

Appendix 5.1. MON 810 Literature Review – Food/Feed

MON 810 literature review (July 2011)

Appendix 5.1 - Food/Feed

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Review of peer-reviewed publications

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

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(La Paz <i>et al.</i> , 2010)	<p>Objective: To determine the stability of the inserted DNA, the variability in the genomic DNA flanking the insert, and the expression of the <i>cryIAb</i> mRNA encoded by the insert in multiple generations of MON 810 was analyzed.</p> <p>Experimental Design: Southern blots were performed on three different varieties containing MON 810. Blots were hybridized with three different probes corresponding to the 35S promoter, the 3' end of the <i>cryIAb</i> coding sequence, and to the 3' flank region. SNP analysis was performed on 28 different MON 810 varieties. Each variety was assayed in triplicate by PCR, followed <i>Cell</i> endonuclease digestion and subsequent analysis by capillary microelectrophoresis. Cytosine methylation was analyzed by bisulfite sequencing of genomic DNA extract from the V7 leaves of seven different MON 810 varieties. Symmetrical and asymmetrical cytosine methylation was examined in the 35S promoter, in the <i>cryIAb</i> coding region, and in the 5' flank region. The accumulation of <i>cryIAb</i> mRNA was evaluated in two MON 810 varieties using a previously validated quantitative real-time PCR assay.</p> <p>Results: Southern blot banding patterns were identical for each of the three varieties tested. No SNPs or indels (insertions or deletions) were found within the inserted DNA, and no SNPs or indels were found within 500 bases of the inserted DNA in either flank in the 28 MON 810 varieties that were tested. There was very little difference in cytosine methylation of the inserted DNA in leaves of different varieties and at different developmental stages. The level of <i>cryIAb</i> mRNA accumulation is decreases over the course of leaf development.</p>	<p>The results indicate that the inserted DNA and the locus surrounding the inserted DNA in MON 810 is no less stable than endogenous maize genes. There was very little difference in cytosine methylation of the inserted DNA in leaves of different varieties and at different developmental stages. The authors noted that the level of <i>cryIAb</i> mRNA accumulation decreases over the course of leaf development. They attribute this to a developmental and/or tissue-specific effect on the expression of the 35S promoter.</p>	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 – Protein expression

