The Deliberate Release of Transgenic Plants - Biology, Hazards and Safety

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This report is a collection of articles published by the Agency BATS in scientific journals, on the safety issues pertaining to the deliberate release of transgenic plants.

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Scope of the Report

The safety assessment of transgenic plants is a challenging and fascinating intersection of many disciplines of study, such as agronomy, molecular biology and ecology. Authorizations for the release of transgenic plants are granted after the examination of scientific evidence about the nature and likelihood of potential hazards. Most of the transgenic organisms being introduced into the environment are modified forms of familiar organisms with a long history of safe use, such as crop plants. However, the permanence of certain types of genetic information that encode novel traits in released organisms might lead to harm, if this information is transferred and expressed across geographical boundaries or taxonomic classes. Assessing the safety of releases is an activity of fundamental importance for the protection of environmental and human health; it is worthwhile to appreciate the broader background against which safety assessments are being performed.

This report is a collection of three papers that have been previously published in scientific journals. The first paper (Part I) explains in what ways the risk assessment for the planned releases of transgenic organisms is distinct from the classical risk assessment for hazardous processes involving toxic compounds, radioisotopes or pathogenic organisms that have an intrinsic hazard. In the second paper (Part II), a flow chart for the comprehensive safety assessment of deliberate releases is proposed, based on impact analysis, hazard and damage scenarios, and a final safety appraisal. The last paper (Part III) compares the safety of plant genomic alteration using recombinant DNA technology with older methods such as cross breeding, mutation breeding or somaclonal variation. Independent of the method used, the process of plant genomic alteration produces a range of phenotypes (primary or secondary) from which the breeder then selects for progeny that display the proper agronomic effect and that are safe. Experience gained from traditional plant breeding, selection technology, and knowledge about natural rates of DNA variation can be combined to define the safety baseline for assessing transgenic plants

PREFACE

The Safety of Genetically Modified Organisms

Over the past few decades, our increasing understanding of gene function and regulation at the molecular level has resulted in the explosion of the genetic technologies. Gene splicing techniques enable scientists to insert specific traits into useful crop plants such that desirable plant phenotypes can be obtained in less time and space compared with traditional breeding methods. While plant genomic alteration is not new, the genetic engineering approach distinguishes itself from older methods of breeding (e.g. cross breeding, induced mutagenesis, plant cell culture) by proposing novel plant traits from virtually any source. The problem of species barriers and sexual compatibility of plants can now be circumvented as genetic information encoding bacterial toxins, antibiotic resistance, or herbicide tolerance is introduced into the host plant genome using various techniques of DNA transfer.

Safety before the release of genetically modified organisms

Before field releases of genetically modified organisms can take place, the instigators of the release must receive the approval of the competent authorities in the country of release. Because the organism cannot be recalled once released, its safety must be demonstrated prior to release. Until here, the precautionary approach is harmonious and the logic infallible. However, the risk emphases of safety assessments for deliberate releases, as well as the definition of acceptability, can differ considerably from country to country - even within the European Community. In its original intention, the directive 90/EEC/220, regulating deliberate releases, was drafted to be broad enough to accommodate differences in values and emphases of the various member nations while ensuring the proper protection of human health and the environment. In practice, the open-ended nature of the directive has proven problematic for achieving consensus during the approval process of market-products. Each country has its own information requirements for the risk assessment as well as its own definitions of acceptability of release. For the French, the safety of release is determined based on the familiarity and knowledge of the genetic construct in the modified organism. This contrasts with countries like Denmark and Sweden which require ecological data in their risk assessment[1].

What are the risks?

Scientific judgment supports the premise that genetically modified organisms do not differ fundamentally from their unmodified counterparts, if properties were the sole basis of contention. Most genetic transfers are confined to one or two genes and result in organisms similar to those created by other methods of genetic alteration used in the past. Unlike the pathogenic organisms used in some contained, industrial settings, genetically modified organisms are the transformed version of familiar organisms classed in the ‘no risk’ biological hazard class or generally recognized as safe (GRAS). This lack of a direct relationship between an intrinsic property of the modified organism and its potentially harmful consequences is the key to understanding why approaches to risk assessments might vary. What is being assessed, in reality, is the possible future fate of the genetic information carried by the organism. Natural processes such as pollen flow or horizontal gene transfer might serve as vehicles for the dispersal of unwanted genetic information into the environment. It is hypothesized, for example, that soil bacteria might acquire the antibiotic resistance information of the marker gene in transgenic plants and that this information might be taken over by pathogens causing human disease. One plausible outcome scenario would be the erosion of antibiotic potency in health care. In the event that transgenic crops are planted on a large scale, the cumulative effect of an improbable event might still need to be considered.

Probability and damage potential

The probability of unlikely events and the damage that they might incur to the environment are usually discussed during the safety assessment. However, it can be perilous to emphasize the probability aspect of hazards at a detriment to being up-front about the types of damage that they can incur. Most of the risks cited for transgenic organisms are associated with low likelihoods that are all too easily manipulated by highly politicized groups. On the other hand, low probability numbers may also trouble those involved with the regulation of transgenics because the

numbers do not speak for themselves. Unlike the regulation of the chemical industries that relies on the calculated probability for a certain number of injuries or deaths per year of plant operation, there is no common currency for expressing the types of environmental damage potentially caused by the release of modified organisms.

Transplastomic plants: how much safer?

There has recently been a lot of excitement over the success at engineering herbicide resistance in the tobacco chloroplast genome[2]. This triumph was hailed as 'a rare piece of good news' for the promotion of genetically modified crops because it offers a tangible resolution to the problem of transgene dispersal through pollen flow in the environment[3]. Transgenes introduced into the chloroplast, unlike nuclear transformed plants, are not found in the pollen of the plant because chloroplasts are inherited maternally. What will be the real-life implications for the risk assessment of transplastomic plants? While chloroplast transformation can effectively lower the probability that transgenes might escape via pollen flow, it is still not an absolute guarantee against the dispersal of unwanted traits to the environment. In some plants, chloroplasts are inherited paternally and therefore present in pollen. Moreover, the emergence of volunteer or feral populations of transgenic crops is still possible. Can the use of transplastomic plants rescue the risk assessment from the characteristic stalemates encountered in describing rare events?

Instead of discussing even lower probabilities for gene flow from transgenic plants, it might be more useful and realistic to devote precious financial and human resources to the elaboration of putative consequences of rare events and the criteria for their acceptability. The nuances involved in speaking about low probability events will be critical when the inserted traits confer a distinct selective advantage to an organism, e.g. drought resistance in plants. Whether a plant is chloroplast- or nuclear-transformed, an honest analysis of the consequences of rare events would help to steer the risk assessment and public debates in the right direction.

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Planned Releases of Genetically Engineered Organisms into the Environment: the Evolution of Safety Consideration

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Abstract

Issues of safety and risk have taken the foreground in discussions on the deliberate release of genetically modified organisms. In most cases, the organisms being introduced into the environment are modified versions of familiar organisms with a long history of safe use and are expected to have no direct adverse effects for human health or for the environment. However, there is legitimate concern about the environmental fate of the these organisms, in particular, about the genetic information which they carry. In the past, discussions of technological risk have often been based on the terminology and logic of the familiar risk-assessment strategy developed for characterizing risks from hazardous chemical processes. While the direct transfer of this assessment model to evaluating contained biotechnological processes has been successful, attempts at molding the model to the requirements of open systems have been unsatisfactory. To be meaningful, the safety evaluation for environmental releases must accommodate the distinguishing features of this open system: the lack of an intrinsic hazardous property, the lack of quantitative thresholds for adverse effects, and the lack of a common currency in which to express potential damages. A survey of risk assessment strategies in the chemical and biotechnological sectors is presented here. This will provide the necessary background to understanding the current situation of assessing and communicating the risks associated with the re-introduction of familiar organisms into environments where they were already naturally present.

Introduction

Biotechnology is the term given to processes which make use of biology to improve human material welfare. Products of biotechnology range from foodstuffs, such as cheese and alcoholic beverages, to drugs for the protection of human health, such as antibiotics, interleukins, interferons, or vaccines. Breeding is one of the oldest forms of biotechnology practiced by farmers to select for desired traits in plants and animals. Although traditional biotechnology has a long history (10,000 years) of safe application, this track record is apparently not enough to validate the applications of ‘modern’ biotechnology which proposes the modification of organisms through the modification of gene signals or through the transfer of genetic information encoding specific characters.

The ambitions of genetic engineering appear to be far greater and much quicker to achieve than what was previously possible with traditional biotechnology, due to the accuracy and ease of application of well-honed molecular biological methods. The rapidity of pace, the fear of future harm, and the ethical issues on the nature of life itself, all contribute to the current unease regarding the widespread application of genetic engineering. With modified organisms targeted for release, there is also concern about their long-term impact on ecosystem processes. While all concerns are legitimate, they may be shaped by the culture of the individual or they may reflect a state of incomplete knowledge about a given situation. The positive and negative consequences of technology are inextricably bound together, thus posing a challenge to regulators to move beyond the paradox, so that mandatory rules are drafted to be commensurate with the actual technological risks.

