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Can–Omics inform a food safety assessment?

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ABSTRACT

Omic technologies can in principle allow visualization of the all of changes that take place when the genetics, nutrition or environment of an organism is altered. Targeted compositional analysis is today a key component of the food safety assessment paradigm in which known nutrients, anti-nutrients, toxicants, allergens, and other molecules of potential biological importance to humans or animals are quantitatively analyzed. This allows safety assessors to compare the composition and safety of one food with closely related counterparts. Omic technologies measure many analytes—some of which are unidentified—but the analysis often sacrifices one or more of the characteristics of validated analytical methods currently used for food analysis. Databases that would allow the safety assessor to interpret differences are not currently available. There is also no reason to believe that the targeted compositional analysis in use today does not provide the evidence needed to ensure food safety, nor is there any current reason to believe that omics can add value to the safety assessment process. The regulation of transgenic crops is far more rigorous than is justified since they present no new risks compared with traditional breeding, and are more precisely defined and better understood than their non-transgenic equivalent.

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1. Introduction

In recent years new transgenic crop varieties possessing a variety of useful agronomic traits have been developed using modern biotechnology (Brookes and Barfoot, 2007, 2009). An important characteristic of transgenic plants is that well-defined genes that encode specific desirable traits are inserted into crop plants *in vitro* to produce new varieties. Plant varieties developed using conventional methods of plant breeding are typically not subject to pre-market regulatory review; however, transgenic crops are required to pass a pre-market review before they can be widely cultivated. A discussion of the reasons for the singling out of transgenic crops for special regulatory review are largely beyond the scope of this paper and have been discussed elsewhere (Bradford et al., 2005; Parrott, 2005; Chassy, 2008; Graff et al., 2009).

One component of safety assessments is the evaluation of safety for transgenic crop as food or feed. The safety review seeks to establish that foods and feeds produced using modern molecular methods of *in vitro* gene insertion are as safe as any other in the market; in particular, that a new variety is as safe as a conventional counterpart, often called a comparator (Kuiper et al., 2001; Kok and Kuiper, 2003; König et al., 2004). Since safety is a relative concept, the degree of safety sought must be defined; in the USA, that the degree of safety is a reasonable certainty of no harm (21 US CFR 170.3(i)).

A cornerstone of the food safety assessment is the Substantial Equivalence paradigm that asserts that differences in safety between a new variety and its conventional comparator can arise only from differences observed between them (OECD, 1993; Kuiper et al., 2001). The comparator selected is usually a variety that is very similar to the parental strain from which the transgenic was developed (Kok and Kuiper, 2003), although in the case of crop varieties in which the composition has been intentionally altered, it is conceivable that alternative comparators could be selected (Kok and Kuiper, 2003; ILSI, 2004). A primary focus of comparative assessment has been a thorough evaluation of crops composition (Ridley, 2010 this issue; Ryals, 2010 this issue; Davies, 2010 this issue; Herman et al. 2009). Typically, 50–100 or more individual components are determined for each crop variety; the selection of analytes follows recommendations of the OECD Consensus Documents that describe key nutrients, anti-nutrients, and other compounds of biological interest for many crops (OECD, 2001–2009). This evaluation of crop composition is sometimes referred to as “targeted analysis”; it should be noted that the “target” of the analysis typically comprises greater than 95% of the crop composition. (ILSI, 2004, 2008; Ridley, 2010 this issue; Ryals, 2010 this issue; Davies, 2010 this issue; Herman et al. 2009).

The emergence of omic technologies over the last two decades has contributed powerful discovery tools for medical, pharmaceutical and biological research. Transcriptomic (van Dijk, 2010 this issue), proteomic (Baginsky, 2010 this issue; Thelen, 2010 this issue; Sancho, 2010; Natarajan, 2010; Teshima, 2010 this issue; Rouquié, 2010 this issue) and metabolomic (Ryals, 2010 this issue; Davies,

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2010 this issue) analysis of crops plants have been described. To date, omic technologies have not been formally applied to the food safety assessment of transgenic crops. The requirements and current status of omics applied to food safety assessment have been previously discussed (ILSI, 2004, 2008). This paper describes the current applicability of omic technologies to food risk assessment. It concludes that the paradigm currently applied to transgenic crops is sufficiently robust to ensure that new crop varieties are safe for consumption, and also finds that omics technologies could add little relevant new data to current safety assessment data.

