

Date: Fri, 19 Jul 2002 00:24:46 +0200
From: Klaus Ammann <Klaus.Ammann@ips.unibe.ch>
Subject: Debate 2002'0717 a: University of Newcastle report summaries: no significant horizontal transgene transfer detected in human guts
X-Sender: kammann@ubecx.unibe.ch
To: klaus.ammann@ips.unibe.ch
X-Mailer: QUALCOMM Windows Eudora Version 5.1

Dear friends,

first apologies for this lengthy piece, but things are complex about horizontal gene transfer. We need a step by step approach and if we obey the rule of not employing antibiotic resistance marker genes which are still in use in animal and human medicine, then there is no reason for a total ban as foreseen in the EU and also in Switzerland.

Horizontal transgene transfer in humans from GM food to bacteria ? These will be the headlines of the next days due to some documents and publications from the University of Newcastle.

We already saw in 1999 in British boulevard journals the headlines such as: Genetic Engineering will cause Super-Meningitis, triggered by a simple phone call from a journalist who did not disclose that he was calling for an interview. Dr. Heritage announced the intentions of doing some research on the question whether horizontal gene transfer could happen in the mouth of human beings. And his worries also included meningitis bacteria which survive in small portions in the complex environment of the mouth of many humans. And from there it is not far to conclude that a meningitis bacterium receiving all sorts of antibiotic resistance genes will trigger a super meningitis...

see April 19, 1999, from the

BBC: http://news.bbc.co.uk/1/hi/english/health/newsid_328000/328578.stm
more information at: <http://www.applesforhealth.com/meninbacteria1.html>

When you check publications from Dr. Heritages Lab (actually most of the work is derived from a thesis at the University of Leeds by Chandler), then you see that the concerns did not actually prove correct, but still, a certain prudence and an evaluation of antibiotic marker genes case by case seems to be recommendable. A short comment about the concerns from Dr. Heritage at: <http://www.botanischergarten.ch/debate/biofutur.pdf>

In the University of Leeds publications I saw there was a clear intention to go ahead with more realistic scenarios, including human test persons. The University of Newcastle now issued reports on those matters and the results are not worrying. Those results do not justify the total ban, which will take place in the European Union at the latest in the year 2008. They do justify a prudent approach in the sense of the precautionary approach (I hate the word principle here) and a step by step decision making process.

But according to some NGO's such as Friends of the Earth, the German BUND and Greenpeace these are alarming results published in reports of the University of Newcastle. Again this is a case of superficial, but professional scare mongering: Go to the original reports, given here as links and also read, if you do not have enough time, the summaries given below with some of the significant statements highlighted.

If you really take the time to dig into the original articles, then things look different and not at all so alarming. I know of many early small lab studies with in vitro conditions where the fate of transgenes have been studied, showing the same tendencies in data. (Bt, RR-Resistance)

It is still worthwhile to state that thorough (published !) studies are undertaken late, at a stage where GM food is introduced for years already.

But under realistic conditions horizontal gene transfer is of no worry, provided the food is following the whole usual path and ends there where our back has lost its decent name. It is certainly advisable to avoid resistance genes from antibiotics still in use in animal and human medicine.

At the BAGECO 7 in Bergen, Norway there was a research group showing that

Klaus

more comments for most of the links given below.

if you want to work on the downloaded ppt files, please right click the mouse and get edit slides

+++++

<http://www.botanischergarten.ch/debate/Flintetal.pdf>

<http://www.botanischergarten.ch/debate/Flintetal.ppt>

Flint H., Mercer D., Scott K., Melville C. and Glover A.
Survival of digested DNA in the gut and the potential of genetic transformation of resident bacteria

FSA Project Code FSG 01007, 1.6.1998 - 1.10. 2001, Report

Two significant sentences from the summary:

"The [half time] survival of DNA in the human mouth in vivo was only for 6 seconds, and the concentration had decreases 100fold after 60 seconds, the DNA being much less

stable than with in vitro conditions."

"We did not detect transformation in vitro using linear DNA that possessed only a single region of matching sequence, which is, arguably, the most likely state of GM DNA in food. We did however detect transformation of genes that were flanked on both sides by sequences that match the bacterial chromosome."