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Kamath <i>et al.</i> , 2010)	<p>Objective: This study was initiated to quantify the expression of <i>cry1Ab</i> gene in target plant tissues (whorl leaf and stem) for <i>C. partellus</i> and <i>S. inferens</i> in multiple MON 810 hybrids over two seasons.</p> <p>Experimental Design: Seven MON 810 hybrids and one conventional maize hybrid (control) were planted in a total of 14 locations during two seasons. A total of three MON 810 hybrids were tested at four locations each during the dry season (October to March) and seven MON 810 hybrids were tested at ten field locations during the wet season (May to October). At each location, the MON 810 hybrid and the conventional maize hybrid were planted in two replicated plots. Concentrations of Cry1Ab protein were measured in leaf and stem sampled at respectively 5 and 3 sampling time points.</p> <p>Results: In the <u>dry season</u> trial the concentrations of Cry1Ab protein in whorl leaf, averaged across the three hybrids, ranged from 50.05 -12.11 µg/g dry weight of tissue (ppm) at 15 days after emergence (DAE). The leaf Cry1Ab concentration gradually declined to a mean of 38.13 -5.56 ppm at 30 DAE and then to 21.10-7.12 ppm at 60 DAE. The concentration of the Cry1Ab protein in the tissues of the conventional hybrid were below the limit of quantification. In the stem the mean Cry1Ab concentration ranged from 9.26- 3.23 to 3.47-2.46 ppm between 15 and 60 DAE. A two-factorial (hybrids, sampling time points) ANOVA revealed no significant differences between the three hybrids with respect to Cry1Ab concentrations in whorl leaves or stem.</p> <p>In the <u>wet season</u> the concentrations of Cry1Ab protein for whorl leaf averaged across the seven hybrids, ranged from 19.30-9.92 ppm at 15 DAE to 11.08 -4.66 ppm at 60 DAE. Similarly mean Cry1Ab concentration in the stem across seven hybrids ranged from 14.28-4.98 to 4.69-1.54 ppm between 15 and 60 DAE. A two-factorial (hybrids, sampling time points) ANOVA revealed no significant differences between the seven hybrids in Cry1Ab concentrations in whorl leaves or stem. However a two-factorial (hybrids, seasons) ANOVA between three common hybrids grown in wet and dry season for Cry1Ab contents in leaf revealed significant difference between the seasons at all sampling time points except at 90 DAE, indicating a possible seasonal preference of the three hybrids for leaf expression of Cry1Ab. But, the same three hybrids did not show a significant difference in stem concentrations of Cry1Ab between the two seasons.</p>	<p>In this study, all MON 810 hybrids contained highest concentrations of Cry1Ab in whorl leaf. These leaf concentrations should provide good control especially when viewed with the MIC₉₀ data for both borers. This is ideal from a plant protection perspective since the neonates of both <i>C. partellus</i> and <i>S. inferens</i> feed by first scraping the leaf lamina before migrating towards the stem. Similarly the concentrations of Cry1Ab in the stem at 15 and 60 DAE in both seasons were high in the context of the sensitivity of <i>C. partellus</i> and <i>S. inferens</i> to Cry1Ab protein.</p> <p>The concentrations of Cry1Ab observed in the target tissues of the seven MON 810 hybrids relative to the sensitivity of <i>C. partellus</i> and <i>S. inferens</i> to Cry1Ab protein, indicate a strong likelihood of effective management of these two pests in both maize growing seasons.</p>	Environment	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Expression of the insert	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 Study

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Delgado and Wolt, 2010)	<p>Objective: To estimate the long-term exposure of fumonisin B₁ (FB₁) in nursery swine diets and associated toxicological adverse effects on negative productivity potential using quantitative exposure assessment (deterministic and stochastic modeling).</p> <p>Experimental Design: Six different feeding scenarios differing in the source of maize (<i>Bt</i>-maize, non-<i>Bt</i>-maize and distillers dried grains with soluble - DDGS) in diets were modeled to assess variation in FB₁ exposure¹. FB₁ concentrations in the different sources were determined by review of the literature.</p> <p>Results: Diets where the maize fraction was entirely from <i>Bt</i>-maize showed the least FB₁ exposure (exceeding the first level of concern in 35% of occasions), whereas a blended diet or diets using non-<i>Bt</i> grain and DDGS sources more commonly exceeded this threshold (95% of occasions).²</p>	Based on these estimates, under blended maize source feeding conditions, swine populations in nursery facilities may frequently exhibit incipient effects (<i>i.e.</i> , LOC1) of FB ₁ toxicity; however, impacts on production efficiency remain uncertain.	Animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Toxicology	There are no changes to the conclusions of safety of the initial risk assessment

¹ Specific maize varieties are not given.

² Results are dependent on the assumptions regarding FB₁ concentrations in the different maize sources.

