Codex guidelines for GM foods include the analysis of unintended effects

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In response to the increased delivery of genetically modified (GM) foods to international markets, the Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology of the Codex Alimentarius Commission (Rome) agreed in March on principles for the human health risk analysis of GM foods[1]. These principles dictate a case-by-case premarket assessment that includes an evaluation of both direct and unintended effects. They state that safety assessment of GM foods needs to investigate direct health effects (toxicity), tendency to provoke allergic reactions (allergenicity), specific components thought to have nutritional or toxic properties, the stability of the inserted gene, nutritional effects associated with genetic modification and any unintended effects that could result from the gene insertion. Of particular note, the task force broadens risk assessment to encompass not only health-related effects of the food itself, but also the indirect effects of food on human health (*e.g.*, potential health risks derived from outcrossing).

Unintended effects of the product

The Codex's aim is to anticipate not only the direct risks, but also the indirect/unanticipated risks that the products of modern agriculture might pose for human health. All of the methods used for breeding or manipulating plant traits, including self- and cross-pollination, the generation of hybrids or haploid breeding, mutational breeding (including X-rays or chemicals) and advanced biotechnologies (including protoplast fusion and/or recombinant DNA technology), have the potential to generate unanticipated effects in plants.

In conventional breeding programs of spring barley, for example, different degrees of a temporary breakdown of the resistance to powdery mildew by a sudden relief of soil water-stress have been attributed to the genetic background rather than the specific allele[2]. There have also been reports that a traditionally bred squash caused food poisoning[3], a pest-resistant celery variety produced rashes in agricultural workers (which was subsequently found to contain sevenfold more carcinogenic psoralens than control celery[4]) and a potato variety Lenape contained very high levels of toxic solanine[5] (which was subsequently withdrawn from cultivation).

The use of tissue culture in plant breeding has also often resulted in somaclonal variation of plant lines and irregular phenotypes or field performance. Somaclonal variations are mutational and chromosomal instabilities of embryonic plants regenerated from tissue cultures. These instabilities may result from activation of dormant transposons in the chromosome[6]. The consequent genetic variability is known to persist for many generations and is difficult to eliminate by backcrossing.

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For plants generated by recombinant technology, unanticipated effects may additionally arise from the process of introducing foreign genes or as a result of the effects of environmental factors/genetic background on the expression of the transgene(s)[7].

Complex multicopy patterns of transgene integration at the same locus, as well as position effects caused by random integration, are often associated with instability in transgene expression[8]. Random insertion of DNA sequences can cause modification, interruption or silencing of existing genes as well as activation of silent genes[9,10]. Safety aspects have been discussed for a transgenic maize line following the observation of integration of recombinant DNA into a retrotransposon[11,12].

Table 1 list examples of unanticipated phenotypes observed in transgenic crops in the field. A comparison of data from documents prepared for notification of GM rape, maize, tomato, soybean and potatoes (exhibiting mainly pest resistances) suggested that environmental factors like heat were more important than genetic modification in influencing variation in the expression of antinutrients[13]. Epigenetic transcriptional silencing has been reported for a complex transgene in rice[14] and epigenetic variations in *Arabidopsis* disease resistance have been attributed to DNA methylation[15]. Environmental stress factors that influence methylation patterns and/or chromatin conformations have been suggested as explanations for gene silencing of transgenes in the field[16]. The presence of a pathogen can induce host defense gene silencing mechanisms[17] also affecting transgenes. And environmental signals have been shown to modulate mRNA stability and translation through modulation of the phosphorylation of components of the mRNA 5′-cap-binding complex, ribosomes and mRNA-binding proteins[18].

Unintended effects mediated via the environment

In addition to investigating health risks directly associated with food products, the broadening of the Codex risk assessment to include indirect effects now encompass effects of novel foods on the environment that may have an indirect impact on human health. This concept has a precedent in agricultural practice (*e.g.*, sustainability[19]) and embraces the view of human "health as an integrating index of ecological and social sustainability" outlined in a report from a joint World Health Organization (Geneva, Switzerland) and the National Agency for the Protection of the Environment (Rome, Italy) seminar in 2000 on potential environmental hazards of GM crops[20].

