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Briefings for MOP 4

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Genetic engineering & omitted health research

The Cartagena Protocol on Biosafety gives a central role for risk assessments to identify and evaluate the possible adverse effects of living modified organisms (LMOs) on the conservation and sustainable use of biological diversity, taking also into account risks to human health. Discussions on risk assessment under the Protocol have so far centred on the need for guidance materials (especially on specific aspects, which have been deemed insufficient), capacity-building and sharing of experiences. Parties have also been asked to identify LMOs or specific traits that may have adverse effects, so that appropriate measures regarding the treatment of such LMOs or traits can be taken.

The potential risks to human health, however, need to be examined more closely. In particular, there are worrying aspects raised by the lack of data, due to omitted research, leaving us with no answers to important questions. Thus, the scientific database on which one can make judgments on GM food and feed safety is still inadequate, and there is urgent need for further investigation. Given the lack of scientific certainty due to insufficient relevant scientific informationand knowledge in relation to human health risks, the Precautionary Principle must apply.

This briefing extracts from the following fully-referenced publication - Genetic Engineering and Omitted Health Research: Still No Answers to Ageing Questions, by Terje Traavik (GenØk-Centre for Biosafety, Norway) and Jack Heinemann (INBI-Centre for Integrated Research in Biosafety, New Zealand), TWN Biotechnology & Biosafety Series 7, 2007.

Introduction

Some of the most crucial scientific questions concerning the health effects of genetic engineering (GE) and genetically engineered organisms (GEOs) were raised up to twenty years ago. Most of them have still not been answered at all, or have found unsatisfactory answers. We believe, as Mayer and Stirling said, "in the end it is often the case that those who choose the questions determine the answers". Will another twenty years pass before societies realize the urgent need for public funding of genuinely independent risk- and hazard-related research? The time for such investment is now, so that a new scientific culture with working hypotheses rooted in the Precautionary Principle can discover other, possibly even more important questions of safety.

In the specific context of food or feed safety assessment, "hazard" may be defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The hypothetical hazards of whole GE foods, i.e. those hazards that have been realized so far, fall into a few broad categories.

First, there are those either related to the random and inaccurate integration of transgenes into recipient plant genomes, uncertainty with regard to direct or indirect effects of the polypeptide product of the transgene, or uncertainty with regard to DNA types and circumstances promoting uptake and organ establishment of foreign DNA from mammalian gastro-intestinal tracts. The second are those that might come from the purposeful production of potential hazards such as allergens or powerful pharmaceutical products.

Do we know that any GE food/feed is safe for consumption?

For a composite material like food/feed, reductionist approaches testing single components *in vitro* are highly unsatisfactory and cannot clarify important safety issues. In spite of the obvious need, very few studies designed to investigate putative effects of GE nucleic acids or food/feed on potential animal or human consumers have been published in peer-reviewed journals. A consensus has emerged that the effects observed in some published studies must be experimentally followed up. To this day, this has not been done.

Most of the animal feeding studies conducted so far have been designed exclusively to reveal husbandry production differences between GEOs and their unmodified

counterparts. Studies designed to reveal physiological or pathological effects are extremely few, and they demonstrate a quite worrisome trend: Studies performed by the industry find no problems, while studies from independent research groups often reveal effects that should have merited immediate follow-up, confirmation and extension. Such follow-up studies have not been performed. There are two main factors accounting for this situation: The lack of funds for independent research, and the reluctance of producers to deliver GE materials for analysis.

Can we rely on the transgenic DNA sequences given by GE food/feed producers?

If the transgenic DNA sequences given in the notifications differ from the inserted sequences found in the GEPs, the risk assessments made prior to approval of the GEPs for marketing do not necessarily cover the potential risks associated with the GE plants (GEPs).

The most thoroughly studied transgenic events are:
* Bt-transgenic maize Mon810
* Bt- and glufosinate-transgenic maize Bt176
* Glyphosate-transgenic maize GA21
* Glufosinate-transgenic maize T25 (Liberty Link)
* Glyphosate-transgenic soybean GTS 40-3-2

Even amongst the most thoroughly studied and some of the oldest commercial GEPs, recent independent work has revealed that rearrangements occur in transgene inserts and the nature of the rearrangements varies. Deletions (Mon810, GA21, Bt176), recombination (T25, GTS 40-3-2, Bt176), tandem or inverted repeats (T25, GA21, Bt176) as well as rearranged transgenic fragments scattered through the genome (Mon810) have been reported.