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Steinke <i>et al.</i> , 2010)	<p>Objective: To evaluate the effects of genetically modified maize on performance of lactating dairy cows over 25 months.</p> <p>Experimental Design: Thirty-six dairy cows were assigned to two feeding groups and fed with diets based on whole-crop silage, kernels and whole-crop cobs from MON 810 or its isogenic not genetically modified counterpart (CON) as main components. The study included two consecutive lactations.</p> <p>Results: There were no differences in the chemical composition and estimated net energy content of MON 810 and CON maize components and diets. CON feed samples were negative for the presence of Cry1Ab protein, while in MON 810 feed samples the Cry1Ab protein was detected. Cows fed MON 810 maize had a daily Cry1Ab protein intake of 6.0 mg in the first lactation and 6.1 mg in the second lactation of the trial. Dry matter intake (DMI) was 18.8 and 20.7 kg/cow per day in the first and the second lactation of the trial, with no treatment differences. Similarly, milk yield (23.8 and 29.0 kg/cow per day in the first and the second lactation of the trial) was not affected by dietary treatment. There were no consistent effects of feeding MON 810 or its isogenic CON on milk composition or body condition.</p>	This long-term study demonstrated the compositional and nutritional equivalence of MON 810 maize and its isogenic control when fed to lactating cows for two consecutive lactations.	Animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Animal performance	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Swiatkiewicz <i>et al.</i> , 2011)	<p>Objective: To estimate the effect of feeding pigs with genetically modified soybean meal (SBM) and maize on fattening results and fate of transgenic DNA in pig tissues.</p> <p>Experimental design: The experiment was carried out on 48 fatteners divided in four groups receiving feed mixtures according to requirement of growing and finishing pigs. The feed mixtures were 1) control (non-GM) SBM and maize; 2) SBM from 40-3-2 and control maize; 3) maize from MON 810 and control SBM; and 4) SBM and maize from 40-3-2 and MON 810, respectively. The experiment lasted from about 30 kg to 110 kg of their body weigh. At the end of fattening, all pigs were slaughtered and right half of carcass was evaluated. Growth, carcass yields and meat composition were measured and digesta, organs and meat were sampled for detecting DNA 170 or 172 bp fragments by PCR for MON 810 or 40-3-2, respectively.</p> <p>Results: Growth, carcass yields and carcass composition were unaffected by diets. DNA fragments were detected in digesta from the stomach and duodenum but not from other, lower gastrointestinal tract segments. Fragments were also not detected from organs or loin muscle. Histopathology of muscle and organs were unaffected by diets.</p>	<p>Animal performance was not affected by feeding on MON 810 maize and diets were of similar nutritive quality. Presence of transgenes only in upper portions of the digestive tract, confirms the efficiency of digestion process in the gastrointestinal tract. The GM feeds did not cause any histopathological changes in the examined tissues.</p>	Animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Toxicology	There are no changes to the conclusions of safety of the initial risk assessment

