

Agricultural Biotechnology Stewardship Technical Committee

Science Narrative - The Performance of Poultry and Livestock Fed Biotech Crops compared to their Conventional Counterparts

I. Description of the topic, sub-topics and background information.

As the adoption of biotech crops grew from 1996 to 2000, the animal production industry and related associations began receiving questions regarding the performance of animals fed biotech crops as compared to the conventional counterparts of the same crop. The biotech industry reviewed the existing animal performance data with the animal industry, industry associations as well as commodity grower associations in late 1999 and early 2000.

II. Review of research on the issue.

Numerous studies with beef and dairy cattle; broiler and layer chickens; swine; sheep and catfish have shown there is no difference in animal performance due to the consumption of biotech crops compared to their conventional counterparts. Biotech and conventional corn, soy, canola and sugar beets have been compared in these studies. This review will be restricted to the published studies on corn and soy as these crops have been the only crops of interest to the industry.

Corn

Poultry:

Aulrich et al² fed Bt and non-Bt corn grain in a five-day study to laying hens and measured no differences in nutrient composition, body weight, digestible organic matter and protein as well as metabolizable energy.

Brake and Vlachos¹⁰ conducted a 38-day broiler study comparing Bt and non-Bt corn and measured no differences in mortality, body weight, feed intake while measuring an improvement ($p < .05$) in feed conversion. Carcass data was not different between groups with the exception of breast meat yield that was improved ($p < .05$) in broilers fed Bt corn. Slight differences in overall composition of the diets may have been the cause for these improvements with the Bt corn.

Halle et al²⁸ fed Bt and non-Bt corn in a 35 day broiler trial. There were no differences in body weight gain, feed intake, feed conversion or protein digestibility.

Sidhu et al⁶⁴ measured no difference in growth, feed efficiency and fat pad weight between broilers fed diets containing glyphosate-tolerant corn and diets containing conventional corn.

Mireles et al⁴⁸ conducted two studies to compare nutrient composition and availability in Bt and non-Bt corn. The first study measured true metabolizable energy (TME) and amino acid digestibility. There were no differences for TME or amino acid digestibility between the two corn sources. The second study was designed to measure the performance of broiler chickens fed starter feeds. No differences were seen for the parameters that were measured between Bt and non-Bt corn (weight gain and feed efficiency).

Lactating cows:

Faust and Miller²⁰ fed green chop from Bt or non-Bt corn to lactating cows for 14 days. No differences were measured in feed intake, milk yield, milk composition or udder health.

Two diets containing Bt or non-Bt corn silage were fed with grass silage and supplement in a five-week crossover test by Mayer and Rutzmoser.⁴⁴ There were no differences measured between diets for intake, milk production and milk composition (fat, protein, lactose, urea).

Folmer et al²³ conducted research designed to compare the ruminal fermentation parameters and lactational performance between four balanced diets containing Bt or non-Bt corn silage either from early maturity or late maturity hybrids in a 4 X 4 Latin square design. No differences were measured between Bt and non-Bt diets at either maturity for rumen fermentation characteristics (in-situ NDF digestibility, rumen VFA concentration, rumen pH), milk production or milk composition. The animals fed early maturity hybrids (Bt and non-Bt) did have improved ($P < .005$) total rumen VFA and efficiency of production than later maturity hybrids (Bt and non-Bt).

The effect of feeding glyphosate tolerant and non-glyphosate tolerant corn silage and corn grain fed in identical mixed rations to lactating dairy cows was determined by Donkin et al.¹⁵ No differences were measured between groups for dry matter intake, milk production, milk protein yield, lactose yield or milk fat yield. Likewise, no differences were measured in milk composition (percentage of: fat, protein, lactose, solids not fat, somatic cell count or milk urea nitrogen).

Beef and sheep:

Animal performance of beef cows grazing Bt or non-Bt corn crop residue has been compared over a two-year period.^{59,60} There was no difference in animal performance in either year of the two-year study.

Two trials were conducted to evaluate the utilization of corn silage and corn residue by Folmer et al.²² An absence of significant European corn borer pressure resulted in similar grain yield and residue corn between Bt and non-Bt corn. In trial 1, twenty-three acres of Bt corn residue and twenty-one acres of non-Bt were divided into 3 pastures each and stocked with 8 or 9 steers per pasture to result in equal stocking rates. Average daily gain (avg. 0.28 kg/d) was similar between both corn sources. In addition, 16 steers were allowed access to either 7 acres of Bt or non-Bt corn residue. No preference was shown for grazing either field.

In trial 2, 128 steers were fed diets containing either Bt or non-Bt versions of two hybrids as corn silage at a 90% inclusion rate with 10% supplement. The steers were fed in a 2 x 2 factorial design and performance parameters were measured. Dry matter intake was higher ($P < .05$) for steers fed Bt than non-Bt corn silage (8.61 vs. 8.32 kg/d respectively). An interaction ($P < .05$) was observed between genotype and the Bt trait for daily gain and feed efficiency. For hybrid A, steers receiving the Bt version had improved daily gain over those receiving the non-Bt version. For hybrid B, there was no significant difference in daily gain between the steers fed the Bt vs. the non-Bt version. Steers fed both versions of hybrid A had improved ($P < .05$) feed efficiency when fed the Bt version than when fed the non-Bt version. No differences were measured in feed efficiency between steers fed the Bt and the non-Bt versions of hybrid B. Improved ($P < .01$) daily gain and feed efficiency were measured for steers fed hybrid B compared to hybrid A, although an interaction was present. The authors concluded that while hybrid genotype appeared to affect performance, there was no consistent effect on performance of growing steers due to the presence of the Bt trait.