Issues of risk and safety in biotechnology have taken the foreground in discussions on the deliberate release of genetically modified organisms (GMO). As a first step, the logic of the familiar risk assessment model for chemical processes was adopted to describe the situation of deliberate releases; after all, the methodology had been

successfully applied to evaluate contained biotechnological applications for safety. This approach soon proved to be unsatisfactory for several reasons. Hazardous chemical processes and hazardous contained biotechnology applications satisfy the underlying condition for quantitative risk assessments: both have intrinsic hazards which can be identified, characterized and described either quantitatively or qualitatively. The toxicity of chemical substances or the pathogenicity of production organisms are properties which can be directly correlated with specific hazards. On the other hand, organisms targeted for releases usually have no known direct adverse effects for human health and the environment. However, the materialization of hazard can be affected by the scale of release, the potential for organisms to proliferate beyond geographical boundaries, and the potential for the inserted genetic information (e.g. antibiotic resistance) to cross taxonomic classes.

The types of damage potential often forecasted in the worst case scenarios for deliberate releases are usually not new, but have already occurred as a result of more traditional activities of agriculture. There is legitimate fear, for example, that the presence of antibiotic marker genes in modified crop plants might exert selective pressure on pathogenic organisms to become resistant to antibiotics, thereby reducing the efficacy and usefulness for the clinical treatment of infections using this class of drugs. This concern should also extend to other sources for the environmental presence of antibiotics, such as the use of antibiotics in animal feed for prophylaxis, chemotherapy and growth promotion. It is difficult to express the damage potential for deliberate releases in terms of a common currency, especially when naturally occurring background processes such as pollen flow, gene transfer and gene acquisition are the vehicles for damage in hazard scenarios.

It is the task of the environmental risk assessment to sift the facts about risks from the perceptions about risks. Ideally, this assessment should be as objective and scientifically-based as possible, but maintaining the transparency and comprehensiveness necessary to encourage effective communication about risks. ***The purpose of this paper is to provide some background on the logic, pattern and evolution of the chemical risk assessment model: how it has been effectively applied to evaluating the safety of contained biotechnology applications; and how the situation of open systems might require another approach for evaluating potential hazards.***
With this background, it might become easier to understand the current predicament of assessing and communicating the risks associated with the re-introduction of modified, familiar organisms into environments where they were already naturally present.

Risk Assessment for Chemical Processes

The dissemination of known hazardous substances is legally regulated in order to avoid untoward exposure and adverse effects to people and to the environment. Good industry safety practices rely on the systematic use of risk assessment schemes to identify, assess and control the risks from hazards. Although risk is never zero, it can be made very small through specific control actions at each stage of hazard evolution over time by modifying wants, changing the technology and preventing initiating events. In its formal structure, the risk assessment scheme for chemical processes focuses primarily on the management of risk through the identification and prevention of initiating events. What follows will be a brief

introduction to the logic and arrangement of tools used to arrive at an unambiguous characterization of risk for chemical processes. The risk assessment strategies which have been proposed for biotechnological applications, contained or open, owe a lot to the concepts expounded originally for debating the risks from hazardous substances.

Endorsed models for the quantitative risk assessment of chemical processes contain the stages shown in Figure 1. The first stage is the system description and provides details on the process: its background, objectives, and material requirements. Once this has been established, the identification of all hazards relevant to the process operation can be performed by answering the two questions: (1) what dangerous situations exist within a plant or a process operation; and (2) how these situations may materialize. All situations in which the potential for harm might exist must be considered, including the sequence of events which could transform this potential into an accident. A number of hazard identification techniques, including scenario analysis, checklists, Hazard Operability Studies (HAZOP) can be used to ensure the comprehensiveness and level of detail required[4]. Categories of danger range from plant accidents resulting in serious injury and death such as explosions, fires, or the dispersal of chemicals to the less visible, but potentially harmful effects, from long-term, low-dose exposure during normal operation.

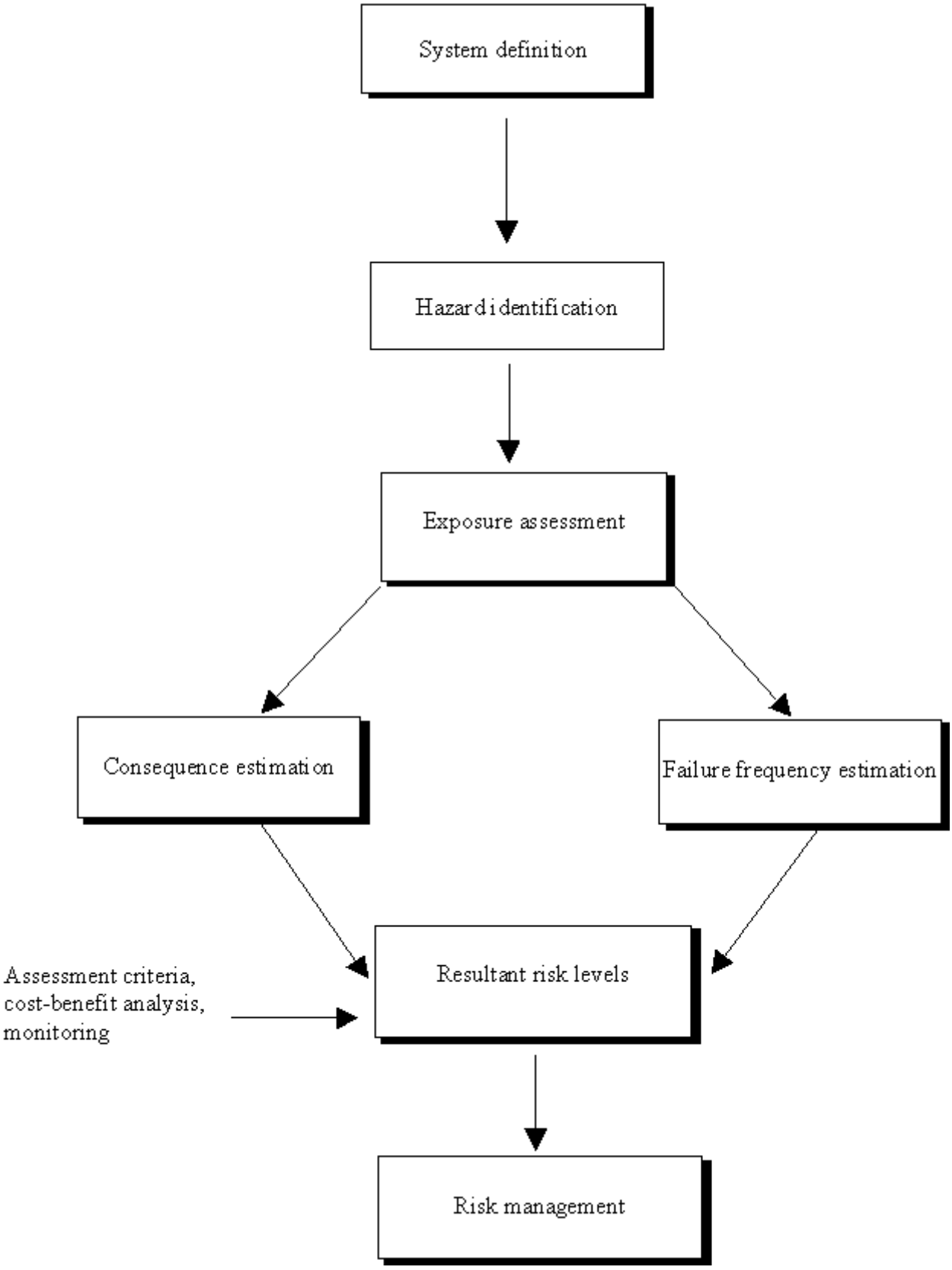

Fig. 1. Riskassessment model for chemical process

In the next stages of the risk assessment, conditional probabilities of harm are calculated based on the maximum amplitude of damage and the probability of

occurrence. Consequence estimation uses two types of models to assess the effects on man, animals and the environment from exposure to identified hazards: (1) physical models are used to evaluate the effects from the production of overpressure during an explosion, the dispersion of airborne flammable or toxic materials, the creation of high levels of thermal radiation from various types of fires; and (2) toxic effect models are used to assess the adverse health effects to man from exposure to toxic substances. These models have their weaknesses and strengths, but it is enough to mention here that the intrinsic properties of chemical substances, i.e. flammability, toxicity or thermodynamic properties, can evolve into hazards. The degree to which the processing system can prevent dangerous situations is given by the overall failure frequency rate, calculated from failure data for individual components or, when available, for similar processes.

The outcome from the probabilistic calculations of the preceding stages of the risk assessment is then compared to the official risk criteria which provide the limits of death or injury from known industrial hazards. Depending on the result of this comparison, the overall risk associated with operating a chemical process may be broadly acceptable, conditionally acceptable or intolerable. If the risk is judged unreasonable, specific actions taken during risk management may succeed in reducing the risk to 'acceptable' levels.