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

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Adel-Patient <i>et al.</i> , 2011)	<p>Objective: To investigate the immunologic and metabolomic impacts of Cry1Ab administration to mice, either as a purified protein or as the Cry1Ab-expressing MON 810 maize.</p> <p>Experimental design: Humoral and cellular specific immune responses induced in BALB/cJ mice after intra-gastric (i.g.) or intra-peritoneal (i.p.) administration of purified Cry1Ab were analyzed and compared with those induced by proteins of various immunogenic and allergic potencies. Cry1Ab protoxin was produced from the <i>B. thuringiensis</i> 407- strain harboring the pHT315Ωcry1Ab plasmid. Possible unintended effects of the genetic modification on the pattern of expression of maize natural allergens were studied using IgE-immunoblot and sera from maize-allergic patients. Mice were experimentally sensitized (i.g. or i.p. route) with protein extracts from GM or non-GM maize, and then anti-maize proteins and anti-Cry1Ab-induced immune responses were analyzed. In parallel, longitudinal metabolomic studies were performed on the urine of mice treated via the i.g. route.</p> <p>Results: Weak immune responses were observed after i.g. administration of the different proteins. Using the i.p. route, a clear Th2 response was observed with the known allergenic proteins, whereas a mixed Th1/Th2 immune response was observed with immunogenic protein not known to be allergenic and with Cry1Ab. This then reflects protein immunogenicity in the BALB/c Th2-biased mouse strain rather than allergenicity. No difference in natural maize allergen profiles was evidenced between MON 810 and its non-GM comparator. Immune responses against maize proteins were quantitatively equivalent in mice treated with MON 810 vs. the non-GM counterpart and no anti-Cry1Ab-specific immune response was detected in mice that received MON 810. Metabolomic studies showed a slight “cultivar” effect, which represented less than 1% of the initial metabolomic information.</p>	<p>The results confirm the immunogenicity of purified Cry1Ab without evidence of allergenic potential. Immunological and metabolomic studies revealed slight differences in mouse metabolic profiles after i.g. administration of MON 810 vs. its non-GM counterpart, but no significant unintended effect of the genetic modification on immune responses was seen.</p>	Human and animal health	No adverse effects were determined in this study.
			Observed parameter	Feedback on initial environmental risk assessment:
			Allergenicity	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Barros <i>et al.</i> , 2009)	<p>Objective: To compare fungal and mycotoxin levels in MON 810 and its control comparator grown in filed trials in Argentina during two seasons. To evaluate the interaction between genotype and fungicide treatment on fungal infection and mycotoxin concentration.</p> <p>Experimental Design: During the first season, trials were carried out in 7 different locations, and 5 different locations during the second season. MON 810 and its isogenic counterpart were planted under conditions of natural insect infestation following a complete randomized block design. At each site there was a four-row plot 7 m. long with row spacing of 0.75 m. Two applications of fungicide (tebuconazole) were given to each experimental unit. At harvest, samples of grain from each site were analyzed for fungal contamination and levels of fumonisins, deoxynivalenol (DON), and aflatoxin.</p> <p>Results: The mycoflora of the <i>Bt</i> and non-<i>Bt</i> maize genotypes was dominated by three genera: <i>Fusarium</i>, <i>Penicillium</i> and <i>Aspergillus</i>, at similar levels across all locations independent of the maize genotypes. <i>Fusarium</i> species were the most prevalent fungi found. The percent infect was lower in MON 810 than its control. Maize genotypes, fungicide treatment, fungal infection and their interaction did not significantly alter the frequency at which members of either of these genera were recovered.</p> <p>Total fumonisin levels in <i>Bt</i> and non-<i>Bt</i> hybrid were statistically significantly lower ($P < 0.001$), with lower levels in MON 810 compared to control at five of the seven locations evaluated during the 2002/2003 harvest season. Fumonisin levels were not affected by fungicide treatment. Similar results were obtained during the 2003/2004 harvest season, with lower fumonisin levels in <i>Bt</i> maize except at one location. The mean level of fumonisin varied significantly by locations ($P < 0.05$). The contamination level with DON was similar for both MON 810 and the control. DON levels were not affected by fungicide treatment. The mean DON concentrations did not differ significantly across locations ($P > 0.05$). No aflatoxin contamination was observed in either genotype in either harvest season.</p>	<p>It was concluded that fungal contamination and fumonisin mycotoxin levels were statistically significantly lower in MON 810 when compared to its control when grown at several locations in Argentina over two growing seasons. Aflatoxin contamination was not detected in either year at the locations tested There was no significant difference in the level of DON contamination. Application of the fungicide did not alter either the infection or the toxin levels in the MON 810 and control.</p>	Human and animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Toxicology	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Folcher <i>et al.</i> , 2010)	<p>Objective: To assess the impact of <i>Bt</i> maize on mycotoxin contamination in field trials</p> <p>Experimental Design: MON 810 maize and its isogenic non-<i>Bt</i> control were grown in 42 twin plots/year located in 21 fields in various locations in Southwestern France. The trials were carried out in 2005 and 2006 under natural conditions. No insecticides or fungicides were applied during cropping. Meteorological data was recorded to verify whether conditions were appropriate for <i>Fusarium</i> spp. growth and were homogenous over the field trials. Insect infestation was monitored during the trials. Two 1 kg samples of grain were collected from 20 mixed ears collected randomly from each plot (4 kg/field) for mycotoxin analysis. Deoxynivalenol, fumonisin B₁ and B₂ and zearalenone were analyzed in grain samples by LC-MS-MS.</p> <p>Results: <i>Bt</i> maize decreased concentrations of fumonisins by 90% and zearalenone by 50% when compared to levels in the non- <i>Bt</i> control, whereas the concentration of deoxynivalenol was slightly increased.</p>	<i>Bt</i> maize improved food safety by greatly reducing mycotoxin levels in field crops in Southwestern France.	Human health	A slight increase in deoxynivalenol levels in <i>Bt</i> maize was observed. However, in many other trials with <i>Bt</i> maize in other countries, there has either been no change or a reduction in deoxynivalenol levels ³ .
			Observed parameter	Feedback on initial environmental risk assessment:
			Toxicology	There are no changes to the conclusions of the safety of the initial risk assessment.