Daenicke et al.¹¹ compared the digestibility and animal performance of sheep and growing bull calves that were fed Bt corn silage or non-Bt corn silage. There were no differences in the digestibility of organic matter, fat, fiber or nitrogen free extract. Likewise, there were no differences in intake, body weight gain, feed conversion, hot carcass weight, dressing percentage and abdominal fat.

The feeding value of whole plant corn silage and crop residues over a two year period was compared between Bt and non-Bt corn by Hendrix et al.³² Three studies were conducted each year: 1) performance of steer calves fed corn silage, 2) performance of beef cows grazing corn residue and 3) grazing pattern of beef cows when given a choice between Bt and non-Bt residue. There were no differences between steers fed the two corn silage sources for average daily gain or dry matter intake. Feed/gain was greater ($P < .05$) for Bt vs. non-Bt corn silage. There was no difference in weight change between cows grazing the Bt and non-Bt residues. Over the entire observation period, no differences were measured in preference for one corn residue over the other between grazing cows.

Russell et al.⁵⁷ studied the nutritive value of the crop residues from Bt and non-Bt corn hybrids and their effects on performance of grazing beef cows. No differences were measured between the residues from Bt and non-Bt residues for dry matter or organic

matter composition. Over the grazing season, no differences were measured between residues for rates of change of residue composition.

In further work, Russell et al⁵⁸ studied the effects of grazing crop residues from Bt-corn hybrids on the performance of pregnant beef cows. Four hybrids planted on duplicate fields were utilized in the study that was conducted over 2 consecutive years. One hybrid was non-Bt while three hybrids contained the Bt gene (two with Yieldguard ® and one with Knockout ®). Thirty Angus x Charolais x Simmental cows in midgestation were allotted between to two drylots or the eight crop residue fields to strip-graze for 126 days. Biweekly visually estimated body scores were taken with dry alfalfa hay supplemented to maintain a mean body condition score of 5 out of a 9-point scale. Crop residue yields were determined monthly from a 4 square-meter location in each grazed and ungrazed area paddock. On two consecutive days following 2 weeks of grazing, forage selected during a two-hour grazing period by one fistulated steer per field or drylot was harvested from via the rumen cannulae. DMI was calculated from the digestibility of the forage and the fecal output in two cows per field or drylot during the same 2-day period. There were no effects on yields of harvested grain, dropped ears or grain, residue DM or OM over the 2 years. AT grazing initiation, IVOMD as well as ADF and ADL differed ($P < .05$) by base genetics but not by Bt vs. non-Bt hybrids. Rates of change in NDF, ADF, ADL, CP and IVOMD over winter did not differ between hybrids. There were also no differences between hybrids for intakes of forage digestible OM, NDF and ADF. No differences were seen in the amount of hay required to maintain body condition score between hybrids.

Kerley et al³⁷ compared Bt and non-Bt corn fed to beef steers for the last 49 days of the finishing period. Thirty-six crossbred steers were allotted to six pens and fed a 75% corn diet. Growth performance and carcass parameters were measured. There were no differences in corn composition, average daily gain, feed efficiency, yield grade or quality grade between Bt and non-Bt corn hybrids.

Petty et al⁵² compared Bt vs. isogenic non-Bt corn fed as whole plant silage (WPS) and dry rolled grain over a two-year period. Each year corn was grown under isolation and harvested as grain and WPS. A feeding study was performed each year utilizing 56 Angus and Simmental sired steers were blocked and randomly allotted by weight and breed type one month postweaning into eight pens of seven steers each. Growing diets comprised primarily of WPS were fed for 89 and 85 days in years 1 and 2 respectively were followed by finishing diets comprised of 75% dry rolled corn, 15% WPS and 10% supplement for 101 and 84 days in years 1 and 2 respectively. During the grower phase in year one, there were no differences ($P > .05$) in average daily gain or dry matter intake but feed efficiency was improved ($P < .05$) for the steers fed non-Bt corn however this difference was not measured in year 2. There were no differences ($P > .05$) in average daily gain, dry matter intake or feed efficiency during the finishing phase for either year. Steers were harvested each year when they were estimated to be 75% USDA choice as a group. There were no differences ($P > .05$) in carcass characteristics in either year. The investigators summarized that there were no major differences in the feeding value of the Bt-corn compared to its isogenic counterpart.

Petty et al⁵³ have also evaluated herbicide resistant and non-herbicide resistant corn fed as whole plant silage (WPS) and dry rolled grain. A total of 56 Angus and Simmental sired steers were blocked and randomly allotted to treatments by weight and breed type. The grower diet of 90% WPS and 10% supplement was fed for 85 days followed by the finishing diet comprised of 75% WPS, 15% dry rolled corn and 10% supplement for 84 days. Average daily gain, dry matter intake and feed efficiency were not different ($P>.05$) during the grower phase, the finishing phase or over the total duration of the feeding study. Steers were harvested when it was estimated that 75% of the steers would grade USDA choice. There were no differences ($P>.05$) in carcass characteristics.

Swine:

Herbicide tolerant and non-herbicide tolerant corn was compared in swine metabolism studies by Böhme and Aulrich.⁹ The results showed that no differences were measured in protein digestibility, nitrogen free extract (NFE) digestibility or metabolizable energy (ME).

