Appendix 5.1. MON 810 literature review – Food Feed (July 2017)

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Area of the environmental risk assessment: Food/Feed Safety – Toxicology/Animal feeding studies

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Ibrahim and Okasha (2016) [1] Effect of genetically modified corn on the jejunal mucosa of adult male albino rat	***Objective:*** To study the effect of genetically modified (GM) maize expressing the *Bacillus thuringiensis* Cry1A protein (MON 810) on the histological structure of the jejunal mucosa of adult male albino rats using different histological, immunohistochemical and morphometric methods. ***Experimental Design:*** Twenty male adult albino rats were randomly divided into two equal groups: Group I (controls) received a diet of conventional maize meal for 90 days; Group II received MON 810 maize at 30% in the diet for the same duration. Feed consumption and total body weight were recorded throughout the study, however only initial and final body weights were reported and no other in life data were provided. At termination, jejunal specimens were dissected, sectioned and stained with different processes. Haematoxylin and eosin (H&E) staining was used to measure the height of jejunal villi and mean jejunal crypt depth. Periodic Acid Schiff (PAS) staining was conducted to determine the mean number of goblet cells. Immunostaining with monoclonal antibodies against proliferating cell nuclear antigen (PCNA) was used to quantify the number of PCNA-positive immunostained nuclei. Ultrathin sections from jejunal specimens were stained with uranyl acetate and lead acetate and examined by using a transmission electron microscope. Statistical analysis was performed with the *t*-test.	The authors concluded that: *"results from the current study could show that in spite of the assuring reports on GM products, GM-corn has profoundly altered the histological structure of the jejunal mucosa at many levels and revealed several alarming signs as the proliferative and eroded hemorrhagic lesions in addition to several ultrastructural alterations described here for the first time for jejunum under GM-corn influence. Possible mechanisms have been proposed including inflammation associated with goblet cell overexpression and PCNA upregulation. This should motivate the conduction of a more extensive research to reveal the exact mechanism of such unintended effect and how to remodulate the GM crops to avoid their adverse effects"*.	Animal health	MON 810 negatively affects the histology of the jejunum in rats[2]
			Observed parameter	**Feedback on initial environmental risk assessment**
			Toxicity	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] Exp. Toxicol. Pathol. 68:579-588.

[2] This study showed no impact of MON 810 on animal health or growth. The authors used poorly described and insufficiently characterized diets/test materials and non-validated techniques (e.g. electron microscopy, morphometry, and immunohistochemistry) to characterize potential hazards in the absence of any tabulated histological data typically used to assess adverse effects and without any evidence of adverse effects in the animal. The reported techniques are non-standard methods for hazard identification. Blinding, randomization, power analysis for morphometry, and tissue sampling procedures were not included to ensure reduction of bias and data interpretability. The jejunum varies greatly along its length and the observations reported are likely to reflect regional differences across this variable tissue on account of sampling bias rather than an effect of treatment. Lastly, this study contradicts other robust published studies conducted under GLP and/or EFSA/OECD guidance that do not show any impact of MON 810 on health of rats using standardized toxicological evaluation methods.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
	Results: No animals expressed any sign of ill health throughout the experiment and no deaths were reported. There were no observable alterations in behavior, feed consumption or average weight gain. In the MON 810 group, focal structural changes, including distortion, shortening, flattening and fusion of some jejunal villi, were observed. Stratification alternating with shedding of the jejunal surface epithelium, associated with a significantly increased crypt proliferation, was also detected. Erosion in the villi and denuded mucosal surface were evident. Congested blood capillaries and focal infiltration with mononuclear cells were observed. Significant upregulation of PCNA expression, increase in number of goblet cells and a significant increase in both villous height and crypt depth were detected. Marked ultrastructural changes of some enterocytes with focal loss of the microvillus border were observed. Some enterocytes had vacuolated cytoplasm, swollen mitochondria with disrupted cristae and dilated rough endoplasmic reticulum. Some cells had dark irregular nuclei with abnormally clumped chromatin.			

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Zeljenková *et al.* (2016) One year oral toxicity study on a genetically modified maize MON 810 variety in Wistar Han RCC rats (EU 7th Framework Programme project GRACE	***Objective:*** To test genetically modified (GM) insect resistant MON 810 maize in chronic rat feeding trials (GRACE project; 1-year study) and based on the obtained results, to evaluate whether such feeding trials are suited to reveal unintended effects elicited by genetic modification. ***Experimental Design:*** A 1-year feeding trial was conducted with Wistar Han RCC rats (20/group, M/F) exposed to MON 810 maize, a near-isogenic non-GM comparator or an additional conventional maize variety, in accordance with OECD Guideline 452 and under GLP. MON 810 maize was administered in feed at levels of 11 and 33%. Agronomic, morphologic, phenological and health parameters of the plants were monitored and were found to be usual for the region. Feed composition was analyzed, animal body weight and feed consumption were monitored, clinical and ophthalmological observations were recorded, hematology and clinical biochemistry parameters were quantified, and a gross necropsy including the determination of the absolute and relative organ weights and a histopathological analysis were performed. ***Results:*** The detailed quantitative and qualitative analysis of the different components of the diets indicated that fumonisins were present in the control diet but this did not influence the outcome of the study. Male rats fed the 33% MON 810 diets had a lower mean body weight than controls but the difference was not statistically significant. Clinical and ophthalmological observations revealed no alterations in all four experimental groups. The percentage of eosinophils, the number of white blood cells, and the blood P, GLU and CREA levels were modified but	The authors concluded that: *"the 1-year feeding trial performed with a MON 810 maize variety in the frame of the GRACE project shows that the MON 810 maize at a level of up to 33% in the diet does not lead to toxicologically relevant effects in male and female Wistar Han RCC rats. However, more sensitivity would be required to distinctly identify specific potential effect"*.	Animal health **Observed parameter** Toxicity	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
	there was no evidence of an event-related effect or of a link with histological liver or kidney alterations. The macroscopic examination of all animals at necropsy revealed a very limited number of findings (such as colored organ, thickened area on bone, or petechial hemorrhage) in all four experimental groups. The histopathological analysis of the organs and tissues obtained from all animals from the control and 33% MON 810 groups identified a very low number of alterations (e.g. cells infiltration, small dilatation, hyperplasia, degeneration or atrophy, and cysts) in both groups. Since there were no relevant differences in the incidence of histopathological findings between these two groups, it was concluded that the alterations were not event-related. Additionally, no malignant tumors were detected in 33% MON 810 fed rats. The data did not provide any indication that the performance of rat feeding studies with whole food/feed would provide additional information on the safety of the MON 810 maize compared to the compositional comparison of the GM line and its closest conventional comparator. Moreover, the data generated by the GRACE project showed that non-targeted feeding studies may generate outcomes at the level of the variability of the laboratory performing the studies and suggest that animal feeding trials may lack the sensitivity needed to detect unintended effects elicited by the genetic modification.			

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Korwin-Kossakowska *et al.* (2016) Health status and potential uptake of transgenic DNA by Japanese quail fed diets containing genetically modified plant ingredients over 10 generations	***Objective:*** To evaluate 1) the impact of genetically modified (GM) ingredients (GTS 40-3-2 herbicide tolerant soybean meal and MON 810 maize expressing the *Bacillus thuringiensis* Cry1Ab protein) used in quail diets in consecutive generations on bird health; and 2) the potential presence of transgenic DNA in breast muscle, eggs and internal organs. ***Experimental Design:*** The trial consisted of 10 generations of Japanese quail (*Coturnix cot. japonica*). A total of 10,947 eggs were incubated and 8,438 healthy chicks entered the trial, of which 3,960 adult birds were used. These were divided in three groups consisting of 102 females and 30 males per group in each of the 10 generations. The birds were fed a diet containing either GTS 40-3-2 soybean meal and non-GM maize, non-GM soya and MON 810 maize or non-GM soya and maize. Performance traits were monitored throughout the trial. For each generation, at the end of the laying period (week 17), a health examination was conducted, including *post-mortem* necropsy and histological organ evaluation. In addition, tissue samples from breast muscle, gizzard, liver, spleen, duodenum, kidney and heart, and germinal discs from eggs were used to extract DNA, which was subsequently analysed by PCR to detect the presence of transgenes (the *CaMV 35S* sequence for GTS 40-3-2 soyabean meal and *nos terminator* for MON 810 maize) using specific primers. Statistical analysis was performed using the nonaparametric one way analysis of variance (NPAIR1WAY) procedure. ***Results:*** The clinical condition of the birds in the experimental and control groups did not show symptoms of disease or signs of nutritional deficiencies. Sectional examination of birds did not indicate the existence of changes due to pathogens, nutritional factors or of environmental nature. The histopathological changes occurred in all three dietary groups and there was no statistical difference between groups. No transgene amplification was observed in the samples derived from different tissues and germinal disks.	The authors concluded that: "*there was no negative effect of the use of GM soya or maize with regard to bird health status or to the presence of transgenic DNA in the final consumable product*".	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Toxicity	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Chrenkova *et al.* (2016) Effect of crimped maize grain ensiled with high moisture grains of transgenic Bt maize in fattening bulls	***Objective:*** To verify the substantial equivalence of genetically modified (GM) insect resistant MON 810 maize and an isogenic counterpart in the context of a feeding study in Holstein bulls. ***Experimental Design:*** A feeding trial was carried out with 40 Holstein breed bulls for 258 days. The feed consisted of maize silage, lucerne silage, meadow hay, wheat, rape extracted oil meal, minerals and crimped maize grain in a total mixed ratio (TMR) with isogenic (DKC 5143) or transgenic (MON 810) maize. The bulls were fed twice daily at 6 and 16 h in two equal doses. Water was provided *ad libitum.* Detailed dissection of the right half carcass was carried out 24 h after slaughter of the animals to obtain weight and proportion of basic tissues (muscle, fat and bones). Samples (approximately 500 g) were taken from the *musculus longissimus thoracis* et *lumborum*. Chemical parameters of the meat (proteins, fat and total water content) were analysed afterwards, when no more changes in chemical composition of meat are in progress. Organic acids levels were determined by gas chromatography and alcohol by the micro diffusion method. ***Results:*** The TMR contained 138.6-139.8 $g.kg^{-1}$ crude protein, 162.4-155.3 $g.kg^{-1}$ crude fibre, 251.3-264.4 $g.kg^{-1}$ starch, 21.62-28.8 $g.kg^{-1}$ fat, 8.5-9.2 $g.kg^{-1}$ Ca and 3.2-3.7 $g.kg^{-1}$ P. The live weight gain of the bulls was 1.255-1.248 $kg.d^{-1}$ and the net energy of fattening value was 6.4-6.4 MJ in kg dry matter for the MON 810 and DKC 5143 groups, respectively. Feeding of TMR with isogenic or transgenic maize did not influence zootechnical parameters and had no negative effect on feed conversion, growth performance, meat quality and health status. The nutrient compositions of the diets revealed no major differences.	The authors concluded that: *"Feeding TMR with genetically modified MON 810 and isogenic maize to bulls did not influence biochemical and zootechnical parameters, and had no negative effect on health status and growth performance of animals. No significant differences were found between the individual nutrients which corresponded with the results of live weight in slaughter bulls. There was no effect of diet on pH value, chemical composition of meat and its colour"*.	Animal health	No adverse effects were determined in this study.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Toxicity	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
Andreassen *et al.* (2016) Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice	***Objective:*** 1) To investigate whether repeated exposures to a high level of trypsin-activated Cry1Ab protein produced in recombinant *Escherichia coli* (trypCry1Ab) may promote allergic responses (i.e. act as adjuvant) in an anaphylactic food allergy model in mice; and 2) to explore immunogenic and allergenic properties of trypCry1Ab by assessments of specific serum antibodies as well as intestinal gene expression. ***Experimental Design:*** On Days 0, 1, 2, 7, 21 and 28, female C3H/HeJ mice, except those of the vehicle control group, were immunized by intragastric gavage with 10 µg of purified trypCry1Ab alone or together with the food allergen lupex (protein extract from *Lupinus angustifolius*). Cholera toxin (CT) was added as a positive control for adjuvant effect to break oral tolerance. 100 µL blood samples were collected from the lateral vein of each animal on Days 0 and 34. On Day 35, a challenge of lupex was given intraperitoneally and the anaphylactic responses were assessed for 30 min thereafter. Mice were exsanguinated after the 30 min observation period or when reaching the score of 4. Serum levels of mouse mast cell protease-1 (MCPT-1), a marker of anaphylaxis, and lupin-specific antibodies were determined using commercial ELISA; anti-Cry1Ab IgG1, IgG2a and IgE antibodies were detected by *in-house* developed ELISA. Excised spleens were crushed to obtain single cell suspensions. Splenic cells were cultured for 3 d in the presence of Concanavalin A and for 5 d with or without lupex or trypCry1Ab. The amount of cytokine IL-2, IL-5, IL-13, IL-10 and interferon gamma (IFNγ) released into spleen cell supernates were determined by Cytometric Bead Array. RNA was extracted from a segment of ileum and used to determine the expression of the *heat shock protein 70* (*HSP 70*), *MCPT-1*, *IL-9*, *IL-6* and *tumor necrosis alpha (TNFα*) genes. ***Results:*** No indication of immunogenic properties of trypCry1Ab protein was seen after repeated intragastric exposures to one dose, with or without CT as adjuvant. Moreover, the results indicated that trypCry1Ab given by the intragastric route was not able to promote allergic responses or anaphylactic reactions against the co-administered allergen lupex at the given dose.	The authors concluded that: *"the present study supports previous findings suggesting no detectable immunogenic, allergenic or adjuvant capacity of the trypCry1Ab protein after intragastric exposure, within the limitations of the model, the doses and the protein contexts applied"*.	Animal health **Observed parameter** Allergenicity and toxicity	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
Osborne *et al.* (2016) Grain and biomass nutrient uptake of conventional corn and their genetically modified isolines	***Objective:*** A three-year field study was conducted in the northern USA maize belt to evaluate the impact of genetically modification on maize plant residue, grain yield, grain nutrient composition and stalk nutrient composition independent of glyphosate use. ***Experimental Design:*** A total of 18 maize hybrids from three different genetic platforms with corresponding single-, double- and triple-stacked modifications for insect and herbicide (glyphosate) resistance were used (Cry1Ab, Cry3Bb1, GR[3], Cry1Ab-Cry3Bb1, Cry1Ab-GR, Cry3Bb1-GR, Cry1Ab-Cry3Bb1-GR and control). The experiment was established in the spring of 2005 and conducted as a completely randomized design, replicated four times, within a two-year maize/soybean rotation with each phase of the rotation present each year, in two adjacent areas. At physiologically maturity, whole plant samples were collected from the two middle rows (in October 2005, 2006 and 2007). Subsamples of grain and biomass were digested with nitric acid and analysed by inductively coupled plasma atomic emissions for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), sulfur (S), zinc (Zn) and sodium (Na). Additionally all biomass samples were analysed for acid detergent fiber (ADF), acid detergent lignin (ADL) and neutral detergent fiber (NDF) fractional composition using a Ankom^{200} fiber analyser. Hemicellulose and cellulose composition were determined by subtraction. Ground residue and grain samples were milled further for total carbon (C) and nitrogen (N) analysis by dry combustion. ***Results:*** Average grain yields were similar to the regional average for maize production in the northern maize belt. There were no significant differences in grain C among the	The authors concluded that: "*this side by side comparison of 18 different maize hybrids with insecticidal and herbicide resistance traits grown under the same soil, environment and agronomic management did not result in any significant difference in above ground biomass or grain yield among the hybrids evaluated*"	Environmental protection **Observed parameter** Plant composition	No adverse effects were determined in this study **Exposure processes in non-target organisms** There are no changes to the conclusions of the safety of the initial risk assessment.

[3] GR : glyphosate resistance

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
	base hybrids and trait combinations, averaged over hybrid families and over all three years of evaluation. Grain K levels were significantly lower in triple-stack and Cry3Bb1-GR double-stack hybrids compared to other trait combinations and base hybrids. Orthogonal contrasts were used to compare base hybrids and trait combinations across hybrid families and years. There was no significant difference in plant biomass yield; however there was an increased K levels in biomass of triple-stack and Cry3Bb1-GR double-stack hybrids. These same trait combinations also had significantly lower C levels than the other combination/base hybrids. Results of pair-wise comparisons within hybrid families indicated that there was a significant difference in how certain traits or trait combinations affected biomass nutrient concentration compared to the conventional (non-GM) hybrid. Hemicelluloses were shown to be significantly lower for the two GR single stack hybrids as well as the two triple-stack hybrids compared to their respective conventional base hybrids. The addition of the GR gene resulted in an approximate 7% reduction in hemicellulose content compared to the respective conventional hybrids, while there was a 15% decrease for the one triple-stack hybrid compared to only a 4% reduction for the another hybrid. No other significant differences were observed in Mn, Mg, Na, Zn or S composition or in ADF, NDF or ADL and cellulose fractional components relating to digestibility and cell structure among trait combinations, averaged across hybrid families and over years. Similarly, pair-wise comparisons within hybrid families comparing trait packages with the conventional base hybrid showed no significant differences in mineral nutrient content or digestibility/cell structure.			

References

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