

EFSA statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed

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1. Background

Regulation (EC) No 1829/2003 on GM food and feed foresees labelling requirements. Decisions on labelling are taken by risk managers with the aim of offering the consumer an informed choice and are outside the scope of EFSA's work. With the letter of 15 March 2007 (ref. SANCO/E1/SG/cc(2007)D/510144), the European Commission informed EFSA about a petition to label food products (such as meat, milk and eggs) from animals that have been fed with genetically modified feed. There were no references to a potential safety concern or to a risk assessment of an existing GMO product. In this context, however, the Commission was interested in the potential for transgenes or their products to be incorporated into animal tissues or products such as eggs and milk, in view of the fate of recombinant protein and DNA within the gastrointestinal tract of livestock. EFSA was asked to provide a reply and has therefore prepared the following literature survey.

2. Literature survey on the fate of recombinant DNA of GM feed

To date, no recombinant DNA sequences have been found in any organ or tissue sample from animals fed GM plants (Flachowsky *et al.*, 2007). In considering whether recombinant DNA from GM plants, or the derived proteins, can end up in animal tissues, milk or eggs, several aspects have been investigated. These include (1) the fate of the recombinant DNA and protein during the feed processing and ensilaging; (2) the fate of the recombinant DNA and protein in the gastrointestinal tract of animals fed with the GM feed; (3) the potential absorption of the digested pieces of DNA or protein into animal tissues/products and (4) the potential of biological functionality of absorbed DNA and protein fragments.

2.1 *The fate of the recombinant DNA and protein during the feed processing and ensilaging*

Before consumption by the animal, the GM plant is processed into feed via different treatments. Studies have shown that mechanical treatments had no influence on the stability of DNA, while the process of extraction and toasting (desolventizing) resulted in highly fragmented DNA (Flachowsky *et al.*, 2007). Forage conservation by ensilaging causes a degradation of DNA to small fragments of about 200 bp (Wiedemann *et al.*, 2006, Lutz *et al.*, 2006, Flachowsky *et al.*, 2007, CAST, 2006).

2.2. The fate of recombinant DNA and protein in the gastrointestinal tract of livestock

In considering the fate of recombinant protein and DNA within the gastrointestinal track of livestock, several aspects need to be taken into account:

2.2.1. Recombinant DNA and proteins in GM feed are not different from other DNA and proteins in the diet

In principle, all feed (and food) contains considerable amounts of DNA and proteins, being essential nutrient sources for animals (and humans) after digestion. Thus, the gastrointestinal tract of animals (and humans) has always been exposed to foreign DNA, proteins and protein fragments from the diet. The DNA introduced into crops through recombinant DNA technology is not different from other sources of DNA in the diet and is considered equivalent to DNA from existing food organisms that have always been consumed (Jonas *et al.*, 2001, CAST, 2006).

2.2.2. Digestion of DNA and proteins into fragments

DNA and proteins are released from plant material by normal digestion processes that take place in the gastrointestinal tract. Ingested DNA and proteins are rapidly cleaved into small fragments by the mechanical processes of mastication along with buccal and gastrointestinal enzymatic digestions and acid hydrolysis. DNA is digested into fragments and nucleotides; proteins into polypeptides, oligopeptides, and amino acids. A number of recent reviews discuss this process (Beever and Kemp, 2000, Jonas *et al.*, 2001, Lutz *et al.*, 2005, CAST, 2006).

2.3. Survival of recombinant plant DNA in the human gastrointestinal tract

Although this statement is focussed on the fate of recombinant DNA and protein in the gastrointestinal tract of livestock, data are also available on the fate of recombinant plant DNA in the gastrointestinal tract of humans (Netherwood *et al.*, 2004). In this study, human volunteers, of whom twelve were healthy and seven had undergone ileostomies (a resection of the terminal ileum and diversion of digesta via a stoma to a colostomy bag), were given meals containing GM soya containing the *epsps* recombinant gene. For the seven ileostomists, the amount of recombinant DNA that survived passage through the small bowel varied between individuals, with a maximum of 3.7% recovered at the stoma of one individual. The recombinant DNA did not survive passage through the intact gastrointestinal tract of healthy human subjects fed GM soya. Three out of seven ileostomists showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel before

their involvement in these experiments. The authors concluded that gene transfer to the microflora did not occur during this feeding experiment.