		Biological Systems	
	Chemical Compounds	**Contained Applications**	**Deliberate Release**
Hazard	Flammability Toxicity: Impacts on human health and the environment	Pathogenicity, environmental impacts	Not apparent: Potential environmental impacts and/or food toxicity
Exposure Assessment	Quantitative data and models available for the dispersion and behavior of the chemical substance in different organisms and environments	Based on considerations of infection dose, host range, transmission mode, dispersion mode and dynamics	No predictable genotype-phenotype relationship; the relevance of scale to potential hazard not known
Consequence Estimation	Adverse effects related to toxicity Quantitative indicators of loss (deaths per year)	Adverse effects related to the organism pathogenic properties (e.g. mortality, morbidity); potential spread and persistence in the environment	Endpoints for adverse health effects or 'environmental damage' given by risk acceptance and tolerability levels
Failure Frequency Estimation	Quantitative probabilistic calculations Incremental safety gain for increased cost	Given by the safety analysis of the technical system Incremental safety gain for increased cost	**Not applicable: No containment (probability of occurrence given by natural processes)**
Monitoring	Chemical concentration	Organism concentration, epidemiology	Effect on ecosystems, biodiversity

Risk Management	Technical measures, good maintenance practice, organizational measures	Technical measures, good maintenance practice, organizational measures	**Not applicable: Expert and societal debate needed to decide on the risk acceptance levels**

Table 1. Risk-Assessment Stages for Chemical Compounds, Contained and Open Biological Applications

Contained Biological Processes

Contained biotechnological applications operate under sterile conditions, which means that contact of the culture fluid at any stage of the process with the surrounding environment is restricted. At the industrial scale, process components consisting of carefully sealed pumps, vessels, and pipes resemble the equipment for chemical processes[1]. Analogous also to chemical conversions, intrinsic hazards of two types can be identified for closed systems in biotechnology: technical hazards related to the process maintenance and operations, such as high pressure steam used during sterilization or toxic solvents used during downstream processing; and biological hazards from the pathogenic properties of the production organism. The risk assessment model for chemical processes is adaptable to the assessment of closed biotechnological applications, because the underlying assumption is verified that apparent hazards exist, that they can be identified and characterized with probabilistic calculations (see Table 1). The remainder of this section will focus mainly on the risk assessment of hazards arising from the biological system. In contrast to the high temperatures and pressures characteristic of industrial chemical conversions, the technical hazards from biological processes are comparatively mild because of the physiological conditions required for bioconversions.

According to internationally recognized guidelines, organisms are classified into four hazard classes, based on their pathogenic properties and their impacts on the environment (Table 2). Class 1 organisms are harmless, while Class 4 organisms represent a high risk to human health. Organisms in Class 2 and 3 are of minor and moderate risk, respectively. An example of Class 2 organisms are bacteria of the genus Salmonella, some of which cause typhoid fever and food poisoning. These hazard classes also delineate the stringency of containment measures, expressed as the corresponding level of safety at which the production facility must operate (Figure 2). Beginning at Safety Level 2, the facility must include in its design the means to inactivate the production organism at the interface of the containment with the environment.

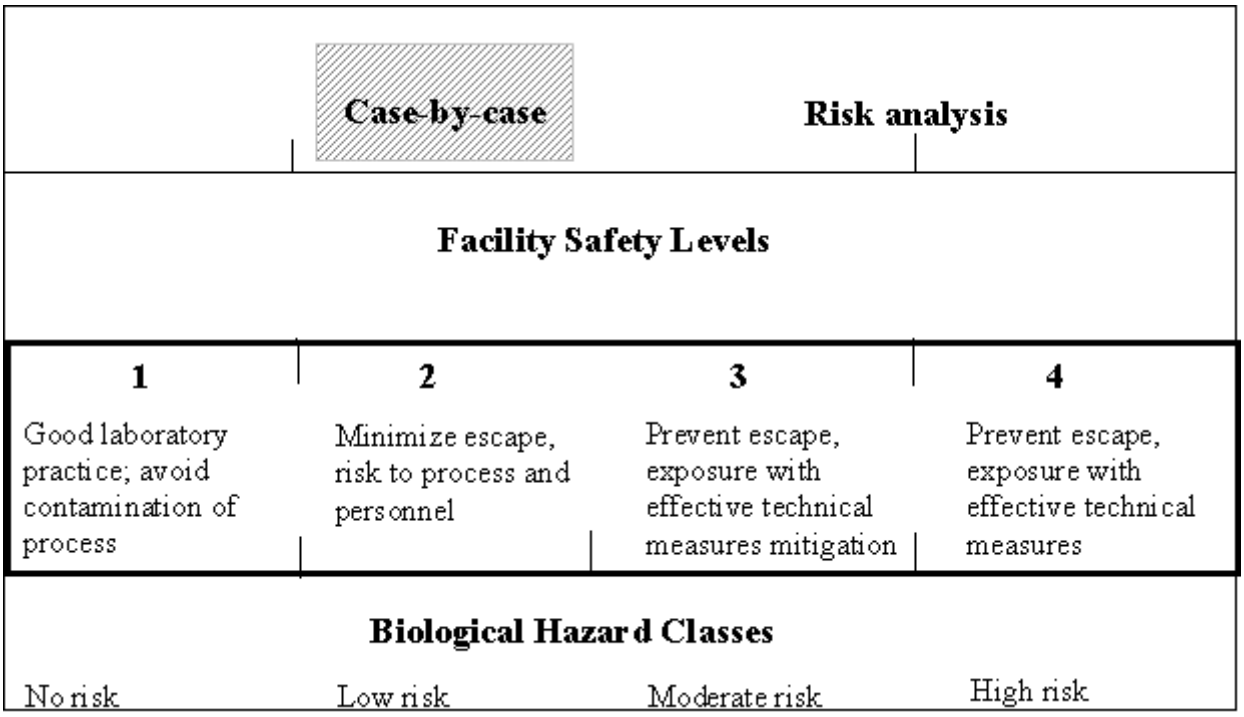

Fig. 2. Hazard categories of the biological system and the corresponding facility safety measures

Biological risk assessments for contained applications are not strictly required for Class 1 organisms, are formally carried out on a case-by-case basis for Class 2 organism and are consistently applied for Class 3 and 4 organisms[1]. After the hazard class of the organism has been determined, the first activity of the risk assessment is to identify the suite of causal events belonging to plausible scenarios which describe the various routes of accidental release. These scenarios indicate the vulnerable points of the system that may become initiating events for major accidents affecting the integrity of containment, such as failure of exhaust air or waste water inactivation. As are available for chemical processes, detailed methods have also been developed for the characterization of possible incident scenarios, such as the Failure Mode Effect Analysis (FMEA) and the Event Tree Analysis.

Subsequent to the scenario elaboration is the determination of the probability that escape might occur (failure frequency rate), how the organisms might be dispersed and what the damage potential might be (exposure and consequence assessments). Airborne dispersion models may be used to predict the concentrations of organisms as a function of time since release and their position with respect to the release site. Further spread through different media such as water and soil could also be assessed based on models developed to predict the scope of contamination.

Unlike the numerical results expected of chemical process risk assessments, the risks associated with contained biological systems are given descriptively. Criteria categories exist for the qualitative characterization of risk-reduced or elevated-from exposure to hazardous organisms. These risk criteria cover the range of pathogenic properties of an organism: lethality, morbidity, transmission, contagiousness, dose of infection, availability of medication.

For the risk assessment of contained processes which use genetically modified organisms, the hazard classes are generally considered applicable. However, the new genetic information must be given due consideration based on information about: the recipient or host organism, the donor organism(s), the vector used, the inserted trait and any empirical data available on the physiology or phenotype of the modified organism. The hazard identification stage for GMOs needs to also examine the possible effects resulting from the technique used for gene insertion, such as pleiotropic or mutational effects. If the modified strain is derived from an industrial strain with a history of long-term optimization, then knowledge and experience with the unmodified strain can be used to definitively classify the modified strain.

Type of Hazard	**Impacts**
Pathogenicity	Toxicity of metabolic products
	Pathogenicity of a genetically modified organism compared to the wild-type strain
	Characterization of pathogenicity: • Type of disease caused, mechanisms, invasiveness, virulence, availability of therapies • transmission, infection mode • infection dose • host range • survival outside host • vectors for transmission • stability • antibiotic resistance
Environmental Impacts	Survival, growth/decay, dispersion
	Impacts on animals, plants, cycling of bioelements
	Impact on ecosystems
	Invasion of managed or unmanaged habitats
	Gene transfer
	Possibility for monitoring

Table 2. Hazards Considered for the Categorization of Industrial Production Organisms

The Challenge of Safety Assessments for Open Systems

The recommendations by the National Academy of Sciences to focus the safety assessment of GMOs on the product itself - and not on the process which produced it - are an attempt to acknowledge the substantial equivalence of organisms modified by recombinant DNA techniques and those modified by older methods. Scientific judgment supports the premise that modified organisms cannot be distinguished from their unmodified counterparts if properties were the sole basis of contention. It is highly unlikely, for example, that a proven non-pathogenic organism would acquire pathogenic properties, unless pathogenicity-related factors (e.g. virulence, host

range, or transmission) or toxic products were deliberately introduced. Most transfers are confined to one or two genes and result in organisms not fundamentally different from those created by other methods of genetic alterations commonly used in the past, such as induced mutagenesis. Proper expression of the introduced genes normally results in specific target effects like the overproduction of valuable metabolites, tolerance to herbicide or resistance to pests in plants, or even resistance to low temperatures for fish species used in pisciculture.

