

Appendix 5.1. MON 810 Literature Review – Food/Feed

MON 810 literature review (July 2016)

Appendix 5.1 - Food/Feed

Table of contents

Area of the environmental risk assessment: Food/Feed Safety – Toxicology / Animal Feeding Study	2
Area of the environmental risk assessment: Food/Feed Safety – Allergenicity studies of the protein or the whole food/feed.....	7
Area of the environmental risk assessment: Food/Feed Safety – Crop compositional studies	9
References.....	10

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Czerwiński <i>et al.</i> The use of genetically modified Roundup Ready soybean meal and genetically modified MON 810 maize in broiler chicken diets. Part 1. Effects on performance and blood lymphocyte subpopulations, (2015 -a)	<p>Objective: To determine whether feed containing grains from genetically modified (GM) MON 810 maize expressing the <i>Bacillus thuringiensis</i> Cry1Ab insecticidal protein and Roundup Ready GTS 40-3-2 herbicide tolerant soybean affects performance and cell-mediated immunity in broiler chicken.</p> <p>Experimental Design: Four cultivars of maize were grown in Poland: 2 containing the MON 810 event, Bacilla and PR39 F56, and 2 non-GM near-isogenic counterparts, Clarica and PR39 F58. Eight experimental diets were formulated to meet or exceed the nutrient requirements for broilers: conventional or GTS 40-3-2 soybean meal was combined with Bacilla, PR39 F56, Clarica or PR 39 F58 maize. Samples of each feed were analyzed in four replicates to determine chemical composition. A total of 192 one-day-old Ross 308 feather-sexed broiler females were randomly assigned to 8 treatments of 24 birds each and fed experimental diets <i>ad libitum</i>. From Day 8 to 28 of age, 18 chickens per group were housed in individual cages and performance parameters (feed intake, body weight gain and feed conversion ratio, FCR) were measured. Prior to slaughter, the chickens were weighed and anaesthetized; in eight randomly selected chickens per group, 10 ml of blood from the heart were taken and kept at 4°C until analysis. The chickens were killed by cervical dislocation, the gonads were examined to verify the sex, the spleen and liver were excised and weighed. Peripheral blood mononuclear cells (PBMC) were isolated from blood samples, according to a standard procedure. The amount of T-helper and T-cytotoxic cells was determined by Flow Cytometry. Distribution analyses were performed using SAS software, while statistical analysis was performed using three-way analysis of variance.</p> <p>Results: The chemical composition of the different feeds was within ranges reported in the literature. The use of GM soybean meal in broiler diets had no adverse effect on either</p>	The authors concluded that: <i>'The use of genetically modified Roundup Ready soybean meal (GTS 40-3-2) and/or genetically modified maize (MON 810) in broiler chicken diets did not negatively affect feed efficiency and blood lymphocyte subpopulations of the birds.'</i>	Animal health	No adverse effects were determined in this study
			Observed parameter	Feedback on initial environmental risk assessment

	<p>feed intake or body weight gain, but improved the feed-to-gain ratio as compared to non-GM soybean meal. In contrast, feed intake and body weight gain of broilers fed GM maize was lower as compared to birds fed conventional maize, while the feed-to-gain ratio was not affected. Neither GM soybean nor maize affected liver weight. Spleen weight was about 13% greater in birds given GM feed in comparison with conventional maize, while it was not affected by other dietary treatments. The percentage of circulating lymphocytes in their entire population averaged 10 to 17% and 6.5 to 12.2% (T helper and T cytotoxic, respectively) for all groups except that fed conventional Clerica maize and non-modified soybean meal (31 and 20.6%, respectively).</p>			
--	---	--	--	--

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
<p>Czerwiński <i>et al.</i> The use of genetically modified Roundup Ready soybean meal and genetically modified MON 810 maize in broiler chicken diets. Part 2. Functional status of the small intestine (2015 -b)</p>	<p>Objective: To determine whether feed containing grains from genetically modified (GM) MON 810 maize expressing the <i>Bacillus thuringiensis</i> Cry1Ab insecticidal protein and Roundup Ready GTS 40-3-2 herbicide tolerant soybean affects the morphology and functional status of the small intestine in broiler chicken.</p> <p>Experimental Design: Four cultivars of maize were grown in Poland: 2 containing the MON 810 event, Bacilla and PR39 F56, and 2 non-GM near-isogenic counterparts, Clarica and PR39 F58. Eight diets were formulated to meet or exceed the nutrient requirements for broilers: conventional or GM GTS 40-3-2 soybean meal was combined with Bacilla, PR39 F56, Clarica or PR 39 F58 maize. A total of 192 one-day-old Ross 308 feather-sexed broiler females were randomly assigned to 8 experimental treatments, 24 birds each, and fed experimental diets <i>ad libitum</i>. From Day 8 to 28 of age, 18 chickens per group were kept in individual cages and performance parameters were measured individually. On Day 30, eight chickens per group were killed by cervical dislocation and three segments of the small intestine (2 from the jejunum and one from ileum) were excised. Epithelial cells from one of the two segments from jejunum were isolated and used to perform the Comet assay according to a standard procedure. The remaining segments from jejunum and ileum were fixed and paraffin-embedded samples were prepared. The slides were used to perform 1) haematoxylin-eosin staining to determine the morphological status of the small intestine; 2) TUNEL staining to evaluate the number of apoptotic cells; and 3) immunostaining to detect proliferating crypt cells. Statistical analysis was performed by using three-way analysis of variance.</p> <p>Results: The functional status of the small intestine was analysed by morphological evaluation of jejunum and ileum sections (villus length, crypt depth and tunica muscularis width), calculation of apoptotic and mitotic indices and measurement of DNA damage in jejunal epithelial cells. There</p>	<p>The authors concluded that: <i>'The functional development and maturation of the small intestinal epithelium is not greatly affected by the use of genetically modified Roundup Ready soybean meal (GTS 40-3-2) and/or genetically modified maize (MON 810) in broiler diets.'</i></p>	Animal health	No adverse effects were determined in this study
			Observed parameter	Feedback on initial environmental risk assessment
			Animal physiology	There are no changes to the conclusions of the safety of the initial risk assessment.

	<p>were no statistically significant differences in morphological parameters of the jejunum and ileum except the width of the <i>Tunica mucosa</i> in the jejunum, which was greater in birds fed GM maize and conventional soybean meal than in those given conventional maize and soybean meal. GM soybean meal had no effect on the apoptotic and mitotic indices in the crypt area or in villi tips, while GM maize did not affect these indices in the crypt area, but increased the apoptotic index in the villi tips of the jejunum. Neither GM soybean meal nor GM maize induced significant DNA damage in the epithelial cells of the jejunum.</p>			
--	---	--	--	--

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
<p>Sartowska <i>et al.</i> Genetically modified crops in a 10-generation feeding trial on Japanese quails - Evaluation of its influence on birds' performance and body composition, (2015)</p>	<p>Objective: To verify the safety and possible influence of genetically modified (GM) feed ingredients on Japanese quails in a 10-generation experiment.</p> <p>Experimental Design: The study consisted of 10 generations of Japanese quails (<i>Coturnix cot. japonica</i>) bred in 3 parallel feeding groups (i) GM soya (40-3-2) and non-GM maize, ii) GM maize (MON 810) and non-GM soya and iii) non-GM soya and maize, from 2010 to 2013. This experiment evaluated bird performance indices, including reproduction, survival rate, growth, egg production, body composition and the basic chemical composition of breast muscle and egg yolk. A total number of 8,438 healthy quail chicks were used in the study. Each group consisted of 6 repetitions of 17 females and 5 males, resulting in 102 females and 30 males in each of the 3 feeding groups, across the 10 generations. During the early laying period (from age 7 to 16 weeks) egg production was evaluated. At the age of 16 weeks, eggs were collected for incubation in order to form the next generation. Detection, identification and quantitative determination of the GM were done by PCR methods.</p> <p>Results: The different feeds used did not influence any of the biological hatch indices, survival rate or body weight of young or adult quails. The GM maize group showed a better laying percentage and a higher egg mass production compared to the other groups. Higher relative weight of breast muscle and gizzard in birds fed GM maize was observed compared to the control group, whereas live body weight and the relative weights of liver and heart were not different among groups. The GM soya group showed reduced average egg mass compared to the other groups, whereas the overall egg production level was the same as in the control group. Meat from the GM soya group showed higher protein and lower fat levels compared to the control group. In the case of egg yolk, its chemical composition in the experimental groups did not differ from the control group.</p>	<p>The authors concluded that: <i>“even though some differences were found among the feeding groups, none could be judged as a negative influence of GM maize or GM soya in feed on the birds or final consumer products over 10 generations of Japanese quails”</i>.</p>	Animal health	No adverse effects were determined in this study.
			Observed parameter	Feedback on initial environmental risk assessment
			Animal physiology	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Food/Feed Safety – Allergenicity studies of the protein or the whole food/feed

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Mathur <i>et al.</i> Lack of detectable allergenicity in genetically modified maize containing "Cry" proteins as compared to native maize based on <i>in silico</i> & <i>in vitro</i> analysis (2015)	<p>Objective: To assess the allergenicity, using <i>in silico</i> searches and <i>in vitro</i> methods, of genetically modified (GM) maize seeds containing <i>Bacillus thuringiensis</i> (Bt) Cry1Ab, Cry1Ac and Cry1C protein sequences, involving determination of specific IgE binding pattern to GM/non-GM maize antigens using maize-sensitized patients sera.</p> <p>Experimental Design: GM maize seeds containing Cry1Ab, Cry1Ac and Cry1C proteins along with their non-GM maize seed were procured from the developer, Metahelix, India. For the <i>in silico</i> analysis, sequence homology searches were performed against two allergen specific databases – Allergen Online of Food Allergy Research and Resource Program (FARRP) and Structural Database of Allergenic Proteins (SDAP). FASTA alignment was performed to compare all possible contiguous amino acid segments of each of the 3 proteins against the sequences listed in the databases. The extent of similarity was calculated as percent similarity and <i>E</i> (expectation) score. The <i>E</i> score reflected the degree of similarity between a pair of sequences based on matches of identical or functionally similar amino acids. For the bioassay, a qualitative evaluation of proteins in GM maize seeds was conducted to evaluate the presence/absence of Cry1Ab, Cr1Ac and Cry1C proteins in GM maize. A pepsin digestion assay (Simulated Gastric Fluid Digestion – SGF) was carried out to determine the stability of food protein in a gastric fluid environment. For the immunoassay, a total of 39 patients with suspected food allergy by history and Skin Prick Test (SPT) from two allergy clinics were selected. Blood samples were drawn from all food sensitised patients (n=39) and healthy volunteers (n=11) (controls). The presence of IgE against the GM and non-GM maize extracts was evaluated in the sera of all patients and volunteers by ELISA protocol.</p> <p>Results: For the <i>in silico</i> approaches, the results indicated Cry1Ab and Cry1Ac proteins did not reflect sequence</p>	The authors concluded that: “Based on <i>in silico</i> tools, it is reconfirmed that Cry1Ab, Cry1Ac and Cry1C protein sequences are non-allergenic, with no cross reactivity to known allergens ... these transgene protein sequences present no appreciable changes in endogenous protein expression of GM and non-GM maize seeds as analysed by specific IgE and immunoblot using native maize allergic patient sera”.	Human health	No adverse effects were determined in this study.
			Observed parameter	Feedback on initial environmental risk assessment
			Allergenicity and toxicology	There are no changes to the conclusions of the safety of the initial risk assessment.

	<p>similarity with known allergens but Cry1C protein exhibited similarity to a common known allergen from <i>Candida albicans</i>. However, the probability of assuming Cry1C protein as allergenic was nullified due to an E score and percentage similarity below the thresholds. These results also did not adhere to the criteria as laid down by Codex for positivity of allergenic cross-reactivity by any of the three transgenic proteins. There was no significant variation in protein content in the GM and non-GM maize extracts ($p>0.05$). SGF revealed reduced numbers of stable protein fractions in GM compared to non-GM maize, which might be due to a shift of constituent protein expression. Specific IgE values from patients showed significant differences in non-GM and GM maize extracts. Heterogeneity of sera samples in binding to different protein fraction(s) of non-GM and GM maize extracts was reflected. Protein fraction at 28, 33 and 78 kD were unique to Case No. 1, 4 and 3, respectively, while protein fractions at 41 and 48 kD were recognized in both Cases No. 2 and 5. Case No. 5 also showed binding of 68 and 88 kD protein fractions. In non-GM maize extracts, 7 protein fractions of 28, 33, 41, 48, 68, 78 and 88 kD approx. were recognized as IgE binding by selected five sera samples on Immunoblot. These same seven fractions were also observed as IgE binding by the same five cases in GM maize extracts of Cry1Ab, Cry1Ac and Cry1C. This signifies absence of variation in IgE binding by selected sera samples against GM and non-GM maize antigen extracts. Four of the reported IgE binding proteins were also found to be stable by SGF.</p>			
--	--	--	--	--

Area of the environmental risk assessment: Food/Feed Safety – Crop compositional studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Vidal <i>et al.</i> Comparative study of transgenic and non-transgenic maize (<i>Zea mays</i>) flours commercialized in Brazil, focussing on proteomic analyses (2015)	<p>Objective: To identify differences, by using a proteomic approach, between two maize flour samples: one obtained from genetically modified (GM) MON 810 maize, expressing the <i>Bacillus thuringiensis</i> Cry1Ab insecticidal protein, and the other one obtained from non-transgenic maize.</p> <p>Experimental Design: Fourteen maize flour samples were obtained from a commercial market in Rio de Janeiro, in the southeastern region of Brazil. DNA from GM maize was extracted and used to perform nested PCR to confirm the presence of the MON 810 event. Protein extracts from GM and non GM flour samples were analysed by 2-D difference gel electrophoresis (DIGE). In-gel digestion of protein spots (from 2-D PAGE preparative gel) showing changes in intensity was performed. The peptides obtained through enzymatic digestion were identified by mass spectrometry (nESI-QTOF MS/MS).</p> <p>Results: The 2-D DIGE technique revealed 99 differentially abundant protein spots (47 with higher and 52 with lower abundance) between GM and non-GM flour samples. Of these 99 spots, 66 could be observed and collected from 2-D PAGE preparative gel to identify the proteins through mass spectrometry. The differences in protein levels between the transgenic and non-transgenic samples could arise from genetic modification or as a result of an environmental influence pertaining to the commercial sample. The major functional category of proteins identified was related to disease/defence and, although differences were observed between samples, no toxins or allergenic proteins were found.</p>	The authors concluded that: <i>'The presented data would serve as an exploratory study into the use of proteomic techniques for the determination of safety or even for a simple comparison between different maize flours commercialized in Brazil. The use of proteomic technology for this purpose should be considered on a case-by-case basis, due to the complexity and duration of the assays involved and the importance in relation to GM food'</i> .	Human health	No adverse effects were determined in this study
			Observed parameter	Feedback on initial environmental risk assessment
			Dietary composition	There are no changes to the conclusions of the safety of the initial risk assessment.

References

- Czerwiński J, Bogacki M, Jalali BM and Konieczka PS, S., 2015 -a. The use of genetically modified Roundup Ready soyabean meal and genetically modified MON 810 maize in broiler chicken diets. Part 1. Effects on performance and blood lymphocyte subpopulations. *Journal of Animal and Feed Sciences*, 24, 2015, 134-143.
- Czerwiński J, Słupecka-Ziemilska M, Woliński J, Barszcz M, Konieczka P and Smulikowska S, 2015 -b. The use of genetically modified Roundup Ready soyabean meal and genetically modified MON 810 maize in broiler chicken diets. Part 2. Functional status of the small intestine. *Journal of Animal and Feed Sciences*, 24, 2015, 144-152.
- Mathur C, Kathuria PC, Dahiya P and Singh AB, 2015. Lack of Detectable Allergenicity in Genetically Modified Maize Containing “Cry” Proteins as Compared to Native Maize Based on In Silico & In Vitro Analysis. *Plos One*,
- Sartowska KE, Korwin-Kossakowska A and Sender G, 2015. Genetically modified crops in a 10-generation feeding trial on Japanese quails. Evaluation of its influence on birds’ performance and body composition. *Poultry Science*, 00:1–8, 1-8.
- Vidal N, Barbosa H, Jacob S and Arruda M, 2015. Comparative study of transgenic and non-transgenic maize (*Zea mays*) flours commercialized in Brazil, focussing on proteomic analyses. *Food Chemistry*, 180 (2015), 288-294.