WHO
World Health Organization
Regional Office for Europe

ANPA
Agenzia Nazionale
per la Protezione dell'Ambiente

Release of Genetically Modified Organisms in the Environment: is it a Health Hazard?

Report of a Joint WHO/EURO – ANPA Seminar

World Health Organization, Regional Office for Europe
European Centre for Environment and Health
Rome-Italy
7-9 September 2000

This report is neither intended to be conclusive nor to reflect a WHO position on the matter. Rather, it is a contribution to the discussion on the health consequences of the release of Genetically Modified Organisms in the environment, provided for the scientific community at large as a basis for future thinking and planning in this area. Comments, suggestions and criticisms will be encouraged.

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INTRODUCTION

It is generally recognized that potential effects on human health of:

- the consumption of foods derived from biotechnology
- the release of genetically modified organisms (especially plants) in the environment

are public concerns.

Biotechnology has been applied to foods since the beginning of the 1990s. On one hand, public health could benefit enormously from biotechnology. It would have e.g. an immense potential for devising new ways of increasing the nutrient contents of foods, decreasing allergenicity in foods, and improving the efficiency of food production. The use of the technology in foods is therefore spreading rapidly. On the other hand, great public mistrust is prevailing, as reflected in new expressions such as "Frankenstein Foods". Many consumer groups and some scientists are claiming that foods derived from biotechnology should not be marketed. Several WHO Member States are also moving in this direction.

In order to respond to this concern, the Codex Alimentarius Commission, at its 23rd session held on 28 June-3 July 1999, established the Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology. The objective of the task force is the development of standards, guidelines or recommendations, as appropriate, for foods derived from biotechnology or traits introduced into foods by biotechnology, on the basis of scientific evidence, risk analysis and with regard, where appropriate, to other legitimate factors relevant to the health of consumers and to the promotion of fair trade practices. The first meeting of the Task Force was held in Japan in March 2000. FAO and WHO expressed their intention to organize a series of scientific expert consultation to support the work of the Task Force.

In June 2000 the First Joint FAO/WHO Consultation on Foods Derived from Biotechnology was held in Geneva. It addressed the overall safety aspects of foods derived from genetically modified plants and focused on the applicability of substantial equivalence as a general guidance for scientific risk assessment. Conclusions and recommendations of this consultation are attached in Annex 4. "Environmental safety" of Genetically Modified Plants (GMPs) and socio-economic issues were not included in the scope of the consultations.

Responses to the concerns on the potential effects on human health of the release of Genetically Modified Organisms (GMOs), especially plants, in the environment are so far very scarce.

Therefore, a WHO/EURO seminar on "Release of Genetically Modified Organisms in the Environment: is it a Health Hazard?" was held at the World Health Organization (WHO) Regional Office for Europe, European Centre for Environment and Health, Rome Division, on 7-9 September 2000, in collaboration with the Italian Environment Protection Agency (ANPA).

A total of 25 scientists, including authors of discussion papers, participated in the Seminar. The complete list of participants is given in Annex 1.

Dr Roberto Bertollini, Acting Coordinator of WHO/EURO, Division for Technical Support, opened the Seminar. In his statement, Dr Bertollini emphasized the clear separation that must be present between the different elements of the risk analysis procedure, especially between risk assessment and risk management as shown in Figure 1. He strongly recommended the participants to consider

the seminar as a "hazard identification seminar" and to try to answer the question: "does the release of genetically modified organisms in the environment cause adverse effects on human health?"

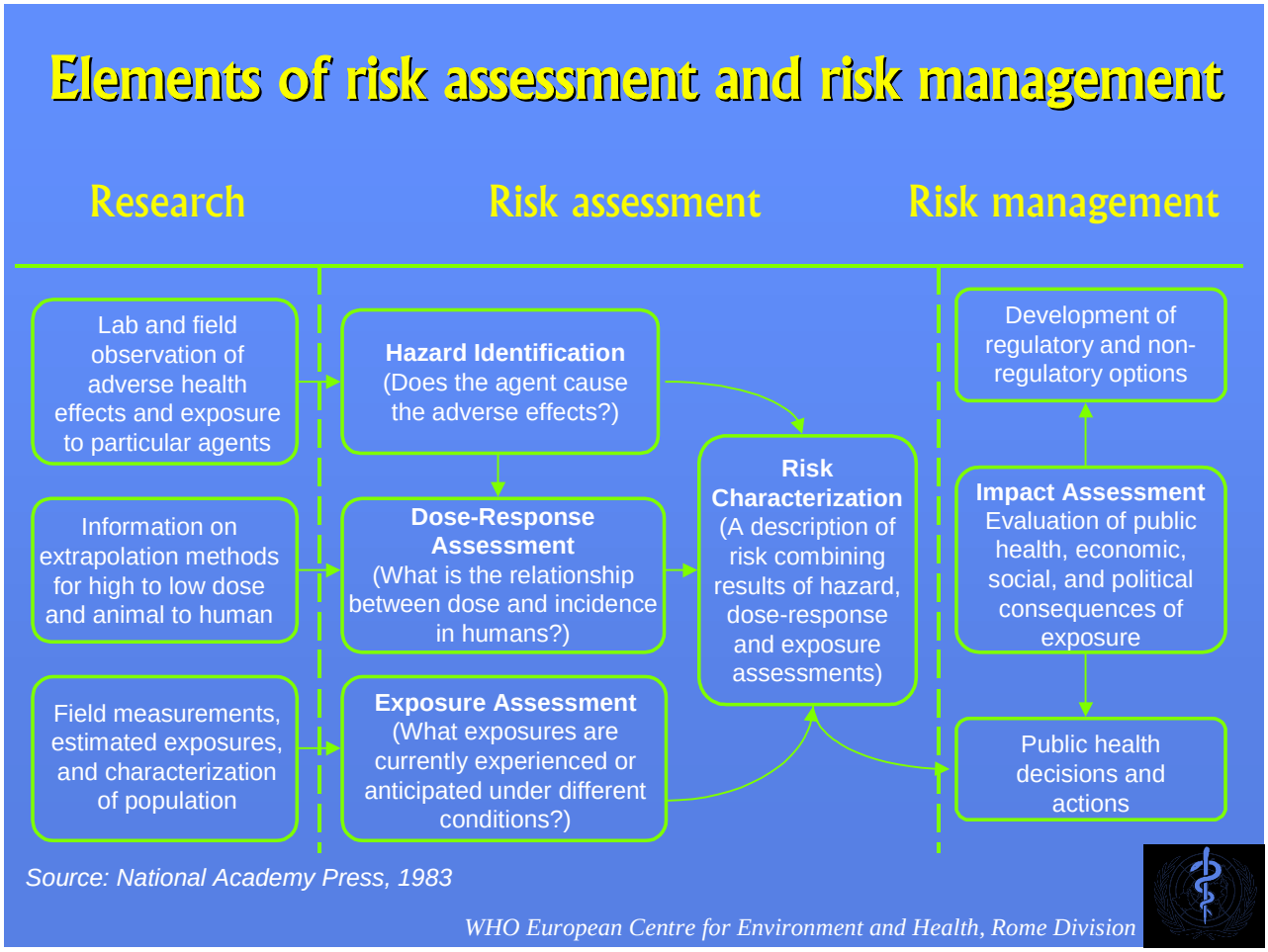

Figure 1: Elements of risk assessment and risk management

Dr Bertollini further informed the participants about the newly established Health Impact Assessment (HIA) Programme within WHO/EURO. This programme aims at enabling Ministries of Health, local health departments and other health institutions to coordinate, and when necessary to implement, assessments of health impacts of a variety of policies. It should provide consistent and coherent advise, make available operational guidelines providing the necessary tools and methods to carry out HIAs, support implementation of case studies, develop institutional capacity and human resources, and provide an international agreed framework for HIA, reflecting legislation and norms. Activities in one sector indeed often impact on the objectives of other sectors. Economic or social activities by public or private actors are known to affect health, positively and negatively, through changes of other systems. The health sector is indeed in the unique position of informing of the health consequences of various other activities, as illustrated in Figure 2.

Dr Onufrio, member of the Board of Management of the Italian National Environment Protection Agency (ANPA) welcomed the participants on behalf of the Agency and of the Italian Government.

In his presentation, Dr Onufrio informed the participants that ANPA is a technical-scientific agency based on the principles of autonomy, technical reliability, independence and organizational flexibility, subjected to the supervision of the Ministry of the Environment.

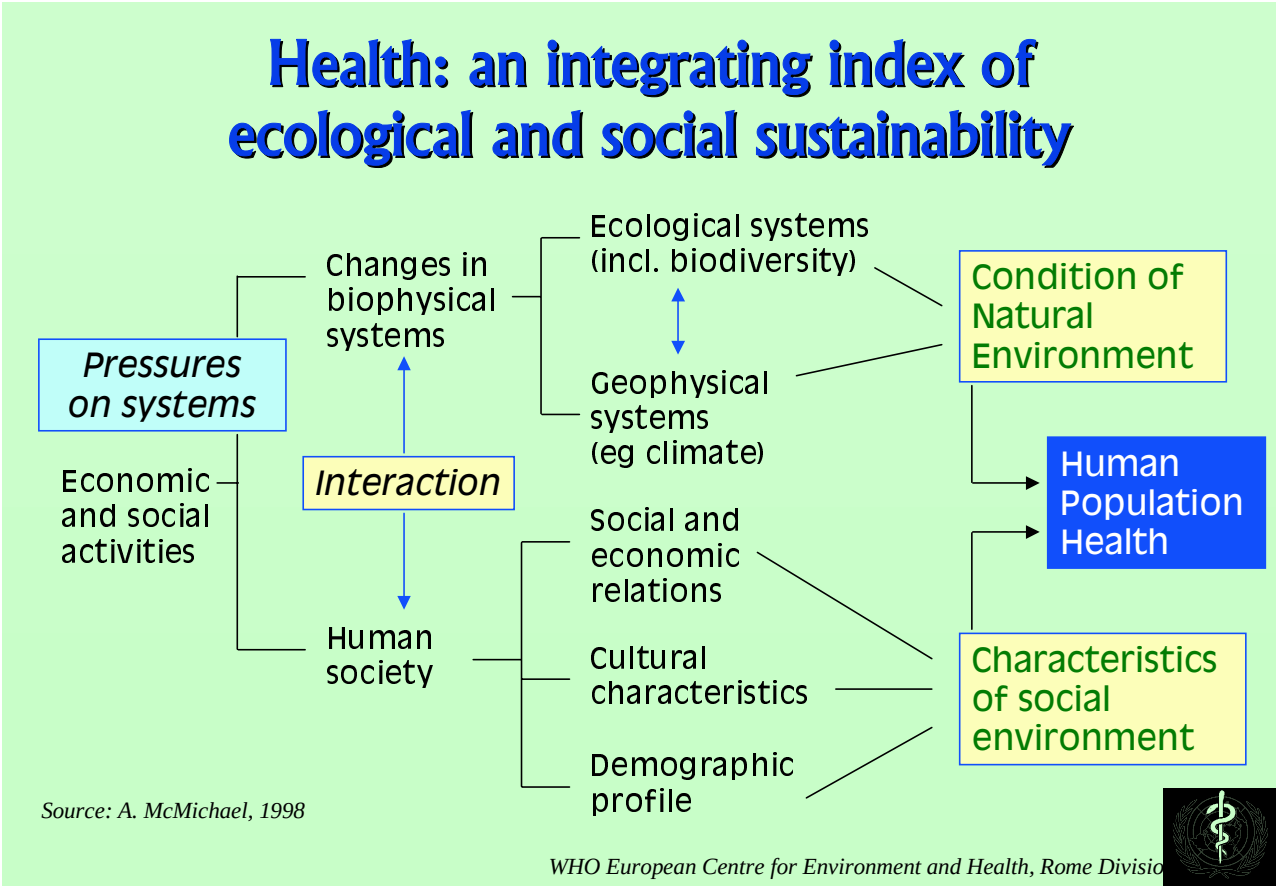

Figure 2: Health: an integrating index of ecological and social sustainability

Its main areas of activity are: to provide of technical and scientific support for the development of environmental legislation; to collect, process and publicize environmental information; to provide guidance and co-ordination for regional and provincial environmental agencies (ARPA-APPA) on the implementation and enforcement of national environmental laws; to develop strategic guidelines for achieving sustainable development and finally education and training on environmental issues; to provide environmental inspectors with new skills and innovative tools to identify and characterise hazards and take the appropriate measures to avoid environmental damages and prevent risks for the human health.

