Comments to the response of Monsanto to the German safeguard measure on MON810 maize

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Abstract

On the basis of justifiable assumptions that the cultivation of MON810 maize in Germany poses a risk for the environment the German Federal Office of Consumer Protection and Food Safety (BVL) in April 2007 temporally suspended the selling of MON810 maize seeds until Monsanto, as authorisation holder, submits an environmental monitoring plan according to Directive 2001/18/EC. For post market environmental monitoring the Directive envisages case-specific monitoring as well as general surveillance.

Monsanto submitted an environmental monitoring plan in May 2007. The plan does not contain any measures for case-specific monitoring. According to Monsanto there is no need for this because the cultivation of MON810 poses no risk to the environment. However, the risk assessment conducted by Monsanto is unbalanced. The risk studies performed by Monsanto are old and do not comply with the requirements of a present-day risk assessment. Data from independent scientists are dealt with in a biased way. For instance, studies showing negative effects of Bt maize are either not included by Monsanto or declared as irrelevant due to methodical flaws. Monsanto on the other hand fails to discuss methodical flaws of studies showing no effects of Bt maize. Monsanto's bias is also mirrored in the way the company deals with the existing scientific uncertainties. Even though Monsanto cites several publications in which the authors emphasize what is not known with certainty, the company neither mentions these uncertainties nor appreciates the assessments of the authors, who recommend that further studies are necessary to reduce uncertainties.

For general surveillance Monsanto plans two measures in the main, namely a farmer survey and an analysis of the annual reports by five existing monitoring networks. Both these are of limited value for measuring the environmental impacts of MON810 maize. The limited value of farmer surveys has already been criticized by the BVL. The authority wrote in its Order that farmer surveys are not appropriate for delivering statistical valuable data about environmental effects.

Given the conflicts of interests Monsanto's approach is not surprising. However, it is surprising that the BVL not only rates the monitoring plan submitted by Monsanto as adequate, but also lifts the temporally ban on MON810 seed sales.

As the data provided by Monsanto cannot allay concerns about environmental risks, and further studies published after the submission of the environmental monitoring plan by Monsanto report negative effects on non-target organisms, it can still be justifiably assumed that cultivating MON810 maize in Germany poses a risk for the environment. Moreover, as the monitoring measures intended by Monsanto are insufficient for protecting the environment against possible undesirable effects of MON810 maize in time, the BVL should reconsider the deregulation of MON810 seed sales. On all account the BVL should commit Monsanto to carry out case-specific monitoring.

Zusammenfassung

Aufgrund der berechtigten Annahme, dass der Anbau des gentechnisch veränderten Maises MON810 eine Gefahr für die Umwelt darstellt, hat das BVL im April 2007 den Verkauf von MON810 Saatgut untersagt, bis Monsanto einen Umweltmonitoringplan gemäß Richtlinie 2001/18/EG vorlegt. Die Richtlinie sieht dazu ein fallspezifisches Monitoring sowie eine allgemeine Überwachung vor.

Monsanto hat im Mai 2007 einen Umweltmonitoringplan vorgelegt. Maßnahmen für ein fallspezifisches Monitoring sind darin nicht enthalten. Aus Sicht von Monsanto ist ein fallspezifisches Monitoring nicht notwendig, da der Anbau von MON810-Mais kein Risiko für die Umwelt darstelle. Die Risikoeinschätzung des Konzerns ist jedoch unausgewogen. Die von Monsanto selbst durchgeführten Risikostudien sind meist alt und entsprechen nicht mehr den Ansprüchen an eine moderne Risikoabschätzung. Daten aus unabhängigen Studien behandelt Monsanto parteiisch. So werden Studien, in denen Umweltwirkungen festgestellt wurden, von Monsanto entweder nicht berücksichtigt oder aufgrund methodischer Mängel als nicht relevant eingestuft. Bei Studien hingegen, in denen keine Effekte beobachtet wurden, unterlässt es Monsanto, methodische Mängel zu diskutieren. Die Befangenheit des Konzerns zeigt sich auch beim Umgang mit der bestehenden Unsicherheit. Obwohl Monsanto mehrere Studien zitiert, in denen die Unsicherheit des Wissens betont wird, geht Monsanto weder auf diese Unsicherheit ein noch berücksichtigt das Unternehmen die in den Studien geäußerten Empfehlungen, vor einem großflächigen Anbau mehr Untersuchungen durchzuführen.

Für die allgemeine Überwachung plant Monsanto eine Befragung der Landwirte, die MON810-Mais anbauen, sowie eine Analyse von Jahresberichten von fünf bestehenden Monitoringprogrammen. Beide Maßnahmen sind jedoch nur eingeschränkt dienlich, um Umweltwirkungen von MON810-Mais zu erfassen. Den limitierten Wert von Befragungen der Landwirte hat bereits das BVL kritisiert. So schrieb das Bundesamt in seinem Bescheid, dass Fragebögen nicht geeignet sind, statistisch auswertbare Daten zu Umweltwirkungen auf Agrarflächen und deren Umgebung zu liefern.

Angesichts der Interessenskonflikte von Monsanto ist das Vorgehen des Konzerns wenig überraschend. Überraschend ist hingegen, dass das BVL die von Monsanto vorgesehenen Maßnahmen als ausreichend einstuft und das Anbauverbot aufgehoben hat.

Da aufgrund der von Monsanto vorgelegten Daten Umweltrisiken nicht ausgeschlossen werden können und neuere Studien, die nach der Einreichung des Überwachungsplans von Monsanto veröffentlicht worden sind, von negativen Wirkungen auf Nichtzielorganismen berichten, besteht weiterhin die berechtigte Annahme, dass der großflächige Anbau von MON810 eine Gefahr für die Umwelt darstellt. Da die von Monsanto vorgesehenen Überwachungsmaßnahmen zudem unzureichend sind, die Umwelt frühzeitig vor etwaigen unerwünschten Wirkungen des MON810-Mais zu schützen, sollte das BVL die Aufhebung des Anbauverbots überdenken und in jedem Fall ein fallspezifisches Monitoring verlangen.

Comments to the response of Monsanto to the German safeguard measure on MON810 maize

Introduction

In April 2007, the German Federal Office of Consumer Protection and Food Safety (BVL) temporarily suspended authorisation for the distribution of MON810 maize seeds for commercial planting in Germany until Monsanto, as authorisation holder, submitted an appropriate plan for monitoring environmental effects of the cultivation of MON810 maize (BVL 2007). The BVL justified the temporarily suspension mainly for two reasons. First, the BVL cited a selection of newer scientific papers, which indicated the potential of negative impacts of MON810 maize cultivation on non-target organisms such as predators, parasitoids, butterflies and soil organisms. Second, the BVL argued that the farm questionnaires normally used by Monsanto for general surveillance are not sufficient for collecting information for statistical evaluation about effects on the environment and especially on non-target organisms.

In May 2007 Monsanto submitted several documents to the BVL including a response to the scientific papers cited by the BVL, the environmental risk assessment from the application for renewal of the authorisation for continued marketing of MON810 maize in the European Union, and an environmental monitoring plan.

The BVL accepted the monitoring plan submitted by Monsanto In December 2007.

The documents submitted by Monsanto were analyzed on behalf of Greenpeace in Germany in February 2007.