Several recent findings argue that such environmental effects could/should be sup-

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Table 1 Selected examples of unanticipated effects arising in transgenic crops

Transgenic crop	Unanticipated effect	Reference
Barley	Transgenic barley lines containing the *BAR* gene, the *uidA* gene and the gene for a heat-stable β-glucanase exhibited unchanged levels of β-glucanase but were inferior to conventional barley in a number of genetic backgrounds and environmental conditions	32
Canola	Seed-specific overexpression of phytoene synthase resulted in up to a 500-fold increase in levels of α- and β-carotene, but not of lutein, the predominant carotenoid in control seeds	33
Maize	The stems of *Bt* maize contain more lignin than controls with complex effects on degradation and consumption in the food chain.	34
Oilseed rape	Oilseed rape plants containing the bialaphos tolerance gene (*BAR*) regulated by the cauliflower mosaic virus (CaMV) 35S promoter become sensitive to the herbicide after infection with CaMV	35
Potato	Transgenic potato lines from three cultivars expressing a kanamycin resistance marker showed unexpected changes in phenotypic and yield performance. Changes attributed to epigenetic/genetic events occurring during tissue culture phase of transformation	36
	Potato plants transformed with lectin genes to enhance insect resistance exhibit lower levels of leaf-glycoalkaloids with potential consequences for nontarget insects and food or feed uses	37
Rice	Transgenic rice containing soybean glycinin gene exhibited 20% increase in protein content (presumably due to elevated glycinin) but also a 50% increase in vitamin B6	38

ported by evidence (*e.g.*, the need to inhibit outcrossing from plants containing biopharmaceuticals; http://www.worldbiosafety.net/paper/05-Rainer%20Fischer.doc) in health risk assessment of GM crops. The introgression of transgenic DNA into traditional landraces of maize in Mexico (for review, see ref. 21), recently confirmed by the Mexican government[22], shows that gene flow may be commonplace for certain crops in certain locations, and the effects of foreign genes in certain backgrounds could pose health risks, although these concerns remain speculative[23]. The risk of outcrossing and gene transfer could also affect crop biodiversity, especially that of landraces, and may compromise the planting of crops by farmers who wish to remain GM-free (*e.g.*, organic farmers). Indeed, the coexistence of GM crop agriculture and organic agriculture (which does not tolerate GM use above specific thresholds) is likely to be difficult for certain plants in specific areas[24]. As a consequence, the wish for regions with restrictions on planting of GM organisms (GMOs) and GMO-free foods has already been expressed in different areas[25,26].

Conclusions

Both conventional methods of breeding and recombinant technology can affect the expression of genes and raise questions about food safety. Phenotypic variability in a novel crop can also result from environmental/epigenetic factors as well as the genetic background in which a trait is expressed. Clearly, risk assessment must account for the effects of transgene-specific factors, environmental signals and genetic background on phenotype. The expression level of a gene, rather than the sequence of the protein product, can often determine phenotypes that contribute to natural variation[27].

In any risk assessment, however, it is important to differentiate between hypothetical and proven risks. And, to date, no food-derived health problems have been identified with the use of GM plants. However, it must be acknowledged that occasional pleiotropic, unintended safety relevant effects in organisms produced with traditional or modern biotechnology can occur and need to be addressed.

The decision by the Codex to include unintended effects (*e.g.*, environmental health risks) in the risk assessment is an important new development. The link between environment and human health operates through the exposure of humans to environmental hazards, where such hazards may take many forms, wholly natural in origin or derived from human activities and interventions. There have been several attempts to conceptualize environmental–human health interactions[28,29]. Indicators for environmental health and methods for the consideration of the burden of disease from environmental risk factors are presently harmonized to support and monitor policy on environment and health for many developments[30,31]. These concepts may be useful in the analyses of effects of GM organisms for food production. Such assessments need to compare different approaches to food production, such as conventional, organic or GM technologies, and may also prove valuable in assessing regional differences (health relevant decreases or increases of pesticide use according to local agroecological situation) in the impacts of modern methods of food production.

The Codex's approach to GM crops will be inherently linked to agreements at the World Trade Organization (Geneva, Switzerland). Codex principles do not have a binding effect on national legislation, but are referred to specifically in the Sanitary and Phytosanitary Agreement of the World Trade Organization (SPS Agreement), and can be used as a reference in case of trade disputes. This has particular relevance in the light of the recent complaint brought by the United States, Canada and Argentina to the WTO against the EU *de facto* moratorium on GM crops.

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