2.4. *The potential absorption of the digested fragments of DNA or protein into animal tissues*

Rapid breakdown of DNA and proteins during normal digestion as described in section 2.2 is expected to minimize the opportunity for absorption of intact DNA or protein. In 2006, the Council for Agricultural Science and Technology (CAST) published a paper entitled “Safety of Meat, Milk, and Eggs from Animals Fed Crops Derived from Modern Biotechnology” (CAST, 2006). This report indicates that no *intact* or *immunologically reactive fragments* of recombinant plant proteins or DNA have been detected in samples of meat, milk, eggs, lymphocytes, blood, and organ tissue from production animals fed crops modified for agronomic traits using recombinant DNA technology. In the following paragraphs an overview is given of research in the area of absorption of DNA and protein via the gastrointestinal tract:

In rodents:

For proteins, there was an initial case report, where purified and orally administered ovalbumin (non-recombinant) was detected in plasma and lymph fluid of rats in minute amounts of 0.007-0.008% of the dose (Tsume *et al.*, 1996). Similarly, for DNA, it was reported that purified *M13* phage DNA (non-recombinant) that was dosed in pharmacologically high concentrations, was detected in white blood cells of mice (Schubbert *et al.*, 1997).

In farm animals:

Under normal conditions in both ruminants and monogastric farm animals, digested proteins are mostly absorbed as free amino acids and also as di- and tripeptides. The results of studies with dairy cattle, growing calves, broiler chickens, and swine have not detected the presence of recombinant protein in products and tissues from farm animals fed currently available genetically modified crops (CAST, 2006). Ash *et al.* studied the fate of genetically modified protein from Roundup Ready soybeans in laying hens (Ash *et al.*, 2003), Chowdhury *et al.* studied the fate of genetically modified protein from Bt11 in calves (Chowdhury *et al.*, 2003); Jennings *et al.* analysed the fate of recombinant DNA and protein from YieldGard corn in broilers (Jennings *et al.*, 2003) and from Roundup Ready soybean meal in swine (Jennings *et al.*, 2003); and Yonemochi *et al.* studied the fate of recombinant DNA and protein from Starlink corn in dairy cows and in broilers (Yonemochi *et al.*, 2002). All of these authors failed to detect recombinant DNA or proteins that were encoded by recombinant DNA in the tissues of animals fed on transgenic plant material.

For recombinant DNA further studies have been undertaken to determine whether or not fragments of recombinant DNA could be detected in animal tissues and food products such as meat, milk and eggs and these have been reviewed by the CAST

taskforce (CAST, 2006). A list of studies is given in the review by Flachowsky *et al.* (Flachowsky *et al.*, 2007), including details on the source of the DNA, the animal species tested, the results of the detection of recombinant DNA and the results of the detection of non-recombinant DNA. It was concluded that even when highly sensitive PCR and Southern blot methodologies were used, no fragments of recombinant DNA from single-copy transgenes were detected in samples of meat, milk, eggs, skin, duodenal tissue, leukocytes, lymphocytes, blood and organs tissue obtained from animals fed with currently available genetically modified crops. As an exception, very small fragments (of 106 and 146 bases) of the recombinant *cry1a(b)* and *cp4epsps* genes (3500 and 1800 bases pairs respectively) were found in conventional milk samples, but these sequences were also found in organic milk samples. The source of the detected DNA is suggested to be a faecal or airborne contamination or directly from the natural environment via the soil bacteria *B. thuringiensis* and *Agrobacterium sp.*, which are the source organisms of the *cry1a(b)* and *epsps* sequences (Agodi *et al.*, 2006).

The CAST review notes that there are reports of fragments from natural multicopy plant genes (*e.g.* chloroplast genes) found in certain animal tissues and fluids (CAST, 2006).

In view of the data described above it is clear that the uptake of DNA fragments or proteins fragments from the intestinal tract into the body is a normal physiological process for animals.

The fact that no intact recombinant DNA has been detected in meat, milk and eggs may be explained because (1) the recombinant sequence is unlikely to stay intact during digestion and (2) the recombinant sequence is present in the GM plants only as a single or low level copy, which makes potential absorption a rare event and therefore difficult to detect. It is noted that this may change if, for example, plants in which the recombinant DNA is incorporated into chloroplast or mitochondrial DNA are released in the future since organelle DNA is present in multiple copies per cell. However, as stated below, this would not imply a safety concern. Emphasizing the critical influence of gene copy number and sensitivity of detection (Alexander *et al.*, 2007), it is also noted that no technique is currently available to enable a valid and reliable tracing of animals products (meat, milk, eggs) when the producer animals have been fed a diet incorporating GM plants.

Flachowsky *et al.* (2007) also concluded that the results of recent publications agree that most of the DNA in a diet is degraded in the gastrointestinal tract, but that some DNA fragments have been found in animal tissues. These fragments came from “natural” plant DNA fragments and were found in some animal species but not in others. No residues of recombinant DNA or protein were detected in any organ or tissue sample including eggs and milk obtained from animals fed with GM feed.

2.5. *The potential of biological functionality of absorbed DNA and protein fragments*

After digestion into fragments and potential absorption into animal tissues, the hypothesis that absorbed DNA or protein from animal feed would be functional in animal tissue is highly questionable in view of the small size of the fragments. In addition, regarding their potential functionality after absorption, the following aspects need to be taken into account:

- Foreign DNA or proteins are degraded by endogenous restriction nucleases or proteolytic enzymes respectively, these enzymes being part of natural defence systems that have evolved to destroy foreign DNA and proteins (Jonas *et al.*, 2001, Alexander *et al.*, 2007).
- For recombinant DNA to become functional, a genomic integration would be the pre-requisite for expression. The probability of such a horizontal gene transfer from a plant or bacterial gene into the animal genome can be considered very low because the principal mechanisms for DNA incorporation into a genome is via homologous recombination.
- Furthermore, for incorporated DNA to be functional, the absorbed DNA must be inserted in a transcriptionally active region. Unless it carries its own expression system, incorporated DNA will also need a location in juxtaposition with an appropriate promoter, transcriptional site and ribosomal binding site (Beever and Kemp, 2000).
- Currently, there is no evidence that any plant proteins are expressed in tissues of animals that have consumed plant material. Indeed, no plant gene (or fragment thereof) has ever been detected in the human genome or that of any other animal species (Beever and Kemp, 2000).
- Also in the context of the studies mentioned above, where multicopy plant DNA was found in certain animal tissues, neither chloroplast DNA nor maize genes are present endogenously within the wild-type poultry genome (Klotz *et al.*, 2002).
- It has also been reported that when mice were fed for eight generations with large amounts of a unique recombinant DNA construct, no functional expression was observed nor germline transfer of this orally-administered DNA (Hohlweg and Doerfler, 2001).

Conclusions

(1) Biologically active genes and proteins are common constituents of foods and feed in varying amounts. After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans.

(2) To date, a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been

detected in tissues, fluids or edible products of farm animals like broilers, cattle, pigs or quails.