Weber et al⁷³ compared grower-finisher performance and carcass characteristics from pigs fed Bt, the non-Bt isogenic counterpart or commodity-sourced (CS) corn. For animal performance, no differences were measured in average daily gain, average feed intake, or feed efficiency between pigs fed any of the 3 corn sources. Pigs fed Bt and the non-Bt corn were not different in carcass weight however pigs fed CS corn had heavier carcass weights and higher dressing percentages ($P<.05$) than the other two groups. Pigs fed the isogenic control had less ($P<.05$) percent lean, greater backfat depth at the 10th rib and P2 location than pigs fed diets containing Bt or the CS corn. Pigs fed the non-Bt had greater ($P<.05$) backfat depth at the last lumbar vertebrae than pigs fed CS corn. Marbling scores were highest ($P<.05$) for pigs fed Bt and non-Bt corn. Weber et al concluded that Bt corn had no adverse effects on growth performance or carcass characteristics.

In-Vitro:

Faust²⁰ compared *in-vitro* digestibility between corn silages derived from Bt and non-Bt corn which were ensiled at two different stages of maturity. No differences were measured in cell wall, true or dry matter digestibility regardless of stage of maturity.

Russell et al⁵⁷ studied the nutritive value of the crop residues from Bt and non-Bt corn hybrids. No differences were measured between the residues from Bt and non-Bt residues for *in-vitro* digestible dry matter. The *in-vitro* organic matter digestibility in residues selected by steers after two weeks of grazing also did not differ.

Soybean Meal

Padgett et al⁵¹ demonstrated the compositional equivalence between conventional soybeans and glyphosate tolerant soybeans. Hammond et al³⁰ reported results from feeding trials comparing soybean meal derived from glyphosate-tolerant soybeans and

soybean meal from the conventional counterpart in broilers, catfish and dairy cows. No differences were measured in feed intake, body weight gain, feed efficiency, breast meat composition and fat pad thickness in broilers. Catfish fed diets comparing both soybean meals exhibited no differences in weight gain, feed efficiency or meat composition. In the dairy cow study comparing the two soybean meal sources, no differences were measured in feed intake, milk yield, milk composition, dry matter digestibility and rumen fermentation end-products between cows fed diets containing either soybean meal.

Science Narrative – The Composition and Quality of Meat, Milk and Eggs from Animals fed Biotech Crops

I. Description of the topic, sub-topics and background information

The animal production industry and related associations have received questions regarding the composition and quality of meat, milk and eggs (MME) derived from animals fed biotech crops. There are three published studies that can be referenced where quality parameters were measured. The animal production industry and the biotech industry do not believe that there should be any difference in the quality of MME from substantially equivalent biotech crops. At the same time, more evidence would be desirable to assist both industries in responding to potential questions or claims around quality differences.

This topic is one of market acceptance. Although opponents have not raised the issue, the industry would like to be prepared for such questions should they arise.

II. Review of research on the topic.

Some nutrient components from feed/forage may be passed on to milk, meat or eggs.³⁶ Changes in the composition of the crop as a result of conventional breeding or genetic engineering may change the composition of these animal products.¹² However, the limited research conducted to date has shown no difference in composition of milk or fish fillets.^{20,30}

Lactating cows that were fed green chop Bt corn for 14 days showed no difference in the amount or quality of milk compared to cows fed green chop from the conventional counterpart.²⁰

Lactating cows fed complete diets containing soybean meal from glyphosate tolerant soybeans showed no difference between it's conventional counterpart in the amount or quality of milk.³⁰ Quality parameters included fat, protein, lactose and somatic cell count.

The same soybean meal from glyphosate-tolerant soybeans was also included in complete diets fed to catfish in another study. No differences were measured in fillets for proximate analyses (moisture, protein, fat and ash) between the biotech-derived meal and it's conventional counterpart.³⁰

Science Narrative –Attempts to Detect Transgenic DNA and Protein in Meat, Milk and Eggs and the Digestive Fate of DNA and Protein

I. Description of the issue, sub-issues and background information

Questions regarding the digestive fate of DNA and protein from biotech crops have been raised from a number of perspectives. The animal production industries and related associations have asked for documentation on whether the DNA or protein from biotech crops can be detected in meat, milk, and eggs. Consumer groups have asked whether direct human consumption of the DNA or protein in plant biotech products impacts human health and whether human consumption of animal products (e.g. meat, milk or eggs) from farm animals fed the biotech crops are safe. The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO)^{19,74}, the US Food and Drug Administration (FDA)⁶⁹ and the U.S. Environmental Protection Agency (EPA)⁶⁸ have each stated very clearly that the consumption of DNA from all sources – including plants improved through biotechnology– is safe, given the long history of safe consumption of DNA. Likewise, the safety of the introduced protein(s) in biotech crops is established on a case-by-case basis according to the safety assessment principles and processes outlined by the FAO/WHO^{17,18} and OECD^{49,50} and regulatory agencies around the world. However, even with the safety of the DNA and protein introduced into biotech products based on strong scientific principles and pre-market regulatory assessments, the advent of new analytical technology like polymerase chain reaction (PCR) leaves unanswered the question of detectability. Therefore, reviewed here are numerous studies that have been conducted to address the question of whether the transgenic DNA or protein is detectable in animal products. Also reviewed is information on the digestive fate of DNA and protein in animals and humans. This digestive fate information further explains why it is safe to consume these macromolecules and why studies to date have not detected the transgenic DNA or protein from biotech sources in the meat, milk or eggs of animals fed these agricultural biotechnology products.