The lack of a direct relationship between an intrinsic property of the modified organism and its potentially harmful consequences limits the usefulness of the key stages of the endorsed risk assessment scheme for chemical processes, such as exposure assessment and consequence estimation (Table 1). The source of potential hazards is rarely the organism itself, but instead the environmental fate of the genetic *information* which is carried by the modified organism. During the operation of contained processes with Class 2 organisms, a low rate of escape is tolerated and taken into account during the exposure assessment. For a Class 1 GMO introduced into the environment, it is not so clear what a corresponding threshold for adverse effect would be. Potential hazards arising from the fate of the inserted genetic information in the environment may still need to be examined from the perspective of scale: as the scale of use of GMOs increase, low probability events may still occur with an observable frequency. *This implies that new risk criteria based on levels of tolerable damage and not on calculations of likelihood would have to be discussed before the implementation of any risk management strategy.*

The preceding discussion would seem to suggest that while most releases will be benign, generic arguments for the safety of all introductions must be rejected due to a lack of irrefutable evidence that no harm will occur. At the present moment, this rhetorical paradox is resolved by requiring the case-by-case environmental safety assessment for all deliberate releases. General concepts from the risk assessment model developed for chemical processes have been valuable in discussing the safety of deliberate releases, but inconsistencies are encountered if the various stages of chemical hazard analysis are directly applied. In an earlier paper, we described another approach for endorsing the safety of GMOs on a scientific basis. This methodology consists of a two-stage environmental safety evaluation adapted to the features of open systems. The first stage is the scientific safety assessment which uses scientific methods, data and models to describe the damage potential; the second stage is the risk assessment where the consideration of essential benefit *vs.* risk are debated (Fig. 3).

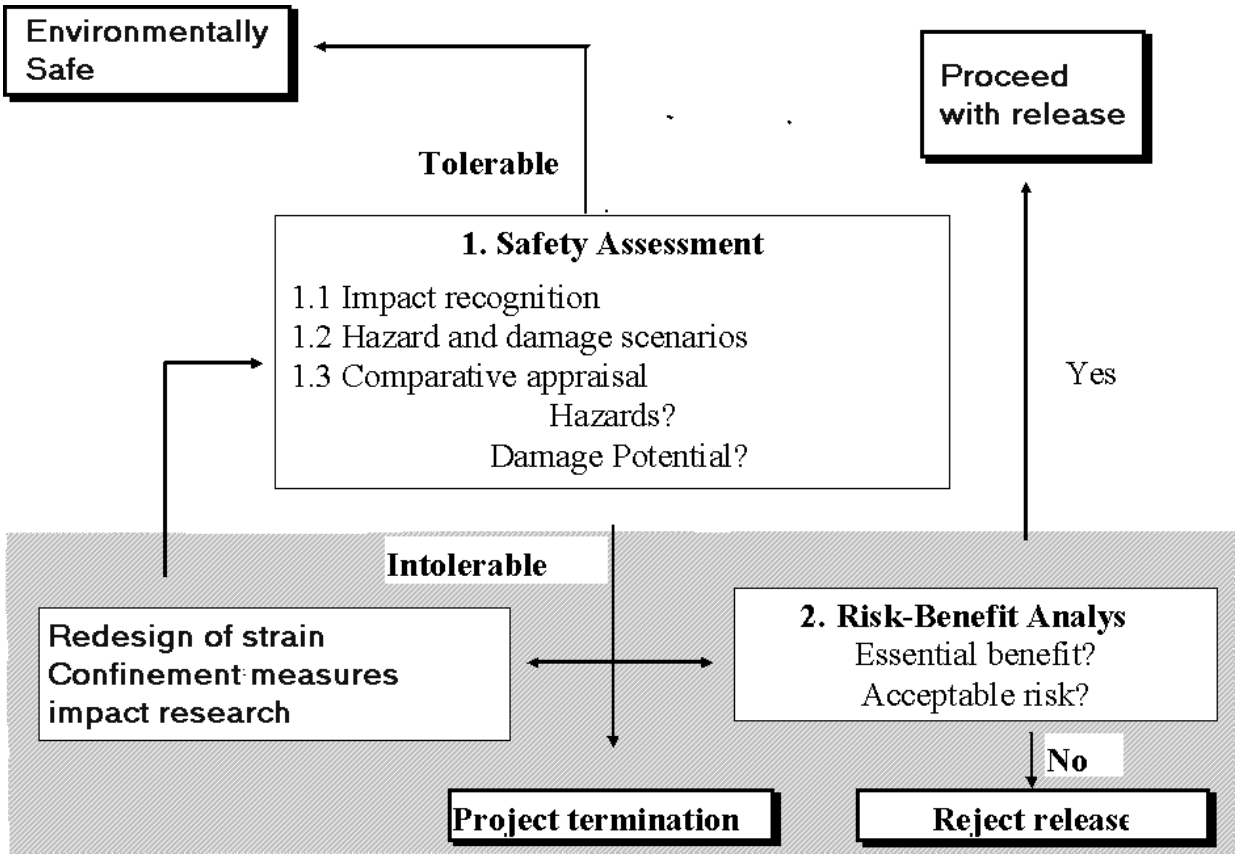

Fig. 3. The environmental safety evaluation for deliberate releases with genetically modified organisms

The Safety Assessment

Most authorizations for release have been made on the basis of safety arguments alone. The working definition of safety states that a "safe" condition or process is associated with tolerable damage or acceptable risk hazards not significantly different from background levels. During the safety assessment, scientific methods, models and data are used to obtain information and knowledge on potential hazards and on the damage consequences, if hazards were to materialize (a hypothetical probability=1). This is achieved in the three separate steps of safety assessment: (1) impact recognition; (2) hazard and damage scenario elaboration; and (3) conclusions about safety by comparison to tolerated background hazards arising from ubiquitous natural processes.

In contrast to the assessment model for chemical processes, the safety assessment for deliberate releases begins with impact recognition rather than hazard assessment (Figure 3). As mentioned earlier, there are no apparent hazards associated with the reintroduction of familiar organisms into their native environments or into agricultural systems. Thus, the potential impacts of deliberate releases are defined as the list of unwanted future outcomes related to the presence of modified organisms in the environment. Covering both the short- and long-term, impact aspects may include: increased allergenicity of crop plants hosting genes from other species; the loss of genetic diversity through large-scale planting of modified crops; and unwanted

vertical or horizontal transfer of genetic information. The ultimate endpoints for the scope of impact recognition are the body of drafted regulatory guidelines which must be fulfilled prior to approval for release.

Once the most important impact aspects of an environmental release have been identified, plausible problem scenarios are constructed to describe all possible causally or conditionally related states, events and actions which may lead to damage. A hypothetical scenario is given in Figure 4, describing one possible environmental fate for the genetic information encoded in the antibiotic-resistance marker gene present in modified food crops. During plant transformation, only a small percentage of the recipient plant cells actually take up the introduced genes, and many desirable traits are not easy to detect before the plant has fully developed. Marker genes linked to the genes for desirable traits are therefore used as selectable markers in order to distinguish the successfully transformed plant cells from the non-transformed ones.

It is well known that bacteria can exchange genetic information amongst themselves, and there is valid concern that soil or intestinal bacteria in contact with the marker gene-containing plant source might acquire antibiotic resistance then transfer this genetic information further to pathogens in the environment. Widespread resistance in pathogen populations would have drastic implications for human medicine: the efficacy of prescribed antibiotic therapies for infectious diseases will likely be limited, morbidity will likely increase and the periods during which individuals are infectious will also likely increase. With respect to this scenario, it would be worthwhile to mention that, whenever possible, the marker genes conferring antibiotic resistance to plants are preferably chosen from the library of antibiotics which are not commonly used in the clinical treatment of human diseases.