In order to strengthen its informative capacity, ANPA created a network named “SINANET”, composed of the National Topic Centres (CTN), the Regional Focal Points (PFR), and the Principal Reference Institutions (IPR), and placed among its priorities the creation of the National System for Information and Environmental Controls, whose entire structure has been designed with reference to the European EIONET system established by the EEA.

Concerning the issues of biotechnologies and GMOs, ANPA is dedicating considerable efforts and resources to the investigation of these problems, and is a member in a Committee established by the Ministry of the Environment, together with the Operative Ecological Body of the Army of Carabinieri, with the special purpose of assessing the ecological and human health effects generated by the experimentation and successive release of GMOs in the Italian ecosystems.

To this aim, ANPA has established an interdepartmental unit composed of experts whose aim is to tackle issues related to biotechnologies both at a normative and a scientific level, and to involve, co-ordinate and support the Regional and Provincial Environmental Agencies, to carry out

inspections of transgenic crops and to implement a national action plan for monitoring and assessing the environmental impact of GMOs in Italy.

ANPA is grateful to WHO for the efforts it has dedicated on this occasion and looks forward to a follow-up meeting with the purpose of integrating the present scientifically-oriented discussion with a more "public-oriented" debate which should provide the public with a clearer knowledge of GMOs-related issues and problems.

At the end of the informal opening ceremony, the participants elected:

- Dr Jennifer Thomson as Chairperson
- Dr David Andow as Vice-Chairperson
- Dr Othmar Kaeppeli as Rapporteur
- Dr Angelika Hilbeck as Vice-Rapporteur

SCOPE AND PURPOSE OF THE SEMINAR

The traditional framework for risk assessment and management, drawn from expertise with chemical products, involves a methodological progression through a rigorous sequence of analytical steps. The biological and ecological phenomena related to the environmental releases however, are not easy to fit into this quantitative approach, due to the current limited insight into the complexity of the phenomena and the scarcity of relevant data. In addition Environmental Risk Assessment usually identifies direct and indirect environmental effects but makes limited references to human health.

For this reason the WHO European Centre for Environment and Health – Rome Division organized the seminar "Release of Genetically Modified Organisms in the Environment: is it a Health Hazard?" with the objective to relate the health and the environmental components of the hazard identification associated with GMOs (plants and micro-organisms).

The category of hazards associated with the release of GMOs in the environment to be dealt with by the seminar participants, and for which human health effects should be identified or excluded, were restricted to:

- alteration of gene pool;
- alteration of ecosystem structure and function;
- development of resistances.

The following were excluded by definition from the scope of the seminar:

- conflict of interest;
- legal liability of damage;
- problems related to international trade and economic hazards;
- socio-economic hazards.

TERMS OF REFERENCE OF THE SEMINAR

The seminar participants were asked:

- to review scientific work, especially in the field of gene flow;
- to provide the WHO European Centre for Environment and Health – Rome Division with scientific support in relation to the potential human health hazards of the release of Genetically Modified Organisms in the environment, taking into consideration work done by academy, national authorities, WHO, FAO and other international organizations and other relevant international fora;
- to review hazard characterization provisions within existing strategies for the Environmental Health Risk Assessment of Genetically Modified Organisms, focusing on the human health component, and evaluating scenario based risk assessment strategies;
- to make recommendations on further research needs and priorities for hazard characterization provisions within the Risk Assessment.

THE SCIENTIFIC SESSIONS OF THE SEMINAR

Abstracts of each presentation were prepared by the authors themselves. They solely reflect their point of view. The sections "discussion/issues raised", which follow each abstract, have been drafted by the secretariat according to key issues raised and book marked as such during the final discussion, further edited by the author of the papers and submitted for final review to all participants.

Activities of International Organizations (WHO, FAO, UNEP, ICGEB, OECD) related to biotechnology

A first session of the seminar was dedicated to the activities of International Organization in relation with biotechnology. Representatives of the Headquarter of the World Health Organization (WHO), Food Safety Programme (FOS); of the Food and Agriculture Organisation (FAO); of the United Nations Environment Programme (UNEP); of the International Centre for Genetic Engineering and Biotechnology (ICGEB); of the Organization for the Economic Cooperation and Development (OECD) were invited to present their activity.

Updated reviews of their activities are available at the following web pages:

WHO/FOS: Safety of food derived from modern biotechnology page (http://www.who.int/fsf/GMfood/index.htm)

FAO: FAO and CBD Biosafety Protocol page (http://www.fao.org/sd/rtdirect/rtre0034.htm)

UNEP: Convention on Biological Diversity – UNEP secretariat (http://www.biodiv.org/)

ICGEB: Biosafety page (http://www.icgeb.trieste.it/biosafety/)

OECD: (http://www.oecd.org/subject/biotech/)

Session a) Risk Assessment

"The fundamentals of science-based environmental risk assessment of GMOs"
(presented by Othmar Kaeppeli)

Abstract

Environmental risk assessment has a long tradition for several technical systems (e.g. chemistry or nuclear power). Good industrial safety practices and engineering safety codes have led to development and application of systematic approaches, methods and tools for environmental risk assessment. A risk assessment process generally involves the following steps: (1) system description, (2) identification of hazards, (3) development of accident scenarios, (4) consequence estimation, (5) probability estimation of hazardous events occurring, (6) risk estimation in terms of both consequences and probabilities, and (7) assessment of risks by reference to established risk criteria or protection goals.

When the risk assessment methodology from well-established technical areas (e.g. chemistry, nuclear energy) is applied to the assessment of environmental risks of transgenic plants the following insights are possible:

- Thorough system analysis indicates that mechanistically, the insertion of a gene is related to genomic variation mechanisms also known to occur with other breeding techniques, particularly with plant biotechnology methods, which form the basis for genetic engineering.
- Due to mechanistic similarities, the risks of transgenic plants can be considered to be within a familiar risk frame also associated with other breeding techniques. Therefore, an important criterion for the validity of comparative risk assessment is accomplished.
- For the analysis of risks related to intentionally introduced traits (target effects) the scenario method is a useful tool. Decision-making on the acceptability of hazards can be done in a systematic and transparent way with the help of reference scenarios based e.g. on technology alternatives. In this way acceptable risk levels can be discussed on a rational basis.
- Unintended effects are managed by traditional breeding control strategies. Additionally, improved hazard recognition and knowledge on environmental interactions continuously evolve from progress in ecology and molecular biology.
- In living, self-reproducing systems probability has a different rank as compared to non-living systems. Therefore, the damage potential should be the primary criterion for hazard acceptability evaluations when GMOs are involved.

Discussion/Issues raised

- There was general agreement that risk assessment can be done on *a case-by-case* basis only, because the risks relate to the traits and genetic elements (e.g. markers) introduced.
- Some of the participants questioned the *mechanistic similarity between naturally occurring genomic variation mechanisms and gene insertion by genetic engineering*. However, molecular biology method evolved that enable to identify genetic polymorphism and underlying mechanisms involved in somaclonal variation. Insertions and deletions in particular were identified as genomic instability forms.

- The comparative risk assessment may in a first instance relate to conventional breeding. But an expansion to *multiple model comparisons* may be necessary as knowledge on ecological interactions accumulate.
- A *damage oriented risk assessment* should be given priority because the meaning of probability in a living system is not the same as in a non-living system. Further, damage extent considerations are often neglected. Sometimes the risks are even attributed to the biological processes involved. e.g., pollen flow is not a risk *per se*. The risk depends on the genes involved and the damage potential related to their spread.
- The issue of *randomness of conventional breeding versus the randomness of genetic engineering* was raised. The mechanisms responsible for genomic variation are all undirected and random. Currently this is also true for the insertion of a gene by genetic engineering. However the place of insertion can be determined after the insertion took place.
- A systematic approach for risk assessment allows a better identification of risk related research, because missing knowledge necessary for risk evaluation becomes apparent. The participants repeatedly mentioned the *need for research* on special issues.

"Current experiences with environmental risk assessment (ERA)"
(presented by Guy Van den Eede)

Abstract

The current state of the art in the field of ERA for GMOs does not allow for the elaboration of unique, standardized and validated methodologies for conducting quantitative risk assessments. Today, ERAs for GMOs are based on a mixture of qualitative and (some) quantitative data as they emerge from modelling, experience and judgemental reasoning. Based on these data, current methods in ERA for GMOs rely on good scientific judgement and common sense to assess the combination of factors that might contribute to a risk. Although current methods do not strive for mathematical precision, they are scientifically sound and consistent in so far as the underlying information and data are assembled and/or processed accordingly. Consequently, it is anticipated that the inter-comparison of ERAs will become more quantitative in the future as the database improves and other recommendations made in this report are adopted.

The following may be considered as key elements in the risk assessment process:

- Expert judgement.
  Expert judgement ought to be fully appreciated in the ERA process for GMOs.

- Data generation.
  High quality review and test data provide the basis for decision-making. Data should be collected/generated in such a way that they can be interpreted in a Hazard/Harm (HH)-oriented model. There is a need for a systematic collection and storage of a thoroughly investigated set of information so that HH and the concomitant risk analysis can be performed on an internationally accepted basis. There is also a need to establish the minimal data set that is required (e.g. provision of full DNA sequence) and experts should agree on the methods for data analysis (e.g. analysis and assessment of expected and unexpected Open Reading Frames (ORFs).

- Common risk assessment methodologies.
  Appropriate guidance to perform ERAs for GMOs should be provided, particularly with regard to hazard identification. Checklists for risk assessment that are sufficiently detailed and flexible to guide experts through the process could be elaborated.