Effects on predators and parasitoids

«As Bt plants express endotoxins during the whole season, potential tritrophic exposure of predators via prey feeding on Bt plants may be increased in comparison to Bt sprays if the Bt sprays are not applied throughout the growing season. The implications of exposure on the performance of these nontarget organisms are still not clear; however, data show that long-term exposure to Bt toxins can occur in the field.»

OECD 2007

Exposure to non-target organisms

Given that MON810 maize is expressing the Cry1Ab toxin during the whole season, there is a great potential for exposure of non-target organisms to Cry1Ab toxin. Since risk is a function of both the potential harm (hazard) and the likelihood of occurrence (exposure), for risk assessment it is important to know which non-target organisms are exposed to the Cry1ab toxin in the field. The BVL takes the importance of exposure data into consideration in its Order and cites three field experiments showing that long-term exposure of non-target organisms to Cry1Ab toxin can occur in the field (Harwood et al. 2005, Zwahlen & Andow 2005, Obrist et al. 2006).

Regarding the three field experiments cited in the BVL Order Monsanto acknowledges that there is a potential for predators and parasitoids to be exposed to Cry1Ab toxin produced by MON810 maize. However, Monsanto downplays the results of the three field experiments by stating that the magnitude of secondary exposure is generally much less than that of primary exposure via direct feeding on MON810 maize. Thus, according to Monsanto the exposure risk of MON810 maize to predators and parasitoids would be much less than to those trophic levels that directly feed on MON810 maize.

Monsanto's opinion has to be contested for three reasons. *First*, Monsanto does not consider that at least some entomophagous arthropods can also be exposed to Cry1Ab toxin through direct routes, namely by direct feeding on plant substrates such as pollen, nectar or plant sap (Dutton et al. 2003). *Second*, data from scientific literature shows that secondary exposure can be similar or even elevated compared to primary exposure. E.g. the herbivorous spider mite *Tetranychus urticae* was found to contain three times the concentration of Cry1Ab in Bt maize (Obrist et al. 2006). Therefore spider mite predators might be exposed to elevated Cry1Ab toxin levels. Also, non-target arthropods licking dew or honeydew might be exposed to elevated Cry1Ab toxin concentrations. Obrist et al. (2005) showed that the faeces of the thrips *Frankliniella tenuicornis* contained 8 times more Cry1Ab toxin than Bt11 maize leaves. *Third*, due to the potential variability in the susceptibility to Cry1Ab toxin the significance and ecological consequences for toxin transfer through the food web can only be assessed adequately by further investigations. For example, Harwood et al. (2005) showed that spiders (*Araneae*), ladybird beetles (*Coccinellidae*) and damsel bugs (*Nabidae*) collected from Bt11 maize fields contained on average between 0.42 to 2.53 µg

Cry1Ab toxin/g fresh weight. They conclude that these toxin concentrations are significant and that the ecological consequences of the toxin transfer should be further investigated during the risk assessment of existing transgenic varieties.

Species selection

Entomophagous arthropods in the maize crop represent a diverse group, which encompasses a wide ecological and taxonomic spectrum with the classes of *Insecta* and *Arachnida* (Dutton et al. 2003). The available literature on maize demonstrates that several species of Ladybird beetles, ground beetles, rove beetles, hoverflies, green lacewings, pirate bugs, damsel bugs and spiders as well as various parasitoids are regularly present in maize and their function as pest regulators is repeatedly reported (Dutton et al. 2003). As the BVL points out in its Order, only a few of these species have been adequately investigated in laboratory studies regarding potential non-target effects of Bt maize. Citing Lövei & Arpaia (2005) the BVL writes that for important pest regulators such as rove beetles, spiders, parasitic wasp and hoverflies risk assessment data are missing or inadequate.

Lövei & Arpaia (2005) show that laboratory data on the impact of Cry1Ab maize on natural enemies are limited. Regarding species selection their review reveals that till 2005 non-target effects of transgenic Cry1Ab maize had been addressed in peer-reviewed laboratory studies only for five species, namely the Ladybird beetle *Coleomegilla maculata*, the two pirate bugs *Orius majusculus* and *Orius insidiosus*, the lacewing *Chrysoperla carnea* and the parasitoid wasp *Cotesia flavipes*. One of these species, *C. maculata*, does not occur in Europe (Dutton et al. 2003).

Commenting the review by Lövei & Arpaia (2005) Monsanto is not at all addressing the limitation of available laboratory data for risk assessment. Monsanto has in other words not explicitly responded to the concerns expressed by the BVL with regard to missing or inadequate risk assessment data.

Effects on Chrysoperla carnea and Cotesia flavipes

Lövei & Arpaia (2005) reveal in their review that for two out of the five species tested (see above) negative effects have been recorded in scientific literature. These two species are *Chrysoperla carnea* and *Cotesia flavipes*.

In the case of *C. carnea* Lövei & Arpaia (2005) cite five studies showing negative effects and three studies showing no negative effects. According to Monsanto, in all cases where a negative effect was observed it was either indirect or due to problems associated with the testing system. However, this view of the contradictory evidence omits to recognize that there is an ongoing scientific debate as to whether recorded negative effects of Cry1Ab occur through indirect bitrophic and tritrophic pathways rather than directly from the Bt toxin itself (Andow et al. 2006, Hilbeck & Schmidt 2006). According to Andow et al. (2006) additional research is necessary for the scientific controversy to be resolved.

In the case of *C. flavipes* Lövei & Arpaia (2005) cite Prütz & Dettner (2004), who showed that the number and size of the parasitoid wasp emerging from *Chilo partellus* hosts fed Bt176 maize leaf material were reduced compared to hosts fed leaf material from non-GM maize. According to Monsanto the observed tritrophic effect was likely due to parasitoid interactions with poisoned or weakened target pests. However, this view is biased as it does not exactly reflect the conclusions of Prütz & Dettner (2004), who state that it remains unclear whether the observed effects on the parasitoid were direct effects of the Cry1Ab toxin or indirect effects of a poisoned or weakened host.

Field trials

Monsanto writes on page 23 of the technical dossier and page 9 of the response to the German safeguard measure that «field studies conducted over the past decade by industry and the academic community, and reported in the peer-reviewed literature on registered insect-protected crops that produce a variety of Cry1A proteins, including Cry1Ab, have demonstrated that these crops have no adverse effects on biodiversity, tested populations of natural enemies, and other ecologically important non-target arthropods.» Monsanto cites 32 publications on this. Although Monsanto is writing on «field studies conducted over the past decade» 12 of the cited publications aren't field studies at all (see Table 1). A closer look at the remaining 20 publications reveals that most of them are of limited relevance for risk assessment of MON810 maize cultivation under German field conditions. Five of the 20 field studies cited are of little relevance, because they were not conducted with Bt maize but with Bt cotton. Another six field studies are of limited relevance, because they were carried out in the United States. Data from these trials are not necessarily transferable to ecosystems outside the United States. As they do not adequately encompass German species and environments, they are not sufficient for predicting consequences on biodiversity and the functioning of ecosystems in Germany.

Nine field trials cited deal with the release of maize under European environmental conditions. As one of these nine publications has been published in a non peer-reviewed journal and was not available, the further analysis was done with the remaining eight publications.