The transgenic modification techniques are prone to introduce such rearrangements because exogenous DNA transfer in plants elicits a "wound" response, which activates nucleases and DNA repair enzymes. This may result in either degradation of the incoming DNA, or insertion of rearranged copies into the plant DNA. In addition, the nature of the DNA constructs used to make transgenic plants may influence the rearrangement tendencies for a given transgenic event. Some genetic elements in the constructs may act as "hotspots" and elicit recombination at high frequencies.

While it was earlier assumed that integration of transgenic constructs took place at random locations in the recipient plant genome, it has now become apparent that integration sites are often concentrated in or near elements such as retrotransposons (T25, Mon810, GA21) and repeated sequences (Bt11 maize), and this poses additional risks.

Firstly, by introducing a new promoter or new enhancer motifs, transgenic insertions into, or close to, such elements may lead to altered spatial and temporal expression patterns of plant genes located close to and even far from, the insert. Secondly, a strong retrotransposon LTR promoter may upregulate the transgene expression level. Thirdly, defective retrotransposons may start "jumping" under the influence of transacting factors recruited by the insert.

All these events may have unpredictable effects on the long-term genetic stability of the GEOs, as well as on their nutritional value, allergenicity and toxicant contents. These putative processes represent areas of omitted research with regard to health effects of GEOs.

Are transgenic DNA and proteins taken up from the mammalian GIT (gastro-intestinal tract)?

If DNA and proteins from GEOs persist in, and are taken up from the mammalian GIT, this could theoretically, as will be further explained below, ultimately lead to development of chronic disease conditions. The fate and consequences of DNA persistence and uptake is, however, not extensively studied, and therefore represents yet another area of uncertainty connected to GEPs.

It has generally been claimed that DNA and proteins are effectively degraded in mammalian GITs. This has been based on assumptions that have never been systematically examined. A restricted number of recent publications have demonstrated that foreign DNA and also proteins may escape degradation, to persist in the GIT and even to be taken up from the intestines and transported by the blood to internal organs in biologically meaningful versions. These findings should not have come as such a surprise, since scientific articles from the 1990s strongly indicated that this was an area of omitted research, as stated by a number of reports.

Briefly summarised, there is evidence that relatively long fragments of DNA survive for extended periods after ingestion. DNA may be detected in the faeces, the intestinal wall, peripheral white blood cells, liver, spleen and kidney, and the foreign DNA may be found integrated in the recipient genome. When pregnant animals are fed foreign DNA, fragments may be traced to small cell clusters in foetuses and newborns. The state of GIT filling, and the feed composition may influence DNA persistence and uptake. Complexing of DNA with proteins or other macromolecules may protect against degradation.

So far only two published reports have investigated the fate of foreign/transgenic DNA in humans. The consequences of DNA persistence and uptake thus represent yet another area of omitted research. Extrapolating from a number of experiments in mammalian cell cultures and in experimental animals, it is conceivable that in some instances insertion of foreign DNA may lead to alterations in the methylation and transcription patterns of the recipient cell genome, resulting in unpredictable levels of gene expression levels and products. Furthermore, even small inserts may result in a so-called "destabilisation" process, the end-point of which may be malignant cancer cells.

The BSE/new variant Creutzfeld-Jacob's Disease epidemics caused by prion proteins painfully illustrated the phenomenon of protein persistence, uptake and biological effects. Two recent publications indicate that this phenomenon may be more general that realized. A hallmark

of prion diseases and a number of other debilitating, degenerative diseases, e.g. Alzheimer's and Huntington's diseases, is deposition of "amyloid fibrils". Recent studies indicate that any protein can adopt a confirmation known as "amyloid" upon exposure to appropriate environmental conditions. Whether those conditions are more likely when proteins are expressed in different species and at very different concentrations, as is often the case for GE food/feed that are already in the marketplace, is unknown.

The consequences of protein persistence and uptake will vary with the given situation. Generally speaking, there is a possibility that toxic, immunogenic/allergenic or carcinogenic molecules may gain entry to the organism via cells in the gastrointestinal walls. The persistence of the Bt-toxin Cry1Ab in faeces means a potential for spread on the fields through manure. The ecological effects, e.g. on insect larvae and earthworms, are at the moment an issue of sheer speculation.