There are no reliable protocols for the safety assessment of whole foods. In 1993, the Organization for Economic Co-operation and Development (OECD) introduced the concept of *substantial equivalence* according to which conventional and GM foods are to be compared with respect to toxicity and nutritional qualities. This concept is also introduced in the European legislation where it is used for defining risk assessment methodologies as well as for labelling requirements.

When assessing the impact on human and animal health the following elements require specific attention:

- Allergenicity.
- Intended and unintended toxic effects (direct as well as indirect).
- The mixing (through gene transfer or through physical means) of traits that are destined to remain contained as they serve a particular purpose.

Discussion/Issues raised

- Environmental risk assessment (ERA) for GMOs is still far from providing a *standardized methodology* that is based on data on occurrence probabilities and on data from environmental effect analyses.
- A large number of *small-scale field trials* have been carried out worldwide, but the experiments have not always contributed to a better insight in the risk evaluation of commercial applications of GMOs.
- For the evaluation of applications of biotechnology a balanced consideration of both the possible associated risks and the perceived social benefits has been advocated in order to take into account wider public concerns.
- Depending on the circumstances, risk assessors might take into consideration intellectual and/or cognitive differences between the parties/stakeholders involved in the decision process, and tailor the risk reporting accordingly.
- The key factor is the identification of possible hazards/harms (HH). Estimation of the frequency of the realization of the hazards and estimation of the respective magnitudes are relevant for small-scale releases but insignificant for commercial releases.
- The participants discussed post-release monitoring (incl. the development of appropriate protocols). Against the opinion of the majority, one of the participants, considering the current lack of knowledge in many areas, expressed the view that post-release monitoring is a bad choice to address safety concerns as GMOs are self-replicating, and once released into the environment it will never be possible to recall them, should a problem arise. Therefore, several safety questions which are still unclear have to be answered before a release takes place. Post-release monitoring cannot address safety concerns, it can only help to identify problems without offering a solution.

"Health Impact Assessment (HIA)"
(presented by Mike Joffe)

Abstract

There is little or no evidence at present on which one could base a Health Impact Assessment (HIA). On one hand there are anxieties, for example concerning possible health effects of Bovine Somatotrophin (BST), while on the other many scientists maintain that GM Foods are substantially equivalent to the naturally occurring form, apart from the consequences of the introduced gene. It is, however, possible to outline a structure into which evidence could be fitted once it becomes available, and to guide research aimed at obtaining such evidence. HIA is "a structured method for assessing and improving the health consequences of projects and policies in the non-health sector". As a process, HIA needs to involve key stakeholders, and relate to policy development. As a technical procedure, HIA takes a broad view, including benefits as well as hazards, and examining a range of determinants including for example effects of a capital project on transport needs and on employment/training. Vulnerable population sub-groups need explicit consideration. Methodological development is still underway in the HIA area. One approach is to consider the standard four-stage Risk Assessment model, and to extend it by studying the effects of a number of policy options on exposure levels, which is the variable element among the four. Examples of HIAs given included one on the new runway at Manchester Airport, which was strong in terms of process; on EU tax harmonization in relation to tobacco, which was a more technical exercise; and a model devised to address the health effects of pollution reduction in Westminster (central London).

Discussion/Issues raised

- Some of the participants shared the view that assessment should always be considered in relation to other policy options;
- The issue was raised of benefits and/or risks of a policy often falling differentially on sub-groups of the population; e.g. an additional runaway may benefit air travellers, who are relatively prosperous, but disadvantage local residents who are typical less well off;
- There always is a socio economic context;
- The fact that some countries lack universal health service coverage was mentioned as an important factor to consider when assessing risk;
- The efficiency/inefficiency of the current approval/control system was questioned. Some of the unexpected hazards have been uncovered by the current system of approval and control, e.g. nut allergy following insertion of a gene for a non-allergenic protein derived from Brazil nuts into soybean. However, in the case of BST the human health hazard were discovered after some years of use.

Session b) Gene Transfer

"Safety considerations when planning, constructing and developing new GM plants"
(presented by Francesco Sala)

Abstract

The long tradition of plant breeding and mutant induction and selection has steadily improved human nutrition and welfare through plant genetic alteration and adaptation to agricultural and industrial needs. This has not been exempt from risks: any new hybrid, by bringing together two full genomic sets, may express unexpected and undesired traits (e.g., production of toxins which were not produced by the parental plants) and mutants may carry a number of uncontrolled and potentially risky mutations besides the one(s) selected for.

All this has traditionally been perceived by the public as entailing minimum risk and high advantage to humanity.

Perception of risks in the case of transgenic plants is different: they are asked to be fully safe for human health and for the environment.

A realistic proposal is that we accept transgenic plants if their ratio risks vs. benefits is equal or better than that accepted in traditional agriculture.

Consequently, enhancing the scientific evaluation of risks and benefits of transgenic plants is of primary importance. Many of the risks that are attributed to transgenic plants are actually common to all cultivated plants. Others may arise from the integration of the foreign gene(s).

Furthermore, many topics of public concern may not have a scientific base, but scientists have the duty to face them and find appropriate acceptable alternatives. In fact, just as necessary is the creation of trust. It is that which the European consumers, in particular, appear to lack. The deep-rooted cultural fears of genetic manipulations, together with the past experience of the aggressiveness of some agro-business companies, has contributed to the success of the fight against the "Frankenstein food".

Here are examples of health concerns raised by transgenic plants and of possible approaches to their solution:

- Allergenicity. The foreign gene is felt as a potentially allergenic factor. But this can be verified by analyzing the physical and chemical characteristics of the foreign protein. Scientists consider this sufficient to warrant allergenic-free transgenic plants.
- Presence of an antibiotic-resistance gene. The large majority of the presently cultivated transgenic plants is endowed with a gene carrying resistance to an antibiotic, usually neomycin and kanamycin. This is perceived as a possible cause of antibiotic resistance in humans, although the allegation has no scientific bases. In fact, it is well recognize that it is the selective pressure imposed by the use and abuse of antibiotic in therapy (and the use of antibiotics as food additives in livestock nurseries) that determines the success of resistant microorganisms in our gut. Nevertheless, this is a typical case in which it is strongly advisable to give an answer to the public concern by proposing alternative solutions. We may use alternative (more acceptable) marker genes, or knockout the marker gene upon its exploitation in the selection step.

- Viral promoter sequences. Concern has been raised on the effect on human health of the spread, by horizontal gene transfer, of viral promoters used to activate transgenes. This allegation does not have solid experimental bases: it is true that "horizontal gene flow occurs in nature through distantly related organisms, but it also true that this may not endanger our health: every day we eat meat and vegetables, but we are unable to find transgenes in our genome.
- Escape of foreign genes through pollen dispersal. It is feared that transgenic pollen may transfer the foreign gene to sexually compatible plants. Indeed, there are restrictions to the success of gene transfer through pollen dispersal: pollen grains must reach a sexually compatible plant, cross pollination will not occur if the species is strictly autogamous, and the expression of the foreign gene must give a selective advantage. Strategies may be worked out in those cases in which gene transfer through pollen dispersal cannot be ruled out. These include the integration of the foreign gene into the chloroplast genome, the use male-sterile transgenic plants, the release of allogamous fertile plants in regions where sexually compatible plants are absent and the cultivation in the greenhouse under strictly controlled conditions.
- Escape of foreign genes through seed dispersal. Transgenic crop plants will spread their seed in the environment. The situation must be evaluated case by case. It is documented that cultivated plants are very poor competitor to wild plants. In most cases, seed dispersal will not turn out to be a problem, in others, concern should be faced with appropriate strategies. In some cases the use of sterile transgenic plants is recommended. Approval for commercialization should not be granted if concern of cross-pollination and environment protection is not fully answered.
- Effects of transgenic plants on natural habitat and biodiversity. Agriculture is not nature! Since it appeared, and at an increased rate in the last century, agriculture destroyed forest land, reduced biodiversity and promoted environmental pollution. More environmentally friendly approaches are needed for both traditional and genetically modified crops. Furthermore, it is not demonstrated that transgenic plants, per se, may reduce plant biodiversity in natural habitats.
- Modification of the soil microorganism (bacteria and fungi) and fauna (larvae) population. It is important that more conclusive data are produced on this specific topic. If this risk is verified, than it could be faced with the use of inducible promoters that will allow expression of the gene only when, or where, needed. On the other hand, beneficial effects may come from the use of transgenic plants that are planned to reduce or eliminate the use of chemicals such as insecticide, fungicides, fertilizers, phytoregulators and other chemicals.

In conclusion, it should be made clear to the public opinion that genetically modified plants are not to be intended as a unique case to be globally accepted or rejected. Rather, points of concern should be analyzed independently for each new transgenic plant. The best argument in favor of transgenic plants is the precision they become altered by introducing one or a few genes by comparison with classical plant breeding and mutagenesis. In most cases, this allows careful analysis of risks. If these are above the acceptable level or are not well defined, transgenic plants should not be accepted for commercialization. In all other cases there is no reason to consider them, in principle, more dangerous to human health and the environment as compared to traditional crops.

Discussion/Issues raised

- The question on technology shaping introduced into regulation was addressed by some of the participants. Regulations could in some cases define the most acceptable technological approaches. For instance, attention should be paid to the use of inducible promoter: in many cases, these would eliminate environmental or health concern.
- The discussion also focused on the consideration that risk analysis should have about local agricultural policy. For some participants risk analysis should indeed consider the vicinity of

sexually compatible plants, and encourage the use of mixed populations of transgenic and non-transgenic plants to eliminate negative effects on soil fauna.

- The issue of the level of expression and dosage with vaccines produced in plants was also raised, and some participants stated that the problems of expressing sufficient quantity of vaccines in transgenic potato and tomato are being solved by the present research in USA and China. Over dosage will not represent a problem in the treatment of humans to induce immunization to infectious diseases.
- A fair large time of discussion was spent on the management of social acceptability of GM plants. Some participants suggested to the scientists working on the construction of GM plants that they consider the need to satisfy social acceptability before planning a GM plant and not after having produced it.
- The speaker was asked about technological alternatives to the currently used approaches to gene transfer. Several options are indeed available when planning a GM plant. There are options in the choice of promoters, site of integration (nuclear or chloroplast genome), method of gene integration, selection of GM cells and many others. The selection of appropriate options has profound effects on risks acceptance.

"Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria – are there new data to fuel the debate?"
(presented by Kornelia Smalla)

Abstract

Presently, the majority of genetically modified plants tested in the field or already commercialized contain bacterial antibiotic resistance genes which are often used to select for transformants. The mechanism, which most likely contributes to a horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria, is termed "natural transformation". Prerequisites for natural transformation are the availability of free DNA, the development of competence, the take-up and stable integration of the captured DNA. Long-term persistence of transgenic plant DNA was observed under microcosm and field conditions. Microbial activity was pinpointed as an important biotic factor affecting the persistence of free DNA in soil. PCR-based detection of transgenic DNA allows a sensitive and specific detection of transgenic DNA in environmental samples. However, so far there was no experimental evidence that horizontal gene transfer of genetic material from plants to bacteria can occur at all. Only recently, the ability of *Acinetobacter sp. BD413* (δnptII) to capture and integrate transgenic plant DNA based on homologous recombination could be demonstrated under optimized laboratory conditions. Present data suggest that transformation of competent bacteria by transgenic plant DNA in soil and in the rhizosphere occurs at very low frequencies, if at all. However, it cannot be ruled out that hot spots, e.g. the digestive tract of insects, exist which might promote gene transfer events. Given the fact that antibiotic resistance genes, often located on mobile genetic elements, are already widespread in bacterial populations and that horizontal gene transfer 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. The control of the antibiotic resistance problem very clearly lies in a reduction of the selective pressure by prudent use of antibiotics.

Discussion/Issues raised

- A large portion of the discussion stressed again the different value of basic research versus interpretation papers. There is indeed a large number of papers discussing the issue of horizontal gene transfer (HGT) e.g. from plants to bacteria. However, the vast majority of publications in the field are based on interpreting original data of others, and this is not always made clear. The number of publications providing original data on HGT is surprisingly low, and there is a need to expand our knowledge on the following issues:

  - How important is natural transformation in different environmental habitats?
  - Which proportion of indigenous bacteria is able to take up non-specific DNA, and is sequence homology always required to achieve stable integration of the DNA? What are the conditions under which different kinds of bacteria reach the competence state?
  - Natural reservoirs of antibiotic resistance genes and selective pressure.

- While PCR analysis of DNA extracted directly from all kind of samples (soil, rhizosphere, sewage, insect gut, faeces, saliva, foods) allows a sensitive and specific detection of transgenic DNA, detection of HGT under field conditions remains difficult due to limitations of techniques currently available. Unequivocal proof of HGT from plants to bacteria requires the isolation and characterization, which is often difficult due to high background levels of resistant bacteria. The strategy to monitor the transfer of complete genes might fail because transformation often involves the stable integration of short DNA fragments.
- Horizontal gene exchange can be seen as a natural phenomenon for bacterial adaptation and for successful colonization of ecological niches. Bacteria possess different and very efficient mechanisms of exchanging DNA: transformation, transduction, conjugation and mobilization. A particularly important role is plaid by mobile genetic elements (MGE) which endow their host bacteria with genetic variability and flexibility in response to environmental stress and thus promote genome plasticity. MGE provide a location where catabolic and anabolic genes can be assembled to provide the response to environmental stresses. Environmental factors stimulating horizontal gene transfer processes need to be better understood in order to inhibit gene exchange (e.g. of antibiotic resistance genes or transgenic DNA) or to stimulate the spread of MGE (e.g. to disseminate biodegradative genes in natural populations). A better understanding of the diversity, maintenance and transfer functions of MGE, the acquisition and spread of new phenotypic traits will provide an important scientific basis for biosafety evaluations and thus will support science-based decision making.

"Environmental risks of crops with transgenic virus resistance"
(presented by Alison Power)

Abstract

Most of the major food crops worldwide have now been genetically engineered for virus resistance via the insertion of viral genes into the plant genome. Potential ecological risks associated with the

widespread adoption of engineered virus resistance fall into three major categories: recombination between transgenes and wildtype viruses; interactions between transgene products and wildtype viruses, such as synergies or transcapsidation; and transgene movement from transgenic crops to wild relatives via hybridization. In all of these categories, both the probability of the event and the degree of hazard that might result from that event need to be assessed. Evidence to date suggests that the probability of occurrence is high for virus-transgene recombination and virus-transgene product interactions, unless particular gene constructs are deliberately avoided. Potential hazards due to these events include increased viral host range, modifications in virulence, and changes in transmission, any of which could provide a selective advantage that would allow the recombinant virus to spread. However, there are few data available to assess these potential hazards.

Transgene movement from transgenic crops to wild relatives via hybridization is also highly probable, and again the hazards are not well understood. Studies are in process to assess the potential hazards associated with movement of transgenic virus resistance from cereal crops to wild crop relatives. Barley Yellow Dwarf Virus (BYDV) is one of the most economically important diseases of cereal crops worldwide, and it is among the most prevalent of all viral diseases. Transgene movement from cereal crops expressing transgenic resistance to BYDV may pose particularly high risks because of the paucity of natural resistance to BYDV in some wild relatives such as wild oats. Accumulating evidence suggests that both the probability of transgene transfer to wild relatives and the fitness advantages of the transgenes are likely to be high for some cereals targeted for transgenic BYDV resistance. The movement of transgenes for BYDV resistance into weedy annual grasses like wild oats or wild barleys may result in both agronomic and ecological hazards, and may have implications for human health. In terms of agronomic hazard, acquisition of BYDV resistance by these weeds may make them more significant competitors with cultivated cereals. This could require increased use of herbicides to control weed populations, potentially exposing workers and consumers to higher levels of these chemicals. In terms of ecological hazard, increased fitness of wild species through the acquisition of transgenic resistance could result in the release of these species from ecological constraints normally imposed by infection with BYDV, resulting in significant negative impacts on native grassland ecosystems.

Discussion/Issues raised

- The author emphasized that recombination among viruses and between viruses and transgenes appears to take place with relatively high frequency, but most of these data come from lab experiments. It is extremely difficult to study recombination, or other viral processes, in the field.
- Some participants commented that it is helpful to distinguish between the probability of the risk occurring and the damage caused. Given the relatively high probability of many of these risks, it is probably most useful to concentrate on evaluating the potential consequences and degree of damage.
- The author provided several examples of technology shaping that might reduce some of the risks associated with transgenic virus resistance, including avoiding the use of viral genes that encode replicase, helper component proteins, or movement proteins.
- There was some discussion of how transgenic resistance may resemble the phenomenon of crossprotection, where inoculation with one virus can protect against infection by a second virus. The possible mechanisms were discussed.
- Participants asked whether plant viruses, including recombinant viruses containing transgenes, posed any direct risk to humans and whether there are any interactions between plant and animal viruses. It was agreed that there is no evidence of direct risk of these viruses to humans.

"Transgene fate in the gastro-intestinal tract and in the environment"
(presented by Claudia Sorlini)

Abstract

The Author summarizes the key worries about transgenic food (obtained from GMP and GM microorganisms) in the following:

- the possibility of transgenes transfer from microorganisms and vegetables content in food to the gastro-intestinal microflora;
- the spread of the transgenes in the environment from animal and human feces;
- the possibility of interaction between transgenes and mammalian cell DNA;
- the negative effects on human health from the expression of proteins (known or not) ingested with food.

Horizontal gene transfer (HGT) is a known phenomenon that naturally occurs between bacteria. It has been demonstrated also in gastro-intestinal tract. This phenomenon has been observed also from genetically modified bacteria to gastro-intestinal bacteria in vivo experiments.

Horizontal gene transfer from plants to microorganisms was evidenced only under laboratory conditions. On the other hand, the possible transformation of gastro-intestinal microflora by free DNA has received until now a scarce attention, because free DNA is considered unlikely to survive the action of gut nucleases.

Fate of foreign DNA in gastro-intestinal tract: results of investigations on foreign DNA (sequences present in constructs used for plants transformation) in gastro-intestinal tract demonstrate that, in opposition to what is generally believed, 5% of DNA can survive in large fragments to the gastro-intestinal digestion. DNA has been recovered from different parts of the gut, blood or spleen and liver of the rats and in the feces, after oral administration.

Interaction between foreign DNA and mammalian cell DNA: fragment of foreign DNA was found covalently linked to DNA extracted from spleen of rats. Also in rare cells of three fetuses, the foreign DNA was found in chromosomal association with both chromatids. “Is maternally ingested foreign DNA a potential mutagen for the developing of fetuses?”

Regarding health risk related to transgenic food, the Author presents some examples of damage to health (allergic reactions and modification of the gastro-intestinal tract of experimental animals).

In conclusion:

- HGT from modified to natural bacteria in gastro-intestinal tract has been observed.
- HGT from modified vegetable food to gastro–intestinal tract has not been demonstrated in vivo experiments.
- Transgenic DNA can survive to gastro-intestinal digestion and spread in the environment.
- Foreign DNA seems to interact with mammalian cell DNA.

Which is the frequency of these phenomena and which are the consequences on humans, other animals and environment? It has not been enough clarified.

The findings suggest continuing research in order to:

- deepen the knowledge about HGT and the environmental fate of transgenic DNA (both free and into bacteria) eliminated with the feces that could be spread in the soil or reach the water decontamination plant;
- develop investigations on the interaction between transgenic DNA of food and mammalian cell DNA;
- improve the investigations on the allergenic activities of proteins, known and unknown, contained in the transgenic food.

Discussion/Issues raised

- Is gene transfer between microorganisms a natural process?
  Gene transfer between microorganisms is a natural process that can occur in the environmental matrices and in gastro-intestinal tract. HGT can happen by transformation (free DNA- bacteria), conjugation (direct contact between two bacteria) and transduction (transfer mediated by a phage). HGT has been evidenced also between distantly related bacteria. Recent investigations showed that not only gene but also transgene can transfer between bacteria.
- Is gene transfer between plants and microbes a natural process?
  Microbial transformation by plant DNA fragments has been never demonstrated in natural environments and in gastro-intestinal tracts, although the high homology of some sequences, detected in plants and bacteria, suggests that some gene exchanges could occur during the evolution. Until now only under laboratory conditions, plant transgene transfer to bacteria was evidenced.
- Integration and activity of the foreign DNA.
  It is known that some disease can be caused by the insertion of viral DNA into mammalian cell DNA (for example human Adenovirus Ad12 DNA induces tumours by this mechanism, as studied in experiments with *Mesocricetus auratus*). For this reason investigations were carried out in order to verify if other foreign DNAs can insert into mammalian DNA: segments of foreign DNA (DNA viral of M13, and plasmidic DNA containing the gene of GFP, that can be present in constructs used for plant transformation), orally administered to mice, have been found to be covalently linked to DNA extracted from spleen, and, when administered to pregnant mice, were found in chromosomal association with both chromatids of foetuses. The activity of these inserted sequences is however not known.

"Inter/intra species gene transfer from GM plants to other plants"
(presented by Joaquim Machado)

Abstract

The Author underlines the importance of the methodology chosen to study gene transfer from genetically modified plants to other plants, recognizing the complexities of such area of genetic studies. The theoretical tools for the analysis of gene flow in specie's populations is the determination of population genetic structure by statistical examination of the frequencies of the allelic variants of individual traits in each population.

The common statistical approach (F-statistics) is not advisable for purposes of answering gene flow questions on an ecological time scale, being only descriptors of historical genetic structure and not sensitive to rare alleles. The result could be and evaluation that ignores on-going dynamics relevant to the interest of ecologists.

The Author highlights the importance of the use of Artificial Life-type simulation software when experiments with real living systems are difficult for practical or ethical reasons. At the same time, much can be learned about algorithms working in real species by comparison with the artificial ones.