Two of the eight publications addressed the potential exposure of honeybees and non-target butterfly species to Bt maize. Lang et al. (2004) examined the temporal occurrence, spatial range and dispersion of maize pollen, and potential Bt maize pollen densities in field margins in South Germany. Babendreier et al. (2004) quantified the amount of pollen that honeybee larvae ingest during development by exposing them exclusively to maize pollen under near-field conditions in Switzerland. Both studies provide helpful information in the evaluation of the exposure of honeybees and butterflies to Bt-maize pollen but they do not examine adverse effects potentially resulting from exposure.

Another three of the eight publications are about field trials in France and Hungary. Bourguet et al. (2002) conducted a one-year field study in South France to evaluate the effects of MON810 and Bt176 maize on non-target species. Their results indicate that MON810 maize has no drastic toxic effect on predators and parasitoids. However, due to the short duration of the field test and the limited number of replicates small effects may have remained undetected. Bourguet et al. (2002) therefore write: «We cannot conclude that Bt corn has no effect on non-target species.» In Hungary a three-year field trial on non-target effects of

MON810 maize on foliage-dwelling arthropods was conducted by Toth et al. (2004) and Arpas et al. (2005). The impact of MON810 maize was assessed by a spider web survey and by whole plant visual sampling. In the spider web analysis the number of individuals found in web samples of non-transgenic (isogenic) maize outnumbered that of MON810 maize plots in almost every insect order. As these differences were not statistically significant, the authors cannot conclude that web samples of MON810 maize differ from isogenic maize. However, according to Arpas et al. (2005) further studies are necessary for a complete analysis of their field trial. In addition to the incompleteness of the analysis it should be noted that spider web content does not necessarily reflect the natural proportions of arthropods occurring in maize fields. Furthermore, it remains uncertain to what extent results from field tests in Hungary are suited for the risk assessment of MON810 cultivation in Germany. As Hungary lies within the Pannonian biogeographical regions the results from Toth et al. (2004) and Arpas et al. (2005) may not necessarily be transferable to the Atlantic and Continental biogeographical regions of Germany.

3 out of the 8 publications deal with field trials addressing potential non-target effects of Bt maize in Germany. Volkmar & Freier (2003) studied the effect of Mon810 maize cultivation on the spider communitiy. The data from the two-year field trial did not reveal any clear signs of effects related to MON810 maize. However, as the study was limited to two years, it may possibly have missed long-term effects (Meissle & Lang 2005). Ludy & Lang (2006a) compared the foliage-dwelling spider fauna of Bt176 maize fields to non-transgenic maize fields. In this three-year study no consistent effect of Bt716 maize on individual numbers and species richness of spiders was found. However, Ludy & Lang (2006a) acknowledge that the statistical power of their study was relatively small. The authors write: «Future field studies should be conducted on longer temporal and larger spatial scales and in higher replication.» In addition to the remaining uncertainties due to the low statistical power of the study it has to be noted that the field trial was conducted with Bt176 maize. Risk assessment should be done on a case-by-case basis. Absence of evidence of adverse effects in field trials with Bt176 maize is no evidence for absence of adverse effects in trials with MON810 maize.

The third publication is about a three-year field trial conducted by Eckert et al. (2006) to detect potential effects of MON810 maize cultivation on the community of arthropods on and between husk leaves of maize ears. No significant effects could be observed over the three years in the field. However, due to the small plot size (0.25 ha) and small replication number (5), the statistical power of the study was limited. Moreover, the arthropod community studied represents only a fraction of the arthropods present in maize fields and therefore can only be part of a comprehensive risk assessment (Eckert et al. 2006).

Table 1: Publications cited by Monsanto as field trials demonstrating the absence of non-target effects

Publication	Plant	Event	Study Location	Country
Daly & Buntin 2005	Maize	MON810	Field	USA
Dively & Rose 2003	Maize	Bt11	Field	USA
Dively et al. 2005	Maize	VIP3xBt11	Field	USA
Head et al. 2001*	Maize	MON810	Laboratory	
Head et al. 2005*	Cotton		Field	USA
Lozzia et al. 1998	Maize	Bt176	Laboratory	USA
Naranjo et al. 2005+	-	-	-	-
Naranjo 2005a	Cotton		Field	USA
Naranjo 2005b	Cotton		Field	USA
Orr & Landis 1997*	Maize	Bt176	Field	USA
Pilcher et al. 1997*	Maize	MON810	Field	USA
Pilcher et al. 2005*	Maize	Bt11 / Bt176	Field	USA
Torres & Ruberson 2005	Cotton		Field	USA
Whitehouse et al. 2005	Cotton		Field	Australia
Arpas et al. 2005	Maize	MON810	Field	Hungary
Babendreier et al. 2004	Maize	conventional	Field	Switzerland
Bakonyi et al. 2006	Maize	MON810	Laboratory	
Bourguet et al. 2002	Maize	Bt176 / MON810	Field	France
Eckert et al. 2006	Maize	MON810	Field	Germany
Freier et al. 2004++	*Maize*	*?*	*Field*	*Germany*
Heckmann et al. 2006	Maize	MON810	Laboratory	
Lang et al. 2004	Maize	Bt176 / MON810	Field	Germany
Ludy & Lang 2006a	Maize	Bt176	Field	Germany
Ludy & Lang 2006b	Maize	Bt176	Laboratory	
Meissle et al. 2005	Maize	MON810	Laboratory	
Romeis et al. 2004	Maize	Bt11	Laboratory	
Romeis et al. 2006b+	-	-	-	-
Toth et al. 2004	Maize	MON810	Field	Hungary
Vercesi et al. 2006	Maize	MON810	Laboratory	
Vojtech et al. 2005	Maize	MON810	Laboratory	
Volkmar & Freier 2003	Maize	MON810	Field	Germany
Wandeler et al. 2002	Maize	Bt11 / Bt176	Laboratory	

Italic: Non peer-reviewed publication; +: Review article ++: Study was not available for analysis; *: Study conducted or sponsored by industry

Effects on carabid beetles

Carabid beetles belong to the most abundant invertebrates in European agroecosystems and are essential in the control of pest organisms. Carabids may be impacted by Cry1Ab toxin through preyed herbivores and through the consumption of maize plant tissues or residues. Zwahlen & Andow (2005), a publication cited by the BVL, showed that some carabid beetle species take up the Cry1Ab protein in the field. In a laboratory experiment with the carabid beetle *Poecilus cupreus* Meissle et al. (2005) found that larvae had a significantly higher mortality when fed with MON810 maize-fed *Spodoptera littoralis* than when fed with non-Bt corn-fed prey. According to Meissle et al. (2005) the observed effects are most likely indirect effects due to reduced nutritional prey quality. However, direct effects cannot be excluded. Even though Monsanto cites Meissle et al. (2005) the observed negative effects are not further discussed. On the contrary, Monsanto presents Meissle et al. (2005) as one of the publications, which demonstrate that Bt-maize has no adverse effects on non-target organisms. This is not in agreement with assessments found in scientific literature. According to Zwahlen & Andow (2005) the results of Meissle et al. suggest that some adverse effects of Bt-maize on carabid beetle abundance may be possible. They therefore recommend further investigations.

Effects on non-target butterflies

«We conclude that possible effects of Bt maize on European butterflies and moths must be evaluated more rigorously before Bt maize should be cultivated over large areas.»