II. Review of research on the sub-issues.

DNA is the basic building block of all life and is in every cell of all animals, plants and microbes. Therefore, nearly all food and feedstuffs contain DNA, and animals and people have safely ingested DNA over the course of natural history. When animals and humans consume food or feed, normal chemical processes break the macromolecular components of the food or feed (e.g. DNA, protein, complex carbohydrates, lipids) into nutritionally beneficial subunits (e.g. nucleotides, amino acids, sugars). The digestive systems of humans and animals have evolved to be effective in degrading DNA and proteins in food or feedstuffs into the constituent components and re-utilizing the resulting nutrients for synthesis of new macromolecular components for growth, maintenance and reproduction. The effectiveness of digestive degradation of macromolecules is evidenced by the long history of safe consumption of DNA and proteins by mammals. All DNA and protein, including DNA and protein from plants improved through biotechnology, are made up of the same building blocks and are

sensitive to the same digestive processes. Although the transgenic DNA introduced into biotech plants is no different from the native plant DNA, experiments were conducted to test for both endogenous and transgenic DNA given the perception that may exist that they are different. The research related to whether DNA or protein from biotech crops can be detected in products from animals fed these crops is reviewed in order to address a series of questions:

- a. Have the proteins introduced into ag biotech products been detected in farm animal products?
- b. Has DNA and/or transgenic DNA been detected in farm animal products?
- c. What is the metabolic fate and safety of ingested proteins, including biotech proteins?
- d. What is the metabolic fate and safety of ingested DNA, including transgenic DNA?

a. Have the proteins introduced into ag biotech products been detected in farm animal products?

Four studies conducted by Novartis/Syngenta demonstrated that biotech proteins were not detectable in samples from animals fed biotech crops. Using a sandwich ELISA assay for the Cry1Ab protein introduced into BT176 corn, studies were conducted on samples from poultry (muscle, liver, egg whites, egg yolks, mid gut tissue) and ruminants (milk, muscle, spleen) fed this insect-protected product. The Cry1Ab protein was not detected in any of the samples from the four studies. Presently, only the milk assay results from one of the four studies²⁰ have been published.

A poster at the 2000 Poultry Science meeting held in Montreal presented data showing that the CP4 EPSPS protein in Roundup Ready[®] soybeans could not be detected by a double antibody sandwich ELISA specific for this protein (Strategic Diagnostic Inc.) in tissues and eggs of laying hens.¹ Not surprisingly, the raw Roundup Ready[®] soybeans, soybean meal and complete diets were positive for CP4 EPSPS protein. By comparison, whole egg, egg white, liver and fecal samples were all negative for the biotech protein. The poster concluded that the digestive process of the laying hen effectively breaks down the CP4 EPSPS protein from the soybean meal portion of the diet such that no modified protein is detectable in the liver, eggs, or feces.

A Japanese government study has concluded tests attempting to detect transgenic protein and DNA in samples from chickens fed a diet containing StarLink[®] maize.³⁵ Press reports from the Japan Ministry of Agriculture, Forestry and Fisheries study stated that neither the Cry9C gene nor protein could be detected in the muscles, livers or blood of chickens that had been fed for up to seven weeks with StarLink[®] maize. The ministry said it would release by July, 2001, the outcome of similar tests being conducted on chicken eggs, dairy cattle and pigs.

Weber and Richert presented a poster at the 2001 Midwest meeting of the American Society of Animal Sciences (ASAS) and American Dairy Science Association (ADSA) in Des Moines, IA showing that all samples of pork loin muscle tissue from grower-finisher pigs fed Bt corn (YieldGard[®]) had no detectable levels of intact or

immunologically reactive fragments of the Cry1Ab protein, using a competitive immunoassay for the Bt protein.⁷² Their data showed that the growth performance and carcass characteristics of pigs fed Bt corn was statistically similar to that observed for pigs fed conventional corn.

Recently, Monsanto studies have provided further support for the conclusion that biotech proteins cannot be detected in animal products. Monsanto studies have not detected the Cry1Ab protein in chicken breast meat, beef brisket and dairy milk from animals fed YieldGard[®] corn grain. It is noteworthy that the competitive ELISA format used in the Monsanto studies was specifically designed to detect both intact protein and immunologically reactive fragments of the Cry1Ab protein. The Monsanto studies are currently being summarized into reports for publication.

b. Has DNA and/or transgenic DNA been detected in farm animal products?

Questions concerning the digestive fate of transgenic DNA are not new and were originally asked in the mid-1970's following the advent of recombinant DNA methodologies.⁴³ These early studies evaluated the degradation of DNA from the functionally-crippled *E. coli* cells and plasmids used for recombinant DNA research. It was shown that the bacterial chromosomal transgenic DNA was rapidly degraded in the stomach and small intestine, attributable to stomach acids and pancreatic nucleases. The plasmid DNA was even less stable, possibly owing to its smaller size. In a more recent study, direct analysis of the stability of the transgenic DNA that encodes the *bar* gene in rapeseed was demonstrated to be completely broken down into nucleotides in digestive fluids isolated from swine, chickens, and cows within one hour at 37° C and pH 1.5.⁵⁴ This rate of degradation is similar to other DNA molecules studied (see below).