In addition to the appropriate statistical methodology, new procedures on how using molecular markers on gene flow are now available or under development, contributing to efficient science-based studies on Population Genetics: multiple RAPD markers, cytoplasmic markers and markers genes, improve the capacity of detecting introgression and estimating allele frequencies and fitness.

The author highlights the importance of a better understanding of phenotypic and genotypic definitions of landraces, in order to better estimate risks related to gene transfer.

The author suggested the following for conclusions:

- Conclusions on effects of inter/intra species gene transfer and gene flow, should be always obtained based on robust scientific methodology of Population Genetics and Evolution, using adequate Statistical Models, and highly-informative markers, concentrating efforts on estimating gene effects, avoiding a priori predictions based on gene origins.
- The use of Articial Life-type algorithmic software should be considered as an efficient way of simulating gene transfer and gene flow phenomena in different genetic backgrounds and environmental conditions.
- Whenever possible, those studies should emulate as much as possible current and on-going agricultural systems and breeding methodologies.

Discussion/Issues raised

Some participants questioned the validity of models, such the artificial life-type simulation software presented by the speaker. According to the speaker ethology, since the very beginning, based indeed its conclusions on observational and empirical studies and experiments. Nevertheless, Population Genetics and Ecological Genetics offer a more appropriate scientific infrastructure towards the understanding of the dynamics of interaction among life forms. The dynamics of pollen dispersion and gene flow, and also the several subsequent evolutionary forces act during decades and even centuries, can be studied using powerful models. There is nothing wrong with models, provided they are well constructed. Medicine (where virtually no public perception pressures exist regarding the ecological impact of medical sciences on life forms) is plenty of models, as a simple visit to our family doctor can demonstrates. We feel better based on models described by our doctor, take medicines based on biochemical models (even with unknown side effects!), and suffer surgery based on models.

Some participants raised the issue of the illogic of making projections on gene flow. The speaker reply was that it is not appropriate to use the term “illogic” to state that it is “illogic to make projection on gene flow”, unless we consider also as completely “illogic” to make projection on dangerous consequences of pollen transfer and transgene flow. Most part of the considerations on

hazards, regarding GMOs, is based on popular perceptions, not on logic. According to the speaker the only logic procedure to determine hazards regarding gene flow is to build consistent models based on:

- Strong previous evidence of directed, and not imagined, GMO hazard.
- The current knowledge of Population Genetics, "accelerating" the necessary time span required to understand parameters as "allel frequency", "genetic drift", "allel fixation" etc, via available algorithms adapted to be studied in computers. Obviously, small and medium-scale "real" experiments can be designed to understand pollen flow and gene flow, but currently, the only way to simulate "nature" is using simulation programmes.

The Genome is a game, as all the recent scientific discoveries clearly indicate. However and obviously, we still need to be responsible on promoting the necessary ways to control accidents and abuses. This is also true regarding drug transborder traffic, GMO-derived blue cheese quality in the supermarkets, special cosmetics enriched by liposomes and vitamin E (even considering that we should not use it around our eyes, according the instructions; nevertheless, we always have other variant - and other price! - this time "safe" to be applied on the eyes region).

The Genome is a game, with logic, statistical, and probabilistical rules. No matter how we define the importance of Nature, genetic rules can always be applied. We have been supporting our taxonomic classification based on phenotypic parameters and descriptions. Life forms always transfer genes, not forms. Life can be compared to a beautiful and complex "origami" where, no matter how many parameters could be defined and controlled, until now it is impossible to preview exactly the final phenotype. This does not mean danger, considering the evolution of life in this planet.

Homo sapiens is part of the game, as an egocentric component of Nature. But we know more and more on the genetic rules. We should base our understanding on pollen transfer and gene flow, on genetics and not on phenotypes, very dynamic by its own nature.

Some participants asked about the use of natural markers. According to the speaker it is impressive to see how "natural markers" could be used to provide more and more details on pollen transfer and gene flow. Most part of the hazardous consequences, even imagined, could be better examined, if supported by information on the gene dynamics studies in populations, where the spread and fixation of transgenes could be established or at least estimated. According to the speaker the GM are safe, and for this reason, those kind of studies are not promoting curiosity and necessity, the mothers of invention.

The issue of post-GMO breeding practices was raised. It was stated by the speaker that right now the first consequences of genetic contamination with GMO pollen, or seed mixture, a somewhat frequent, even easily controlled issue in seed production, are being observed. Several commercial consequences, as seed importation from countries where GMOs are already released, to countries where this is not still permitted, for example, are being discussed by governmental officials in order to promote transborder commercial exchanges of seeds. The main problem e.g. in South America, nowadays, is how to certificate laboratories, to assure the quality of GMO detection tests.

According to the speaker, another interesting issue is the understanding on property laws, regarding the use of patented transgenes in different genetic backgrounds, for recurrent selection practices, for example. An exciting issue for lawyers should be the contamination of a maize landrace by a

commercial maize GMO neighbour crop. Would be possible for a third-part breeder to develop commercial inbreeds by selfing that landrace?

The session ended with an extensive discussion on the lack of data on landraces. If there is "lack of data on landraces", right now, this means that "nothing", or just very few studies were being conducted before. According to the speaker this is not a reason to delay GMOs development since:

- As demonstrated in the main paper, GMO's are not a threat to landraces, by itself. In fact, many other cultural, agricultural, and economic development conditions are responsible for the disappearance of landraces.
- A landrace, if alogamous, is an open-genetic system. Considered as very important for cultural reasons, or even for small-scale subsistence, should be protected by special breeding methodologies, described in grad studies texts, and amenable to be applied even by the small farmers, and not by impeding GMO crops development. Otherwise, those landraces will step by step disappear, no matter the existence of GMOs. In southern Brazil, there is an NGO dedicated to landless people agricultural development, using plant-breeding methodologies to preserve crop landraces. It is a marvellous example of applying Science on the benefit of small farmers.

Session c) Soil as ecosystem

"Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis"
(presented by Guenther Stotzky)

Abstract

Insecticidal proteins produced by various subspecies of *Bacillus thuringiensis* bind rapidly and tightly on clays, both pure mined clay minerals and soil clays, and on humic acids extracted from soil. This binding reduces the susceptibility of these proteins to microbial degradation, and the bound toxins retain their biological activity. Both purified toxins and toxins released from the biomass of transgenic *Bt* corn and in root exudates of growing *Bt* corn exhibit binding and persistence in soil.

Biomass of transgenic *Bt* corn decomposes less in soil than does biomass of isogenic non-*Bt* corn. This lesser decomposition does not appear to be related to differences in the C/ ratios of Bt and non-Bt corn. Preliminary studies indicate that Bt corn has a higher content of lignin, which may be involved in the differences in decomposition. The toxins do not appear to have any consistent effects on organisms (earthworms, nematodes, protozoa, bacteria, fungi) in soil or *in vitro*. The toxins are not taken up from soil by non-*Bt* corn grown in soil in which *Bt* corn has been grown or into which biomass of *Bt* corn has been incorporated. Larvicidal activity of purified toxins was detected 234 days after its addition to non-sterile soil; activity of toxin released in root exudates of Bt corn was detected 120 days after harvest of the plants; activity in soil amended with biomass of bt corn was detected more than one year after addition. In all cases, these were the longest times studied, and persistence is probably longer.

These studies on the interaction of insecticidal proteins with two types of surface-active particles (clays and humic acids) that differ greatly in composition and structure demonstrate further the importance of surface-active particles to the biology of natural habitats. These studies also confirm and extend previous observations on the influence of clays and other surface-active particles on the

activity, ecology, and population dynamics of microbes (including viruses) in soil and other natural habitats, as well as on the transfer of genetic information among bacteria by conjugation, transduction, and transformation.

Moreover, the results obtained with these proteins indicate their potential environmental importance when bound on surfaces in soil. For example, the persistence of the bound toxins from *Bt* could pose a potential hazard to nontarget organisms and result in the selection of toxin-resistant target insects and, thereby, negate the benefits of using a biological, rather than a synthetic chemical, insecticide. However, the persistence of the bound toxins could also enhance the control of target pests. These aspects require more extensive study.

In addition to suggesting potential hazards and benefits of bound toxins from *Bt*, the results of these studies emphasize that caution must be exercised before transgenic plants and animals genetically modified to function as "factories" for the production of vaccines, hormones, antibodies, toxins, pharmaceuticals, and other bioactive compounds are released to the environment. Because of the large differences in the chemical composition and structure between clays and humic acids, these studies can serve as models for the potential fate and effects of other biomolecules, which are also chemically and structurally diverse, that will be introduced to soil from such factories. As with *Bt* plants, where only a portion of the plants is harvested (e.g., ears of corn, bolls of cotton, kernels of rice, potatoes) and the remainder of the biomass is incorporated into soil wherein the toxins released from disintegrating biomass are rapidly bound on surface-active particles, some of the biomass of these plant factories will also be incorporated into soil. With transgenic animal factories, faeces, urine, and subsequently even carcasses containing bioactive compounds will eventually reach soil and other natural habitats (e.g., surface and ground waters). If these bioactive compounds bind on clays and humic substances - and as many of these compounds are proteinaceous, they most likely will - they may also persist in natural habitats. If they retain their bioactivity, they could affect the biology of these habitats. Consequently, before the use of such plant and animal factories (and, probably, also microbial factories), the persistence of their products and the potential effects of the products on the inhabitants of soil and other habitats must be thoroughly evaluated.

Discussion/Issues raised

- The participants demonstrated high interest in the studies revealing the presence of significantly higher levels of lignin in Bt corn. Especially the question about the “substantially equivalence” of Bt corn with higher lignin content was raised.
- The participants considered too speculative at this stage of the studies to link the high content of lignin directly with the genetical modification (construct)
- Some participants raised the issue of the origin (exudates or cell lysis) of the toxin and of the persistence of bio molecules in general in soil. The author of the background paper confirmed that he release, persistence, and biological activity in soil of the toxin in root exudates of Bt corn was demonstrated with 12 different hybrids representing three different events. Although some toxin was probably released from sloughed and damaged root cells, the major portion was derived from exudates, as there was no discernable root debris from plants grown in hydroponic culture. Regardless of the proportion of toxin released as exudates or from lysed cells, the toxin is present and persists in the rhizosphere soil of Bt corn.
- One participant was of the view that transgenic plants and animals expressing biological active peptides that are new to the given organism pose a specific health hazard. The proteins released through decaying organic material or urine/faeces might be bound to the soil matrix and thus

presented to wildlife. As any release into the environment is an introduction of the transgene into the gene pool, the transgene and the possibly hazardous pharmaceutical compound will eventually show up in wild relatives and/or food crops, with far reaching health consequences.

Session d) Resistances

"Monitoring for early detection of resistance"
(presented by David Andow)

Abstract

Insect resistance management (IRM) can be characterized as either responsive or pre-emptive. Responsive strategies respond to the widespread occurrence of field resistance, while pre-emptive strategies attempt to avoid or delay resistance before it occurs in the field. Most IRM strategies have been responsive, but recently greater attention has been paid to pre-emptive strategies, especially for transgenic insecticidal crops.

