British Journal of Nutrition (2002), **87**, 529–531
DOI: 10.1079/BJN2002596

Invited commentary

Degradation of transgenic DNA from genetically modified soyabean and maize in human intestinal simulations

Until May 1997, approval to market transgenic plants for food purposes was not regulated by international legislation in Europe. Before that date, applicants wishing to market transgenic plants sought approval under a voluntary scheme. In the UK, an opinion on the safety of such plants for food use was obtained from the Advisory Committee on Novel Foods and Processes. From May 1997, however, in countries belonging to the European Community permission to market transgenic material for food use was subject to the Novel Foods Regulation EC/258/97 (Commission of the European Communities, 1997*a*). This regulation lays down a structured approach to the safety assessment of foods or food ingredients that have not hitherto been used for human consumption to a significant degree within the European Union. These fall into six broad categories, and include food or food ingredients that are generated using *in vitro* recombinant DNA technology as the first category. This part of the regulation specifies consideration of: 'foods and food ingredients containing or consisting of genetically modified organisms within the meaning of Directive 90/220/EEC' (Commission of the European Communities, 1990). The prominence given to foods or food ingredients containing genetically modified ingredients seems interesting with hindsight. Given the public pressure against the introduction of recombinant DNA technology into food production and the self-imposed industry moratorium on the commercial planting of genetically modified plants in the UK until farm-scale trials have been evaluated thoroughly, there is little prospect of large-scale production of transgenic crops in this country. Of course, this does not preclude the use of imported transgenic crops from outwith the European Community in our food.

At present, no legislation at European level considers specifically the use of transgenic plant material as animal feed, although Directive 90/220/EEC on deliberate release into the environment of genetically modified organisms requires the safety assessment of transgenic plants to include animal feeding studies if the novel plant material is to be used for that purpose (Commission of the European Communities, 1990). It is envisaged that, with the forthcoming establishment of the European Food Agency, feeding transgenic plant material to animals will have specific legislative regulation.

To assist applicants who may wish to market transgenic plants for food use, the European Commission has published a guidance document on how applications for approval should be structured, including numerous 'decision trees' (Commission of the European Communities, 1997*b*). These demonstrate the requirements for safety assessments of a variety of novel foods and include an explicit requirement to consider the potential for transfer of genetic material from genetically modified organisms.

The explicit requirement to consider the potential for gene flow has been the focus for many of the concerns that have been articulated following the introduction of the Novel Foods Regulation. This has led to intense debate, which has polarised opinion on the use of recombinant DNA technology. Much of what has been written, for and against the use of transgenic plants in food, has been, and is: 'full of sound and fury, signifying nothing'. Some applicants for approval to market transgenic plants have made extrapolations regarding the potential for gene flow; some opponents have cited scientific evidence out of context to support a contrary view. Thus, this has become an area of science and technology of intense public interest; it is also an area with surprisingly little peer-reviewed publications.

The application, made under the voluntary scheme, to obtain marketing consent to a maize line that resisted attack by the European corn borer (*Ostrinia nubialis*) was originally rejected by the UK Advisory Committee on Novel Foods and Processes, but this decision was overturned at European Community level (Commission of the European Communities, 1997*c*). The objection was that, in addition to a gene encoding the crystal toxin gene from a strain of *Bacillus thuringiensis*, these plants carry a copy of a gene encoding resistance to β-lactam antibiotics, including ampicillin. This and similar concerns prompted the Ministry of Agriculture, Fisheries and Food to initiate research to study the potential for gene flow from transgenic plants. At its inception, the Food Standards Agency took over management of this programme. The paper by Martín-Orúe *et al.* (2002) in this present issue of the *British Journal of Nutrition* is part of the outcome of that programme.