³ Ostry *et al.*(2010) . A review on comparative data concerning *Fusarium* mycotoxins in *Bt* maize and non-*Bt* isogenic maize. *Mycotox Res* 26:141–145.

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Randhawa <i>et al.</i> , 2011)	<p>Objective: Evaluate the potential cross-reactivity of Cry proteins, including Cry1Ab, using bioinformatics search tools.</p> <p>Experimental design: The amino acid sequences of the Cry proteins, including Cry1Ab, were compared to all allergens in the AllergenOnline database using FASTA3 algorithm and NCBI Entrez database using BLASTX algorithm. Additionally, search for similarity in domains of the allergen and Cry proteins was conducted.</p> <p>Results: No significant alignment or sequence similarity was observed between the Cry proteins and known allergens revealing there is no potential risk of allergenic cross-reactivity.</p>	None of the proteins were found positive for potentially allergenic cross-reactivity. Hence, the criteria for potential cross-reactivity have not been reached. This demonstrates that any significant risk of cross-reactivity for those who are allergic to known allergens is not expected.	Human health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Allergenicity	There are no changes to the conclusions of safety of the initial risk assessment

Area of the environmental risk assessment: Food/Feed Safety – Protein/DNA fate in digestive tract

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Guertler <i>et al.</i> , 2010)	<p>Objective: The objective of this study was to investigate the fate of transgenic <i>cry1Ab</i> DNA and the encoded Cry1Ab protein during the metabolic degradation of dietary feed components in dairy cows and a potential transfer to blood, milk, feces or urine.</p> <p>Experimental Design: A 25-month long-term feeding trial was conducted on thirty-six Simmentaler cows allocated in two groups fed diets containing either genetically modified maize (MON 810, N=18) or the near-isogenic maize variety (N=18). The nutrients and energy contents of both maize varieties were comparable, ensuring equivalent feed conditions. Feed samples were collected weekly, whereas samples for feces, blood and milk were collected monthly, urine samples were taken bimonthly. All samples were analyzed for <i>cry1Ab</i> DNA by means of end-point PCR (feces, blood, urine) and quantitative real-time PCR (feed, milk). A sensitive and highly specific ELISA, optimized to quantify immunoreactive fragments of the Cry1Ab protein, was used to determine the recombinant protein in the collected samples.</p> <p>The decision limit ($CC\alpha$) and the decision capability ($CC\beta$) of the ELISA were determined according to the guidelines of the European Commission Decision 202/657/EC.</p> <p>Results: Non-transgenic feed samples were free of recombinant DNA and protein within the limit of detection, while in transgenic feed samples both, a 206 bp fragment of <i>cry1Ab</i> and immunoreactive fragments of the Cry1Ab protein were present. Cows fed <i>Bt</i>-MON 810 maize had a daily Cry1Ab protein intake of 6.0 mg in the first lactation and 6.1 mg in the second lactation of the trial. In contrast, all blood, milk and urine samples were free of recombinant DNA and protein. The <i>cry1Ab</i> gene was not detected in any fecal sample, whereas immunoreactive fragments of the Cry1Ab protein were detected in feces from all cows fed transgenic feed.</p>	The authors conclude that “milk of dairy cows fed genetically modified corn for 25 months should be classified not different from milk of cows fed non-transgenic corn.”	Animal/Human 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.