Bt maize has been genetically engineered to express Cry toxin from genes from Bacillus thuringiensis. Pre-emptive resistance management for Bt maize is based on the high-dose plus refuge strategy. A central feature of this approach is the 20% structured refuge for susceptible corn borers.

Monitoring is the first step in the design of adaptive IRM: this paper concentrates on methods to monitor the frequency of resistance in natural populations.

Models suggest that for monitoring to be useful, dominant resistance should be detected at frequencies <0.0001, while recessive resistance should be detected at frequencies <0.005. Five monitoring methods are given serious consideration, late larval discriminating concentration assays, neonate discriminating concentration assays, late-planted in-field Bt sweet maize screens, late-planted in-field Bt field maize screens, and F2 screen. Test stock and molecular methods may become useful once resistance is detected. Bayesian statistics are summarized for each method, and the relative costs are compared.

Effective discriminating concentration assays have not been verified for European corn borer. These assays are not very precise unless resistance is common (>0.1) and they may be cost-effective only when screening common, dominant resistance. Thus they will not be useful for early detection of resistance, but may be helpful for documenting control failures and the breakdown of resistance management.

The in-field screens should be able to detect dominant resistance at frequencies <0.0001 at relatively low cost (< USD 2,000.00/sample): this is the best method for monitoring for dominance. They have been used to monitor resistance in European corn borer and corn earworm in Minnesota.

The F2 screen has been used in North America, the Philippines, France and Germany, and has proven reliable, consistent and precise. It is the most cost-effective method for detecting recessive resistance, and for about USD 5,000.00 per sample, it can estimate resistance <0.005. The F2 screen should be useful for early detection of resistance to Bt maize in European corn borer. With some minor improvements, the in-field screen should prove useful for early detection of dominant resistance, but when resistance becomes common, the F2 screen should remain a cost-effective

monitoring tool for recessive resistance. Monitoring common resistance may become important for improving resistance management if resistance has a significant fitness cost.

One of the conclusions suggested by the author was that there will be risks that cannot be well-characterized prior to commercialization. Hence there is a need for monitoring and some continued oversight.

Discussion/Issues raised

- Gene-stacking and IRM. One participant asked if gene-stacking would reduce the need for a refuge. Under the assumption that there is no cross-resistance to the "stacked" toxins, e.g., that the resistance mechanisms are independent, then gene-stacking would result in a delayed evolution of resistance, and a smaller refuge might be as effective as a larger refuge for a non-stacked product. If there is cross-resistance then a similar sized refuge would be needed for the stacked product. Thus the issue of refuge size depends on the assumption of cross-resistance. Because it is not now possible to predict cross-resistance a priori, this is an empirical issue. Consequently, the precautionary assumption would be to assume some degree of cross-resistance and retain refuge sizes similar to non-stacked products. Additionally, the stacked products will be deployed in an environment where non-stacked products are also used. Because the non-stacked products could provide a pathway for sequential evolution resistance to each of the stacked toxins (in contrast to the requirement of simultaneous evolution of resistance if only the stacked product were being used), it will be essential to maintain similar sized refuges for both stacked and non-stacked products.
- Differencies in phenology between resistant and susceptible insects may jeopardize current pest resistance management approaches. This is certainly a possibility that could jeopardise present IRM plans. For ECB and Bt corn, however, this threat may be mitigated by the fact that second generation ECB phenology is spread out widely in time. Thus, slow developing ECB are likely to emerge at a time when susceptibles are also emerging, and those that emerge too late will be unlikely to produce progeny that can survive the winter. As both the possibility of delayed development and phenological dilution by susceptibles are somewhat hypothetical, this concern amplifies the need for effective monitoring of resistance evolution, the main point of the presentation.
- Spatial arrangement of refuges is important for pest resistance management. In general, a larger, closer refuge is more effective for IRM. The requirement in the US that the refuge is located within 800m (1/2 mile) for Bt corn and Bt potatoes is essential.
- Comparison between resistance after Bt-spray and Bt-crops. In the absence of concrete scientific information about resistance to Bt-crops, the information on resistance to Bt-sprays is essential for projecting evolution in Bt-crops. Expression of Bt toxin in crops is different from expression in sprays, so the evolutionary process will not be the same. The primary utility in the comparison is in the analysis of resistance mechanisms and the genetic characterization of the inheritance of resistance.
- Monitoring. Monitoring is essential because the assumptions of the IRM plan may be faulty, and unanticipated effects leading to faster resistance evolution may arise.

Session e) Impact on non-target fauna

"Impact of GM plants on non-target arthropod fauna"
(presented by Tanja Schuler)

Abstract

The overall impact of GM plants on non-target arthropods is likely to depend to a large extent on how the crops are managed, e.g. when a herbicide is applied or what measures are used to control non-target pests. Large-scale experiments are currently underway to establish if growing herbicide tolerant crops will affect wildlife through changes in agronomic practice and what role Bt plants can play in integrated pest management systems.

There needs to be an overall consensus about the standard for comparison. It is unrealistic to compare for example the effect of Bt plants on populations of non-target arthropods solely with a situation where no pest control is applied. Based on the information available to date there is no indication that Bt plants will be more disruptive to biological control than conventional pest control based on insecticides. So far it has not been possible to compare GM crops with organic farming methods of pest control since organic farming regulations do not permit GM crops as part of organic rotations.

It is important to study any potential negative side effects of GM plants. The risk assessment should involve several tritrophic systems with target and non-target pests. A three-tiered testing scheme is recommended for the risk assessment:

- the first tier involves small scale bioassays initially representing a worst-case scenario and which should identify potential hazards, including assessments of sub-lethal effects;
- the second tier is represented by experiments with populations either in laboratory or on a small scale in the field;
- the third and most realistic tier consists of large scale field trials.

However, it is probably impossible to test all possible interactions in pre-approval trials and subtle long-term effects on non-target populations will only be detectable by monitoring on a large scale over several years at the same locations. Additional post-approval monitoring therefore seems advisable.

Discussion/Issues raised

It was commented that although herbicide tolerant crops may give farmers theoretically the possibility to delay and reduce herbicide applications, farmers might choose not to take up this option in practice.

A question was raised regarding a comment in the background paper that referred to studies which reported positive effects of GM crops on non-target arthropods. It was explained that this comment referred to field studies by Johnson & Gould and Hoy et al. Johnson & Gould observed synergistic interactions between low-expressing Bt tobacco and a species of parasitic wasp. Hoy et al. reported higher populations of beneficial insects in Bt potato plantings at several sites and over several years compared to insecticide treated standard plots. The highly skilled, labour and time intensive effort involved in identifying the wide range of non-target arthropods found in crops was emphasized.

"Review on non-target organisms and bt-plants"
(presented by Angelika Hilbeck)

Abstract

A summary about the previous work of the author on the impact of transgenic Bt-corn and microbially produced Bt-preparations on an important biocontrol agent, the green lacewing (*Chrysoperla carnea*) was firstly provided.

The results of all conducted experiments (three series using different Bt-delivery systems) consistently demonstrated the susceptibility of immature *C. carnea* to Bt proteins (Cry1Ab toxin, Cry1Ab protoxin, and Cry2A protoxin) whether ingested in prey or directly. The rate of mortality varied with the method of Bt-delivery. Prey-mediated mortality of immature *C. carnea* was highest when the prey's food source was transgenic Bt-corn. This was despite the fact that the concentration of the Bt-toxin Cry1Ab was lower in Bt-plants than in all other Bt-incorporating diets. In context, these results suggested complex triple-interactions, among Bt-proteins, herbivores, and plants, all contributing to the observed prey-mediated mortality of *C. carnea*.

Secondly, a report was delivered on a scientific review by the author and her colleagues that analysed previous published and regulatory (carried out by biotech companies for commercial approval) studies also testing the impact of Bt-preparations or Bt-plants on nontarget insects, primarily natural enemies. Most of them reported the finding of no significant effect. For the first time, studies submitted to regulatory authorities (e.g. EPA) for commercial approval were critically assessed one by one. It was concluded that EPA's approval of insect-resistant crops with regard to nontarget side effects was based on questionable testing procedures.

Major criticism was expressed because:

- only one tested long-term exposure to the Bt toxin. In the one chronic study, some adverse effects on non-target insects were observed;
- none considered effects of the toxin in the food-chain, in other interactions among plants, or on insects feeding on them and their natural enemies;
- for most, if not all, methods applied, it needs to be demonstrated whether the Bt toxins were ingested by the target test species;
- all regulatory tests were modelled according to testing guidelines for assessing the ecotoxicity of industrial chemicals (e.g. pesticides) – none were designed to test the risks of releasing living organisms into the environment. It was noted that testing procedures designed for pesticides and their mode of release alone are not sufficient for assessing effects of transgenic plants on nontarget organisms.

Discussion/Issues raised

- the presentation emphasized the need of scientific rigorous and robust data;
- the transportation of Bt protein in plants is not understood. Based on a report by the author of the background paper Bt was not found in Bt maize phloem sap;
- due to the constitutive expression of Bt in transgenic Bt-plants, the number of nontarget organisms potentially affected or coming into contact with Bt proteins of transgenic Bt-plants is extended than previously when Bt-insecticides were used.

CONCLUSIONS AND RECOMMENDATIONS[1]

In general:

- the participants recognize that hazards to human health may arise from the release of genetically modified organisms in the environment, therefore there is a need for risk analysis before release;

- the participants felt that specific hazards might be identifiable for certain groups of e.g. genetically modified organism, crops, traits. However risk analysis of GMOs must be carried out on a case by case basis;

- the participants agreed that risk assessment must be done to account for environmental and genetic variability;

- the participants recognize the existence of gaps of knowledge therefore continued research and significant increase of funding on biosafety related research and capacity building is required to address present and future needs.

- the participants agreed, with one exception, that technological innovation could be designed (technology shaping) to include the criteria of enhancing inherent safety;

- the participants agreed that risk analysis should always consider alternatives including non biotechnological ones (comparative risk assessment covering all alternatives);

- with one exception, all participants recommended post release monitoring (incl. the development of appropriate protocols) broad enough to capture unexpected consequences, including building capacity in developing countries;

- the participants agreed that risk analysis must be strengthened, in order to involve all stakeholders;

In particular the discussion focused on DNA transfer, development of resistance and non-target effects with the following conclusions:

- the participants concluded that gene transfer might pose a hazard if genes coding for proteins affecting human health are involved;

- the participants recognized that DNA transfer and expression should be further investigated in various ecosystems;

- the participants were of the view that resistance build up may require changes in pest management strategies bearing human health hazards;

- the participants agreed that non-target effects as they relate to agronomic practices and biodiversity issue should be investigated.