Have the protein contents of GE food been altered in unpredictable ways?

Transgenes or upregulated plant genes may give rise to toxicants, anti-nutrients, allergens and, putatively, also carcinogenic or co-carcinogenic substances. The concentration of a given transgenic protein may vary according to the location(s) in the recipient host cell genome of inserted GE construct DNA, and to environmental factors influencing the activity of the transgenic regulatory elements, e.g. the 35S CaMV promoter. The biological effects of a given transgenic protein, e.g. the Cry1Ab Bt-toxin or the ∝-amylase inhibitor from beans when expressed in peas, may be unpredictably influenced by post-translational modifications, alternative splicing, alternative start codons for transcription, chimeric reading frames resulting from integration into the reading frame of a plant gene, and complex formation with endogenous plant proteins.

The influence of foreign DNA insertion on endogenous plant gene expression patterns may vary with local environmental factors, the actual insertion site(s), the number and stability of the inserts, transgenic promoter effects, methylation patterns of the insert(s), and post-transformational mutations in the transgenic protein coding as well as in regulatory sequences. Even a single nucleotide change may affect the properties of a protein, or it may create a new transcription factor-binding motif. Detailed studies of these phenomena under authentic conditions are lacking, and hence we are confronted with yet another area of omitted research.

Could GE food/feed cause allergies?

One of the major health concerns related to GEPs is that the transgenic product itself, e.g. a Bt toxin, changed expression of endogenous plant genes, or chemical reactions that occur during the cooking of novel foods, may result in exposure to *allergenic* compounds. The risk assessment of allergens often follows an *allergenicity decision tree*. These "trees" are based on *in vitro* tests comparing a limited number of structures, usually only one, of the transgenic protein with known allergens. Hence, these comparisons are hopeful that the protein isolated for the test matches all proteins produced from the same gene in the GEP. But in fact, this is unlikely because allergenicity tests are usually carried out with bacteria-, not *in planta*-produced versions of the transgenic protein. Glycosylation invariably takes place in plants, but not in bacteria, so this form of post-translational modification of both the transgenic protein and endogenous proteins would not be tested. Allergenic characteristics of proteins, and also their resistance to degradation in the organism, can be affected by glycosylation. Other protein modifications may also take place, adding to the unpredictability of transgenic products.

Another important question related to allergenicity is whether post-marketing surveillance can provide useful information about allergens in GE foods. For a number of reasons this is not likely to happen. Treatment of allergy is symptomatic, whatever the cause may be. The allergic case is often isolated, and the potential allergen is rarely identified. The number of allergy-related medical visits is not tabulated. Even repeated visits due to well-known allergens are not counted as part of any established surveillance system. Thus, during the October 2000 Starlink episode, it proved very difficult to evaluate Starlink (containing Bt-toxin Cry9C) as a human allergen. An additional reason for this was that the ELISA tests, used by FDA, that found no anti-Cry9C antibodies in suspected human cases, were dubious because bacterial, recombinant antigens were used instead of the Cry9C maize versions that the individuals had been exposed to.

Case: Bt toxins in Bt-transgenic GEPs

It is very important to be aware of the fact that the Bt-toxins expressed in GEPs have never been carefully analysed, and accordingly, their characteristics and properties are not known. What is clear from the starting point, however, is that they are vastly different from the bacterial *Bacillus thuringiensis* protoxins, used in organic and traditional farming and forestry for decennia. The difference is evident already at the gene level, since the versions found in GEOs are engineered to produce active Bt toxins. By extrapolation, these have a number of potentially unwanted biological characteristics, ranging from solubilization of the protein under natural conditions and effects on insect and mammalian cells, to persistence and non-target effects in the environment. In addition, the post-translational modifications that may influence conformations, cellular targets and biological effects of GEP-expressed Bt-toxins are unknown, and hence we once more identify an area of omitted research.

During the last few years a number of observations that may be conceived of as "early warnings" of potential health and environmental risks, have appeared in the literature. Most of them have, however, not been followed up by extended studies.