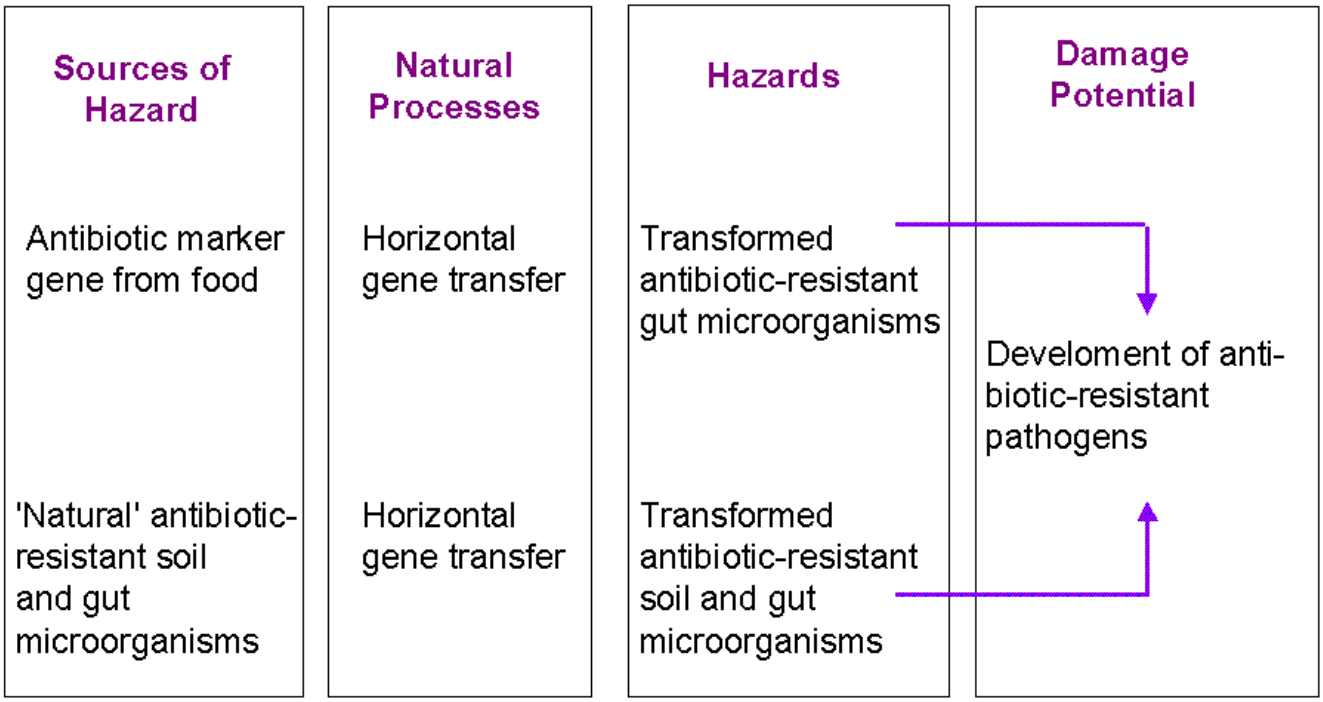

Fig. 4. Hazard and damage scenario for the horizontal gene transfer of antibiotic resistance from plants to microorganisms

Unlike the risk characterization stage of chemical process assessments where numerical figures exist as quantitative indicators of loss (*e.g.* deaths per year), there is no common currency in open systems for quantifying and, hence, characterizing the potential damage from deliberate releases. The plight of the damage appraisal might be rescued with additional information provided by retrospective scenarios which consider alternate pathways in the background, which could, through the same natural process, result in the same damage potential. In a recent article on the medical consequences of antibiotic use in agriculture, it was reported that the prophylactic use of antibiotics in animal husbandry has been a crucial driving force for the development of antibiotic resistance in certain pathogenic bacterial species. There is some evidence that the problem of antibiotic resistance in humans has been exacerbated by the prophylactic or growth-stimulating function of antibiotics in animal feed. A comparative analysis of both the animal feed pathway and the GMO pathway in Figure 4 demonstrates a similar damage potential. It can be concluded that the introduction of antibiotic resistance genes into the environment through GMOs would not be new, for this has been known and tolerated in the past. Such comparisons have great value not only in providing a basis for damage appraisal and for demonstrating likeness between "new" and "old" or the "regulated" and "tolerated", but also in attracting attention to urgent issues of hazards which exist in a dimension outside of genetic engineering. As long as no effective therapeutic alternatives to antibiotics exist, the policies on *all* forms of antibiotic usage in the environment need to reflect the importance of this class of drugs for human health care.

Risk Assessment

The analysis of risk is central to any technological debate; numerical values for risk are expressed in common units of damage in the dimension of time, based on the likelihood that a hazard will occur and the extent of damage that this will produce. For technological activities under scrutiny, ‘acceptable risk’ is defined as the unavoidable or manageable risk level associated with the intended benefits of the particular option which has been chosen.

Performing reliable risk analysis for environmental releases is a challenging task and is necessary only if the safety assessment could not provide conclusive or acceptable proof that released organisms will have no significant adverse impacts on human health and the environment (Figure 3). Until now, most decisions about GMOs have been made on the basis of the safety assessment alone. In most cases, the identification of any realistic hazard associated with an open biotechnological application was sufficient for terminating a project in its early stages, thereby avoiding any risk.

More scientific knowledge and experience beyond the current expertise would be required to ensure the accuracy of risk assessments for deliberate releases. The question of threshold for effect or scale beyond which low probability events in biology come to significance would need to be addressed by more research, which, ironically might only be possible through careful monitoring of deliberate releases. On another level, the difficulties in performing good risk assessments can be ascribed to the current predicament that common units do not exist for the potential types of damages forecasted for the environmental use of modified organisms. Risk then becomes a matter of individual convictions, held up against personal yardsticks for tolerability. Because decisions of the scope of environmental releases affect whole

societies, teams of decision-makers consisting of people with various opinions should ideally be assembled to come to some sort of consensual decision, but without straying too far from scientific rationale and evidence. Unlike the incommunicable lofty truths of human existence which vary from culture to culture and from one person to another, scientific truths can be communicated and understood by different people in the same way.

Conclusion

Promising biotechnological applications are being planned and carried out beyond the contained laboratory and production settings. It is now recognized that genetic engineering has the potential to become a valuable tool for environmental management. In most of the cases, modified strains of familiar species are being reintroduced into environments in which they were already present, but this time as optimized agents for bioremediation or for biological pest control. Other agricultural applications include the modification of crop plants to carry desirable agronomic characters difficult to achieve by traditional methods of plant breeding. Plants modified to metabolize nitrogen more efficiently could spare the environment from high fertilizer loads. The problem of excess nitrogen runoff from agriculture is a problem which has been known since the 1960s and has resulted in the eutrophication of estuaries and coastal oceans as well as lakes and rivers.

Most of the organisms planned for release are expected to have no direct adverse effect for human health or the environment, and there is scientific support that genetically modified organisms are not fundamentally different from their unmodified counterparts. However, the environmental release of genetically modified organisms is strictly regulated, and their must be demonstrated prior to release. There is legitimate concern about the long-term effects of modified organisms in the environment, and the demand for a safety assessment that can show that these hazards are not new, but have been previously tolerated in the background is justified.

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Figure Legends

Figure 1. Risk assessment model for chemical processes

Figure 2. Hazard categories of the biological system and the corresponding facility safety measures

Figure 3. The environmental safety evaluation for deliberate releases with genetically modified organisms

Figure 4. Hazard and damage scenario for the horizontal gene transfer of antibiotic resistance from plants to microorganisms

Table 1. Risk assessment stages for chemical compounds, contained and open biological applications

Table 2. Hazards considered of the biological system and the corresponding facility safety measures

How safe is safe enough in plant genetic engineering?

Use of the techniques of molecular biology in plant science has led to increased understanding of natural mechanisms involved in gene transfer, gene acquisition and genetic variability. From a human perspective, the phenotypic variability resulting from genomic plasticity in plants can either be beneficial, when there are improved agronomic features, or harmful, when there are adverse environmental or toxicological properties. The long tradition of plant breeding and selection technology has steadily improved human nutritional welfare, through successive plant genetic alterations, entailing minimal risk. This accepted background level of safety in plant

modification could be used to define the safety baseline for recombinant DNA modification of plants and to evaluate the tolerability of potential deviations from background levels.

Prior to the use of recombinant DNA technology, varieties of crops improved through conventional breeding were, in most cases, not considered for safety regulation. These older techniques used in plant modification were well accepted, as was the allocation of natural settings to the production of useful crops. Within the agricultural system of the cultivated plant, hazards, if any, originated externally; unfavourable climatic conditions or soil chemistry were seen as sources of constraints for the success of agriculture, requiring compensation through the use of agrochemicals. During seed production, the genetic purity of the cultivated species was a prime concern; genetic contamination arising from the introgression of foreign genes found in the pollen of neighbouring plants was minimised by prescribing set isolation distances or insect-proof cages in breeding protocols. For food crops, there was no routine scientific testing for safety, except in certain plants with known toxins (e.g. the glycoalkaloid solanine in potato). Plant breeders developed and selected new varieties of plants based principally on sensory analyses and sometimes on chemical analyses for quality, wholesomeness and agronomic performance.

By contrast, detailed national guidelines have been drafted for the regulation of transgenic plant use. This suggests a shift in the perception of hazards when recombinant DNA techniques have been used in plant breeding for introducing novel traits. Hazards are now seen as originating from the transgenic plant itself, with potentially harmful consequences for the environment. Some of these concerns include:

- The spread of undesirable genetic information (antibiotic or herbicide resistance) in the environment.
- The invasion of natural habitats by transgenic plants.
- The potential allergenicity or toxicity from the transgene protein products for human and animal health.