[1] The seminar recognizes that the hazards discussed are not all unique to GMOs but may also apply to other organisms

ANNEX 1: LIST OF PARTICIPANTS

EXPERTS

Professor David Andow University of Minnesota Department of Entomology 219 Hodson Hall MN 55108 St. Paul USA	Phone: +1 612 6245323 Fax: +1 612 6255299 E-mail: dandow@tc.umn.edu AUTHOR OF BACKGROUND PAPER ON *Monitoring for early detection of resistance*
Professor Philip L. Bereano Washington Biotechnology Action Council Council for Responsible Genetics P.O. Box 352195 98195 Seattle, Washington USA	Phone: +1 206 5439037 Fax: +1 206 5438858 E-mail: phil@uwtc.washington.edu
Dr Inge Broer Scientist, University of Rostock Fachbereich Biowissenschaften/FINAB Doberanerst. 143 D-18053 Rostock Germany	Phone: +49 381 4942071 Fax: +49 381 4942079 E-mail: ib@bio4.uni-rostock.de
Dr Hans-Jorg Buhk Robert Koch Institute Wollankstr. 15-17 D-13187 Berlin Germany	Phone: +49 30 45473000 Fax: +49 30 45473030 E-mail: buhk@rki.de
Dr Angelika Hilbeck Senior Researcher Swiss Federal Institute of Technology Geobotanical Institute and (2) EcoStrat GmbH Zurichbergstr. 38 2. Feldblumenstr. 10 CH-8044 Zurich CH-8048 Zurich Switzerland Switzerland	Phone: +41 1 6324322 / 4303060 Fax: +41 1 6321215 E-mail: hilbeck@geobot.umnw.ethz.ch AUTHOR OF BACKGROUND PAPER ON *Review on non-target organisms and bt-plants*
Dr Michael Joffe Reader in Epidemiology and Public Health Department of Epidemiology & Public Health Imperial College School of Medicine St Mary's Campus Norfolk Place London W2 1PG UK	Phone: +44 207 5943338 Fax: +44 207 4022150 E-mail: m.joffe@ic.ac.uk AUTHOR OF BACKGROUND PAPER ON *Health Impact Assessment (HIA)*

Dr Othmar Kaeppeli
Executive Manager
Agency BATS
Clarastrasse 13
CH-4058 Basel
Switzerland

Phone: +41 61 6909310
Fax: +41 61 6909315
E-mail: kaeppeli@bats.ch

AUTHOR OF BACKGROUND PAPER ON
The fundamentals of science-based environmental risk assessment of GMOs

---

Dr Joaquim Machado
Manager, Biotechnology Special Projects
Novartis Seeds Ltda
Av. Prof. Vicente Rao 90
04706-900 Sao Paulo
Brasil

Phone: +55 11 5327468
Fax: +55 19 5324315
E-mail: joaquim.machado@seeds.novartis.com

AUTHOR OF BACKGROUND PAPER ON
Inter/intra species gene transfer from GM plants to other plants

---

Dr Alison G. Power
Cornell University
Department of Ecology & Evolutionary Biology
Corson Hall
NY 14853 Ithaca
USA

Phone: +1 607 3474095
Fax: +1 607 2558088
E-mail: agp4@cornell.edu

AUTHOR OF BACKGROUND PAPER ON
Environmental risks of crops with transgenic virus-resistance

---

Dr Francesco Sala
Department of Biology
University of Milan
Via G. Celoria, 26
I-20133 Milan
Italy

Phone: +39 02 26604322
Fax: +39 02 26604322
E-mail: francesco.sala@unimi.it

AUTHOR OF BACKGROUND PAPER ON
Safety considerations when planning, constructing and developing GM plants

---

Dr Nancy Schellhorn
Research Scientist, CSIRO Entomology, Australia
CSIRO Cotton Research Unit
Locked Bac # 59
Myall Vale
NSW 2390 Narrabri
Austrialia

Phone: +61 2 67991538
Fax: +61 2 67991186
E-mail: nancy@mv.pi.csiro.au

Dr Tanja Hedwig Schuler
Postdoctoral Entomologist,
Department of Entomology & Nematology, IACR Rothamsted
AL5 2JQ Harpenden
UK

Phone: +44 1582 763133 ext 2488
Fax: +44 1582 760981
E-mail: tanja.schuler@bbsrc.ac.uk

AUTHOR OF BACKGROUND PAPER ON
Impact of GM plants on non-target arthropod fauna

Dr Palarp Sinhaseni
Deputy Director, Research Affairs
Institute of Health Research
Chulalongkorn University
Soi Chulalongkorn 62
Phyathai RD, Pathumwan
10330 Bangkok
Thailand

Phone: +66 2 21881523
Fax: +66 2 2532395
E-mail: spalarp@chula.ac.th

Dr Kornelia Smalla
Senior Scientist, Federal Biological Research Centre for Agriculture and Forestry
Messeweg 11/12
D-38104 Braunschweig
Germany

Phone: +49 531 2993814
Fax: +49 531 2993013
E-mail: k.smalla@bba.de

AUTHOR OF BACKGROUND PAPER ON
Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria - are there new data to fuel the debate?

Dr Claudia Sorlini
Faculty of Agricultural Science Microbiology
Department of Food and Microbiological Sciences and Technologies
University of Milan
Via G. Celoria, 2
I-20133 Milan
Italy

Phone: +39 02 23955822
Fax: +39 02 70630829
E-mail: claudia.sorlini@unimi.it

AUTHOR OF BACKGROUND PAPER ON
Transgene fate in the gastro-intestinal tract and in the environment

---

Dr Guenther Stotzky
New York University
Department of Biology
752 Brown Building
NY 10003 New York
USA

Phone: +1 212 9988268
Fax: +1 212 9954015
E-mail: gs5@is2.nyu.edu

AUTHOR OF BACKGROUND PAPER ON
Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis

---

Dr Jennifer Ann Thomson
Head, Department of Microbiology
University of Cape Town
Private Bag Rondebosch 7701
Cape Town
South Africa

Phone: +27 21 6503269/70
Fax: +27 21 6897573
E-mail: jat@molbiol.uct.ac.za

Dr Terje Traavik
University of Tromso
Norwegian Institute of Gene Ecology
Mh-Breivika
N-9037 Tromso
Norway

Phone: +47 776 44643
Fax: +47 776 45350
E-mail: terjet@fagmed.unit.no

INVITED BUT UNABLE TO ATTEND

Dr Jan van Aken
Greenpeace
Chausseestrasse 131
D-10115 Berlin
Germany

Phone: +49 30 30889914
Fax: +49 30 30889930
E-mail: van.aken@t-online.de

Mr Guy Van den Eede
Sector Head, European Commission
Joint Research Centre
TP 361
I-21020 Ispra (VA)
Italy

Phone: +39 0332 785239
Fax: +39 0332 785483
E-mail: guy.van-den-eede@jrc.it

AUTHOR OF BACKGROUND PAPER ON
Current experiences with environmental risk assessment

REPRESENTATIVES OF INTERNATIONAL ORGANIZATIONS

Dr Giovanni Ferraiolo
Programme Officer, International Centre for Genetic Engineering and Biotechnology (ICGEB)
Area Science Park
Padriciano 99
I-34012 Trieste
Italy

Phone: +39 040 3757364
Fax: +39 040 226555
E-mail: biosafe@icgeb.trieste.it

AUTHOR OF ROOM DOCUMENT ON
Biosafety and the ICGEB

Dr Hiremagalur N.B Gopalan
Task Manager (Environmental Health)
Policy Analysis, Review and Development Unit
Division of Environmental Policy Development and Law
United Nations Environment Programme (UNEP)
P.O Box 30552
Nairobi
Kenya

Phone: +254 2 623246
Fax: +254 2 623861
E-mail: hiremagalur.gopalan@unep.org

AUTHOR OF THE ROOM DOCUMENT ON
UNEP activity report

Dr Peter Kearns
Organization for Economic Co-operation and Development (OECD)
2, Rue André Pascal
F-75775 Paris Cedex 16
France

Phone: +33 1 45241677
Fax: +33 1 45241675
E-mail: peter.kearns@oecd.org

INVITED BUT UNABLE TO ATTEND

Dr Yasuyuki Sahara
Sustainable Development and Healthy Environments
Protection of the Human Environment
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland

Phone: +41 22 7914324/3557
Fax: +41 22 7914807
E-mail: saharay@who.ch

AUTHOR OF THE ROOM DOCUMENT ON
WHO and Biotechnology in Food Safety

Mr Makoto Tabata
Food Standards Officer, Joint FAO/WHO Food Standards Programme
Food and Nutrition Division
Economic and Social Department
Food and Agriculture Organization
Via delle Terme di Caracalla
I-00153 Rome
Italy

Phone: +39 06 57054796
Fax: +39 06 57053383
E-mail: makoto.tabata@fao.org

Dr George T. Tzotzos
Chief, Biodiversity Unit, Cleaner Production and Environmental Management Branch
United Nations Industrial Development Organization (UNIDO)
P.O. Box 300
A-1400 Vienna
Austria

Phone: +43 1 26026 ext. 4336
Fax: +43 1 260266810
E-mail: george@binas.unido.org

INVITED BUT UNABLE TO ATTEND

Ms Maria Zimmermann
Senior Agricultural Research Officer, Research and Technology Development Service
Research, Extension and Training Division
Food and Agriculture Organization
Via delle Terme di Caracalla
I-00153 Rome
Italy

Phone: +39 06 57055499
Fax: +39 06 57055731
E-mail: maria.zimmermann@fao.org

AUTHOR OF ROOM DOCUMENT ON
Biosafety issues related to biotechnologies for sustainable agriculture and food security

OBSERVERS

Dr Carlos Dora
Regional Adviser, Environmental Epidemiology
WHO European Centre for Environment and Health, Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877541
Fax: +39 06 4877599
E-mail: cdo@who.it

Dr Valeria Giovannelli
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 50072273
Fax: +39 06 50072048
E-mail: valeggg@tin.it

Dr Bettina Menne
Short-Term Professional, Global Change and Health
WHO European Centre for Environment and Health, Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877546
Fax: +39 06 4877599
E-mail: bme@who.it

Dr Giovanni Staiano
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 50072519
Fax: +39 06 50072044
E-mail: staiano@anpa.it

SECRETARIAT

Dr Roberto Bertollini
Director, WHO European Centre for Environment and Health, Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877530
Fax: +39 06 4877599
E-mail: rbe@who.it

Dr Sonia Cantoni
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 50072155
Fax: +39 06 50072048
E-mail: cantoni@anpa.it

Dr Anna Corrado
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 46832808
Fax: +39 06 50072048
E-mail: zacap@tiscalinet.it

Dr Nicoletta Di Tanno
Data Processing Assistant, WHO European Centre for Environment and Health, Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877523
Fax: +39 06 4877599
E-mail: ndt@who.it

Dr Walter Ganapini
President
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 50072043
Fax: +39 06 50072219
E-mail: ganapini@anpa.it

DR GANAPINI WAS REPRESENTED BY DR G. ONUFRI

Dr Susanna Greco
Italian Environment Protection Agency (ANPA)
Via Vitaliano Brancati, 48
I-00144 Rome
Italy

Phone: +39 06 8841360
Fax: +39 06 8841360
E-mail: md3053@mclink.it

Dr Marco Jermini
Regional Adviser, Food Safety Programme
WHO European Centre for Environment and Health
Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877525
Fax: +39 06 4877599
E-mail: maj@who.it

Dr Paola Picotto
Department of Food and Nutrition and
Veterinary Public Health
Ministry of Health
Piazza Marconi, 25
I-00144 Rome
Italy

Phone: +39 06 59943752
Fax: +39 06 59943676
E-mail: p.picotto@sanita.it

Dr Biancamaria Pietrangeli
National Institute for Occupational Safety
and Prevention (ISPESL)
Environmental Department
Via Urbana, 167
I-00184 Rome
Italy

Phone: +39 06 4714260
Fax: +39 06 4744017
E-mail: dipiadir@tin.it

Ms Manuela Zingales
Programme Assistant, Food Safety Programme
WHO European Centre for Environment and Health
Rome Division
Via Francesco Crispi, 10
I-00187 Rome
Italy

Phone: +39 06 4877535
Fax: +39 06 4877599
E-mail: mzi@who.it

ANNEX 2: LIST OF BACKGROUND PAPERS

5021704/08 Othmar Kaeppeli
"The fundamentals of science-based environmental risk assessment of GMOs"

5021704/09 Guy Van den Eede
"Current experiences with environmental risk assessment"

5021704/10 Francesco Sala
"Safety considerations when planning, constructing and developing GM plants"

5021704/11 Kornelia Smalla
"Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria - are there new data to fuel the debate?"