Lang & Vojtech (2006)

«Data on the distribution and hence the exposure of European lepidopteran species in agricultural landscapes on a population level are still lacking, but are essential to complete the risk assessment.»

Gathmann et al. (2006a)

According to the BVL non-target German butterfly species are potentially at risk from the cultivation of MON810 maize. The BVL cites three studies which show that larvae of two North American lepidopteran species, the black swallowtail (*Papilio polyxenes*) and the monarch butterfly (*Danaus plexippus),* are negatively affected when they consume pollen of Bt176 maize (Hansen-Jesse & Obrycki 2000, Hellmich et al. 2001, Zangerl et al. 2001). In addition, the BVL cites Dively et al. (2004), who found an impact on mortality and different fitness parameters for monarch butterfly that consumed MON810 pollen throughout larval development in laboratory and semi-field trials.

As the Cry1A family of toxins is active against lepidopteran species Monsanto acknowledges in its comments to the BVL Order that the Cry1Ab protein expressed by MON810 maize can be toxic to non-target butterflies. However, citing Dively al. (2004) and Sears et al. (2001) Monsanto shows that exposure of monarch and black swallowtail butterfly populations to Bt-maize pollen is very low under field conditions. Considering both toxicity and exposure, Monsanto concludes that MON810 poses no risk to monarch and black swallowtail populations.

Can these results obtained for species occurring in North American landscapes be transferred to Germany? According to Monsanto, it is not possible to test the Cry1Ab protein against all lepidopteran insects, especially those that are endangered or threatened or otherwise protected. Therefore Monsanto assumes that a sensitive species such as the monarch butterfly provides a suitable surrogate species for risk assessment of potential effects on other lepidopterans. On this Monsanto cites Mendelsohn et al. (2003), Romeis et al. (2006a) and Wolt et al. (2005). All three publications agree that generally only a fraction of all possible non-target arthropods can be considered in risk assessment and that therefore it is necessary to select appropriate species to serve as surrogate. Is the monarch butterfly an appropriate surrogate species for German butterfly species? None of the three publications cited by Monsanto address this issue. In contrast, Romeis et al. (2006a) point out that in order to reflect biogeographical variation it is crucial to determine what species are likely to occur in the cropping systems where the transgenic plant is expected to be grown. In other words, risk assessment should encompass species that are of relevance in particular regions. Moreover, Monsanto does not consider scientists who challenge the assumption that Monarch butterfly is a suitable surrogate species for other lepidopterans. E.g., Lang & Vojtech (2006) write that results obtained for species occurring in North American agricultural landscapes cannot be transferred to Europe and that there is an urgent need for comprehensive risk assessment for European butterflies. Scholte & Dicke (2005) recommend that European non-target butterflies should be considered in risk assessment. And a recently published OECD report emphasize that in areas where agricultural land and natural habitats are more closely integrated than in the United States exposure of non-target lepidopterans merits closer scrutiny (OECD 2007).

Monsanto also ignores the prevailing uncertainty regarding the potential exposure of German lepidopteran species to MON810 maize. Gathmann et al. (2006a), for example, write that data at the population level on the distribution and hence exposure of European lepidopteran species in agricultural landscapes are still lacking. As a consequence the final determinants of risk for lepidopteran species at the population level are incomplete and further research is needed (Gathmann et al. 2006b).

In summary, by showing that the monarch and black swallowtail butterflies do not appear to be at risk from MON810 pollen, Monsanto does not prove that susceptible lepidopteran species that might have a high likelihood of exposure to MON810 maize under German landscape conditions are not at risk.

Monsanto refers on page 24 of the technical dossier to opinions of the EFSA's Scientific Panel on Genetically Modified Organisms (GMO Panel) on the safeguard clause on MON 810 maize (EFSA 2004, EFSA 2005, EFSA 2006a) invoked by Austria, Hungary and Greece. It suggests that scientific data reflect the safety of the Cry1Ab protein to non-target lepidopterans, citing the following ten publications: Dively et al. (2004), Eckert et al. (2006), Gatehouse et al. (2002), Gathmann et al. (2006a), Losey et al. (1999), Pons et al. (2005), Rauschen et al. (2004), Sears et al. (2001), Yao et al. (2006), and Zangerl et al. (2001). Five of these publications are concerned with potential effects of Cry1Ab maize on North American butterfly species, namely the black swallowtail *P. polyxenes* and the monarch butterfly *D. plexippus* (Dively et al. 2004, Gatehouse et al. 2002, Losey et al. 1999, Sears et al. 2001, Zangerl et al. 2001). As shown above results obtained with North American lepidopteran species are of limited relevance for risk assessment of German non-target lepidopteran species. Another study cited by Monsanto addresses impacts of a transgenic rice line expressing a fused *cry1ab/cry1Ac* gene on non-target silkworm *Bombyx mori* (Yao et al. 2006). As this study is about Bt-rice and the investigated non-target lepidopteran species is not native to Germany the results of Yao et al. 2006 are of little relevance for risk assessment of cultivation of MON810 maize in Germany. Two other studies cited by Monsanto do not address at all the potential impacts on non-target lepidopteran: Rauschen et al. (2004) investigated the effects of MON810 maize on cicadas and Eckert et al. (2006) monitored the abundance of a diverse set of arthropods but not the abundance of non-target lepidopterans.

Two of the ten publications cited by Monsanto are about potential impacts of Bt-maize on European non-target lepidopteran species. Pons et al. (2005) evaluated the impact of Bt176 maize on cutworms (order *Lepidoptera*, family *Noctuidae*) living in soils in a three-year study at the farm scale in Spain. *Agrotis segetum* was the only cutworm species recorded. No significant differences in Bt and non-Bt maize plots could be found in the case of this non-target lepidopteran. However, it has to be noted that the field study was conducted with Bt176 maize, which has a lower level of Cry1Ab toxin in the roots compared to MON810 maize. In addition, as the field trial was at the farm scale, Pons et al. (2005) recommend that «studies at the landscape scale should be conducted in order to assess risks over longer periods». The second publication cited addressing impacts on European non-target lepidopteran is about a three year field trial with MON810 maize in Germany conducted by Gathmann et al. (2006a). In this field trial the two most frequent non-target lepidopteran species were *Plutella xylostella* and *Pieris rapae*. No statistically significant adverse effects of MON810 pollen on larvae of these two species were found. One reason for the absence of adverse effects is probably the lack of temporal overlap between larval development and pollen shed (Gathmann et al. 2006a). Another reason could be that due to the limited number of replications the statistical power was too low for small effects on the larvae of *P. xylostella* and *P. rapae* to be detected.

In summary, only two out of the ten publications cited by Monsanto address potential impacts of Bt maize on European non-target lepidopteran species, and only one of the two studies was conducted with MON810 maize. Regarding data quality and species number tested, the two publications deliver preliminary results for only three European non-target lepidopteran species. Schmitz et al. (2003) however showed that, for Germany, 96 lepidopteran species occur in farmland areas near maize fields during maize pollen shed. In consequence, the data delivered by Monsanto do not provide the basis for a well-founded risk assessment. In other words, as the available database is small it cannot be concluded that the cultivation of MON810 maize poses no risk to German non-target lepidopteran species.