+++++

<http://www.botanischergarten.ch/debate/Newcastlereport.pdf>
<http://www.botanischergarten.ch/debate/NewcastleReport.ppt>

Technical report on the
Food Standards Agency project G010008
"Evaluating the risks associated with using
GMOs in human foods" –
University of Newcastle
5. July 2002

<http://www.food.gov.uk/multimedia/pdfs/gmnewcastlereport.PDF>

the articles (an extensive summary and reports on two parts of the project) are given as one pdf-file

The conclusions are in fact very simple:

There is no evidence of horizontal transgene transfer, as long as you obey to the 'reality rule' of measuring only potential horizontal transfer of antibiotic marker genes in use and thus widespread in nature - AND provided the test food has been sent through the whole of the human intestines, INCLUDING the colon.

In order to get some clearcut results for the potential release of bacteria with new antibiotic resistance genes transferred from GM food into nature, it was necessary first to find an antibiotic resistance gene which was rare enough in nature so that transfer could be detected, to this purpose a plasmid with a chloramphenicol resistance was constructed, see the Report of the University of Newcastle on page 8:

Initial attempts to measure gene transfer from E. coli into soil bacteria were unsuccessful due to the very high level of Kanr (10⁻²) in the ecosystem. The antibiotic resistance spectrum of soil ecosystems was therefore assessed. The data revealed chloramphenicol resistance (Cmr) was very low (<10⁻⁷) in the soil microbial population. To measure gene transfer a conjugative broad range gram negative plasmid was constructed that conferred Cmr.

From those lines it is clear for me that the experiments again show artificial conditions - conditions which should have been avoided according to the original concept. But even so, with conditions strongly changed in favour of a potential horizontal gene transfer, there is no evidence - sorry Greenpeace, BUND, Friends of the Earth....

page 9

Conclusions: The data presented in this study demonstrated that the AZT-based donor kill system is a highly effective in measuring gene transfer into natural microbial populations. The primary advantage that this system over standard gene transfer methodology is that there is no need to include a marked recipient bacterium and thus gene transfer into the complete microbial population can be assessed. This system can be exploited in studying gene transfer from food grade microorganisms into natural populations, such as the intestinal microflora, into which these prokaryotes are introduced. This methodology is this of considerable value when developing risk assessment protocols for GMOs that are introduced into the human food chain. It was hoped that the strategy could also have been exploited in detecting gene transfer into viable but non- culturable populations, which comprise >90 % of natural microbial ecosystems. This proved not to be feasible as the endogenous fluorescence in natural populations created a high background, the donor cells, although killed did not lyse, and GFP was expressed at very variable levels when gfp was transferred into the recipient bacteria. In the original project we had intended exploiting the AZT kill system to measure gene transfer from the food grade microorganism *Lactococcus lactis*, which is a target GMO, into the microbial ecosystem of the large bowel. In a parallel study, however, H. J. Flint's group showed that *L. lactis* was very rapidly lost from in vitro large colon simulations with no evidence of gene transfer (erythromycin resistance) occurring. For these reasons we did not further explore this system in the large bowel but focussed more on Objectives 2 and 3 as agreed with FSA.

Page 20

Report on Objective 3

These experiments have recently been published in the British Journal of Nutrition (2002) 87 533-542

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

Susana M Martín-Ortue, Anthony G O'Donnell, Joaquin Arino, Trudy Netherwood, Harry J Gilbert and John C Mathers

Abstract

The inclusion of genetically modified (GM) foods in the human diet has caused considerable debate. There is concern that the transfer of plant-derived

transgenes to the resident intestinal microflora could have safety implications. For these gene transfer events to occur, the nucleic acid would need to survive passage through the gastrointestinal tract. The aim of the present study was to evaluate the rate at which transgenes, contained within GM soya and maize, are degraded in gastric and small bowel simulations. The data showed that 80% of the transgene in naked

soya DNA was degraded in the gastric simulations, while no degradation of the transgenes contained within GM soya and maize were observed in these acidic conditions. In the small intestinal simulations, transgenes in naked soya DNA were degraded at a similar rate to the material in the soya protein. After incubation for 30 min, the transgenes remaining in the soya protein and naked DNA were 52 (SEM 13.1)% and 34 (SEM

17.5)% respectively, and at the completion of the experiment (3h) these values were 5% and 3% respectively. In contrast to the soya transgene, the maize nucleic acid was hydrolysed in the small intestinal simulations in a biphasic process in which approximately 85% was rapidly degraded, while the rest of the DNA was cleaved at a rate similar to that in soya material. Guar gum and tannic acid, molecules that are known to inhibit digestive enzymes, did not influence the rate of transgene degradation in soya protein. In contrast guar gum reduced the rate of transgene degradation in naked soya DNA in the initial stages, but the polysaccharide did not influence the amount of nucleic acid remaining at the end of the experiment. Tannic acid reduced the rate of DNA degradation throughout the small bowel simulations, with 21 (SEM 5.4)% and 2