In order to improve the public perception of genetic engineering, it is important to emphasize that the deliberate genetic alteration of plants is not new; what is new, however, is the heightened awareness of biological hazards that were also present in traditional breeding. We owe this new awareness to increased scientific understanding of natural biological mechanisms such as gene transfer, gene acquisition and genetic variability.

When quantitative risk assessments were undertaken to estimate the likelihood of hazards from transgenic plants, it was found that the accumulated experience from traditional breeding could not provide the scientific data needed. Moreover, the models of risk assessment were adopted from those developed for toxic chemicals or contained biotechnological applications with pathogenic organisms, and, as such, proved to be inadequate for endorsing the safety of transgenic organisms in the open environment. Classical risk assessment models are designed to estimate the potential consequences arising from a system failure, based on the presence of an intrinsic hazard (e.g. toxicity or pathogenicity). For modified forms of familiar crops, there is no direct relationship between an intrinsic property and the resulting hazard.

Thus, the risk-based approach for decision-making relevant to genetically modified organisms fails to:

- Take into account that genotype-phenotype relationships are unpredictable, thereby hampering attempts at quantitative and deterministic formulations of risk.
- Acknowledge that low probability biological events could still occur as both scale and time increase, leading to observable damage.
- Provide the foundation for determining the realistic hazards arising from the introduction of novel genetic information into organisms with a safe history of use, such as crops.

An assessment based on tolerability criteria is perhaps more appropriate than the probabilistic approach of risk assessment for determining the safety of transgenic plants. Safety is a relative notion defined by the tolerability of hazards and the acceptability of risk involved in a given situation. This approach can be used to evaluate the safety of expected phenotypic effects or primary effects (desirable agronomic or nutritional qualities in food crops) as well as the unexpected phenotypic effects or secondary effects seen in the progeny of breeding programmes (Box 1). Secondary effects are unpredictable, potentially harmful or not useful agronomically and manifest themselves in the following ways: in unexpected changes in flower colour, physiology, metabolism, or transgene silencing; and in the formation of new, toxic plant metabolic intermediates. In both traditional and mutation breeding, the selection process efficiently eliminated the accidental, but potentially harmful candidates. The question now is whether genetic engineering techniques in plant breeding introduce additional, unexpected hazards that could bypass normal screening procedures. An answer can be provided by examining the three areas:

- The accumulated experience with plant genetic modification in traditional breeding.
- The safety limits of genomic plasticity in plants for both intended and unintended phenotypic effects.
- What this implies for the safety of genetic engineering in plant breeding programmes.

These elements constitute a rational basis for assessing the safety of plants modified with recombinant DNA technology. It is then possible to define an acceptable background safety level, and to evaluate the tolerability of deviations.

Glossary
Aleatoric: Describes a process which displays no evidence of progression in a specific direction or towards a pre-determined goal.
Genomic plasticity: The capacity for the genome to reorganize itself in response to internal and external cues, through processes such as transposition, DNA rearrangement, mutation, or recombination.
Pleiotropy: When one gene affects more than one trait.
Primary effect: The intended agronomic effect (phenotype) expected after performing any technique of plant alteration.
Secondary effect: All other unexpected or unintended phenotypes obtained from any technique of plant alteration.
Selfing: A sequence of plant matings initially involving two genetically dissimilar parents, with subsequent generations mated to only one of the parents.
Somaclonal variation: The rearrangement of plant genetic material during tissue culture and plant regeneration, leading to different characteristics of plants originating from a single cell.
Substantial equivalence[28]**:** Principle used to determination the safety of novel foods. When a new food is found substantially equivalent to an existing food or food component, then it can be treated in the same manner with respect to safety.

Box 1. Glossary of terms

Plant genomic plasticity as the basis for crop breeding

Traditional plant breeding

Until a few decades ago, plant breeding was based on the natural genomic plasticity of plants which could be exploited to create improved varieties. The earliest farmers systematically selected for agronomically useful phenotypes within a given species. By modifying the environment to suit the physiological needs of the growing plant, the farmers could increase the frequency of this phenotype. The intraspecies variability that they observed was the result of unintended genomic alterations by natural molecular mechanisms such as mutation, DNA rearrangement, transposition, or recombination. These mechanisms are partially random generators of genomic diversity, which, along with selective forces in the environment, result in a range of possible phenotypes from a common genotypic background.

Method of plant breeding	Origin of traits	Processes for genomic variation	Selection criteria for desirable agronomic features
Selection	Plant genotype	Background processes	Sensory assessment in landraces.
Cross Breeding (Directed)	Gene pools of parents	Sexual mating and background processes	Sensory assessment in progeny.
In vitro cell culture (Random)	Plant genotype	Induced mutagenesis (increases the frequency of background processes); somaclonal variation	Sensory assessment in regenerated clones.
Genetic engineering (Directed)	Possibly of any source	Gene insertion (e.g. pleiotropy, position effects, insertional mutagenesis); somaclonal variation; background processes	Sensory, molecular, or biochemical analyses in regenerated plants.

Table 1. Genetic variation and selection criteria used in traditional breeding and genetic engineering of crops.

Systematic breeding was used for acquiring superior varieties with greater yield, better disease resistance or higher nutritional value. Controlled matings were carried out between compatible partners or more distant relatives, giving rise to a variable population of progeny which underwent selection according to predefined agronomic criteria such as height, resistance to disease, and processing characteristics. In some wide crosses, the parents used were not suitable for consumption as food due to the presence of natural toxicants; breeders were therefore careful to eliminate the sexual progeny of these crosses that also produced toxicants. Other potentially harmful or unstable phenotypes were likewise eliminated. Because of the amount of genetic information exchanged during cross breeding, several years of backcrossing and selfing (Box 1) were required to dilute out the unwanted traits while selectively conserving the traits of interest.

In vitro culture and somaclonal variation

Scientific progress in plant tissue and cell culture provided breeders with a rapid and efficient means of clonal propagation as well as a new method for rapidly inducing genotypic variation within a given genetic background: the mutation frequency can be

enhanced by irradiating cells in tissue and suspension cultures; or by treating them with chemical mutagens. In addition, the process of regenerating whole plants from the undifferentiated cell state often results in abnormal programming of the genome, thereby leading to increased phenotypic variability in regenerated plants. Very different quantitative and qualitative characteristics can be observed from plants originating from one clones, some of which can be agronomically useful while others can be potentially harmful (e.g. unacceptable toxin concentrations). This range of variability - somaclonal variation - is known to be affected by the tissue used for culture, the constituents of the medium, and the duration of culture. For example, individual plants of *Allium sativum* regenerated from long-term culture or callus culture display altered characteristics, such as in bulb size and shape, clove number, and chromosome number. Despite the unpredictability of somaclonal variation, the high levels of genetic variation that can be generated in a short time and in a small area made it possible for the plant breeder to develop plants that expressed characteristics not found in the typical-breeding gene pools. Another advantage in the selective improvement of popular cultivars through somaclonal variation is the economy in time and effort by working within a constant genetic background rather than creating new varieties. The proven value and adaptation characteristics of the plant to local conditions could often be maintained.

Foreign gene insertion and phenotype variability

Plant modification using recombinant DNA technology is the insertion of a known sequence of foreign DNA into the host plant genome. It is thus quite distinct from mutation breeding, because it is based on initial non-random DNA change and can cross species boundaries (Table 1). The new genetic information is assembled as one or more gene `cassettes' consisting of promoter, coding and terminator regions. Because it is impossible to screen for certain traits in individual transformants (e.g. delayed ripening in fruits), genetic information for selectable marker genes conferring antibiotic resistance or herbicide tolerance are also co-introduced along with the primary target traits.

Early experiments in plant transformation used *Agrobacterium*-mediated gene transfer to introduce foreign DNA into the host plant cell. *Agrobacterium tumefaciens* is a bacterial plant pathogen that causes the crown gall and hairy root diseases. During infection, a section of plasmid, T-DNA, is transmitted by *Agrobacterium* into individual plant cells, usually within wounded tissue. In the lab, the oncogenic genes within the T-DNA can be removed and replaced by virtually any gene of interest targeted for transfer into the recipient plant genome. For plants that are recalcitrant to infection and transformation with *Agrobacterium*, other methods of direct DNA uptake or transfer exist: particle bombardment, electroporation or microinjection. A successful transformation is determined by the proper integration of foreign DNA into the genome, the correct expression of the new genetic information and its inheritance by progeny in a near-Mendelian ratio. Recent studies suggest that particle bombardment might be the best form of plant transformation, when adequate equipment and resources are available.; *Agrobacterium*-mediated transformation has been associated with two potential problems: the transfer of DNA outside the left and right borders of T-DNA and the persistence of *Agrobacterium* on plant tissue after transformation. This new evidence would also have to be considered in assessing the safety of transgenic plants.