Monsanto writes on page 25 of the technical dossier that Lang et al. (2005) support the conclusion that Bt maize does not pose unreasonable adverse effects on non-target wildlife or beneficial invertebrates. However, regarding potential impacts of MON810 maize on non-target butterflies Lang et al. (2005) urgently advise comprehensive field trials with MON810 maize because very little is known about the impacts of MON810 maize on German butterfly species.

Elsewhere in the documents submitted to the BVL Monsanto cites studies, which were conducted with Cry1Ab expressing maize other than MON810 maize. However, where potential effects on non-target lepidopteran species are concerned, Monsanto changes its practice. The company overrides several hazard studies, which examined effects of Bt176-maize on European butterfly species. The laboratory studies not mentioned by Monsanto show that the consumption of Bt-176 maize pollen negatively affects larvae of *Pieris brassicae*, *Pieris rapa*, *Plutella xylostella* (Felke et al. 2002), *Inachis io* (Felke & Langenbruch 2003) and *Papilio machaon* (Lang & Vojtech 2006).

Effects on honeybees

The large quantities of pollen produced by maize flowers attract numerous insect pollinators such as honeybees, and maize pollen may be collected especially when other pollen sources are scarce (O'Callaghan et al. 2005). Therefore, honeybees in some regions may well be exposed to the pollen of MON810 maize if this crop is deployed on a large scale (Sabugosa-Madeira et al. 2007).

To addresses this issue Monsanto evaluated the dietary effects of purified Cry1Ab toxin on honeybee larvae and adults (Maggi & Sims 1994a/b). No significant effects on survival of larvae or adults were observed in these acute toxicity tests. However, as the number of replicates used in these tests was quite small (three replicates per treatment), there was a little chance of detecting real effects (Marvier 2002).

The findings of Maggi & Sims (1994a/b) are in agreement with results published in scientific literature. Hanley et al. (2003) reported no effect of Cry1Ab toxin on honeybee larvae and pupal mortality when larvae were fed Bt11-corn pollen. In the experiments of Babendreier et al. (2005) MON810 maize pollen showed no effect on bee survival. However, as the number of replicates used in the studies conducted by Hanley et al. (2003) and Babendreier et al. (2005) was small (5 and 3 replicates respectively), the power of these risk assessment experiments may be undermined (Marvier 2002).

The studies cited above focused mainly on the survival of bees exposed to Bt-maize pollen or purified Cry1Ab toxin. Babendreier et al. (2007, 2005) gained additional insights on the potential effects of MON810 maize on honeybees. They showed that feeding honeybees with pollen from MON810 maize did not affect the development of their hypopharyngeal gland (Babendreier et al. 2005) or their gut flora (Babendreier et al. 2007).

The data delivered by Monsanto and scientific publications indicate that pollen of Bt-maize do not adversely affect honeybees. However, the need for additional studies in the field may be warranted if stressors such as heat, pesticides, pathogens, and so on are suspected to alter the susceptibility of honeybees to Cry1Ab protein toxicity (Duan et al. 2008). Hitherto unpublished results from field trials in Germany do actually raise suspicion that MON810 maize could affect honeybees if biotic stressors are present (Kaatz, unpublished results). In bee colonies infested with parasitic microsporidia the number of bees and the brood size were significantly more reduced in MON810 maize-fed colonies compared to colonies fed on Bt-toxin-free pollen. The significant differences indicate an interaction of Cry1Ab toxin and pathogen with an effect on the epithelial cells of the honeybee intestine (Kaatz, unpublished results).

Effects on aquatic organisms

Repeated and large-scale use of MON810 maize may lead to exposure of Cry1Ab toxin to non-target organisms in aquatic environments. To address this issue Monsanto performed an acute toxicity test on the water flea *Daphnia magna* (Graves & Swigert 1997). No effects of Bt maize pollen on the mortality of *D. magna* could be observed in this laboratory study. However, the study has several shortcomings.

First, the toxicity test was conducted with pollen of Bt11 maize but not with MON810 maize pollen.

Second, *D. magna* may not be a good surrogate species for aquatic non-target insects, because Bt-maize is more likely to have effects on aquatic organisms through decomposition food chains, involving arthropods shredders and filter feeders, rather than through the primary production chain (algae and water fleas) (Andow & Hilbeck 2004). Independent scientists therefore recommend that aquatic non-target testing of Bt-maize should focus on mayflies, caddisflies and chironomids (Andow & Zwahlen 2004).

Third, Graves & Swigert (1997) used only three replicates. According to Marvier (2002), the use of only three replicates makes it less likely that an effect could be detected, even if it does exist.

Fourth, the duration of the laboratory test was limited to 48 hours. As this exposure time is only designed to test for acute toxicity, Monsanto does not deliver data on chronic toxicity.

Based on the data delivered by Monsanto it remains totally unknown whether aquatic organisms might be impacted by large-scale cultivation of MON810 maize in Germany.

Bt-toxin remaining in the soil of the cultivation areas; impacts on soil organisms and soil functions

«Environmental impacts on soil biota of the Bacillus thuringiensis (Bt) Cry toxin produced by maize plants are poorly known. This is a matter of regret, because about 40-50% of the Bt-maize production enter the soil on 9.1 million ha worldwide.»

Bakony et al. 2006

«Genetically modified Bacillus thuringiensis Berliner (Bt) maize (Zea mays L.) expressing Cry toxins against various target pests is now grown on more than 16 million hectares worldwide, but its potential effects on the soil ecosystem need to be further investigated.»

Zwahlen et al. 2007

Bt-toxin remaining in the soil of cultivation areas

According to the BVL the persistence of Cry1Ab toxin expressed in transgenic Bt maize remains unexplained. Citing Crecchio & Stotzky (2001) and Zwahlen et al. (2003a) the BVL points out that the Cry1Ab toxin could accumulate in the soil, opening the potential for long-term exposure to non-target soil organisms.

According to Monsanto the laboratory study by Crecchio & Stotzky (2001) and the surrogate tissue study by Zwahlen et al. (2003a) are not good indicators of environmental persistence of Cry1Ab. Contrary to the opinion of the BVL Monsanto holds the view that Cry1Ab toxin is rapidly degraded in soil, indicating a lack of exposure to non-target soil organisms. On this Monsanto mainly cites the two publications Sims & Holden (1996) and Dubelman et al. (2005). As will be shown below, the data delivered in these two publications cannot exclude the potential for the persistence of Cry1Ab toxin expressed by MON810 maize in agricultural soil in Germany. Therefore, the concerns expressed by the BVL regarding long-term exposure of non-target organisms remain unresolved.

To reinforce the opinion that Cry1Ab toxin from MON810 maize is rapidly degraded in soil Monsanto cites Sims & Holden (1996). Indeed, the results of this laboratory study, which was done by Monsanto scientists, indicate that in leaf material the Cry1Ab protein is quickly degraded. However, the laboratory study has several flaws. As Sims & Holden (1996) ground, sieved and lyophilized the plant material, they used plant material in a form in which it was readily utilizable by microorganisms. In the field, raw unprocessed plant material will enter the soil or remain on top of it. Moreover, the laboratory study was conducted at constant temperatures of 24-27 °C. As the degradation of Bt-toxins depends to a significant extent on microbial activity, which is reduced at colder temperatures, the data of Sims & Holden (1996) are unrealistic for maize fields in temperate regions with distinct cold seasons.

