Appendix 5.1. MON 810 Literature Review – Food/Feed

MON 810 literature review (July 2013)

Appendix 5.1 - Food/Feed

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Review of peer-reviewed publications

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Sartowska *et al.*, 2012)	***Objective:*** To evaluate the impact of glyphosate tolerant soybeans and *Bacillus thuringiensis* (Bt) insect resistant maize on health, performance and nutritional value of quail after exposure over two generations. These are preliminary results from a nine generation study. ***Experimental Design:*** Japanese quails *(Coturnix cot. japonica)* obtained from in-house breeding were exposed over two generations to feed containing glyphosate tolerant Roundup Ready soybean (A 5403), insect resistant MON 810 maize or a control diet. Diets were balanced according to quail needs and the level of basic nutrients was monitored. The content of modified DNA was analysed by a reference laboratory. Basic production performance was observed in the course of the trial (hatching, bodyweight, laying performance, egg mass and mortality). At the end of the laying period (Week 17), weight of edible products (breast muscle, gizzard, liver and heart) was determined in 12 males and females from each group in each generation. Breast muscle samples were also analysed for basic chemical composition (% dry matter, protein, fat and ash). During Week 17, egg yolk samples were collected for analysis of dry matter, protein, fat, residues of water content and ash. ***Results:*** No treatment-related effects were seen on incubation, hatching parameters and mortality. Feed intake was comparable across all groups, as was laying performance. A significant influence of generation number was found on carcass composition, however it could be explained by different season of the year. For egg yolk composition, the group receiving Roundup Ready soybeans showed lower dry matter and crude fat content compared to the other two groups. Quails exposed to MON 810 maize had higher crude protein content than the other two groups. No differences in crude ash were seen across treatments.	The authors concluded that "*no negative effects of the use of Roundup Ready soybeans or MON 810 maize were found so far in the course of the feeding trial with regards to animal reproduction, health and growth or laying performance, which was maintained at an expected level. Some differences were noted in chemical composition of breast muscle and egg yolk, however no clear tendency was seen for or against any of the diets used. These indices require further research.*"	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Animal performance	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
(Reichert *et al.*, 2012)	***Objective:*** To investigate by histopathological examination the impact of diets containing genetically modified (GM) insect resistant maize MON 810 and meal from soybeans tolerant to Roundup herbicide (MON-40-3-2) on internal organs collected from broiler chickens, laying hens, fattening pigs and calves. ***Experimental Design:*** Feeding experiments were performed on broiler chickens, laying hens, fattening pigs and calves. Four treatments were used: (I) conventional maize and soybean meal, (II) conventional maize and GM soybean meal, (III) GM maize and conventional soybean meal and (IV) GM maize and GM soybean. For the broiler study, 640 one day old Ross 308 chicks, kept for 42 days, were used. For the laying hens, 96 Bovans Brown hens, between 25 and 54 weeks of age, kept individually for 30 weeks, were used. The study on pigs was carried out on 72 fatteners, each group consisted of six gilts and six barrows. Experimental fattening lasted from about 30 to 110 kg of body weight. The experiment on calves was carried out on 40 Polish Black and White HF bulls from 10 to 90 days of age. Immediately after slaughter, the liver, kidneys, spleen, pancreas, duodenum, jejunum and skeletal muscles were collected and fixed in formalin. For broiler chickens, the *bursa of Fabricius* instead of muscles were collected. Samples from 40 broilers and 40 hens, 36 pigs and 20 calves were collected. Results were analysed statistically. ***Results:*** The study revealed morphological changes in many organs, however, the statistical analysis showed no significant differences between treatments. The authors conclude that broilers, laying hens, pigs and calves fed diets containing MON 810 maize and MON-40-3-2 soybean showed no adverse effects on morphology and structure of internal organs and muscles, as assessed histologically.	No harmful effects of MON 810 maize and MON-40-3-2 soybean in feed materials were detected on animal health.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Histopathology	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