2. Assessing the safety of whole foods

No food or feed poses zero risk or is absolutely safe. It is also difficult to evaluate the safety of a plant-derived food since the food can be a mixture of 100s or even 1000s of compounds. The mycoprotein food Quorn is a good example of the difficulty of assessing the safety of a wholly novel food (Miller and Dwyer, 2001). Quorn is a single-cell protein product that can be formed into cheese-, meat- or poultry-like foods. Researchers performed extensive compositional studies, fed the material to animals, rubbed it on their skin, injected it under their skin, and fed it to human volunteers without ill effect being observed. FDA approved the product even though there was no specific test that definitively demonstrated the product was safe to consume; what could be demonstrated was that its composition was similar to other high quality protein foods and that it was apparently innocuous when consumed by living subjects. The FDA did not object to the developers claim that quorn should be considered GRAS in spite of the fact that about one of every 100,000 people that consume Quorn experiences an adverse reaction (Miller and Dwyer, 2001).

Although transgenic crops are categorized as “novel foods,” the safety assessment process applied to transgenic crops, foods and feeds should in principle be much more straightforward than that described for Quorn since only one, or at most a few genes, are inserted and the changes that are introduced are small, well-defined and usually predictable (König et al., 2004; Cellini et al., 2004; Parrott, 2005; Baudo et al., 2006; Batista et al., 2008; Herman et al., 2009). Transgenic crops are not wholly novel foods as was the case with Quorn. In the production of a transgenic crop, the crop composition usually remains largely unchanged, except for the presence of a few well-defined additions. When a gene is inserted into a plant, three questions can be asked about safety for consumption:

1. Is the inserted DNA safe to consume?
2. Is the protein product(s) of the gene, if applicable, safe to consume?
3. Are the intended, and any unintended changes, safe to consume?

Since DNA, including antibiotic resistance makers used in the development of the transgenic plant, is considered safe to consume (Beever and Kemp, 2000; Goldstein et al., 2005; Gay and Gillespie, 2005; EFSA, 2004; Ramessar et al., 2007) the safety assessment focuses primarily on the safety of: (1) any newly synthesized proteins, (2) any newly introduced compounds, and (3) any changes in composition that have occurred in plant breeding. It is noteworthy in this regard that conventional plant breeding produces genetic and compositional changes of similar or greater magnitude than transgenic breeding but resulting cultivars are not required to undergo pre-market safety assessment (Cellini et al., 2004; Parrott, 2005; Herman et al., 2009).

Prior to selection of a gene to insert into a plant, a bioinformatic analysis is performed to ensure that the gene product bears no resemblance (similarity) to potentially toxic or allergenic proteins

(Delaney et al., 2008; Goodman et al., 2008). The safety of inserted proteins is also assessed through evaluation of the digestibility of the protein, and by toxicological evaluation of the protein in laboratory animals exposed by an oral route of administration. Since the great majority of proteins are safe for humans to consume, digestible proteins that do not have a structural resemblance to known allergens or toxins, and which produce no adverse reactions in animal tests, are regarded as safe to consume.

As noted previously, extensive “targeted” compositional analysis is a key element of the safety assessment paradigm. Compositional analysis provides direct evidence that intended changes have occurred as well as revealing if any unintended changes in composition have occurred. The safety and nutritional value of a food or feed can be deduced from the composition (ILSI, 2004, 2008). Moreover, the absence of large unexpected and the presence of intended changes in composition are also interpreted as an indicator that no adverse unintended effects have occurred in the breeding process.