To reinforce the opinion that Cry1Ab toxin from MON810 maize does not accumulate in soil

Monsanto cites Dubelman et al. (2005). This field study was conducted with MON810 maize at three locations in the United States and was sponsored by Monsanto, Syngenta and Pioneer Hi-Bred. No accumulation or persistence of Cry1Ab protein was found in the environment after three years of continuous use (Dubelman et al. 2005). However, the field study has several shortcomings. First, as soil subsamples from each field were mixed to create a single composite, the probability of detecting Cry1Ab was lowered. Second, as soil samples were taken at a distance from maize plants sufficient to minimize inadvertent collection of root tissue with the soil sample, Dubelman et al. (2005) probably missed measuring Cry1Ab toxin concentrations in the rhizosphere. Higher concentrations of Cry1Ab toxin compared to the surrounding bulk soil can be found in the rhizosphere, because MON810 maize continuously releases Cry1Ab toxin via root exudates (Saxena et al. 2002). Third, as Dubelman et al. (2005) removed visible plant or root material from the sample composite, major reservoirs of the Cry1Ab toxin in soil were not considered in the field study. Fourth, it has to be pointed out that the field study was conducted in the United States and therefore the results may not be transferable to agricultural soil in Germany. Data from scientific literature indicate that persistence of Cry1Ab toxins in soil depends on the interactions between many variables such as biotic activity, soil type, crop management practices, and environmental conditions, and therefore may vary between sites and seasons (Clark et al. 2005, O'Callaghan et al. 2005). Moreover, the production of Cry proteins in MON810 maize varies with season and can be influenced by numerous environmental factors. Altogether, the available scientific data emphasize the importance of doing studies on the fate of Cry1Ab toxin under local climatic conditions and with local varieties.

To sort out the concerns expressed by the BVL Monsanto should deliver data on the persistence of Cry1Ab toxin in soils that are typical for maize-growing regions in Germany.

Impacts on soil organisms and soil functions

The BVL expresses concerns about potential negative effects of MON810 maize on soil organisms and soil function. However, according to Monsanto the potential for MON810 maize to be hazardous to non-target soil organisms is negligible. Monsanto writes on page 24 of the technical dossier that peer-reviewed scientific data reflect the safety of the Cry1Ab protein on soil organisms such as collembola, earthworms and nematodes. On page 25 of the response to the German safeguard measure Monsanto writes that several researchers have observed no adverse effects of Bt proteins on soil microflora. As will be shown below for earthworms, nematodes and microflora the data delivered by Monsanto cannot reduce the existing uncertainty regarding potential negative effects. Therefore, Monsanto does not negate the concerns expressed by the BVL.

Impacts on earthworms

Monsanto encloses the following four publications to supports its opinion that MON810 maize poses no risk to earthworms: Vercesi et al. (2006), Evans (2002), Saxena & Stotzky (2001) and Palmer & Beavers (1995). These studies indicate that there are no acute adverse effects of Bt-maize on the two earthworm species *Lumbricus terrestris* and *Aporrectodea caliginosa*. However, based on the data of these four publications sublethal long-term effects cannot be ruled out. Monsanto does not address this uncertainty at all. Nor does it appreciate the opinion of independent scientists, who recommend that further studies be conducted to rule out the possibility of long-term sublethal effects of Bt-maize on earthworms (Vercesi et al. 2006, O'Callaghan et al. 2005, Zwahlen et al. 2003a).

The publications cited by Monsanto are commented on in the following:

Palmer & Beavers 1995 conducted a 14-day acute toxicity test with the earthworm *Eisenia fetida* in an artificial soil substrate. No adverse effect could be found in this laboratory test, which was carried out on behalf of Monsanto. However, the study by Palmer & Beavers (1995) has several flaws. First, the chosen species *E. fetida* is of minor ecological relevance in maize fields (Vercesi et al. 2006). Independent scientists recommend working with earthworm species that are common in areas where transgenic plants are grown, such as *Lumbricus terrestris* and *Aporrectodea caliginosa* in European maize fields (Zwahlen et al. 2003b, Vercesi et al. 2006). Second, the study by Palmer & Beavers (1995) was performed with microbially produced Cry1Ab toxin instead of MON810 maize material. Third, the short duration time of the study does not reflect the fact that, in the field, earthworms are potentially exposed to Cry1Ab for several months. Fourth, experts in earthworm ecotoxicology recommend that the effects of Bt-maize should be evaluated on more subtle life-history characteristics of earthworms than provided by acute toxicity tests (Zwahlen et al. 2003b, Vercesi et al. 2006).

In the laboratory study by Saxena & Stotzky (2001) conducted with Bt11 maize no significant differences in percentage mortality and weight of the earthworm *L. terrestris* could be observed after 40 days in soil planted or not planted either with Bt or non-Bt maize or after 45 days in soil amended or unamended with biomass of Bt or non-Bt maize. However, Saxena & Stotzky (2001) point out that this result should be considered as being preliminary, because only one species of earthworm was evaluated. Therefore, they recommend more detailed studies to confirm the absence of Cry1Ab toxicity.

The publication by Evans (2002) is a review article on impacts of exudates from roots of transgenic Bt-plants. Regarding the impacts of Bt-maize on earthworm the only data covered by Evans (2002) are those delivered by Saxena & Stotzky (2001).

Vercesi et al. (2006) investigated the effects of MON810 maize on important life-history traits (survival, reproduction and growth) of the earthworm *A. caliginosa*. No deleterious effects on survival, growth, development or reproduction in *A. caliginosa* could be found. However, MON810 maize had a significant negative effect on cocoon hatchability. The ecological significance of this negative effect remains unclear. Vercesi et al. (2006) write that «a sensible way to follow up on the results of this and previous studies, and to bolster a sound risk assessment of Bt-corn, would probably be to assess the effects of Bt-corn on earthworm populations in carefully designed field experiments». As the authors point out, so far no published studies of earthworm populations have described the consequences of long-term cultivation of Bt-maize.

The recommendation by Vercesi et al. (2006) for further studies to be made is in agreement with other scientists (O'Callaghan et al. 2005, Zwahlen et al. 2003b). For example, Zwahlen et al. (2003b) showed that *L. terrestris* fed Bt11-maize residues over 200 days had a significant reduction in weight compared to those fed non-Bt-maize. Therefore, Zwahlen et al. (2003b) recommend that studies should be conducted for more than 200 days in order to assess long-term impacts of transgenic Bt-maize on earthworms.

Impacts on nematodes

Monsanto ignores all studies reporting negative effects of MON810 maize on nematodes.

Field tests with MON810 maize conducted in France and Denmark revealed a significant, but transient, decrease in the numbers of nematodes under MON810 maize compared to non-Bt maize (Griffith et al. 2005). The observed effects were site and season specific. In laboratory tests with *Caenorhabiditis elegans*, a slight reduction in body size, number of eggs and rates of reproduction were noted in MON810 maize (Arndt 2006). *C. elegans* also showed a possible sensitivity to MON810 maize in the field, especially in rhizosphere soil (Manachini and Lozzia 2003).

None of these publications was considered in the documents Monsanto submitted to the BVL.

Impacts on microflora

Citing Blackwood & Buyer (2004), Koskella & Stotzky (2002) and Saxena & Stotzky (2001) Monsanto argues that no negative effects of Cry1Ab toxins on soil microflora have been reported in the literature. However, as none of these three studies deals with MON810 maize they do not attest to the absence of negative effects of MON810 maize on soil microflora. In addition, Monsanto does not address the uncertainty expressed by the authors of the studies cited. The authors in all three publications cited recommend further studies be made in order to confirm the absence of negative effects. Moreover, Monsanto omits several studies in which minor effects of *cry1Ab* expressing maize on soil microorganisms have been reported.

One of the three studies cited by Monsanto is the laboratory study with Bt11 maize conducted by Saxena & Stotzky (2001). The results of this study indicate that Bt11 maize is not toxic to protozoa, bacteria and fungi. However, the results should be considered as preliminary, as only total numbers of culturable bacteria, fungi, protozoa were evaluated. Saxena & Stotzky (2001) therefore conclude that «more detailed studies on the composition and diversity of these groups of organisms are necessary to confirm the absence of the effect of the Cry1Ab toxin on biodiversity in soil.»

The in vitro study by Koskella & Stotzky (2002) is not directly about Bt-maize but about microbicidal and microbiostatic effects of Cry toxins purified from various *Bacillus thuringiensis* subspecies. The results of this in vitro study indicate that the purified toxins including Cry1Ab are not inhibitory to a variety of bacteria, fungi, and algae. However, as the effects of these toxins on the biodiversity of the microbiota in soil is probably more complex than simply a microbicidal or microbiostatic effect, Koskella & Stotzky (2002) conclude that «further studies on the effects of these toxins on biodiversity are obviously needed».

Blackwood & Buyer (2004) assessed the effects of Bt11 maize on soil microbial community structure in a growth chamber experiment. Based on their results the authors conclude that Bt effects were small and transient. However, regarding potential long-term effects

Blackwood & Buyer (2004) write: «It is important to note that, while we have concluded that the impacts of Cry protein on the soil microbial community were limited in this experiment, further long-term experiments under a variety of conditions and using a variety of assays are required. Small impacts may be amplified in the field over time, and more detailed analysis may uncover other important changes in soil microbial communities».

Monsanto ignores three studies, which show that *cry1Ab* expressing maize might have at least some minor effects on soil microorganisms. One of the publications omitted is the study done by Brusetti et al. (2004). They showed that Bt176 maize could change microbial communities in the rhizosphere. Similar results were obtained by another publication omitted by Monsanto. In greenhouse experiments Castaldini et al. (2005) detected differences between Bt176 maize and non-Bt maize in rhizospheric eubacterial communities and in mycorrhizal colonization. The third study omitted by Monsanto was conducted with MON810 and Bt176 maize. In microcosm experiments Mulder et al. (2006) observed short but robust differences in the soil community after the addition of Bt-maize straw compared to sister non-Bt conventional lines. Their data suggest that the introduction of Bt-maize has a short-lived influence on abundance, diversity, and ecosystem functioning of the bulk soil bacteria.

Farm questionnaires

The general surveillance program that will be implemented by Monsanto during 2007 consists mainly of a questionnaire to farmers. The questionnaire focuses on four sections:

(1) Maize grown area (size and location of Bt MON810 maize fields, soil characteristics, pest pressure);
(2) Agronomic practice;
(3) Observations on MON810 maize (disease, pest and weed pressure, occurrence of wildlife, performance of animals fed MON810 maize);
(4) Implementation of GM plant specific measures (compliance and stewardship data)

The use of farm questionnaires is in accordance with EFSA (ESFA 2006b) and industry (Tinland et al. 2007, 2006), who consider farm questionnaires a useful tool for collecting data on the performance and impact of a transgenic plant and for comparing the transgenic plant with conventional plants. However, it has to be pointed out that farm questionnaires have several limitations. First, the data collected by farmers are based on evaluations or estimates and not on precise measurement; second, there might be relevant changes caused by MON810 maize which scientifically untrained and unequipped farmers do not attribute to the cultivation of MON810 maize; third, conflicts of interest or social norms might influence the farmers responding; fourth, to be accepted by farmers questionnaires are restricted in length; fifth, farm questionnaires mainly cover agricultural fields and disregard the surrounding environment.

Beside these general limitations additional shortcomings can be found in the farm questionnaire developed by Monsanto. First, the questionnaire does not explicitly cover subsequent years. According to EFSA (2006b) farm questionnaires should observe fields in subsequent years for any unusual residual effects. Second, as the methodology of empirical data survey is subject to several problems, a quality control system should be implemented so as to ensure that the methodology is applied properly (Berensmeier & Schmidt 2007). No quality control system is considered in the documents submitted by Monsanto. Third, according to EFSA (2006b) applicants should establish independent audits to ensure the independence and integrity of all monitoring data. Based on the documents submitted to the BVL it remains uncertain if Monsanto will establish such an independent audit. Fourth, based on the documents submitted by Monsanto it remains unclear how many German farmers will be interviewed in 2007. It therefore also remains unclear whether the number of questionnaires will be sufficient to achieve statistical power in the data collected. Fifth, to improve the quality of the questionnaires farmers should receive supplemental training so that they can detect unexpected environmental effects. In the documents submitted by Monsanto no such training is mentioned.

Use of existing monitoring networks

Directive 2001/18/EC and Guidance note 2002/811/EC (EC 2002) recommend that existing monitoring networks for the general surveillance of transgenic plants should be used whenever they are suitable. In accordance with this recommendation the BVL made available to Monsanto a list of 35 potentially useable networks operating in the field of environmental monitoring in Germany. Monsanto selected five networks, which provide information on relevant monitoring characteristics. These networks are:

- Monitoring of game species (Wildtier-Informationssystem der Länder Deutschlands)
- Monitoring of soil (Bodendauerflächenbeobachtungsprogramm der Länder, BDF)
- Monitoring of common birds (Monitoring häufiger Brutvogelarten)
- Monitoring of butterfly population dynamics (Tagfaltermonitoring)
- Monitoring of bees (Bienenmonitoring)

These networks will be used by employingthe following methodology. Monsanto will analyse the reports published by the selected network on an annual basis in order to establish whether any potential adverse effects of MON810 maize cultivation can be identified.

As existing monitoring networks were not designed for the general surveillance of transgenic plants, the targets, the time, frequency and scale of data collection, sampling, analysis and reporting methods of these programmes are not necessarily suitable for monitoring MON810 maize. Existing networks therefore have to be analysed to see how usable they are for the general surveillance of MON810 maize in practice. According to EFSA (2006b) it is important that consent holders describe the processes and criteria that are used for the selection and evaluation of existing monitoring networks. Despite this, the process of the evaluation and selection is not described in detail by Monsanto. E.g., to analyse the relevance and suitability of the 35 existing networks made available by the BVL Monsanto developed a questionnaire for interviewing the operators of each network. Neither the questionnaire nor the answers of the operators are included in the available documents, which Monsanto submitted to the BVL.

Based on the data submitted by Monsanto it remains difficult to assess whether the five selected networks are suitable for the general surveillance of MON810 maize. In addition, based on the data submitted by Monsanto it remains difficult to assess whether Monsanto's selection is adequate or not. These issues would need further analysis.