(SEM 1.8)% of the naked soya DNA remaining in the presence of the phenolic acid, respectively. [These data indicate that some transgenes in GM foods may survive passage through the SMALL intestine.](#)

Page 22

Report on Objectives 2 and 3

Transgenes in genetically modified Soya survive passage

through the human small bowel but are completely

degraded in the colon

Trudy Netherwood^{1,2}, Susana M. Martín-Ortue¹, Anthony G. O'Donnell², Sally

Gockling^{1,2}, Harry J. Gilbert¹ and John C. Mathers¹

Department of Biological and Nutritional Sciences¹ and Department of Agricultural

and Environmental Sciences², University of Newcastle upon Tyne,

Newcastle upon Tyne NE1 7RU, U.K.

The introduction of genetically modified plants (GMPs) into the human food chain has caused considerable debate with regard to the associated risks to human health. One of the major issues following the inclusion of GMPs in the human diet is the possible transfer of transgenes to the commensurate intestinal microflora and/or the epithelial cells lining the intestinal lumen, both of which could have health implications. Recent data indicate that a significant proportion of the transgenes in GMPs does survive in vitro simulations of the small bowel [7], and bacteriophage M13 DNA gavaged into the mouse intestines is detected in the faeces, blood and liver [3, 4, 8]. The persistence of dietary GMP-derived DNA in humans, however, is unknown. In this study we have evaluated the survival of transgenes in GMPs during passage through the gastrointestinal tract of humans. To track DNA survival through the small intestine seven ileostomists were given

a single meal containing genetically modified Soya (GMS), and the appearance of transgene DNA on the digesta collected from the stoma was monitored. Whilst the amount of transgene that survived passage from the small bowel was highly variable between subjects, the nucleic acid was detected in all seven subjects. In one individual as much as 3.7 % of the transgene DNA was recovered at the stoma. In a second trial, human volunteers with an intact gastrointestinal tract were fed a single meal containing GMS. [No transgene DNA was detected in the faeces indicating that the nucleic acid did not survive passage through the complete intestine.](#)

+++++

and some texts from the University of Leeds, from the Laboratory of Dr. John Heritage J.Heritage@leeds.ac.uk , he kindly sent me some files

<http://www.botanischergarten.ch/debate/DugganSurvival.pdf>
<http://www.botanischergarten.ch/debate/DugganSurvival.ppt>

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

Paula S. Duggan a, Philip A. Chambers a, John Heritage a;*, J. Michael Forbes b
a Division of Microbiology, School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

b Centre for Animal Sciences, Leeds Institute for Plant Biotechnology and Agriculture (LIBA), University of Leeds, Leeds LS2 9JT, UK

Received 5 July 2000; received in revised form 1 August 2000; accepted 2 August 2000

Abstract

To assess the likelihood that the bla gene present in a transgenic maize line may transfer from plant material to the microflora associated with animal feeds, we have examined the survival of free DNA in maize silage effluent, ovine rumen fluid and ovine saliva. Plasmid DNA that had previously been exposed to freshly sampled ovine saliva was capable of transforming competent Escherichia coli cells to ampicillin resistance even after 24 h, implying that DNA released from the diet could provide a source of transforming DNA in the oral cavity of sheep. Although target DNA sequences could be amplified by polymerase chain reaction from plasmid DNA after a 30-min incubation in silage effluent and rumen contents, only short term biological activity, lasting less than 1 min, was observed in these environments, as shown by transformation to antibiotic resistance. These experiments were performed under in vitro conditions; therefore further studies are needed to elucidate the biological significance of free DNA in the rumen and oral cavities of sheep and in silage effluent. fl 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

<http://www.botanischergarten.ch/debate/ChambersFinal.pdf>

A rapid, reliable method for the extraction from avian faeces of total bacteria DNA to be used as a template for the detection of antibiotic resistance genes

Chambers Ph., Duggan P., Forbes M. and Heritage J.

Journal of Antimicrobial Chemotherapy (2001), 47, 239-246

This is the method published which is used to detect potential horizontal gene transfer

events at the Lab of John Heritage University of Leeds.