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References

- AGODI, A., BARCHITTA, M., GRILLO, A. & SCIACCA, S. (2006) Detection of genetically modified DNA sequences in milk from the Italian market. *Int J Hyg Environ Health*, 209, 81-8.
- ALEXANDER, T. W., REUTER, T., AULRICH, K., SHARMA, R., OKINE, E. K., DIXON, W. T. & MCALLISTER, T. A. (2007) A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production. *Animal Feed Science And Technology*, 133, 31-62.
- ASH, J., NOVAK, C. & SCHEIDELER, S. E. (2003) The fate of genetically modified protein from Roundup Ready Soybeans in laying hens. *Journal of Applied Poultry Research*, 12, 242-245.
- BEEVER, D. E. & KEMP, C. F. (2000) Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts and Reviews. Series A, Human and Experimental*, 70, 197-204.
- CAST (2006) Safety of Meat, Milk, and Eggs from animals fed crops derived from Modern biotechnology. *Animal agriculture's future through Biotechnology*, Issue Paper 34.
- CHOWDHURY, E. H., SHIMADA, N., MURATA, H., MIKAMI, O., SULTANA, P., MIYAZAKI, S., YOSHIOKA, M., YAMANAKA, N., HIRAI, N. & NAKAJIMA, Y. (2003) Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. *Veterinary and Human Toxicology*, 45, 72-75.
- FLACHOWSKY, G., AULRICH, K., BOHME, H. & HALLE, I. (2007) Studies on feeds from genetically modified plants (GMP) - Contributions to nutritional and safety assessment. *Animal Feed Science And Technology*, 133, 2-30.

- HOHLWEG, U. & DOERFLER, W. (2001) On the fate of plant or other foreign genes upon the uptake in food or after intramuscular injection in mice. *Mol Genet Genomics*, 265, 225-33.
- JENNINGS, J. C., ALBEE, L. D., KOLWYCK, D. C., SURBER, J. B., TAYLOR, M. L., HARTNELL, G. F., LIRETTE, R. P. & GLENN, K. C. (2003) Attempts to detect transgenic and endogenous plant DNA and transgenic protein in muscle from broilers fed YieldGard Corn Borer Corn. *Poultry Science*, 82, 371-380.
- JENNINGS, J. C., KOLWYCK, D. C., KAYS, S. B., WHETSELL, A. J., SURBER, J. B., CROMWELL, G. L., LIRETTE, R. P. & GLENN, K. C. (2003) Determining whether transgenic and endogenous plant DNA and transgenic protein are detectable in muscle from swine fed Roundup Ready soybean meal. *Journal of Animal Science*, 81, 1447-1455.
- JONAS, D. A., ELMADFA, I., ENGEL, K. H., HELLER, K. J., KOZIANOWSKI, G., KONIG, A., MULLER, D., NARBONNE, J. F., WACKERNAGEL, W. & KLEINER, J. (2001) Safety considerations of DNA in food. *Annals of Nutrition and Metabolism*, 45, 235-254.
- KLOTZ, A., MAYER, J. & EINSPANIER, R. (2002) Degradation and possible carry over of feed DNA monitored in pigs and poultry. *European Food Research and Technology*, 214, 271-275.
- LUTZ, B., WIEDEMANN, S. & ALBRECHT, C. (2006) Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. *J Anim Physiol Anim Nutr (Berl)*, 90, 116-23.
- LUTZ, B., WIEDEMANN, S., EINSPANIER, R., MAYER, J. & ALBRECHT, C. (2005) Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *J Agric Food Chem*, 53, 1453-6.
- NETHERWOOD, T., MARTIN-ORUE, S. M., O'DONNELL, A. G., GOCKLING, S., GRAHAM, J., MATHERS, J. C. & GILBERT, H. J. (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nat Biotechnol*, 22, 204-9.
- SCHUBBERT, R., RENZ, D., SCHMITZ, B. & DOERFLER, W. (1997) Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 961-966.

TSUME, Y., TAKI, Y., SAKANE, T., NADAI, T., SEZAKI, H., WATABE, K., KOHNO, T. & YAMASHITA, S. (1996) Quantitative evaluation of the gastrointestinal absorption of protein into the blood and lymph circulation. *Biological & Pharmaceutical Bulletin*, 19, 1332-1337.

WIEDEMANN, S., LUTZ, B., KURTZ, H., SCHWARZ, F. J. & ALBRECHT, C. (2006) In situ studies on the time-dependent degradation of recombinant corn DNA and protein in the bovine rumen. *J Anim Sci*, 84, 135-44.

YONEMOCHI, C., FUJISAKI, H., HARADA, C., KUSAMA, T. & HANAZUMI, M. (2002) Evaluation of transgenic event CBH 351 (StarLink) corn in broiler chicks. *Animal Science Journal*, 73, 221-228.