(Buzoianu *et al.*, 2012c)	***Objective:*** To investigate whether feeding genetically modified (GM) MON 810 maize expressing the *Bacillus thuringiensis* insecticidal protein (Bt maize) had any effects on porcine intestinal microbiota. ***Experimental Design:*** Crossbred (Large white x Landrace) male pigs were weaned at ca. 28 days and allowed a 6 day acclimatization period, then assigned to diets based on: (i) non-GM isogenic parental line maize (Pioneer PR34N43) or (ii) Bt maize (Pioneer PR34N44 event MON 810) for 31 days (n= 9/treatment). Bt and isogenic parental line maize were grown simultaneously in neighbouring plots in Navarra, Spain over the 2007 season. The maize was tested for chemical, amino acid and carbohydrate composition and for the presence of the *cry1Ab* gene, pesticide contaminants and mycotoxins. Immediately after euthanasia, fecal, ileal and cecal digesta samples were collected from all pigs and stored in sterile containers at 4°C until analysis. *Lactobacillus* and *Enterobacteriaceae* counts were determined as indicators of beneficial and pathogenic bacteria, respectively. Total anaerobic bacterial counts were also performed. Total DNA was extracted from individual cecal digesta samples using the QIAamp DNA stool minikit and microbial composition was established by sequencing of 16S rRNA tags. ***Results:*** Both the Bt and isogenic maize diets had similar proximate compositions and amino acid contents. Fecal, cecal, and ileal counts of the anaerobic bacteria *Enterobacteriaceae*, and *Lactobacillus* were not significantly different between groups. High-throughput 16S rRNA gene sequencing revealed few differences in the compositions of the cecal microbiotas. The only differences were that pigs fed Bt maize had higher cecal abundance of *Enterococcaceae* (0.06 versus 0%; $p < 0.05$), *Erysipelotrichaceae* (1.28 versus 0.17%; $p < 0.05$), and *Bifidobacterium* (0.04 versus 0%; $p < 0.05$) and lower abundance of *Blautia* (0.23 versus 0.40%; $p < 0.05$) than pigs fed the isogenic maize diet.	Bt maize was well tolerated by the porcine intestinal microbiota following **31 days** of exposure. Differences observed were not believed to be of major biological importance and were not associated with any adverse health effects. These data can potentially be extrapolated to humans, considering the suitability of pigs as a human model.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Microbial intestinal flora	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
(Buzoianu *et al.*, 2012a)	***Objective:*** To investigate whether feeding genetically modified (GM) MON 810 maize expressing the *Bacillus thuringiensis* insecticidal protein (Bt maize) to male pigs had effects on growth and health indicators. ***Experimental Design:*** The study included 72 crossbred male pigs weaned at 28 days of age. Four dietary treatments were used: (1) non-GM maize-based diet (isogenic parent line; Pioneer PR34N43) fed up to Day 110; (2) GM maize-based diet (Bt; Pioneer PR34N44 event MON 810) fed up to Day 110; (3) non-GM maize-based diet fed for 30 days followed by GM maize fed up to Day 110; and (4) GM maize-based diet fed for 30 days followed by non-GM maize-based diet fed up to Day 110. Seeds derived from MON 810 maize and the non-GM parent line were grown side by side in Spain. Seed samples were tested for chemical, amino acid and carbohydrate compositions, as well as for presence of the cry1Ab gene, pesticide contaminants and mycotoxins. Bodyweight and daily feed intake were recorded on Days 0, 30, 60 and110 for determination of growth performance. Feed conversion ratios were calculated as average daily feed intake divided by average daily gain. Body composition was determined on Day 80 using dual energy X-ray absorptiometry technology. Samples were taken from various organs for histological examination. Blood, urine and serum samples were also taken for analysis. ***Results:*** Feeding GM maize to pigs from 12 days post weaning up to slaughter did not adversely affect growth, carcass characteristics, bone health or body composition. Although some changes in serum biochemistry were observed, values were all within the normal reference intervals for pigs, did not conform to a pattern indicative of organ dysfunction and were not correlated with differences in organ weight or histopathology. Histological examination indicated the absence of an adverse effect of GM maize at the main site of nutrient digestion and absorption, i.e. the small intestine.	This study indicates that GM Bt MON 810 maize is safe as an ingredient in swine diets. There is little evidence to suggest that adverse health effects should be expected in humans following GM maize consumption.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Animal performance	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
(Buzoianu *et al.*, 2012d)	***Objective:*** To assess the effects of feeding *Bacillus thuringiensis* (Bt) MON 810 maize to pigs for **110 days** on the intestinal microbiota using both culture-dependent and independent methods. ***Experimental Design:*** Forty crossbred male pigs weaned at around 28 days of age and were allowed access *ad libitum* to a non-genetically modified (GM) starter diet during a 12 day basal period. They were then assigned to one of 4 treatments: (1) isogenic maize-based diet (Pioneer PR34N43); (2) Bt maize-based diet (Pioneer PR34N44 event MON 810); (3) isogenic maize-based diet for 30 days followed by Bt maize-based diet until Day 110 or (4) Bt maize-based diet for 30 days followed by isogenic maize-based diet until Day 110. MON 810 and isogenic maize were grown in neighbouring plots in Valtierra, Navarra, Spain. *Enterobacteriaceae*, *Lactobacillus* and total anaerobes were enumerated in the feces using culture-based methods on Days 0, 30, 60 and 100 of the study and in ileal and cecal digesta on Day 110. The QIAmp DNA Stool kit was used to extract total DNA from individual cecal digesta samples. ***Results:*** No significant differences were found between the four dietary treatments for fecal, ileal and cecal counts of *Enterobacteriaceae*, *Lactobacillus* or total anaerobes on Days 30, 60 and 100. The porcine cecal microbiota was dominated by *Clostridiaceae* (9.6%), *Prevotellaceae* (9.1%), *Veillonellaceae* (6.2%), *Ruminococcaceae* (5.2%) and *Bacteroidaceae* (3.8%). No significant differences in relative abundance were detected between treatments for any of the bacterial families. No differences were observed in any bacterial taxa between treatments, with the exception of the genus *Holdemania* which was more abundant in the cecum of pigs fed the isogenic/Bt treatment (3) compared to pigs fed the Bt treatment (2) (0.012 vs. 0.003 %; $p \leq 0.05$).	Feeding pigs a Bt maize based diet for 110 days did not affect counts of any of the cultural bacteria enumerated in the feces, ileum or cecum (i.e., *Enterobacteriaceae*, *Lactobacillus* or total anaerobes) at any time during the study. The composition of the cecal microbiota was also not influenced, with the exception of a minor increase in the genus *Holdemania*.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Microbial intestinal flora	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
(Walsh *et al.*, 2012)	***Objective:*** To evaluate potential long-term (110 days) and age-specific effects of feeding genetically modified *Bacillus thuringiensis* (Bt) maize on peripheral immune response in pigs and to determine the digestive fate of the *cry1Ab* gene and truncated Bt toxin. ***Experimental Design:*** Forty crossbred male pigs weaned at around 28 days of age were allowed access *ad libitum* to a non-genetically modified (GM) starter diet during a 12 day basal period. They were assigned to one of 4 treatments: (1) isogenic maize-based diet (Pioneer PR34N43); (2) Bt maize-based diet (Pioneer PR34N44 event MON 810); (3) isogenic maize-based diet for 30 days followed by Bt maize-based diet until Day 110 or (4) Bt maize-based diet for 30 days followed by isogenic maize-based diet until Day 110. MON 810 and isogenic maize were grown simultaneously in 2007 in Valtierra, Navarra, Spain. Blood samples were collected during the study for haematological analysis, measurement of cytokine and Cry1Ab-specific antibody production, immune cell phenotyping and *cry1Ab* gene and truncated Bt toxin detection. Pigs were sacrificed on Day 110 and digesta and organs samples were taken for detection of the *cry1Ab* gene and the truncated Bt toxin. ***Results:*** On Day 100, lymphocyte counts were higher ($p < 0.05$) in pigs fed Bt/isogenic than pigs fed Bt or isogenic diets. Erythrocyte counts on Day 100 were lower in pigs fed Bt or isogenic/Bt than pigs fed *Bt*/isogenic diets ($p < 0.05$). Neither the truncated Bt toxin nor the *cry1Ab* gene was detected in the organs or blood of pigs fed Bt maize. The *Cry1Ab* gene was found in stomach digesta and at low frequency in the ileum but not in the distal gastrointestinal tract (GIT), while the Bt toxin fragments were detected at all sites in the GIT.	Perturbations in peripheral immune response were thought not to be age-specific and were not indicative of Th-2 type allergenic or Th-1 type inflammatory responses. There was no evidence of *cry1Ab* gene or Bt toxin translocation to organs or blood following long-term feeding.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Haematology, DNA/protein fate	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
(Buzoianu *et al.*, 2012b)	***Objective:*** To investigate whether feeding genetically modified (GM) MON 810 maize expressing the *Bacillus thuringiensis* insecticidal protein (Bt maize) to sows during gestation and lactation had effects on maternal and offspring immunity and to assess the fate of transgenic material. ***Experimental Design:*** Twenty four sows fed on non-GM diet were used in the experiment. On the day of insemination, sows were assigned to one of two treatments; 1) non-Bt control maize diet (PR34N43), or 2) Bt- MON 810 maize diet (PR34N44), and were fed for 143 days throughout gestation and lactation. Immune function was assessed by leukocyte phenotyping, haematology and Cry1Ab-specific antibody presence in blood on Days 0, 28 and 110 of gestation and at the end of lactation. Peripheral-blood mononuclear cell cytokine production was investigated on Days 28 and 110 of gestation. Haematological analysis was performed on offspring at birth (n = 12/treatment). Presence of the *cry1Ab* transgene was assessed in sow's blood and faeces on Day 110 of gestation and in blood and tissues of offspring at birth. Cry1Ab protein presence was assessed in sow's blood during gestation and lactation and in tissues of offspring at birth. ***Results:*** While differences in a limited number of immune parameters were observed in breeding pigs and their offspring in response to maternal intake of Bt maize, the authors consider these differences insufficient to indicate consistent activation of the innate immune system. Likewise, activation of the adaptive immune system (Th2 profile/allergy or Th1 profile/ inflammation) was not observed in the present study. Furthermore, cytokine production was neither significantly different between treatments nor indicative of an immune response to Bt maize consumption. As neither the Cry1Ab protein nor antibodies specific to it were detected in the blood of either sows or offspring, the results support the conclusion that feeding Bt maize to pregnant sows during gestation and lactation does not adversely affect maternal or foetal immune function.	Feeding transgenic maize to sows during gestation and lactation did not result in any adverse effects in immunity. No Cry1Ab or Cry1Ab-specific antibodies were detected in the blood of sows or their offspring.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Haematology, DNA/protein fate	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
(Walsh *et al.*, 2013)	***Objective:*** to investigate the health effects of feeding sows and their offspring with GM maize. ***Experimental Design:*** twenty-four sows and their offspring were fed diets containing GM or non-GM maize from service to the end of lactation. Two dietary treatments were included: (1) non-GM isogenic parent line maize (Pioneer PR34N43) and (2) GM maize (Pioneer PR34N44 event MON 810). Samples from GM and non-GM maize were tested for chemical, amino acid and carbohydrate composition as well as for the presence of the cry1Ab gene, pesticide contaminants and mycotoxins. Individual body weight[1] of piglets in all litters was recorded at birth and weaning and average daily gain was calculated during the suckling period. Blood samples were taken for haematology and biochemical analysis (e.g. serum urea, creatinine, GGT and AST[2]; MCHC, red cell distribution width[3]). Heart, kidneys, spleen and liver were removed, trimmed of any superficial fat or blood, blotted dry and weighed. ***Results:*** The results from the study indicate that feeding GM maize to sows during gestation does not affect body composition, as determined by back-fat depth. Some differences in body weight were observed between the treatments at mid-gestation, but these differences were not present in late gestation. There was a minimal effect of feeding GM maize to sows during gestation and lactation on maternal and offspring serum biochemistry and haematology at birth and body weight at weaning.	The authors conclude there was a minimal effect of feeding GM maize to sows during gestation and lactation on maternal and offspring serum biochemistry and haematology at birth and body weight at weaning.	Animal health	Occasional differences were seen between treatment groups, but the differences were not considered to be adverse by the authors. [4]
			Observed parameter	**Feedback on initial environmental risk assessment**
			Animal performance	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] The authors state that feeding GM maize to sows during gestation does not affect body composition as determined by back-fat depth, and differences in body weight observed between the treatments at mid-gestation were not present in late gestation (i.e., the finding did not persist with continued feeding of GM maize). The lack of persistence with continued treatment suggests the relationship of the finding to treatment is equivocal).

[2] In the discussion section the authors point out that the values were all within the normal reference range and that there were no correlating signs of toxicity in the relative organs (i.e., liver enzyme activity and organ weight). Taken together, they conclude the findings do not, "*...conform to a pattern indicative of either liver or kidney dysfunction...*"

[3] The authors state in the Discussion that differences in hematology parameters were transient and minimal in sows, and thus were unlikely to be of biological significance. They also indicate the difference MCHC noted in offspring were not accompanied by any other changes in haematology, which suggests biological variability as the cause rather than test substance treatment.

[4] Despite not definitively stating there were no adverse effects, the authors make statements supporting that conclusion. Information provided in footnotes 1-3 support this position.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Guertler *et al.*, 2012)	***Objective:*** To assess the potential effects of feeding genetically modified (GM) maize (MON 810) on the gene expression profiles of biomarkers for apoptosis, inflammation and cell cycle of several tissues of cows. ***Experimental Design:*** From 2005 until 2007, a study conducted with 36 Fleckvieh cows, 18 cows fed GM maize (MON 810) and 18 cows fed near-isogenic maize, was carried out at the Bavarian State Research Center for Agriculture, investigating the fate of recombinant DNA and protein. A diet containing maize silage and maize stem pellets was used and maize green tissue was added to ensure high exposure. The presence of transgenic DNA and the Cry1Ab protein in the partial total mixed ration (PTMR) and in single feed components was confirmed by PCR analysis and ELISA. After a period of 25 months which comprise two lactation period for each cow, 10 cows fed transgenic maize and 7 cows fed near-isogenic maize were slaughtered due to operational reasons. Several tissue samples were taken in triplicates from whole tissues (liver, rumen, abomasum, small intestine, large intestine and appendix) for gene expression analysis of major genes of the inflammation, cell cycle and apoptosis pathways. For the determination of potential effects on gene expression level, mRNA expression of genes that play a key role in apoptosis, cell cycle and inflammation were analysed by qPCR. ***Results:*** The mRNA level pattern of selective genes involved in apoptosis (Bcl_X_L, Bax, caspase 6, caspase 8), inflammation (IL1α, IL1β, TNFα, CD8) and of the cell cycle pathway (CDK2, cyclin A, cyclin D1, myostatin) was comparable between the control group and the target group in all analysed tissues. No significant changes in gene expression patterns were perceived in tissues of the gastrointestinal tract and in liver. Feeding GM maize (event MON 810), compared to feeding the near-isogenic maize variety, does not influence the gene expression of biomarkers for apoptosis, inflammation and cell cycle in liver and in the gastrointestinal tract of cows.	Cows fed with GM maize (event MON 810), compared to cows fed with near-isogenic maize variety, did not show any differences in the gene expression of biomarkers for apoptosis, inflammation and cell cycle in liver and in the gastrointestinal tract.	Animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Gene expression	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
(Gu *et al.*, 2013)	***Objective:*** To assess whether response in Atlantic salmon to dietary inclusion of *Bacillus thuringiensis* (Bt) maize expressing Cry1Ab protein differed from the near-isogenic maize line (fish sensitised with 15% soybean meal (SBM) inclusion in diet).[1] ***Experimental Design:*** Bt maize (MON 810) and its near-isogenic non-genetically modified line were derived from Pioneer varieties PR34N44 and PR34N43 grown in Spain. The trial was conducted at the aquaculture research facility in Norway. There were triplicate tanks of 100 fish fed diets containing 20% whole-kernel meal maize. The experiment was carried out using a 2 x 2 factorial design with four diet[2] groups: 1) control, 2) GM-maize, 3) control SBM, and 4) GM-maize SBM. Fish were fed continuously[3] for either 33 or 97 days. The factors GM and SBM inclusion were tested separately and in combination. After 33 or 97 days, blood was collected. Selected organs were weighed. Various physiological responses were assessed to identify potential biomarkers for Bt maize exposure: (1) growth performance and feed utilisation, (2) haematology, plasma clinical chemistry, and relative weights and histomorphology of main organs, (3) Cry1Ab levels and specific antibodies in plasma, (4) digestive and intestinal function and (5) distal intestinal (DI) cell proliferation, oxidative stress and immune responses. ***Results:*** Fish exposed to Bt maize used feed less efficiently, as revealed by lower protein and mineral digestibility and lower lipid and energy retention efficiency. Higher intestinal weight, increased interferon-γ, decreased sodium-glucose co-transporter mRNA expression and increased T-helper cell presence were measured by cluster of differentiation 4 (CD4) proteins in the DI, partly explaining the lower nutrient digestibility and retention. Bt maize seemed to potentiate oxidative cellular	The increase in CD4 protein and IFN-γ mRNA in the DI of Bt maize-fed fish suggest that Cry1Ab protein or other antigens produced due to genetic modification have potential local immunogenic effects in the gastrointestinal tract and may function as biomarkers for MON 810 maize exposure for this species. Long-term observations and more in-depth studies on immune responses and nutrient utilisation may be needed to confirm these results[5].	Animal health	The authors claim, less efficient feed use[6], immunogenic effects localized to the distal intestine, and potentiation of oxidative cellular stress in immune-sensitised fish[7].
			Observed parameter	**Feedback on initial environmental risk assessment**
			Animal performance	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] High levels of soybean meal (SBM) in the salmon diet were previously shown by the authors to produce gut inflammation and underperformance in salmon; this study attempted to test if the additional presence of Bt-maize[1] in the diet would worsen these effects. The authors frequently cite manuscripts alleging Bt toxicity in vertebrates. These papers have been deemed unreliable for informing risk assessment by multiple sources (Snell C, 2012).; as well as the opinions of EFSA (http://www.efsa.europa.eu/en/gmo/gmomeetings.htm?p=40), FSANZ (http://www.foodstandards.gov.au/consumer/gmfood/adverse/Pages/default.aspx, and the French High Counsel on Biotechnology (http://www.food.gov.uk/multimedia/pdfs/acnfp9612a2).

[2] There were a number of potential problems with the test diet:

- Gluten meal is more common in salmon diets, but whole-kernel maize meal was tested instead due to "financial and time restraints"; the effects of this substitution on salmon growth and nutrition were not discussed.
- Yttrium oxide was added to the diets as a biomarker, but according to the MSDS it is a skin, eye, and respiratory irritant.

[3] As fasting in salmon reduces potential diet-induced inflammatory changes in the intestine, feeding the animals continuously meant there were inconsistencies in a) the timing of the last meal and b) the amount consumed just prior to collecting samples. These variables could easily account for the few physiological differences observed in this study.

	stress in the DI of immune-sensitised fish, as shown by increases in superoxide dismutase and heat shock protein 70 mRNA expression[4]. The data suggest that Cry1Ab protein or other antigens in Bt maize have local immunogenic effects in salmon DI. No systemic immune responses could be detected, as indicated by haematology, differential leucocyte counts, plasma clinical chemistry and absence of Cry1Ab-specific antibodies and protein in plasma.			

[4] When a test substance is toxic, there are frequently converging lines of evidence that indicate the mechanism of toxicity or target organ. Instead, in this paper too much emphasis is placed on statistical significance for minor, scattered differences between groups, particularly in the absence of a normal range of values for the endpoints under investigation in the test species. Per a recent publication by EFSA on the topic, "Biological relevance and statistical significance are not necessarily linked."

[5] The startling claims stated in the Title, Abstract, and Conclusions are not supported by the weight of the evidence in the paper, and are instead contradicted by much of the data presented.

[6] This claim is not supported by the data. The authors themselves readily admit in the first line of the Discussion section that, "…growth and feed efficiency after 97 days of feeding did not differ between fish fed Bt-maize or non-GM maize."

[7] The salmon is not an accepted model for testing food and feed safety. Furthermore, the general scientific consensus is that animal models have not been sufficiently validated to accurately predict immunologic effects in humans (Goodman, 2008; Thomas, 2009; Codex, 2009, http://www.fao.org/docrep/011/a1554e/a1554e00.htm).

Area of the environmental risk assessment: Food and Feed – DNA fate

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Fernandes *et al.*, 2013)	***Objective:*** to study DNA degradation, detection and quantification of transgenic maize along the process of broa bread preparation. ***Experimental Design:*** Certified reference material from the IRMM containing 1% and 5% MON 810 maize was used. To prepare incurred maize breads with GM maize, two different types of maize were used: maize semolina, containing MON 810 (20%) purchased from a local market in Portugal; MON 810 maize kernels from crops cultivated in Portugal. The GM content of both samples was determined. Three different maize breads were prepared in a bakery according to the traditional process. During the preparation of maize bread two samples of dough were taken: before and after leavening. In the final baked breads, three samples were taken from different bread parts: crust, under crust and middle soft part of the bread. DNA was extracted from each sample and checked for purity and quality. Qualitative and quantitative PCR were performed. The effect of breadmaking processing on the extracted DNA from the three maize breads along the stages of preparation and location of sampling was performed by agarose gel electrophoresis. ***Results:*** The results showed that dough samples before and after leavening the breads led to shearing and degradation of DNA of high molecular mass. After oven cooking the breads, a decrease of the DNA amount and integrity was noted. The results of PCR amplification of extracted DNA showed that the sequences for the maize invertase gene and for event MON 810 were easily detected. Real-time PCR showed that the part of the bread sampled had a limited influence on quantification.	The process used in the making of broa bread results in DNA degradation, however, DNA from the transgenic event can be detected.	Human/animal health	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			DNA degradation	There are no changes to the conclusions of the safety of the initial risk assessment.

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