In 1998, Klotz and Einspanier published that the CP4 EPSPS gene of Roundup Ready[®] soybeans was not detectable by PCR followed by Southern blot in either the blood of the cow or the milk from these animals.⁴⁰ This publication showed that the highly sensitive method of PCR followed by a Southern was able to detect a small fragment of a highly abundant endogenous chloroplast gene in blood lymphocytes but not milk. Very recently, Einspanier's laboratory has published data from a study in which dairy cows, beef steers and broiler chickens were fed either conventional maize grain or grain from Novartis' BT176 event.^{16,21} The investigators evaluated two DNA detection technologies [standard Polymerase Chain Reaction (PCR) and Light Cycler "real time" PCR]. Although Light Cycler PCR showed advantages for detecting Bt-maize in feed, this technique did not provide additional sensitivity beyond standard PCR methods for animal tissue samples. The presence of even a small portion of the coding region of the Bt gene (Cry1Ab) was not detectable by either standard PCR or Light Cycler PCR in any samples from the cows, steers or chickens fed BT176 maize. Similar to their previous report, using standard PCR technology, a small portion of the coding region of a highly abundant chloroplast gene (tRNA_{leu}) was detectable in lymphocytes of dairy cows and in muscle, liver, spleen and kidneys of chicken, but not in dairy milk, or any tissue samples from steers. It is important to note that plastid genome copy number per cell varies depending on tissue-type, ranging from ~500 to

10,000 copies in roots and leaves, respectively.⁶ Therefore, the copy number of plastid genes is orders of magnitude higher than a transgene in a biotech product, which is typically has only one copy present per haploid genome. In addition, plastid gene sequences are also present in high numbers in the nuclear genome, with sometimes >100 copies of some sequences being observed,³ such that the nuclear copies of plastid genes are an additional source of positive PCR signals. As a consequence, the high copy number of plastid genes and their subcellular localization within organelles could explain detection of these endogenous genes while transgenic DNA fragments are undetected to date.

Khumnirdpetch, *et al*, of Thailand, presented a poster at the 9th Plant and Animal Genome Conference in San Diego, January 2001, in which results from their studies attempting to detect transgenic DNA in broiler chickens were shown.³⁸ Broiler chickens were maintained by commercial standards and fed diets containing meal from either conventional or Roundup Ready[®] soybeans from birth to seven weeks of age. Samples (meat, skin, duodenum and liver) were isolated from the birds at 1, 3, 5 and 7 weeks. Real-time PCR was used to test for the transgenic DNA in the various samples. PCR results of the broiler samples taken over this entire seven-week feeding period were all negative. The authors speculated that the negative detection results suggest that the transgenic DNA in Roundup Ready[®] soybean meal has been fully degraded in the digestive tract of the broilers.

Weber and Richert's poster at the 2001 Midwest meeting of the ASAS and ADSA also included data on PCR studies attempting to detect both the Bt gene and an endogenous corn gene in DNA extracted from 24 pork loin samples (12 fed YieldGard[®] corn and 12 fed a control conventional corn).⁷² PCR, followed by Southern blot analysis for ~200 bp fragments of the cry1Ab and shrunken-2 (sh-2) were uniformly negative.⁷² The sh-2 gene is an endogenous single-copy corn gene. By comparison, an endogenous swine gene (pre-prolactin) was readily detected in all pork loin samples, and spiking corn DNA into the extracted swine DNA also yielded positive results, indicating that the DNA quality and PCR conditions were both favorable for detection of DNA fragments, had they been present in the original samples. The PCR assay coupled with Southern blot was shown to have a limit of detection of approximately 1 to 2.5 pg of target DNA per 1 µg of input DNA, or approximately 1 genome equivalent of the target gene per PCR, the theoretical limit of assay sensitivity.

DNA degradation during the digestive process has been documented from mouse feeding studies with M13 phage DNA and recently reviewed.¹³ From studies feeding purified M13 phage DNA to mice, it was observed that up to approximately 0.1% of that ingested DNA could be detected in their blood.⁶¹⁻⁶³ This extremely high level of DNA observed in the circulation is most likely owing to unique features of this circular, non-methylated phage DNA. Using the M13 data from mice, however, a calculation can be performed to predict the theoretical level of transgenic DNA that might be present in animal tissues, assuming uniform tissue distribution of that DNA in the farm animal. Basing uptake of transgenic DNA in farm animals on the mouse M13 phage data, it can be estimated that approximately 0.002 fg of transgenic DNA (1 femtogram equals one-trillionth of a mg, or 10⁻¹⁵ of a gram) might be present per mg of muscle tissue in the farm animal. No transgenic DNA has been detected in meat,

milk or eggs from farm animals fed biotech products. These results are consistent with the knowledge that there are extremely small amounts of transgenic DNA in plants improved through biotechnology (<0.0004% of the total plant DNA).

However, it is important to remember that even if transgenic DNA is detected by a future study, scientific evidence and opinion concludes that ingested transgenic DNA would not be any different from ingestion of DNA already in foods, which is deemed safe. The safety of ingested DNA cannot only be derived from the long natural history of animal and human consumption of DNA, but it is also significant that, as would be expected because of digestive processes, no intact genes, only relatively small fragments, have yet been detected in animal tissues, regardless of the gene's abundance. Instead, in the published reports describing detection of DNA from ingested plants in animal tissues, only small portions of the entire coding region of these highly abundant chloroplast genes were found.^{16,40} Furthermore, only samples from a fraction of the total number of tested animals are yielding positive detections for these highly abundant gene fragments, suggesting that most of the individual animals are degrading ingested DNA to levels below the most sensitive PCR detection limits. Therefore, the likelihood that a transgenic gene or fragments is absorbed to any significant degree following digestion remains extremely low, especially when the relatively low levels of the transgenic DNA per cell is also considered when compared to the highly abundant endogenous plastid genes.

c. What is the metabolic fate and safety of ingested proteins, including biotech proteins?