+++++

Kornelia Smalla's classic:
k.smalla@bba.de

<http://www.botanischergarten.ch/debate/Smalla20000828.pdf>

Conclusion

Given the fact that antibiotic resistance genes, often located on mobile genetic elements, are already widespread in bacterial populations and that HGT events from transgenic plants to bacteria are supposed to occur at extremely low frequencies and have not yet been detected under field conditions, it is unlikely that antibiotic resistance genes used as markers in transgenic crops will contribute significantly to the spread of antibiotic resistance in bacterial populations. There is no doubt that the present problems in human and veterinary medicine, resulting from the selective pressure posed on microbial communities, were created by the unrestricted use of antibiotics in medicine and animal husbandries, and not by transgenic crops carrying antibiotic resistance markers. Unfortunately, in some European countries the discussion about antibiotic resistance genes in transgenic crops attracts much more public attention than the massive use of antibiotics. We feel that the public debate about antibiotic resistance genes in transgenic plants should not divert the attention from the real causes of bacterial resistance to antibiotics such as the continued abuse and overuse of antibiotics by physicians and veterinarians (Salyers, 1996). The control of the antibiotic resistance problem very clearly lies in a reduction of the selective pressure by prudent use of antibiotics.

+++++

and the way, Greenpeace handles the case:

<http://www.botanischergarten.ch/debate/GreenpeaceDeclaration.pdf>
<http://www.botanischergarten.ch/debate/Greenpeace.ppt>

There is no word in the Greenpeace website that all experiments where transgene DNA has been followed up through the all intestines with highly sensitive methods, there was no sign of any horizontal gene transfer. The reason is that this fact does not fit into the scare mongering scheme.

Its the usual tactics of Greenpeace: Using lots of scientific expressions, putting them into simple boulevard style scary sentences and misleading its own readers. This is another and repetitious abuse of science.

"The study went further to see if this genetically modified DNA could be transferred via bacteria in the [large](#) intestine. In laboratory simulated gastrointestinal tracts, three of the seven samples revealed bacteria had taken on the herbicide-resistant gene. And this was after only one GM meal. There have been no studies of the long term effects of introducing GM food into people's diets."

+++++

The latest article in the Guardian from yesterday

<http://www.botanischergarten.ch/debate/Guardian20020717.pdf>

And it is again the Guardian working to make the news from the reports to look as bad as possible.

Just read the paragraph cited from this article, and you will have to read it at least twice to realize that people with normal stomachs don't show any horizontal transgene transfer.

"The scientists took seven human volunteers who had their lower intestine removed in the past and now use colostomy bags. After being fed a meal of a burger containing GM soya and a milkshake, the researchers compared their stools with 12 people with normal stomachs. They found "to their surprise" that "a relatively large proportion of genetically modified DNA survived the passage through the small bowel". None was found in people who had complete stomachs."

What the Guardian author Vidal actually wants to say is very simple: no horizontal gene transfer in human faeces going through the whole NATURAL intestine sequence, including the colon.

If you do not know what a "small bowel" is, then you have no idea that these human test persons do not have normal digestion anymore.... In medical language, these patients are called ileostomists. Just have a look at

<http://www.cancerhelp.org.uk/help/default.asp?page=3926> and

<http://www.virginiamason.org/dbSurgery/sec3206.htm> and then you will know how 'significant' results obtained by those deplorable human ilestomic test persons really are.

I just leave it to you to decide whether this is bad or misleading journalism.

+++++

and finally some food for thought from the excellent BAGECO 7 in Bergen, Norway
June 2002

see <http://carl.im.uib.no/nyheter/Bageco7/>

Some selected contributions on horizontal gene transfer, especially interesting the poster and oral contribution from from Anne Mercier, Pascal Simonet et al.

The research group is working on a very special case of bacterial infection on a higher

plant where in the infestation zone there might be some horizontal gene transfer possibility involving transgenes.

Poster owner: Mercier, Annette

CONTROL OF HORIZONTAL GENE TRANSFER AND GENETIC VARIABILITY IN *RALSTONIA SOLANACEARUM*

Mercier Anne, Bertolla Franck, Passelègue-Robe Eugénie, Normand Philippe and Simonet Pascal. Center for Microbial Ecology, UMRCNRS 5557, Université Lyon 1-La Doua, Bat. G. Mendel, 69622 Villeurbanne cedex, France. E-mail: mercier@biomserv.univ-lyon1.fr

<http://www.botanischergarten.ch/debate/MercierBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/CrecchioBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/KieselBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/deVriesBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/SikorskiBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/WuertzBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/KayBAGECO7.pdf>

<http://www.botanischergarten.ch/debate/HeinemannBAGECO7.pdf>