2007. Ref. No. 6788-02-13 vom Juli 2007.

http://www.bvl.bund.de/nn_1071104/DE/06_Gentechnik/093_ZKBS/01_Allg_Stellungnahmen/04_pflanzen/Mon810.html