Proteins are hydrolyzed into increasingly smaller fragments in the mammalian digestive system. Intact proteins are, for the most part, not absorbed across the gut wall *per se*.²⁵ One exception would be a special class of milk-borne immunoglobulins (IgA) that are specifically designed to be absorbed to provide passive immunity for newborn mammals.²⁵ A second exception is that very low levels of intact proteins or large fragments of proteins are taken up by mononuclear leukocytes, possibly through macropinocytosis, as part of the immune system surveillance of gut contents.⁶⁷ From a nutritional perspective, proteins are typically classified as either non-digestible (excreted in the feces) or digestible, (absorbed as small peptides and amino acids). The digestible proteins are largely soluble in water or acid whereas the indigestible proteins are typically insoluble, being bound primarily to sugars or fiber. Within the stomach, hydrochloric acid and pepsin denature and fragment the soluble proteins.⁷¹ Later in the small intestine, protein-digesting enzymes secreted by the pancreas and intestinal wall further fragment the protein chains into peptides of decreasing size, typically ranging in length from a few hundred amino acids down to the component individual amino acids and extremely short peptides (two to six amino acids). The stability of the introduced protein(s) in a transgenic plant is routinely determined in an *in vitro* digestive fate study as part of the assessment of allergenic potential.²⁷

Roberts et al evaluated the oral bioavailability of three bioactive peptides of varying size: thyrotropin-releasing hormone, lutenizing hormone-releasing hormone and insulin, that are 3, 10 and 51 amino acid in size, respectively.⁵⁵ This study showed

that the oral bioavailability of these peptides was inversely related to their length such that peptides longer than 10 amino acids were very poorly absorbed intact. In addition, in most cases if small peptides are absorbed by intestinal epithelial cells they are degraded intracellularly to amino acids before being absorbed into the circulation. In another study, it was shown that 0.007-0.008% of ovalbumin orally administered to humans was detectable in their circulation.⁶⁷ The authors concluded that the digestive tract provides a strong barrier to the absorption of macromolecular proteins into the body. Amino acids that enter the blood stream are used to synthesize new proteins. Any non-essential amino acids in excess of nutritional requirements are further degraded to carbon and nitrogen that are oxidized as a source of energy (carbon), or to ammonia (nitrogen), that is converted to urea by the liver and excreted. Although the indigestible proteins pass through the digestive tract undigested, they may be partially fermented by enteric bacteria in the large intestine as a source of energy and nitrogen.

The digestibility of the protein(s) introduced into a biotech plant is routinely assessed by an *in vitro* digestive fate study as part of the safety assessment of allergenic potential.⁴⁷ To date, the proteins introduced into biotech products approved for food and feed usage have been shown to be readily degraded in simulated gastric digestion studies.^{8,47} Hence, the introduced proteins are very unlikely to be detectable in farm animal products consumed by humans. This approach to assess the safety relative to food allergy is consistent with the guidance provided by the International Life Science Institute (ISLI) and which has served as primary source of guidance for regulatory agencies around the world.⁴⁷

d. What is the metabolic fate and safety of ingested DNA, including transgenic DNA?

The FAO¹⁹, WHO⁷⁴, U.S. FDA⁶⁹, and the U.S. EPA⁶⁸ have concluded that there is no inherent risk in consuming DNA, including that derived from biotech crops. A key reason for their conclusion is the long history of safe consumption of significant quantities of DNA from a wide variety of sources including plants, animals and microbes by animals and people.

DNA, a nucleic acid, encodes the fundamental genetic information by which the vast majority of organisms convey instructions for function and survival of self to subsequent generations. Consequently, DNA is an essential component of most living organisms and, as such, is present in nearly all foods and feedstuffs. In biotech crops, the introduced transgenic DNA molecules are made of exactly the same basic chemical components as the endogenous DNA (four ubiquitous nucleotides - adenosine, guanosine, thymidine, and cytosine). Therefore, the addition of transgenic DNA into a plant does not introduce any new chemical entities to foods or feeds. Generally, the total DNA in food contributes less than 0.02% to the total dry matter of the food.⁷⁰ The amount of transgenic DNA in plants improved through biotechnology represents an extremely small proportion of the total amount of DNA in a biotech plant (<0.0004% of the total plant DNA). To help put into context the level of transgenic and total DNA consumed by an animal, it has been estimated that approximately two thirds of a gram (608,000 µg) of DNA is consumed on a daily basis by a 600 kg

animal such as a cow.⁴ If 60% of the feed were from a biotech crop such as BT176 maize, the daily intake of transgenic DNA would be approximately 1.5 µg, which is approximately 0.00025% of the total amount of DNA ingested per day.

The gastrointestinal tract is constantly exposed to foreign DNA that is released from partially or completely digested foods or feeds, ingested microbes, and DNA from intestinal microflora. Ingested food is mechanically disrupted and the released DNA is cleaved through acid hydrolysis and enzymatic digestion (especially by DNase I from salivary and pancreatic secretions) into small DNA fragments and eventually converted to single nucleotides.⁴⁶ The presence of various phosphatases and deaminases continue to destroy the structural integrity of any free DNA. One study with beef steers showed that plant DNA in feed is progressively degraded as it moves through their digestive tract, with over 50% being degraded in the first third of the intestine and 80% having disappeared by the time the digesta reaches the terminal ileum.⁴⁵ DNA given directly to steers was shown to be completely degraded into mononucleotides by the animal's digestive tract in about 4 hours.⁴⁶ The generated nucleotides are readily abundant in food and feed and exceed nutritional requirements of the host⁷⁵ and gut bacteria.⁴⁶ The breakdown products of DNA are absorbed for use in cellular synthetic processes as they can be found in blood and tissues⁴⁶; however, as intact nucleotides they are non-essential nutrients. The nucleotides are typically deaminated before being rapidly absorbed. Once absorbed, they are further catabolized into nitrogenous bases, free bases and other metabolites including sugars and phosphates that are used in cellular biosynthetic pathways.⁶⁵ Interestingly, intestinal epithelial cells have unique salvage pathways for using free nucleotides, owing to their high rate of cell turnover.³¹ Any small polynucleotide DNA fragments that might enter the body would be phagocytized by mononuclear leukocytes and further degraded by cellular enzymes and nucleases.¹³

The fact that the nucleotides of any gene (endogenous or transgenic) are in a precise sequential order to encode for production of a specific protein becomes essentially irrelevant to the digestive processes. The genetic sequence for a protein introduced in a plant is only functional when the DNA (gene) is activated in the plant. The presence of DNA in the diet is so common that it is of virtually no consequence to animals and people consuming plant-derived products. A recent publication describes experiments that directly tested whether extensive feeding of DNA to mice results in detectable expression of mRNA and protein in any of several organs of the animals.³³

Approximately 50 µg of DNA was fed to the mice per day. The DNA fed to the mice encoded the green fluorescent protein (GFP) under the control of one of three strong mammalian viral promoters [human cytomegalovirus (hCMV), Rous sarcoma virus (RSV) or simian virus 40 (SV-40)]. Separate experiments used a "gene therapy" approach with intramuscular injection into mice of the GFP gene coupled with either the hCMV promoter (pEGFP-C1) or the RSV promoter. These gene therapy studies showed clearly detectable expression of the GFP protein and mRNA at the site of injection.³³ By comparison, no GFP protein or mRNA expression was detectable in liver, spleen, blood or intestinal epithelia of 21 animals fed the exact same DNA over a three week period. Also, fragments of the GFP gene were not detectable by PCR analysis of DNA isolated from spleen, liver or tail tip samples from either this three

week feeding study or a separate experiment that involved feeding 50 µg of the pEGFP-C1 DNA per day to mice over eight generations.³³ Therefore, it can be concluded from these studies that gene/promoter constructs clearly capable of functioning *in vivo* when administered via a gene therapy procedure (e.g. intramuscular injection) do not lead to gene expression in somatic cells or detectable integration into the germline of animals when provided orally.

In addition to digestive processes that degrade DNA, feed processing procedures (and food preparation methods) significantly degrades DNA, especially those that involve heating to temperatures greater than 95°C (200°F).^{24,26} The stability of transgenic DNA in maize preserved as silage has been studied.³⁴ The intact transgene was only detectable during the first five days of ensiling, with only small fragments (about 200 bp) of DNA being identifiable using sensitive PCR methods for longer stored silage. The rapid breakdown of DNA during ensiling was not unexpected since this process creates a harsh environment that involves plant tissue being chopped which leads to cell breakage, release of cell contents including the DNA and nucleases, and mild acidic conditions from natural fermentation. Thus, feed generated by ensilage reduces an animal's dietary exposure to intact DNA, including any introduced transgenic DNA, even before ingestion and further degradation by its own digestive system.

On a related issue of DNA uptake by intestinal flora, the concern as such could lead to foreign DNA persistence in the mammalian system. The possibility of plasmid DNA being incorporated via a normal biological process into endogenous gut bacteria is minimized due to the non-conjugative nature of typical plasmids used in recombinant DNA laboratories²⁹ and the low frequency with which unaided transformation (uptake of naked DNA) occurs. Furthermore, beyond the difficulty of unaided transformation is the lack of stable incorporation⁵ for DNA in general. Moreover, the probability of transferring such plasmids into natural bacteria in the gut environment has been calculated to be less than one in one million.⁴² Obviously, both of these assume the plasmid is free rather than incorporated into the plant genome, which would also require that DNA be precisely excised and in a form that was sufficiently homologous to be incorporated into the host genome.