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Paul <i>et al.</i> , 2010)	<p>Objective: To investigate the relative degradation and fragmentation pattern of the recombinant Cry1Ab protein from genetically modified (GM) maize MON 810 throughout the gastrointestinal tract (GIT) of dairy cows.</p> <p>Experimental Design: A 25 months GM maize feeding study was conducted on 36 lactating Bavarian Fleckvieh cows allocated into two groups (18 cows per group) fed diets containing either MON 810 or nearly isogenic non-GM maize as the respective diet components. All cows were fed a partial total mixed ration (pTMR). During the feeding trial, 8 feed (4 transgenic (T) and 4 non-transgenic (NT) pTMR) and 42 feces (26 T and 18 NT) samples from the subset of cows fed T and NT diets, and at the end of the feeding trial, digesta contents of rumen, abomasum, small intestine, large intestine and cecum were collected after the slaughter of six cows of each feeding group. Samples were analyzed for Cry1Ab protein and total protein using Cry1Ab specific ELISA and bicinchoninic acid assay, respectively. Immunoblot analyses were performed to evaluate the integrity of Cry1Ab protein in feed, digesta and feces samples.</p> <p>Results: A decrease to 44% in Cry1Ab protein concentration from T pTMR to the voided feces (9.40 versus 4.18 ug/g of total proteins) was recorded. Concentrations of Cry1Ab protein in GIT digesta of cows fed T diets varied between the lowest 0.38 ug/g of total proteins in abomasum to the highest 3.84 ug/g of total proteins in rumen. Immunoblot analysis revealed the extensive degradation of recombinant Cry1Ab protein into a smaller fragment of around 34 kDa in GIT.</p>	The results of the present study indicate that the recombinant Cry1Ab protein from MON 810 maize is increasingly degraded into a small fragment during dairy cow digestion.	Animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			Protein fate in digestive tract	There are no changes to the conclusions of safety of the initial risk assessment

Publication	Summary of research and results	Conclusions	Protection goal	Adverse effects
(Swiatkiewicz <i>et al.</i> , 2010)	<p>Objective: To determine the fate of recombinant <i>cryIA(b)</i> and <i>epsps</i> genes from MON 810 maize and 40-3-2 soybean meal in the digestive tract of Ross 308 broilers. The possibility of the transfer of transgenic DNA from feed to chicken tissues was also evaluated.</p> <p>Experimental design: Experimental maize-soybean meal diets (isonitrogenous and isoenergetic) were fed for 42 days to four replicates (pens) of 40 birds (20 males/20 females) per treatment group. Four treatment groups included (1) control: isogenic, parental non-GM maize and non-GM soybean meal, (2) non-GM maize and GM soybean meal, (3) GM maize and non-GM soybean meal and (4) GM maize and GM soybean meal. At the 43 day of age, the broilers were slaughtered. DNA was extracted from gut content and tissues and was analyzed for the presence of transgenic fragments using the PCR method, allowing detection of 0.1% genetically modified DNA in total DNA isolated from samples.</p> <p>Results: The transgenic sequences from single-copy genes of soybean (172 bp) and maize (170 bp) were detected only in content of crop and gizzards of broilers consuming feed containing genetically modified maize/soybean. There were no traces of transgenic DNA in duodenum, jejunum, ileum, and caecum digesta, excreta, and in blood, liver, spleen, and breast muscle. Similarly, no small fragments from other single copy genes of soybean and maize (recombinant 35s promoter and NOS terminator, and endogenous lectin and invertase genes) were detected in broiler tissues.</p>	The obtained data indicated that transgenic DNA sequences from MON 810 maize and 40-3-2 soybean are well digested in the gastrointestinal tract and are not transferred to broiler tissues.	Animal health	No adverse effects were detected in this study
			Observed parameter	Feedback on initial environmental risk assessment:
			DNA fate in digestive tract/tissue	There are no changes to the conclusions of safety of the initial risk assessment

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