Methods of plant modification requiring a cell culture stage are exposed to somaclonal variation and its range of phenotypes. Possible, unpredictable modifications in plant features are expected, such as changes in morphology, adaptive characteristics, fertility, and levels of nutrients or toxic compounds. Although the potential for unexpected or harmful phenotypes to develop from the plant regeneration step is not specific to genetic engineering, it is still possible that phenotypic variability might arise from perturbations to the host genomic DNA sequence from foreign gene insertion such as position effects, pleiotropy or insertional mutagenesis. However, it should be emphasized that gene insertion and its possible collateral effects on the genome are a one-time event. This should be contrasted to the continuous nature of background processes for genomic variation (Table 1).

In an experiment designed to distinguish between the impact of somaclonal variation and gene insertion on the observable plant phenotypes in regenerated potato cells, Dale and McPartlan measured the variation in plant performance characteristics in three groups of plants: nontransgenic potato plants established from tuber nodal shoot cuttings; nontransgenic plants regenerated from tuber discs; and *Agrobacterium* - transformed tuber discs containing the *GUS* reporter and the neomycin resistance genes. Plants from shoot cuttings do not go through a disorganized state and were thus the controls; the nontransgenic plants regenerated from undifferentiated tuber discs provided an estimate of the somaclonal variation associated with cell culture. For the plant characters defined in this study (e.g. plant height at flowering), it was found that the mean values were significantly lower for the transgenic plants than for the shoot-culture-derived plants, with values for the tuber disc-derived plants lower than the controls but higher than the transgenic plants. Thus the gene insertion event has observable consequences on plant characteristics. The heterogeneity between individual plants also followed the same trend.

In another field test with transgenic rice plants modified to express the rice stripe virus coat protein, it was possible to attribute the quantitative differences between transgenic and nontransgenic rice plants to cell culture-related somaclonal variation. The transgenic plants were shorter than the nontransgenic plants in height, culm and panical length. Because this suite of traits continued to be present in the progeny of transgenic plants that had lost the integrated gene, it was concluded that the differences in features were the consequences of genomic plasticity during the transformed protoplast regeneration. In most practical applications, secondary phenotype changes in transformant populations are not so easily attributable to either cell regeneration or gene insertion.

Molecular events associated with genetic alterations: position effects, insertional mutagenesis and pleiotropy

Other molecular mechanisms that influence intertransformant variability are related to the process by which new genetic material is integrated into the host genome. Position effects are defined as variability in the transgene expression due to the structural and functional properties of the chromatin regions flanking the DNA integration site. Properties of the chromatin that may influence the expression level of the integrated genes include local- and higher-order structures (e.g. looped domains)as well as neighbouring regulatory sequences that might enhance or silence the activity of the introduced gene. During plant transformation, foreign DNA is

integrated at a random position in the genome, in most cases at a single locus, either as a single copy or as a cluster of tandem copies. The randomness of gene integration could be perceived as exacerbating the problem of unexpected secondary effects. However, the natural internal rearrangements of the genetic code through background transpositional events could also produce profound changes in biology. Many mobile genetic elements, which move around chromosomes without the benefit of homology, are known to carry control sequences that can influence neighbouring genes in the new site of insertion. The contribution of position effects to unintended and unexpected secondary effects in transgenic plant populations may be minimised by developing advanced techniques for site-directed gene insertion. Moreover, the first generation of transformants is usually backcrossed with unmodified elite breeding lines to ensure genetic stability and to attempt to eliminate possible artefacts arising from the gene insertion step, such as the integration of pieces of truncated or rearranged foreign DNA.

Insertional mutagenesis is the modification or disruption of functional genes of the host plant at the site of foreign gene insertion. Inactivation of existing genes may occur when the incoming DNA inserts into coding regions, while activation may result from insertions into the regulatory regions. Any mutation that alters the substrate specificity or rate of enzyme catalysis in plants could readily result in new compounds or an accumulation of toxic metabolites that are normally detoxified through further metabolism. It has been argued that the likelihood of gene inactivation or activation is very low, because of the vast regions of noncoding or repetitive regions in the plant genome (95%). Moreover, it has been observed that compared with mammals, genes in higher plants are organized to be very compact: most introns, 5'- and 3'-untranslated regions are <200 bases apart, with the promoter elements usually close to the site of transcription initiation. Insertional mutagenesis is probably rare, but in any case should not be more likely with genetic engineering than with traditional breeding.

Pleiotropy - defined as the ability of one gene to affect more than one trait - is another source of unexpected or unintended effects seen in the progeny phenotypes of breeding populations. The potentially dramatic and unforeseeable effects of pleiotropy in traditional breeding were demonstrated by the male-sterile lines of the Texas cytoplasm maize hybrid (*cms*-T). During the early 1970s, the massive area in the USA dedicated to the *cms*-T maize was devastated by the southern corn leaf blight, caused by *Bipolaris maydis* race T. The susceptibility to fungal disease was observed only with *cms*-T maize and not in any other varieties. The c*ms*-T maize carry a mitochondrial gene, T-*urf13*, which encodes a 13-kDa polypeptide (URF13) that is a component of the inner mitochondrial membrane. However, URF13 interacts with the pathotoxin produced by *B. maydis* race T to become a channel forming protein, allowing the leakage of small molecules and then loss of mitochondrial function. This example illustrates that pleiotropy is an ubiquitous biological mechanism; for the purposes of the plant breeding safety discussion, it needs to be acknowledged that pleiotropy escapes direct examinations and screenings, regardless of the plant modification method.

The background safety level for plants modified with recombinant DNA technology

The many regulatory guidelines and studies on the assessment of transgenic plants are based on expected (target) agronomic effects in plants. The presence and the expression stability of the inserted genetic information in successive transgenic generations are prerequisites for the reliability of the safety assessment techniques. When the nature of the intended effect is known, environmental safety evaluations or food toxicity and allergenicity testing can be performed based on the impact of the gene product for human health and for the environment (Table 2). For example, a comprehensive safety assessment for a herbicide-tolerant crop would examine the potential impact of this genetic information in the environment as well as the allergenic potential of the gene product, based on tests such as the comparison of the protein's amino acid homology with known allergens.

Hazard	**Safety considerations with traditional breeding**	**Safety considerations with genetic engineering**	
		Intended (primary) effect	*Unintended (secondary) effect*
Allergenicity	Not systematically assessed; trial-and-error.	Source of introduced gene checked: if **allergenic**, check the gene product for allergenicity; If **non-allergenic**, amino acid similarity to known allergens, resistance to digestive degradation and stability to heat and acid are all checked.	Based on substantial equivalence with unmodified plants; biochemical and molecular analyses when possible.
Adverse environmental effects	Concern focused on adverse effects of environment on crop system.	Demonstration of safety required; harmful effects, such as from gene flow, can be assessed in scenarios.	Increased awareness of unforeseeable hazards; implementation of monitoring or containment.
Plant nutrient and toxicant levels	Experience-based selection; chemical analyses sometimes performed, but not routine.	Based on substantial equivalence with unmodified plants; biochemical and molecular analyses	Substantial equivalence with unmodified plants; biochemical and molecular analyses when possible

Table 2. Safety considerations of traditional breeding and genetic engineering of crops

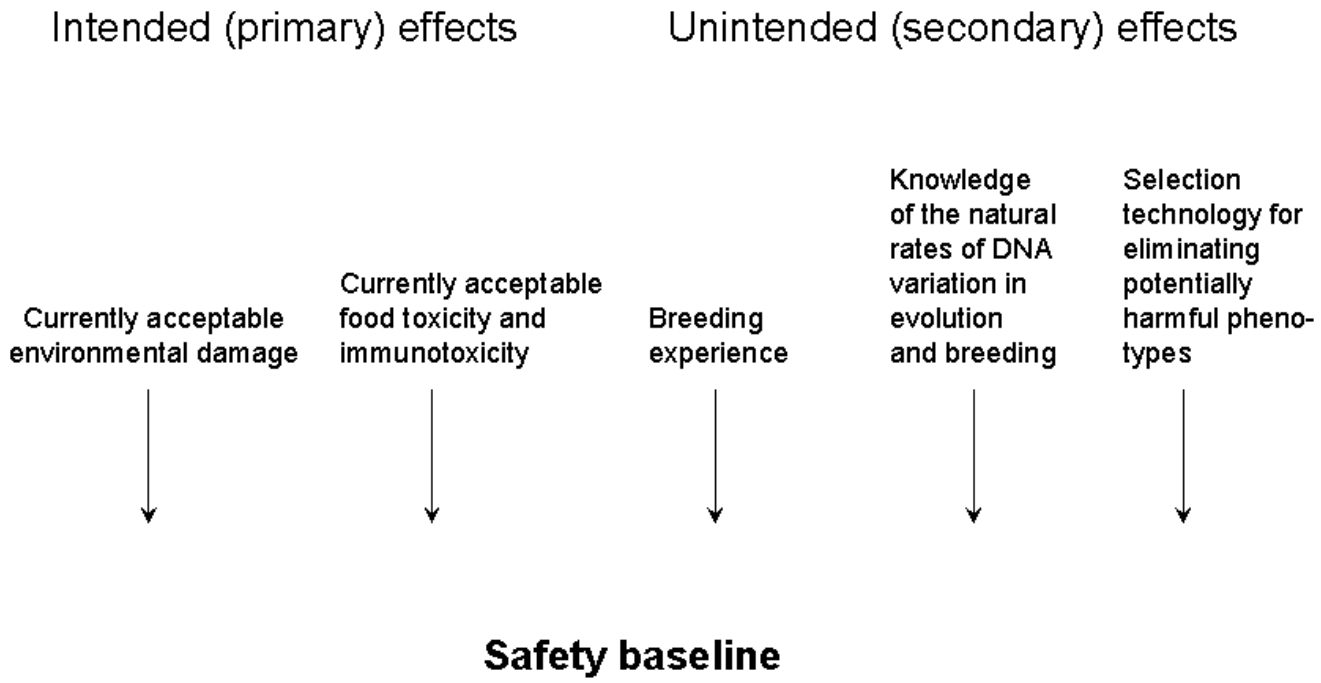
A comprehensive and reliable safety assessment should also address the possibility for adverse effects from unexpected plant phenotypes that may escape notice during safety assessments based on primary traits. It is recognised that only a few plants from a transformant population will behave in the expected way, implying that adequate selection is essential for choosing those plants that satisfy both agronomic and safety criteria. Unexpected plant phenotypes are the result of non-deterministic background molecular processes, such as:

- Mutations.
- DNA rearrangements and recombination.
- DNA replication errors.

Pleiotropic effects are also possible; whether or not they occur with greater frequency in transgenic plants has not yet been conclusively established. The example of the cytoplasm male-sterile Texas maize clearly illustrates that pleiotropy is not specific to genetic engineering. Regarding the possibly harmful phenotypic novelties originating from alterations to the genome by random molecular processes, after selection, the policy of the Animal and Plant Health Inspection Service in the USA presume that the magnitude will not be greater than that observed with traditional plant modification techniques.

As a basis for assessing the impact from unexpected or unintended phenotypic variability on the environmental and food safety of transgenic plants, we propose an examination of the existing, accepted level of uncertainty created by the aleatoric activities of normal processes in the genome. Better clarity might be achieved in the discussion on transgenic plant safety once it is recognised that potential harm from unexpected plant phenotypes has always existed in traditional plant breeding and that the purpose of selection has been to eliminate any potentially harmful progeny. A biosafety baseline could therefore be defined from the abundance of experience in plant selection technology, scientific knowledge about the evolutionary significance of plant genomic plasticity and the understanding of the role intended for recombinant DNA techniques in plant breeding programmes. This baseline would be both a statement of the confidence level in selection technology and of the inherent uncertainty in biology. From such a baseline, levels of tolerable damage and acceptable risk for environmental and food safety could then be derived.

The safety baseline for assessing transformed plants is arrived at based on various considerations. For intended (primary) agronomic effects, safety considerations are based on comparisons with accepted background damage levels from familiar processes. For the saftey assessment of unintended (secondary) effects, experience with plant breeding is used as the main guide.



Box 2. The safety baeline

There is no evidence that foreign gene insertion with recombinant DNA techniques increase the frequency of secondary, nontarget effects in their host plants. Nevertheless, plant breeders working with transgenic plants are aware that random, multicopy gene integration into the host plant genome could influence the phenotype of the primary transformant or its progeny in subsequent generations. The methods customarily practiced in dealing with the uncertain outcomes of plant modification have been both of prevention and comparison (principle of substantial equivalence) with nonmodified plants having a known history of safe use.

Preventative measures aimed at lowering the frequency of unintended effects in transformant populations attempt to stabilize the genetic background of the transgene. For example, mutations may influence the phenotype of a primary transformant or may be revealed only in subsequent generations. This problem is technically managed by introducing the transgene into less advanced breeding material that is then backcrossed to recover the agronomic qualities of the elite lines. Normal crossing, recombination and selection of transformed plants are also carried out to eliminate fragmented copies of the genes or vectors that may have inserted at different locations in the genome. It is known that direct DNA transformations, in contrast to *Agrobacterium*-mediated transformations, create this type of artefact. The successful elimination of nonfunctional fragments of 'junk' DNA could improve the specific expression characteristics of the transgene in transformed plant lines.

The potential impact of unexpected or unintended effects on food safety can be evaluated by performing independent safety assessments on the host and donor plants. This methodology, which is used by the US Food and Drug Administration, assumes that the damage potential from unexpected or unintended genomic alterations falls into one of three categories: plant toxicant levels, nutrient levels, or allergenicity. Risk from potentially altered toxicant or nutrient levels is assessed based on knowledge of the existing levels of these compounds in the transgene donor (Table 2). When the transgene comes from a novel or unusual source for which there is no history of use in plant breeding, such as another species, the principle of substantial equivalence (Box 1) is used to determine food safety. Proteins form the largest class of substances that are being introduced into food with recombinant DNA techniques; at the moment, there is no scientific method for screening new food proteins for immunotoxicity. For both expected target effects and unexpected effects, therefore, immunotoxicity issues are dealt with in safety assessments by examining the properties and history of use of the donor of the new genetic information. If the donor plant for the new gene has known allergenic properties, then it is assumed that the host plant may become newly allergenic. It has also been recommended that novel proteins be compared with known food allergens based on amino acid homology, heat stability, acid stability, stability to digestion and perhaps evaluations of immunogenic responses in animal models.

Conclusions

Since humans began practicing agriculture more than 10000 years ago, they have been remodeling the genomes of useful plants through careful selection and breeding. Many important crops no longer resemble their original parents. The modifications that have taken place in plants have been to a large extent dictated by breeding criteria, reflecting:

- The required role of the particular crop.
- The environment available for growing it.
- The quantity needed.
- The economic feasibility for the grower.

Meeting the challenge of producing agricultural products in a rapidly evolving society and changing environment has been the true driving force for the development of a variety of plant breeding techniques for introducing novel traits into domesticated plants.

The application of genetic engineering to plant breeding may be one source of urgently needed creative solutions in agriculture, where the technology needs at least to ensure a steady rate of food production while sparing ecosystems from unnecessary harm. The prodigious yield increases seen in the major world crops during the 'Green Revolution' were achieved at a high cost to the environment. High inputs of fertilizers, pesticide applications, and natural resources were necessary to compensate for unfavourable crop biology or climatic conditions. The awareness gained from this - that cropping systems can be a source of hazard for existing ecosystems - has been transferred to the safety and risk discussion in plant genetic engineering, such that there has been a paradigm shift in the perception of hazards in agriculture. It is good that the resulting awareness could provide the impetus for the systematic use of available molecular biological tools to making agriculture safer for the environment and for human or animal health, with the help of careful monitoring and testing. However, the new technology is sometimes perceived as a source of unusual, unmanageable hazards; it is detrimental that the objective perspective on millennia-old breeding practices has been lost.

Safety and risk assessments of transgenic plants, performed to fulfil the requirements of regulatory guidelines, are designed to identify the potential hazards based on the impact of the primary trait on the environment and for food safety. For example, these studies might focus on evaluating the consequences arising from the transfer of undesired genetic information from transgenic crops to neighboring plant populations. When such studies are undertaken, it would certainly help improve the public perception of genetic engineering to emphasize that gene flow is not a risk but is a natural process; it is the nature of the introduced genetic information that will have a bearing on the type of consequences which could occur. In the safety assessment and in public debate, it would also be instructive to highlight other existing (and currently accepted) environmental sources of similar genetic information which lie outside the realm of genetic engineering. For example, there is legitimate concern that the presence of antibiotic marker genes in modified crop plants might be transferred to pathogenic organisms, which might then become resistant to antibiotics; genetic engineering might therefore be perceived negatively as a technology that exacerbates the problem of widespread antibiotic resistance. However, a more realistic and responsible assessment of the environmental presence of antibiotics should also acknowledge their extensive use in animal husbandry and also question the desirability of the current practice.

Environmental and food safety assessments rarely deal with the range of secondary, unintended effects that may also arise in plant genetic engineering. These secondary effects may result in potentially adverse plant phenotypes; they are the outcome of random, background molecular and genetic phenomena within the plant genome,

whose frequency is increased during plant regeneration from cell culture. The gene insertion event has been associated with position effects or insertional mutagenesis at the site of insertion, although these mechanisms are not specific to genetic engineering but are observed also in traditional breeding and in naturally-occurring DNA rearrangements (transposition) in organisms. From a purely genetic perspective, the inherent genomic plasticity of organisms creates the variability that maintains evolution. What this implies for the safety discussion is that genetic engineering cannot be made 'safer' than biology itself, but that a biosafety baseline can be defined as the limit for levels of tolerable damage and acceptable risk with transgenic plants. The abundance of experience in plant selection technology, coupled to scientific knowledge about the evolutionary significance of genetic variation, should be a reminder that potential adverse effects can be managed with good scientific and experimental practice.

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