There is also no evidence for the transfer of intact genes to humans from bacteria in the gut or from any food source.⁵⁶ In fact, the DNA remaining after digestion is small random pieces of DNA regardless of the food material. Thus, the fundamental question is not related to any effects from the presence of transgenic-DNA, it is related to whether such DNA will be incorporated into the host cells in a functional way. There is no precedence for DNA being incorporated into host cells beyond the use of the basic nucleotide building blocks as nutrients. And, in fact, acid hydrolysis in the stomach is expected to depurinate most adenosine and guanosine nucleotides rendering the DNA sequence of questionable value.³⁹

A series of papers from Prof. Walter Doerfler's laboratory^{13,14,33,61,62,63} have addressed questions on the fate of ingested DNA. In preliminary work,⁶² the Doerfler team fed mice circular M13 bacteriophage DNA (~ 7.2 Kb) and were able to detect small DNA fragments in certain organs and tissues. These fragments were mostly 200 - 400 bp in size, although up to 1.7 Kb fragments were detected in the feces and up to 500 bp fragments were detectable in the blood. These DNA fragments were detectable within

2-7 hours after feeding. The sum of all of the DNA fragments recovered from all tissues and feces could account for 2-4% of the total M13 fed to the mice, with only 0.01% detectable in the blood. Therefore, 96-98% of the ingested DNA was presumably digested quickly and completely to very small and undetectable pieces. Furthermore, *in vitro* incubation of intact M13 DNA in blood demonstrated complete elimination within 6 hours. The conclusions from this pioneering work were consistent with the general understanding that DNA is a normal component in food and subjected to extensive degradation during digestion. The authors stated “*The implication that a random mixture of DNA including gene fragments or intact genes of animal, plant or microbial origin should have been constantly excreted by innumerable organisms over millennia does not appear startling given the complexities of evolution. This barrage of linear DNA fragments, i.e. of recombinationally highly active DNA fragments in Nature should mitigate any concerns that one might have had in the past about biological consequences of experiments carried out with recombinant DNA over the course of the past two decennia.*” Earlier research⁴⁶ complements these reports on studies on the fate of naked DNA demonstrated complete and quick digestion in the intestine.

In follow-up studies, a re-cloning experiment with DNA from mouse splenocytes appears to have isolated one M13-positive 1.3 kb fragment clone from 10⁹ clones screened.^{16,63} Moreover, this one was covalently linked to an 80-nucleotide fragment with 70% homology for the IgE receptor gene. Additional chimeric molecules of M13 DNA fragments were also found associated with bacterial DNA (authors suggest from normal gut bacteria) and with rearranged lambda phage DNA. These later findings raise serious questions about the technical conduct of these studies and suggest that the results are possibly common cloning artifacts (as cautioned when creating genomic libraries by Maniatis.⁴²). That concern is independent of the question of why an ‘altered’ IgE sequence (or even how the IgE sequence was altered) was found in the same clone.

Finally, a recent report⁶³ from Doerfler’s laboratory suggests that ingested foreign DNA can be associated with chromosomes and cross the placenta to the fetus, which led the authors to make the claim, that foreign DNA is a potential mutagen. This last report, using a plasmid containing the GFP gene for green fluorescent protein (pEGFP-C1) in addition to M13, confirmed that DNA fragments (maximum size is 0.82 kb) persist in the digestive track and can penetrate the intestinal wall. The authors extended the previous work by stating that the fragments were found in the cell nuclei by using *in situ* hybridization and even linked to chromatids of the parent and offspring. They also report the persistence of an M13 fragment in one fetus (a few cells) for up to three months. A conflicting study, on a separate topic⁶⁶, showed that DNA could not cross the placenta and is not taken up by fetuses without the addition of lipopolyamines (synthetic additive), an essential added component. Furthermore, follow-up studies from the Doerfler laboratory failed to detect fragments of the GFP gene by PCR analysis of DNA isolated from spleen, liver or tail tip samples from mice fed the pEGFP-C1 DNA over eight generations, showing that the ingested DNA never became integrated into the mouse germline.³³

The Doerfler work has been critically reviewed and questioned⁴ not only in regard to the feeding of large quantities of purified DNA to animals but also in regards to some of the

methodology. In particular, a question is raised about the possibility of methodological errors given the infrequent observations of incorporated M13 DNA and that identifications were only after powerful amplification methods. Interestingly, even with high doses of foreign DNA (M13), no reports of adverse effects on the animals were presented. Further confounding the issue was the question of whether these fragments might be fully explainable by normal immuno-surveillance mechanism involving mononuclear phagocyte ingestion of foreign materials from the digestive tract.

The final question that might be raised is: if any DNA, endogenous or transgenic, were incorporated into a cell, would this have any deleterious health consequence? For such an outcome, it is required that the transgenic gene would need to be incorporated intact and that the host cell would have the cellular machinery necessary to express the biotech protein product (discussed in Beringer⁷). This has never been shown before, and considering that we routinely eat many plant and animal products containing DNA, there is no evidence that any such DNA molecules have ever been incorporated into mammalian hosts nor any such natural products have had a deleterious effect (reviewed in Beringer⁷). Also as reviewed above, no GFP protein or mRNA expression was detectable in liver, spleen, blood or intestinal epithelia of the mice fed the pEGFP-C1 DNA over a three week period. This data fairly convincingly supports the conclusion ingesting a gene/promoter construct that clearly is capable of functioning *in vivo* when administered via a gene therapy procedure (e.g. intramuscular injection) does not lead to gene expression in somatic cells in animals when provided orally.³³

Conclusions

DNA and protein contained in foods and feeds are typically rapidly degraded upon consumption by the normal digestive processes. To date, the transgenic DNA and/or protein from biotech crops fed to farm animals have not been detected in the raw food products derived from these animals. This result is expected based on the rapid and effective degradation systems that animal possess. Transgenic DNA has been deemed to be safe for consumption as it is made up of the same building blocks as plant genomic DNA. In addition, the proportion of transgenic DNA relative to the total plant genomic DNA consumed by humans or farm animals is extremely small. Even if some DNA or protein survived digestion and ended up at some extremely low level in animal tissues, this would not present a safety concern for human consumption.

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