The issues surrounding the use of transgenic plants in

food continue to hold the interest of both scientists and the public at large. The problem of potential gene flow was one of the main items discussed at the open hearing on criticisms of the risk assessment for T25 genetically modified maize held by the UK Advisory Committee on Releases to the Environment on 20 February 2002 (Advisory Committee on Release to the Environment, 2002). The Secretariat of the Advisory Committee on Release to the Environment has yet to publish the outcome of that hearing. Also in February 2002, the Royal Society reported on the use of genetically modified plants for food use and human health (Royal Society, 2002). The penultimate section of this report reviews current scientific opinion on the fate of transgenic DNA in the digestive system. In their previous report, the Royal Society concluded that most ingested DNA is broken down in the intestinal tract (Royal Society, 1998). The report reiterates that view but acknowledges that subsequent research demonstrates the transfer of biologically active transgenic DNA from plants to other cells, both eukaryotic and prokaryotic, in the gastrointestinal tract. Duggan *et al.* (2000) studied the potential for the β-lactamase gene to transfer from transgenic maize to the commensal microbial flora of animals using such maize as feed material. It was concluded that, using an *in vitro* model, biological activity of DNA sequences survived for only a very short period when the target molecules are exposed to sheep rumen contents. Einspanier *et al.* (2001) looked for the presence of plant DNA sequences in leucocytes of cattle and chickens fed on transgenic maize that had been genetically modified to resist insect attack. Short chloroplast sequences were discovered in the peripheral lymphocytes of cattle and in muscle, liver, spleen and kidney tissue taken from chickens fed on the maize. No DNA derived from the inserted cassette in the maize was found in any sample. The absence of transgenic DNA in animal cells even if those cells can be shown to carry plant-derived sequences is not surprising given that both conventional and transgenic plant material will contain chloroplast DNA and that the organelle genome is present in multiple copies in cells. In any food or feed, conventional plant-derived sequences will be present in vast excess compared with the DNA inserted into transgenic plant cells. It is noteworthy that plant DNA was found only in lymphocytes in samples taken from cattle. These cells form part of our first-line host defences and it is not surprising that they may be found to contain 'foreign' material.

The paper by Martín-Orúe *et al.* (2002) in this present issue of the *British Journal of Nutrition* contributes positively to work in this field. The authors have studied the fate of transgenic DNA in gastric and small intestine models. The oral cavity does not possess enzymes or environmental conditions conducive to the breakdown of ingested DNA although there is a rich and complex microflora within the oral cavity (Henderson & Wilson, 1998). Other workers have examined the fate of ingested DNA in the oral cavity (Mercer *et al.* 1999, 2001). Martín-Orúe and colleagues have concentrated their efforts upon those areas of the digestive tract where, either because of the environmental pH or because of the presence of digestive enzymes, breakdown of DNA is most likely to occur. The results presented from *in vitro* tests demonstrate that transgenic DNA has the potential to survive passage through the gut. This is a significant finding because it demonstrates that not all DNA present in food is digested. There remains, therefore, the potential for gene flow. Gebhard & Smalla (1998) have demonstrated that, under idealised conditions, bacteria can take up and integrate DNA fragments derived from transgenic sugar beet. This required: bacteria that were genetically competent to be transformed; a functional host recombination system; the presence in the recipient bacterial cell of a deleted copy of the target transgene and DNA fragments of sufficient size to undergo recombination once they had been taken up into the bacteria. Nevertheless, gene flow has been shown to occur, albeit under idealised and artificial circumstances. The absence of evidence for gene flow in more realistic simulations must not be taken for evidence that gene flow cannot occur. The paper presented by Martín-Orúe and colleagues, with its tantalising potential for gene flow, reinforces the need to move from models of the natural situation to *in vivo* studies.

John Heritage
Division of Microbiology
University of Leeds
Leeds LS2 9JT
UK

References

Advisory Committee on Releases to the Environment (2002) ACRE open hearing on the criticisms of the risk assessment for T25 GM maize on-line. http://www.defra.gov.uk/environment/acre/hearing200202.htm

Commission of the European Communities (1990) Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. *Official Journal of the European Communities* L **117**, 15–27.

Commission of the European Communities (1997*a*) Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal of the European Communities* L **043**, 1–6.

Commission of the European Communities (1997*b*) Commission recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. *Official Journal of the European Communities* L **253**, 1–36.

Commission of the European Communities (1997*c*) Scientific committee on food opinion on the potential for adverse health effects from the consumption of genetically modified maize (*Zea mays* L.) (Expressed on 13 December 1996). Updated to include references 21 February 1997. http://europa.eu.int/comm/food/fs/sc/oldcomm7/out02_en.html

Duggan PS, Chambers PA, Heritage J & Forbes JM (2000) Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiology Letters* **191**, 71–77.

Einspanier R, Klotz A, Kraft J, Aulrich K, Poser R, Schwägele F, Jahreis G & Flachowsky G (2001) The fate of forage plant

DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology* **212**, 129–134.

Gebhard F & Smalla K (1998) Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* **64**, 1550–1554.

Henderson B & Wilson M (1998) Commensal communism and the oral cavity. *Journal of Dental Research* **77**, 1674–1683.

Martín-Orúe SM, O'Donnell AG, Ariño J, Netherwood T, Gilbert HJ & Mathers JC (2002) Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *British Journal of Nutrition* **87**, 533–542.

Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA & Flint HJ (1999) Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* **65**, 6–10.

Mercer DK, Scott KP, Melville CM, Glover LA & Flint HJ (2001) Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. *FEMS Microbiology Letters* **200**, 163–167.

Royal Society (1998) *Genetically Modified Plants for Food Use and Human Health*. London: The Royal Society.

Royal Society (2002) *Genetically Modified Plants for Food Use and Human Health – An Update*. Policy document 4/02. London: The Royal Society.