Although animal studies are exceedingly difficult to perform and have little power when completed (ILSI, 2003, 2004, 2007, 2008), over two-hundred animal studies on whole transgenic foods and feeds have also been conducted in order to provide an additional level of assurance that a new crop variety is safe (EFSA, 2007). While a number of reports, beginning with Ewen and Pusztai's (1999) flawed experiments, have claimed adverse effects resulting from feeding transgenic crops to laboratory animals, none of these studies has been proven to have revealed any negative effect(s) that was the result of the transgenic breeding process nor have these studies been repeatable. Studies that make claims of adverse effects upon consumption of GM crops are often poorly designed, inadequately documented and conducted, suffer from experimenter bias, and make conclusions that are not rooted in any meaningful biological differences (EFSA, 2007; ILSI, 2004).

The safety of the inserted gene and its products, taken along with the compositional analysis are the keystones of an effective safety evaluation. It should also be noted that the rigorous screening for absence of changes in a long list of key phenotypes that is performed throughout the development process, as well as the extensive back-crossing into elite varieties that is required to produce a commercial cultivar, doubtless serve as an effective screen against unintended and undetected changes being present in a new transgenic variety. More importantly, change in and of itself does not increase risk since changes can be beneficial, neutral or deleterious. This is evidenced by fact that the crop varieties in use today differ significantly in composition from historical stock (Ridley, 2010 this issue; Ryal, 2010 this issue).

3. Can-Omics inform a safety assessment?

As noted previously, the Omic technologies are powerful analytical tools for discovery research. The potential applicability of Omics to safety assessment is evaluated in the following paragraphs.

3.1. Transcriptomics

The current status of transcriptomic analysis of food crops has been reviewed (van Dijk, 2010 this issue). Transcriptome profiling tools allow the researcher to identify differences in the transcription of genes representing a significant portion of the genome between two or more samples of a plant. The research reported to date demonstrates that reproducibility is a major challenge for transcriptomic analyses. Duplicate analysis of a sample can produce different differential gene expression patterns, and it is clear that variables such time of sampling, method of mRNA isolation,

and differences in soil and weather during cultivation, can have profound impact on the expression patterns observed. Perhaps more important is that different varieties of the same crop can display very different gene expression patterns when cultured side-by-side in the same field (van Dijk, 2010 this issue; Batista et al., 2008; Baudo et al., 2006).

It is not immediately apparent how differences in the expression of genes observed in two varieties of the same food or feed crop can be interpreted from a safety perspective. If careful transcriptomic analysis reproducibly points to significant differences between two varieties in the expression of one or a series of genes what does that tell the regulator about safety? For a variety of reasons, differences in transcription do not necessarily translate into differences in the proteome or metabolome. The transcriptome is therefore not necessarily predictive of the composition of final food or feed that will be consumed. Catalogues of gene expression patterns that correlate with “safe” and “unsafe” samples of a crop plant do not exist since: (1) to date, virtually all examples of commonly eaten crops are equally safe when consumed as intended, and (2) no extensive databases of “normal transcriptome” patterns have been compiled as has been done for compositional analysis of crops. If a plant is known to produce an anti-nutrient or an allergen, using expression analysis to evaluate expression of the genes associated with biosynthesis of these components would not be predictive for the quantitative measure of anti-nutrient content. Transcription analysis targeted in this way would also be simply an alternative, and likely an inferior alternative, to a direct analysis of a constituent, such as a protein, that is currently analyzed through composition studies.

An underlying motivation for the suggestion that transcriptomics could be useful in safety assessment arises from concerns that unintended changes may have occurred as a result of transgene insertion (Lantham et al., 2006; Wilson et al., 2006). The hypothesis to be evaluated here is that transcriptomics could be a tool for revealing unintended effects. The comparatively few studies reported to date indicate that transgene insertion is *less* likely to elicit major changes in the transcriptome than other modalities of crop breeding as, for example, radiation mutagenesis (Batista et al., 2008; Baudo et al., 2006). It has been reported that radiation mutagenesis produces numerous random DNA mutations in plant genomes including 4 bp to 8 kb deletions, inversions of up to 1.5 kb, insertions of ~200 bp of DNA, multiple frame-shift mutations, and premature stop codons (Wilkinson and Crawford, 1991; Bruggemann et al., 1996; Vizir and Mulligan, 1999; