

Appendix 5.1. MON 810 Literature Review – Food/Feed

MON 810 literature review (July 2012)

Appendix 5.1 - Food/Feed

Table of contents

Area of the environmental risk assessment: Food/Feed Safety - Molecular Characterization Studies.....	2
Area of the environmental risk assessment: Food/Feed Safety – Animal Feeding Studies.....	3
Area of the environmental risk assessment: Food/Feed Safety – Composition/Nutrition Studies	10
References.....	15

Review of peer-reviewed publications

Area of the environmental risk assessment: Food/Feed Safety - Molecular Characterization Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Neumann <i>et al.</i> , 2011)	<p>Objective: To investigate the genetic stability of MON 810 maize by PCR measurements using Scorpion primers.</p> <p>Experimental Design: 567 individual seeds of MON 810 maize from the same variety were collected from several fields in Germany. DNA alterations amongst individual seeds of MON 810 maize were investigated using Scorpion-PCR by a two-step procedure. First, a large number of individual plant seeds were screened with real-time PCR using event-specific Scorpion primers. Second, samples whose real-time PCR Ct values differed strongly from the mean of the Ct values were screened by SYBR green and sequenced. Briefly, genomic DNA was extracted from seeds and cloned using deferent Scorpion primers for the 5' and the 3' region of the insert. Real-time PCR was conducted under reaction conditions optimized for each Scorpion primer followed by sequencing. Further, reliability of the method was studied by determining several parameters that are required for a validation, such as specificity, cross-reactivity, relative repeatability standard deviation (RSDr) and relative reproducibility standard deviation (RSDr).</p> <p>Results: None of the selected samples showed any alterations in the sequence of the 5' plant-to-insert or 3' insert-to-plant junction. The results of the screening supported the notion that no post-transformational changes occurred and that the MON 810 construct is stable, which is in accordance with the certification for MON 810 maize. Further, the results of the validation supported that the Scorpion method was reliable and fulfils the criteria for a Phase 1 validation.</p>	The results indicate that the MON 810 construct was stable in maize seeds collected from several field trials. Further, several validation parameters studied showed that the Scorpion probe PCR technique is reliable and reproducible.	Environment	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment
			Genetic stability	There are no changes to the conclusions of safety of the initial risk assessment

Area of the environmental risk assessment: Food/Feed Safety – Animal Feeding Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Stadnik <i>et al.</i> , 2011)	<p>Objective: To evaluate the effects of MON 810 maize and 40-3-2 soybeans as dietary components on the quality of breast and thigh muscle of broilers.</p> <p>Experimental Design: Four groups of broilers (n = 20 males and 20 females) were fed with non-GM maize and soybean (controls), non- GM maize and 40-3-2 soybean, MON 810 maize and non-GM soybean or MON 810 maize and 40-3-2 soybean, respectively. Each experiment was conducted in quadruplicate. The nutrient content of the diets was formulated to meet the nutrient requirements of growing broilers. At 43 days of age, 4 broilers from each group were sacrificed after 12 h of fasting. Breast and thigh muscles were collected and stored at -24°C until analysis. The pH of minced meat homogenate was analysed and the water holding capacity (WHC) was assessed using a centrifuge method. Oxidation-reduction potential (ORP) of muscles was determined by a modified Nam and Ahn method (2003)¹. The colour parameters of a freshly cut muscle surface were measured using a spectrophotometer according to the recommendations of the American Meat Science Association. Lipid oxidation was assessed by analysing the values of thiobarbituric acid reactive substances. The data were statistically analysed using one way of analysis of variance.</p> <p>Results: No statistically significant differences were observed in the pH values or the water holding capacity of breast and thigh muscle in the broilers consuming GM or non-GM feed. There were only slight changes in the ORP and colour measurement in breast and/or thigh muscles of broiler consuming different diets. The broilers fed GM diets exhibited improved lipid stability of breast and thigh muscles.</p>	The MON 810 maize used as feed component in the broiler diet did not affect the physico-chemical parameters of broiler meat such as pH, WHC, ORP and meat colour. Further, broilers fed GM diets exhibited improved lipid stability of breast and thigh muscles.	Animal and human health.	No adverse effects were detected in this study.
			Observed parameter	Feedback on initial environmental risk assessment
			Wholesomeness	There are no changes to the conclusions of safety of the initial risk assessment.

¹Nam K.C. and Ahn D.U. (2003). Effects of ascorbic acid and anti-oxidants on the colour of irradiated ground beef. *J. Food Sci.* 68:1686–1690.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Rossi <i>et al.</i> , 2011)	<p>Objective: To compare the effect of MON 810 maize and isogenic lines on the growth of weaned piglets.</p> <p>Experimental Design: MON 810 maize and its isogenic lines were grown at two farms in Italy. After a pre-experimental period of 13 days, 128 weaned piglets weighing 8.8 ± 1.27 kg live weight (LV) were randomly assigned to 4 groups of 32 animals each (16 castrated males and 16 females) and fed experimental diets containing 33% of either MON 810 or isogenic maize from each farm for 35 days. The experimental period was divided in 2 phases i.e. Days 0-14 and 15-35. Fourteen and 35 days after the start of the study, feed remaining in the feeders was weighed to calculate intake. Animals were also individually weighed on Days 0, 14 and 35. The average daily gain (ADG) during three feeding phases (Days 0-14, 15-35 and 0-35 after the start of the experimental period) was recorded for each replicate. The adjusted feed conversion ratio (F:G) was calculated by dividing the total daily feed intake by the total weight of live and dead piglets in each group. All piglets that died during the study were weighed within 24 h of death. Composition analysis of MON 810 and non-Bt maize was also performed. Data were statistically analysed using a completely randomised block design with a factorial arrangement.</p> <p>Results: MON 810 maize had the same chemical composition as the isogenic maize but a lower content (about 73%) of fumonisin B1 (FB1). All the diets were isonitrogenous and isoenergetic. ADG with MON 810 maize was higher for the entire study period (Bt: 396.4 g/day, isogenic: 374.1 g/day, $p < 0.05$) and between day 15 and 35 (Bt: 464.1 g/day, isogenic: 429.1 g/day, $p < 0.05$). The ADG of the whole period decreased linearly ($p < 0.05$) with respect to the FB1 content of the diet. Final weight was higher in piglets fed the diet containing MON 810 maize (Bt: 22.68 kg, isogenic: 21.83 kg; $p < 0.05$). No differences in feed intake or in F:G ratio were observed, although F:G ratio decreased linearly ($p < 0.05$) with the FB1 content of the diet.</p>	<p>Weaned piglets fed MON 810 maize performed better than piglets fed conventional maize. The authors stated that this was related to a lower FB1 content in MON 810 maize.</p>	Animal and human health	No adverse effects were detected in this study
		<p>The authors further stated that these findings, along with results of previous studies, suggest that including MON 810 maize in diets for weaned piglets does not have any detrimental effect and can improve performances due to lower FB1 levels.</p>	Observed parameter	Feedback on initial environmental risk assessment
			Wholesomeness	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Swiatkiewicz <i>et al.</i> , 2011)	<p>Objective: To estimate the effect of feeding swine with genetically modified soybean meal and maize on fattening results and fate of transgenic DNA in swine tissues.</p> <p>Experimental Design: Forty eight swine weighing around 30-110 kg were used. All animals received isonitrogenous and isoenergetic feed mixtures containing, or not, genetically modified (GM) Roundup Ready soybean or MON 810 maize meal. The animals were divided into four groups: I – controls (conventional soybean and maize meal); II – GM soybean and conventional maize meal; III – conventional soybean and GM maize meal; IV – GM soybean and maize meal. Proximal analysis was conducted according to AOAC standard methods. Meat colour was measured with a colorimeter Minolta CR-310. Water-holding capacity was estimated according to the Grau and Hamm method and meat acidity was recorded with a pH meter. Further, at termination, samples of digesta from the stomach, duodenum, jejunum, caecum and colon, as well as samples of the liver, spleen, lung, <i>longissimus</i> muscle and blood were collected from six swine of each group and submitted to DNA extraction and PCR analysis.</p> <p>Results: No statistical differences were observed across dietary treatments in any of the performance parameters, carcass measurements, meat quality indices or chemical analysis results. Roundup Ready soybean and MON 810 maize DNA was not detected in the digesta of the distal intestine, blood, liver, spleen, lung or muscle. Histopathological analyses showed no differences between controls and the groups receiving GM feed in their diet.</p>	<p>The authors concluded that “<i>glyphosate-tolerant soybean meal (Roundup Ready) and insect-resistant MON 810 maize did not affect the fattening results and carcass and meat quality of fattened pigs. The transgenic DNA was not detected in any tissues and content of distal parts of alimentary tract. The presence of transgenes only in the content of the stomach and duodenum confirmed the efficiency of digestion process in the gastrointestinal tract. The GM feeds did not cause any histopathological changes in the examined issues.</i>”</p>	Animal and human health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment
			Wholesomeness	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Walsch <i>et al.</i> , 2011)	<p>Objective: To evaluate the effects of insect-resistant <i>Bacillus thuringiensis</i> (Bt) maize (MON 810) feeding in pigs on the peripheral and systemic immune responses and investigate the <i>in vivo</i> fate of transgenic DNA and protein.</p> <p>Experimental Design: Two experiments were conducted. In the first one, 32 crossbred male pigs of about 28 days of age were assigned to one of two treatments: (1) Genetically modified (GM) MON 810 maize (Pioneer variety PR34N44) or (2) non-GM isogenic parent line (Pioneer variety PR34N43). Corn sources were grown at, and purchased from independent adjacent farms.¹ Pigs were housed in individual pens in four rooms with 8 pigs/room.² A non-GM starter diet was fed for the first 6 days post-weaning, then the experimental diets containing 39% of MON 810 or non-GM maize were given <i>ad libitum</i> for 31 days. On Day 31, 10 pigs per treatment were sacrificed. The heart, liver, spleen, kidneys, semi-tendinosus muscle and entire gastrointestinal tract were removed. Blood samples were collected on Days 0 and 29, from which plasma was stored for analysis of anti-Cry1Ab IgG and IgM. Peripheral blood mononuclear cells (PBMC) were isolated, cultured and the culture supernatants were analysed for cytokines using ELISA. Tissues and digesta samples were analysed for <i>cry1Ab</i> gene by PCR and Cry1Ab protein by commercial ELISA. In the second experiment, 20 pigs blocked by weight and litter and housed in a single room in individual pens were fed with starter diets from Days 0 to 7 post-weaning and experimental diets from Days 7 to 35. Bodyweight and feed consumption were recorded on Days 0 and 30. On Day 35, 10 pigs from each treatment were sacrificed and spleen samples were collected for splenocyte isolation. Ileal samples were collected for isolation of lamina propria and intraepithelial lymphocyte (LPL and IEL respectively). All data were analyzed as a completely randomized block design using SAS software.</p> <p>Results: Feeding MON 810 maize had no effect on growth performance or bodyweight of pigs.</p> <p>Production of IL-12 and IFNγ from mitogenic stimulated PBMC ($P < 0.10$) was decreased following 31 days of MON 810 treatment. While Cry1Ab-specific IgG and IgA were not detected in the plasma of MON 810 maize-fed pigs, the detection of <i>cry1Ab</i> gene and Cry1Ab protein was limited to the gastrointestinal digesta (and were not found in any organ or blood sample). IL-6 and IL-4 production from isolated splenocytes was increased ($P < 0.05$) in response to feeding MON 810 while the proportion of CD4⁺ T cells in the spleen decreased.</p>	<p>Short-term feeding of MON 810 to weanling pigs has shown no adverse effect on growth performance or bodyweight. Maize-derived DNA, either of intrinsic or recombinant origin, was largely degraded in the gastro intestinal tract (GIT). There was no evidence of the <i>cry1Ab</i> gene or Cry1Ab protein translocation to organs or plasma.</p> <p>The Cry1Ab protein was detected in GIT digesta but at very low concentrations. Dietary exposure of MON 810 maize did induce some alterations in localized and peripheral immune responses in weanling pigs which require further investigation. However, the lack of Cry1Ab specific Ig production in plasma suggests that the immune response was not allergenic. The lack of recombinant DNA or protein translocation to tissues or changes in growth should help offer assurance to consumers as to the safety of GM</p>	Animal and human health	Authors indicate that there were some significant differences in cytokines but these were inconsistent and authors conclude they may be due to endotoxins. They did not feel there was a negative effect on health.
			Observed parameter	Feedback on initial environmental risk assessment
			Wholesomeness, dietary fate of the DNA, and allergenicity	There are no changes to the conclusions of safety of the initial risk assessment

¹ Corn sources were from independent farms and no indication if similar agronomic practices were used at both sites. Corn sources were tested for mycotoxins but this can be variable within batches of corn. A mycotoxin inhibitor was added to the diet that suggests previous issues with mycotoxins.

² Authors do not indicate if 4 pigs/treatment per room and if room was then a blocking factor in the model, which would be the only appropriate statistical design.

	<p>In the ileum, the proportion of B cells and macrophages decreased while the proportion of CD4⁺ T cells increased in MON 810 maize-fed pigs. IL-8 and IL-4 production from isolated IEL and LPL were also increased ($P < 0.05$) in response to feeding MON 810 maize.³ Anti-Cry1Ab IgF or IgM were not detected. Endogenous or transgenic genes were not detectable in white blood cells or organs of either GM or non-GM fed pigs. Cry1Ab protein was similarly not detected except for low levels in digesta.</p>	<p>ingredients.</p>		
--	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	---------------------	--	--

³ Cytokine results were not consistent between tissues or Experiment #1 & 2; however, no changes in cytokines are related to allergenicity.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Walsch <i>et al.</i> , 2012)	<p>Objective: To evaluate the effects feeding of insect resistant <i>Bacillus thuringiensis</i> (Bt) MON 810 maize on the growth performance, intestinal morphology and organ pathology of pigs.</p> <p>Experimental Design: Thirty-two crossbred weanling male pigs were weaned at approximately 28 days of age and randomly assigned to one of the two dietary treatments: (1) non-genetically modified (GM) isogenic parent maize line (Pioneer PR34N43) and (2) 38.9% GM maize (Pioneer PR34N44 event MON 810). Maize was grown at separate farms located 70 km apart.⁴ Pigs were housed in individual pens in four rooms with both treatments in a room. A non-GM starter diet was fed <i>ad libitum</i> for the first 6 days post-weaning during an acclimatisation period and either the non-GM or MON 810 experimental diets were fed for the remaining 31 days of the study. Proximate and amino acid analyses were performed on the diets. Bodyweight and feed disappearance were recorded on a weekly basis (n = 16 per treatment) and 10 pigs per treatment were sacrificed on Day 31 for the collection of organ, tissue and blood samples. Blood chemistries for assessing liver and kidney function were determined and tissue/organ samples were processed for histopathological examination. All the data were analysed as a completely randomised block design with initial weight as a block⁵ using the GLM procedures of SAS. The level of significance for all tests was $p < 0.05$ and trends were reported up to $p = 0.10$.</p> <p>Results: Pigs fed MON 810 maize- consumed more feed than control pigs during the 31 day study ($p < 0.05$) and were less efficient at converting feed to gain during days 14–30 ($p < 0.01$). The weight of kidneys of the pigs fed MON 810 maize was higher than that of control animals ($p = 0.06$). However, no corresponding histopathological changes or alterations in the blood biochemistry were seen. There was no treatment-related difference in the morphology of the small intestine. However, duodenal villi of MON 810 maize-fed pigs tended to have fewer goblet cells/μm of villus compared with control pigs ($p < 0.10$).</p>	<p>The short-term feeding of MON 810 maize to young pigs demonstrated no adverse effect on the growth performance or intestinal morphology. There were no changes in organ weights except for the increase in kidney weight, which was not associated with histopathological or blood biochemical changes.</p> <p>Due to the similarities in gastrointestinal anatomy and physiology between pigs and humans, similar responses to MON 810 maize consumption could be expected in humans. Therefore, the present findings offer some assurance to consumers regarding the safety of short-term exposure to MON 810 as a food and feed ingredients.</p>	Observed parameter	Feedback on initial environmental risk assessment
			Animal and human health	Toxicology

⁴ No indication of differences in agronomic practices between these farms.

⁵ Authors do not indicate how this was done and based on the design the model is not correct since room should also be a blocking factor.

⁶ There were minor numerical differences in starch, ADF, sucrose and enzyme-resistant starch, but these cannot be shown to be statistically significant due to lack of replication and are probably just normal variability associated with these assays.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Sissener <i>et al.</i> , 2011)	<p>Objective: To follow up on apparent differences in growth, relative organ sizes, cellular stress and immune function in Atlantic salmon fed diets containing MON 810 or non-GM parental maize.</p> <p>Experimental Design: Atlantic salmon, <i>S. salar</i>, received feed containing MON 810 maize or a near-isogenic non-GM line. Mycotoxin analysis and metabolic profiling (with GC-MS) were conducted on the feed to determine possible confounding factors. Fish were placed in tanks (3/diet; 45 fish per tank) and provided with the respective diet <i>ad libitum</i> for 82 days. Tissues from the liver and distal intestine were collected and RNA was isolated. Gene expression profiling was performed by microarray and selected genes were followed by quantitative PCR (qPCR). The scale-normalised intestinal data from microarray was subjected to correspondence and significance analysis (SAM). For qPCR data, cycle threshold values were calculated using the second maximum derivative method in LightCycler® software.</p> <p>Results: MON 810 maize feed contained 90 µg/kg of deoxynivalenol (DON)² while levels were below the detection limit in control feed. Differences were also observed in the metabolite profiles of the two maize varieties, some of which appeared linked with mycotoxin levels. In the fish livers, qPCR revealed some differentially regulated genes, including up-regulation of the gelsolin precursor, down-regulation of the ferritin heavy subunit and a tendency towards down-regulation of metallothionein (MT)-B. Combined with the up-regulation of anti-apoptotic protein NR13, ferritin heavy chain and MT-A and MT-B in the distal intestine, this suggests changes in cellular stress/antioxidant status. This strengthens previous findings. Discrepancies between microarray and qPCR may be the result of artifacts rather than true biological effects.</p>	Observed changes may have been related to mycotoxins based on effects of DON in other species. Information of the effects of mycotoxins in fish is limited. Observed effects are confounded between DON level and GM event.	Animal and human health	Data from the present study suggested that the effects the authors were previously attributing to MON 810 maize may have been due to the presence of mycotoxins in the feed. Contamination with mycotoxins would not be due to the MON 810 event itself but to conditions in the field during cultivation and/or storage.
			Observed parameter	Feedback on initial environmental risk assessment
			Animal gene expression	There are no changes to the conclusions of safety of the initial risk assessment.

¹ Data suggest that there may have been an effect on palatability, reflecting the presence of molds.

² Although several mycotoxins were measured and DON was only moderately elevated, numerous other mycotoxins that can be present were not measured.

Area of the environmental risk assessment: Food/Feed Safety – Composition/Nutrition Studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Balsamo <i>et al.</i> , 2011)	<p>Objective: To compare the leaf proteomic profiles of four Brazilian MON 810 maize varieties and their four non-GM isogenic varieties using two-dimensional gel electrophoresis (2-DE).</p> <p>Experimental Design: The study used seeds from four MON 810 varieties (DKBYG 240, DKBYG 330, DKBYG 350, AGYG 6018) and four non-GM varieties (DKB 240, DKB 330, DKB 350, AG 6018). Plants were grown side-by-side for two years and three protein extractions were carried out from each variety in each year, generating six two dimensional gel electrophoresis (2-DE) patterns from each variety. Genomic DNA of the eight varieties was isolated from leaves using a cetyl trimethylammonium bromide-based method. Samples were subjected to nested PCR to confirm whether they were indeed MON 810. Certified reference material (5% MON 810) was used as a positive control. 2-DE coupled to mass spectrometry was used to compare the leaf proteomic profiles. Protein identification was performed using the Mascot Software Database (MSDB). Physiological parameters and total protein content of leaves was determined; chlorophyll content was analysed using spectrophotometry. The data were analysed by one-way analysis of variance and compared by the <i>F</i>- test, followed by a <i>post-hoc</i> test of Tukey's multiple range.</p> <p>Results: There were some differences between the four MON 810 varieties and their non-GM isogenic counterparts with regard to physiological parameters such as aerial part weight, main leaf length and chlorophyll content. These differences were not constant among all MON 810 and non-GM counterparts. The number of detected spots did not differ significantly between GM and non-GM isogenic varieties. Twelve exclusive proteins were observed in two of four MON 810 varieties and these leaf proteins were variety-specific.</p>	Leaf proteomes of four varieties of MON 810 were similar to those of non-GM counterparts.	Animal and human health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment
			Compositional change	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Coll <i>et al.</i> , 2011)	<p>Objective: To assess possible unintended effects of the MON 810 transgene in different commercial varieties using proteomic analysis.</p> <p>Experimental Design: Seeds from two MON 810 varieties (PR33P67 and DKC6575) and their corresponding near-isogenic lines (PR33P66 and Tietar) were grown in Catalonia, Spain. Maize grains of 3 plants from different micro-plot were harvested 40 days after flowering. Two biological replicates from the micro-plots were sampled per maize variety. Protein extraction was performed using a modified thiourea/urea protein solubilisation method. Three protein extractions from each sample were analysed by two-dimensional gel electrophoresis (2-DE). ImageMaster Platinum software v.2.0 (GE Healthcare) was used to process and analyse scanned images. Further, spots of interest were manually excised from 2-DE gels, processed and analysed by an ESI-Ion Trap mass spectrometer. Proteins were identified from their peptide mass fingerprint by searching the Mass Spectrometry protein sequence Data Base (MSDB).</p> <p>Results: The PR33P67 and PR33P66 virtual gels had 737 and 698 spots, respectively, including 601 common spots. DKC6575 and Tietar gels had 535 and 478 spots, respectively, with 335 common spots. No quantitative differences were observed in analysed proteins in any of the two MON 810 and non-GM varieties. A total of 4 spots were accumulated at higher levels in DKC6575 than Tietar (1.2% of analysed spots) and 4 spots (0.7% of analysed ones) were differentially accumulated in PR33P67 and PR33P66: 3 up-regulated and 1 down-regulated in the transgenic variety. Re-analysis of 2-DE gels for the possible presence of absolute differences revealed two additional spots below the detection limit in PR33P67 while present in conventional PR33P66 samples. Further, LC-MS/MS analysis showed that at least one presumed identity could be assigned to 9 out of 10 quantitative and absolute differential spots.</p>	<p>The authors concluded that “<i>MON 810 and non-genetically modified milky starchy grains had virtually identical proteomic patterns, with a very small number of spots showing fold-variations in the 1-1.8 range. They were all variety-specific and had divergent identities and functions. Although 2-DE allows the analysis of a limited dataset our results support substantial equivalence between MON 810 and comparable non-GM varieties.</i>”</p>	Animal and human health	No adverse effects were detected in this study.
			Observed parameter	Feedback on initial environmental risk assessment
			Compositional change	There are no changes to the conclusions of safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
<p>(Zhou <i>et al.</i>, 2011)</p>	<p>Objective: To assess the compositional equivalence of grain from MON 810 maize and seed from 40-3-2 soybean to conventional counterparts over multiple seasons, locations and germplasms.</p> <p>Experimental Design: For maize, a total of 74 MON 810 hybrids were grown in Honduras, Europe and Brazil and conventional reference varieties were grown in the US, Mexico, Europe and Brazil from 2000 to 2009. For soybean, a total of 112 commercial 40-3-2 varieties were grown in Canada (2002 - 2005) while conventional reference soybean varieties were grown in US and Canada (2000 - 2009). Normal agronomic practices were followed. Glyphosate treatment of the 40-3-2 samples was performed at commercially relevant rates. For both soybean and maize, the analysis of fat, ash and moisture was conducted using AOAC methods. Protein content was determined using a Kjeldahl technique. Carbohydrates content was calculated. The level of isoflavones in soybean was determined by HPLC; lectin content was determined using hematocrit of lyophilized rabbit blood and trypsin inhibitors were measured using an AOCS method. For all samples, compositional analysis was conducted within 3-9 months of harvest. Meta-analysis was performed using MetaEasy. Statistics were generated by the SAS MEANS procedure.</p> <p>Results: The mean and ranges of analyte levels such as ash, carbohydrate, fat, moisture and protein for MON 810 and conventional maize grain were consistently similar for samples from 2001 to 2009. Likewise, the means and range values for 40-3-2 and conventional soybean analyte levels were consistently similar to each other within each corresponding year from 2000 to 2009.</p>	<p>Assessment of compositional data from a total of 74 MON 810 maize and 112 40-3-2 soybean varieties grown over at least nine growing seasons encompassing diverse geographic areas highlighted the stability in compositional equivalence to conventional reference varieties.</p>	<p>Animal and human health</p>	<p>No adverse effects were detected in this study.</p>
		<p>The authors concluded that <i>“this approach, combined with graphical approaches, provided an effective summary of the overall data set and confirmed the continued compositional equivalence of these important crops to their conventional counterparts over time.”</i></p>	<p>Observed parameter</p>	<p>Feedback on initial environmental risk assessment</p>
		<p>Compositional change</p>	<p>There are no changes to the conclusions of safety of the initial risk assessment.</p>	

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Frank <i>et al.</i> , 2012)	<p>Objective: To investigate the metabolite profiles of MON 810 and herbicide tolerant Roundup Ready maize, and the relative impact of genetic modification versus environmental influence on the metabolite profiles.</p> <p>Experimental Design: Two sets of transgenic crops: (a) MON 810 maize (DKC78-15B, TXP 138-F) and (b) Roundup Ready maize (DKC78-35R; NK603 event) and their respective conventional counterparts were grown in several locations and seasons in South Africa and Germany. A sample of 40 g of maize kernels from each field was frozen and ground. The flour was freeze-dried for 48 h and stored at -18°C until analysis. A metabolite profiling approach based on gas chromatography-mass spectrometry (GC-MS) was applied to analyse a broad spectrum of primary metabolites (e.g. fatty acid methyl esters) as well as of nutritionally relevant minor maize constituents (e.g. tocopherols) by means of multivariate principal component analyses (PCA) and univariate (ANOVA) statistical analyses.</p> <p>Results: There were significant differences between the GM and non-GM maize lines. For the factor genotype, 4% (Bt TXP 138-F) and 3% (DKC78-15B and DKC78-35R) of the total number of peaks detected by GC-MS showed statistically significant differences in peak height ($p < 0.01$) as compared to the respective near-isogenic lines. However, ANOVA for the factor environment (growing location, season) revealed higher numbers of significant differences ($p < 0.01$) between the GM and the non-GM maize grown in Germany (42%) and South Africa (10%), respectively.</p>	<p>Finding of the present study indicate that the majority of differences observed are related to natural variability rather than to genetic modifications. In addition, multivariate data assessment by means of PCA revealed that environmental factors (i.e. growing locations and seasons) were dominant parameters driving the variability of the maize metabolite profiles.</p>	Animal and human health	No adverse effects were detected in this study.
			Observed parameter	Feedback on initial environmental risk assessment
			Compositional change	There are no changes to the conclusions of safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kamota <i>et al.</i> , 2011)	<p>Objective: To evaluate the effects of ensiling insect-resistant <i>Bacillus thuringiensis</i> (Bt) MON 810 maize on degradation of the Cry1Ab protein and compositional quality of silage.</p> <p>Experimental Design: Two studies were conducted with MON 810 varieties in 2008/2009 and 2009/2010, respectively, at the University of Fort Hare, South Africa. One MON 810 maize cultivar (DKC80-12B) and its near-isogenic line (DKC80-10) were grown in the first study while two <i>Bt</i> maize cultivars (DKC61-25B, PAN6Q-321B) and their isolines (DKC61-24, PAN6777) were grown in the second study. At the three-quarter milk-line stage, samples of the maize hybrids were collected by cutting whole plants at 30 cm above the ground, then chopped to <10 mm pieces. A sample of approximately 700 g was ensiled in 1 L glass jars used as mini-silos. The material was compacted and consolidated leaving no headspace and sealed with lids that did not allow gas exchange. Three replicates per treatment for each of the sampling intervals were prepared. The jars were stored at 24 to 28°C in the dark. Destructive sampling was done for fresh non-ensiled material and silages after 2, 4, 8, 15 and 42 days of ensiling for 2008/2009. In 2009/2010, only initial samples and those after 8 and 42 days of ensiling were collected. The samples were analysed for ash-free dry matter (AFDM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and crude protein. Cry1Ab protein was estimated by ELISA. Statistical analysis was performed with two-way ANOVA using GenStat Release 7.22 DE.</p> <p>Results: Silage from two of the MON 810 maize varieties had lower AFDM than their near-isolines, while variety DKC61-25B was similar to its near-isoline. There was no consistent degradation of Cry1Ab protein during the ensiling period; at least 60% of the initial protein concentration remained in the silage at the end of the study. MON 810 maize had higher NDF and ADF than its isolines before ensiling. This remained constant after ensiling in 2008/2009, but was neither influenced by ensiling nor variety in 2009/2010. Crude protein and ADL in MON 810 silages were similar to those in silages derived from the near-isolines.</p>	<p>The findings of the study suggest that modification of maize with <i>B. thuringiensis</i> gene (event MON 810) did not essentially influence the compositional quality of silage. Final Cry1Ab protein levels in MON810 silage were unchanged in one 42-day study or reduced 27-40% in a similar study the following year.</p>	Animal and human health	No adverse effects were detected in this study ⁷ .
			Observed parameter	Feedback on initial environmental risk assessment
			Compositional change	There are no changes to the conclusions of safety of the initial risk assessment.

⁷ This article reconfirms what has already been shown in other studies of Cry1Ab - that ensilement does not lead to complete degradation of the Cry1Ab protein or its activity. These previous studies are noted by the authors in the body of the article.

References

- Balsamo GM, Cangahuala-Inocente GC, Bertoldo JB, Terenzi H and Arisi ACM, 2011. Proteomic analysis of four brazilian MON810 maize varieties and their four non-genetically-modified isogenic varieties. *Journal of Agricultural and Food Chemistry*, 59, 11553-11559.
- Coll A, Nadal A, Rossignol M, Puigdomenech P and Pla M, 2011. Proteomic analysis of MON810 and comparable non-GM maize varieties grown in agricultural fields. *Transgenic Research*, 20, 939-949.
- Frank T, Roehlig RM, Davies HV, Barros E and Engel K-H, 2012. Metabolite profiling of maize kernels-genetic modification versus environmental influence. *Journal of Agricultural and Food Chemistry*, 60, 3005-3012.
- Kamota A, Muchaonyerwa P and Mnkeni PNS, 2011. Effects of ensiling of *Bacillus thuringiensis* (*Bt*) maize (MON810) on degradation of the crystal 1Ab (Cry1Ab) protein and compositional quality of silage. *African Journal of Biotechnology*, 10, 17484-17489.
- Nam KC and Ahn DU, 2003. Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. *Journal of Food Science*, 68, 1686-1690.
- Neumann G, Brandes C, Joachimsthaler A and Hochegger R, 2011. Assessment of the genetic stability of GMOs with a detailed examination of MON810 using Scorpion probes. *European Food Research and Technology*, 233, 19-30.
- Rossi F, Morlacchini M, Fusconi G, Pietri A and Piva G, 2011. Effect of insertion of *Bt* gene in corn and different fumonisin content on growth performance of weaned piglets. *Italian Journal of Animal Science*, 10, e19.
- Sissener NH, Hemre G-I, Lall SP, Sagstad A, Petersen K, Williams J, Rohloff J and Sanden M, 2011. Are apparent negative effects of feeding GM MON810 maize to Atlantic salmon, *Salmo salar*, caused by confounding factors? *British Journal of Nutrition*, 106, 42-56.
- Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz S and Kwiatek K, 2011. Effect of genetically modified, insect resistant corn (MON810) and glyphosate tolerant soybean meal (Roundup Ready) on physico-chemical properties of broilers' breast and thigh muscles. *Bulletin of the Veterinary Institute in Pulawy*, 55, 541-546.
- Swiatkiewicz M, Hanczakowska E, Twardowska M, Mazur M, Kwiatek K, Kozaczynski W, Swiatkiewicz S and Sieradzki Z, 2011. Effect of genetically modified feeds on fattening results and transfer of transgenic DNA to swine tissues *Bulletin of the Veterinary Institute in Pulawy*, 55, 121-125.
- Walsch MC, Buzoianu SG, Gardiner GE, Rea MC, Gelencser E, Janosi A, Epstein MM, Ross RP and Lawlor PG, 2011. Fate of transgenic DNA from orally administered *Bt* MON810 maize and effects on immune response and growth in pigs. *PLoS ONE*, 6,
- Walsch MC, Buzoianu SG, Gardiner GE, Rea MC, Ross RP, Cassidy JP and Lawlor PG, 2012. Effects of short-term feeding of *Bt* MON810 maize on growth performance, organ morphology and function in pigs. *British Journal of Nutrition*, 107, 364-371.
- Zhou J, Harrigan GG, Berman KH, Webb EG, Klusmeyer TH and Nemeth MA, 2011. Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms. *Journal of Agricultural and Food Chemistry*, 59, 8822-8828.