Case: Transgenic, glyphosate-tolerant (Roundup Ready) GEPs

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition. The transgene, *cp4 epsps*, used in genetically modified crops was isolated from an *Agrobacterium* strain. The whole idea is of course the combined use of the GEP and the herbicide. Recent studies indicate that in some cases such GEPs are associated with greater usage of glyphosate than the conventional counterparts. A very restricted number of experimental studies have been devoted to health or environmental effects of the GEPs or the herbicide itself. Some of these may be considered "early warnings" of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings. Consequently: yet another area of omitted research.

Is the 35S CaMV promoter inactive in mammalian cells?

Cauliflower mosaic virus (CaMV) is a DNA-containing para-retrovirus replicating by means of reverse transcription (Poogin et al., 2001). One of the viral promoters, called 35S, is a general, strong plant promoter. It has been used to secure expression of the transgenes in most of the GEOs commercialized so far.

Industry proponents have claimed unconditionally that the 35S is an exclusive plant promoter, and hence cannot, even theoretically, represent a food/feed safety issue.

In addition to studies in yeast and in *Schizosaccharomyces pombe,* there are published studies indicating that the 35S CaMV promoter *might* have potential for transcriptional activation in mammalian systems. And the final proof has become available during the last couple of years. First, 35S promoter activity was demonstrated in human fibroblast cell cultures, thereafter in hamster cells, and very recently one of us (TT) has demonstrated substantial 35S promoter activity in human enterocyte-like cell cultures. Such cells line the surface of human intestines. However, no published studies have investigated 35S CaMV activity *in vivo*, and this is hence an obvious area of omitted research.

Could the use of antibiotic resistance marker genes (e.g. nptII) present health hazards?

The antibiotic kanamycin is used extensively in crop genetic engineering as a selectable marker, *inter alia* in GE oilseed rape event lines like MS1Bn x RF1Bn and Topas 19/2.

A selectable marker is a gene inserted into a cell or organism to allow the modified form to be selectively amplified while unmodified organisms are eliminated. In crop genetic engineering, the selectable marker is used in the laboratory to identify cells or embryos that carry the genetic modifications that the engineer wishes to commercialize. The selection gene is used once briefly in the laboratory, but thereafter the genetically modified crop has the unused marker gene in each and every one of its cells.

There are multiple well-known mechanisms for cross-resistance to antibiotics of a particular type. Kanamycin is a member of the family aminoglycoside antibiotics. There are approximately 17 different classes of aminoglycoside-modifying enzymes. Some of these inactivate up to four different aminoglycosides. Cross-resistance between kanamycin and other aminoglycosides, e.g. gentamycin and tobramycin, was found to vary markedly between isolates. All of the antibiotics mentioned are used to treat human diseases.

In spite of the belief of many genetic engineers that kanamycin is no longer employed in medical applications, there is evidence that the antibiotic is used extensively for some applications.

Concluding remarks: Where do we go from here?

We have discussed in some detail a handful of selected, unanswered risk questions related to the first generation of transgenic GEOs. There are many more risk issues. Among them are issues of Horizontal Gene Transfer (HGT), the new generations of multitransgenic GEOs for pharmaceutical and industrial purposes, safety questions related to GE vaccines, the new nanobiotechnology approaches and the applications of small double-stranded (ds)RNAs (which can cause RNAi) for a number of medical purposes. Furthermore, we have the "questions not yet asked", and we have the problem of whether available methods and regulatory frameworks will be able to pick up and manage the conceived risks once they become reality.

In recent publications it has been demonstrated that the presently used sampling and detection methods may fail to detect GE materials in food and feed. In another article it was demonstrated that HGT events, that potentially carry very serious public health consequences, would not be detected in time for any meaningful preventive actions. And it has been illustrated that the dsRNA techniques are not as "surgically targeted" as initially indicated.

We are therefore left with a high number of risk issues lacking answers, adding up to a vast area of omitted research, and this falls together in time with a strong tendency towards corporate take-over of publicly funded research institutions and scientists.

We must as citizens and professionals join together to reverse the present situation. Publicly funded, independent research grants need to become a hot political issue. That would be the most efficient remedy for chronically unanswered questions and the corporate take-over of science. In conclusion, we once more quote Mayer and Stirling: "Deciding on the questions to be asked and the comparisons to be made has to be an inclusive process and not the provenance of experts alone". But then again, whom should society rely on for answers and advice should the time come when all science resource persons work directly or indirectly for the GE producers?