5021704/12 Alison Power
"Environmental risks of crops with transgenic virus-resistance"

5021704/13 Terje Traavik
"Health hazards of naked DNA"

5021704/14 Claudia Sorlini
"Transgene fate in the gastro-intestinal tract and in the environment"

5021704/15 Joaquim Machado
"Inter/intra species gene transfer from GM plants to other plants"

5021704/16 Guenther Stotzky
"Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis"

5021704/17 David Andow
"Monitoring for early detection of resistance"

5021704/18 Tanja Schuler
"Impact of GM plants on non-target arthropod fauna"

5021704/19 Angelika Hilbeck
"Review on non-target organisms and bt-plants"

5021704/20 Michael Joffe
"Health Impact Assessment (HIA)"

ANNEX 3: LIST OF ROOM DOCUMENTS

5021704/22	Maria Zimmermann, FAO **"Biosafety Issues Related to Biotechnologies for Sustainable Agriculture and Food Security"**
5021704/23	Giovanni Ferraiolo, ICGEB **"Biosafety and the ICGEB"**
5021704/24	Yasuyuki Sahara, WHO/HQ **"Report of the 1st Joint FAO/WHO Consultation on Food derived from Biotechnology, WHO Headquarters, Geneva, Switzerland, 29 May–2 June 2000"**
5021704/25	Yasuyuki Sahara, WHO/HQ **"WHO and Biotechnology in Food Safety"**
5021704/26	Hiremagalur N.B. Gopalan, UNEP **"UNEP activity report"**
5021704/27	Hiremagalur N.B. Gopalan, UNEP **"Capacity Building in Developing countries and countries with economies in transition to facilitate the implementation of the Cartagena Protocol on Biosafety**"
5021704/28	Peter Kearns, OECD **"Safety in Biotechnology News**" **Inter-Agency Network for Safety in Biotechnology (IANB)**
5021704/29	Peter Kearns, OECD **"Biotechnology Update**" **Internal Co-ordination Group for Biotechnology (ICGB)**
5021704/30	Nancy Schellhorn, CSIRO Australia **"Ecological implications of GMO**"

ANNEX 4: CONCLUSIONS AND RECOMMENDATIONS OF THE FIRST JOINT FAO/WHO CONSULTATION ON FOODS DERIVED FROM BIOTECHNOLOGY. GENEVA, SWITZERLAND, MAY-JUNE 2000

Conclusions:

1. The Consultation agreed that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods.

2. The Consultation was of the view that there were presently no alternative strategies that would provide a better assurance of safety for genetically modified foods than the appropriate use of the concept of substantial equivalence. Nevertheless, it was agreed that some aspects of the steps in safety assessment process could be refined to keep abreast of developments in genetic modification technology. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterize hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework. The Consultation was satisfied with the approach used to assess the safety of the genetically modified foods that have been approved for commercial use.

3. The Consultation further agreed that the safety assessment of genetically modified foods requires methods to detect and evaluate the impact of unintended effects, such as the acquisition of new traits or loss of existing traits. The potential occurrence of unintended effects is not unique to the application of recombinant DNA techniques, but is also a general phenomenon in conventional breeding. Present approaches to detect unintended effects are based, in part, on the analysis of specific components (targeted approach). In order to increase the probability of detecting unintended effects, profiling techniques are considered as potentially useful alternatives (non-targeted approach). In order to assess the biological and safety relevance of an unintended effect, the genetically modified plant should first be compared to other conventional varieties and data on it compared to literature data. If the differences exceed natural variations, a nutritional and toxicological assessment is required. This may require an evaluation of specific components of the genetically modified food or of the whole food.

4. The Consultation considered the issue of long term effects from the consumption of genetically modified foods and noted that very little is known about the potential long term effects of any foods. In many cases, this is further confounded by wide genetic variability in the population, such that some individuals may have a greater predisposition to food-related effects. In this context, the Consultation acknowledged that for genetically modified foods, the pre-marketing safety assessment already gives assurance that the food is as safe as its conventional counterpart. Accordingly it was considered that the possibility of long term effects being specifically attributable to genetically modified foods would be highly unlikely. Furthermore, it was recognised that observational epidemiological studies would be unlikely to identify any

such effects against a background of undesirable effects of conventional foods. Experimental studies, such as randomised controlled trials (RCTs), if properly designed and conducted, could be used to investigate the medium/long term effects of any foods, including genetically modified foods. Such studies could provide additional evidence for human safety, but would be difficult to conduct. In this respect, it is also important to recognise the wide variation in diets and dietary components from day to day and year to year.

5. The Consultation recognized that genetically modified foods with intentional nutritional effects may provide improved products for developed and developing countries. The change in nutrient levels in a particular crop plant may impact on overall dietary intake. In such cases, it is important to determine alterations in nutrient content and bioavailability, and their stability with time, processing and storage, as well as to monitor changes in dietary patterns as a result of the introduction of the genetically modified food and evaluate its potential effect on nutritional and health status of consumers. However, an assessment of the impact on nutritional status of consumers is important for all significant dietary changes and not specific to the introduction of genetically modified foods.

6. The Consultation agreed that if a genetically modified food contains the product of a gene from a source with known allergenic effects, the gene product should be assumed to be allergenic unless proven otherwise. The transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the gene transferred does not code for an allergen. The novel proteins introduced into genetically modified foods should be evaluated for allergenicity on the basis of the decision-tree shown in Figure 1. Additional criteria should be considered for the addition to the decision-tree approach when the source of the genetic material is not known to be allergenic. The level and site of expression of the novel protein and the functional properties of the novel protein would be two such criteria.

7. The Consultation considered horizontal gene transfer from plants and plant products consumed as food to gut microorganisms or human cells as a rare possibility, but noted that it cannot be completely discounted. The most important consideration with respect to horizontal gene transfer is the consequence of a gene being transferred and expressed in transformed cells. An important example is the transfer of antimicrobial resistance genes, if it were to occur, from genetically modified foods to gut microorganisms. Important considerations for the assessment of the consequences of the transfer and expression of this gene in transformed cells would be the clinical and veterinary importance of the antibiotic in question, the levels of natural resistance and the availability of effective alternative therapies. In case of genes that confer resistance to drugs important for medical use, the possibility of transfer and expression of these genes is a risk that warrants their avoidance in the genome of widely disseminated genetically modified plants. The Consultation further noted that the antibiotic resistance markers currently used in genetically modified plants have been previously reviewed for safety. It concluded that there is no evidence that the markers currently in use pose a health risk to humans or domestic animals.

Recommendations:

1. While the limitation of animal study methodology when used on whole food has been pointed out, the Consultation was of the view that in specific cases animal testing may be useful. It is recommended that further research and standardization should be initiated in this area.

2. The detection methods for unintended effects based on the analysis of specific components could be supplemented with alternative strategies, such as profiling techniques. These techniques are under development; it is recommended that these methods are further developed and validated. This will be especially important for more complex genetic modifications perhaps involving multiple between-species gene transfers.

3. It will be important to monitor changes in nutrient levels in foods from plants derived by conventional breeding and by genetic modification, and assess their effect on the nutritional status of the population. A number of future food products with specific nutritional changes will be especially relevant to the needs of developing countries, and efforts should be made to improve the dissemination of appropriate methodologies and capacity building in the developing world.

4. It is recommended that integration of nutritional and toxicological expertise needed for the evaluation of genetically modified foods be encouraged and facilitated. This will facilitate R&D in the area of genetic modification of plants and lead to an early identification of relevant safety and nutritional issues.

5. The Consultation encourages the use of alternative transformation technologies, if available and demonstrated to be safe, that do not result in antibiotic resistance genes in genetically modified foods. If further development of technology is required, additional research should be strongly encouraged.

6. It is recommended that consensus documents are developed to facilitate uniform application of the concept of substantial equivalence. These should include guidelines for appropriate design of field trials and the use of appropriate statistical methods to generate and analyse comparative data on genetically modified plants and their conventional counterparts.

7. Communication of the principles involved in the safety assessment of genetically modified foods should be improved. The Consultation concluded that the key message to be conveyed is that substantial equivalence is a concept used to identify similarities and differences between the genetically modified food and a comparator with a history of safe food use which in turn guides the safety assessment process.

8. The Consultation identified the following as the additional issues to be addressed in future FAO and WHO Consultations.
   - Safety assessment specific to genetically modified micro-organisms
   - Safety assessment specific to genetically modified animals (including fish)
   - Safety assessment of functional food, including the nutritional aspects of the genetically modified foods
   - Improved methodologies for the safety study of whole foods.

ANNEX 5: GLOSSARY[2]

- ***Hazard***: A biological, chemical or physical agent, or condition, with the potential to cause an adverse health or environmental effect
- ***Risk***: A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s)
- ***Risk analysis***: A process consisting of three components: risk assessment, risk management and risk communication
- ***Risk assessment***: A scientific based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization
- ***Hazard identification***: The identification of biological, chemical and physical agents capable of causing adverse health or environmental effects
- ***Hazard characterization***: The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents. For chemical agents, a dose-response assessment should be performed if the data are obtainable
- ***Dose-response assessment***: The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response)
- ***Exposure assessment***: The qualitative and/or quantitative evaluation of the likely exposure to biological, chemical and physical agents via different sources.
- ***Risk characterization***: The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment
- ***Risk management***: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of population and for the promotion of fair practices, and if needed, selecting appropriate prevention and control options
- ***Risk communication***: The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, population, industry, the academic community and other parties, including the explanation of risk assessment findings and the basis of risk management decisions.

[2] These definitions are proposed on an *ad interim* basis: they are subject to modification in the light of developments in the science of risk analysis and as a result of efforts to harmonize similar definitions across various disciplines