One of the criteria used for the selection of the existing networks is that networks collect data in areas where MON810 maize is cultivated. However, to implement an adequate general surveillance, more detailed spatial planning is necessary. Without more detailed spatial planning it cannot be ensured that the monitoring sites of the existing networks are congruent with MON810 maize cultivation sites. For example, the use of existing long-term soil monitoring networks makes only sense when the data collection takes place on MON810 cultivation sites. The data available fail to show how Monsanto will ensure that MON810 cultivation sites will be integrated into the infrastructure of the existing networks.

Monsanto plans to implement the selected networks by analysing the reports published by the networks. However, as the existing networks were not developed to detect impacts of transgenic plants it is questionable whether the analysis of the reports is useful for detecting unexpected effects of MON810 maize cultivation. Based on the data available it remains unclear why Monsanto does not use the raw data from the existing network.

In its analysis of reports published by monitoring networks Monsanto has to answer two questions in the main: (1) do changes reported represent an environmental damage; and (2) is it likely that this damage has been caused by MON810 maize cultivation. As Monsanto is not specifying its definition of environmental damage, it cannot be assessed whether Monsanto will adequately analyze the published reports. As it may in most cases be impossible to establish a direct causality between a reported damage and MON810 maize cultivation is has to be concerned that, due to conflicts of interest, Monsanto's analysis might be biased in favour of neglecting potential causalities with MON810 maize.

Analysis of the ongoing literature

For general surveillance Monsanto proposes to complement the annual farm questionnaire with a detailed analysis of the ongoing scientific literature.

In accordance with this proposition the present work scanned scientific literature for publications, which were published after Monsanto's submission. As shown below the scan reveals several publications, which confirm the concerns raised by the BVL in its Order.

Effects on soil organisms

Zwahlen et al. (2007) investigated the effects of Bt11 maize on soil community in an 8-month field study. Their results do not show major changes in the composition of the soil organism community. However, subtle effects on three taxonomic groups were found, with species of Enchytraeidae (annelids), Tullbergiidae (collembolans) and Gamasina (mites) being found in lower numbers in Bt11 maize than in non-Bt maize. To date, no study has investigated the impact of Cry1Ab maize on species of these taxonomic groups (Zwahlen et al. 2007). Whether the relatively subtle effects observed by Zwahlen et al. (2007) lead to significant changes in the soil food web remains unclear. Therefore further studies are needed to assess the impact of Bt-maize on soil organisms.

In a 49-day incubation experiment carried out with field grown stem and leaf residues of MON810 maize Raubuch et al. (2007) showed that the mineralization and incorporation into the soil microbial biomass of MON810 maize was reduced in comparison to the parental non-Bt maize variety.

In a laboratory feeding trial Büchs et al. (2007) showed that larvae of *Lycoriella castanescens* (Diptera: Sciaridae) have a significant longer period until pupation when they were fed with litter of MON810 maize compared to when fed with non-Bt maize litter.

Effects on aquatic organisms

MON810 maize pollen and crop residues can be transported to adjacent streams via wind and water. Through this route water organisms can become exposed to the Cry1Ab toxin. In studies conducted in the USA Rosi-Marshall et al. (2007) showed that 50 percent of filtering caddisflies collected in an agricultural stream during peak pollen shed had pollen grains in their guts and that detrivorous caddisflies were located in accumulations of decomposing corn litter after harvest. Moreover, preliminary laboratory feeding studies showed that the leaf-shredding caddisfly, *Lepidostoma liba*, and the alga-scraping caddisfly, *Helicopsyche borealis*, could be negatively affected when they are fed Bt-maize litter as compared to non-Bt maize litter (Rosi-Marshall et al. 2007). Despite several shortcomings in the experimental setting the results of Rosi-Marshall et al. (2007) do point out that assessment of potential non-target effects from MON810 maize should be expanded to include relevant European aquatic organisms.

Bohn et al. (2008) investigated whether MON810 maize negatively impacts *D. magna*. Their laboratory study demonstrates significant and negative long-term effects after feeding MON810 maize material. Bohn et al. (2008) conclude, that the observed effects of MON810 maize on *D. magna* call for greater attention, not only on the runoff material from transgenic agricultural fields but also on the sensitivity of aquatic nontarget organisms to transgenic products.

Effects on beetles

In a tritrophic study with *Lycoriella castanescens* as prey the two predatory beetles *Poecilus cupreus* and *Atheta coriaria* consumed significantly more larvae fed with MON810 maize litter than those fed maize litter of the isogenic cultivar. Furthermore, beetle larvae fed on prey, which were raised on MON810 maize litter needed longer till pupation (Büchs et al. 2007).

Effects on the snail Helix aspersa

Snails can be exposed to the Cry1Ab-toxin from MON810 maize through direct consumption of plant material and soil in fields that have been cultivated with the transgenic maize. In a laboratory experiment Kramarz et al. (2007a) analyzed the effects of purified Cry1Ab toxin on the common european terrestrial snail, *Helix aspersa*. No detrimental effects could be observed. However, in a study with MON810 maize it was shown that *H. aspersa* could be susceptible to Bt-toxin if biotic stressors are present. Kramarz et al. (2007b) infected *H. aspersa* with nematodes and also fed them either MON810 maize or non-Bt maize. Neither exposure of snails to nematodes nor exposure of snails to MON810 maize alone affected the survival of snails. But infected snails fed MON810 maize had a lower growth rate than animals not exposed to Bt-toxin (Kramarz et al. 2007b).

Effects on the parasitoid Cotesia marginiventris

Ramírez-Romero et al. (2007) showed that parasitism rates, larval survival, development times, adult size and fecundity were significantly affected in *Cotesia marginiventris* developing on *Spodoptera frugiperda* fed MON810 maize. It is noteworthy that these effects occurred despite the relatively low levels of Bt toxin present in hosts. The results of the study by Ramírez-Romero et al. (2007) suggest that Cry1Ab protein as expressed in MON810 may have direct effect on *C. marginiventris*. The authors conclude that «the occurrence of direct effects of Cry1Ab protein on a hymenopteran parasitoid, such as *C. marginiventris*, merits further research because of the importance of these parasitoids as natural enemies in agroecosystems.»

Unexpected variation of Cry1Ab toxin levels in MON810 maize

Two recent studies on Cry1Ab expression in leaves of MON810 reveal toxin levels that differ substantially from those reported by Monsanto to the European authorities in the mid-1990s. While the mean Cry1Ab levels reported by Monsanto ranged from 7.93 to 10.34 μg Bt/g fresh weight, Nguyen & Jehle (2007) report mean levels ranging from 2.4 to 6.4 μg Bt/g fresh weight, and a study commissioned by Greenpeace report mean levels from 0.5 to 2.2 μg Bt/g fresh weight (Lorch & Then 2007). Moreover, Nguyen & Jehle (2007) and Lorch & Then (2007) not only found a high variation between MON810 plants on single fields, but also statistically significant differences between different locations. The reasons for these variations remain unclear and may be due to environmental or agricultural factors. Bruns & Abel (2007) showed that the levels of Cry1Ab toxin are increased in MON810 maize as levels of nitrogen fertilizer used to grow the crop increase.

As the level of Cry1Ab toxins in MON810 tissues affects risk assessment and resistance management plans, more information is needed on the average Cry1Ab toxin production and the way in which this production develops during the growing season.

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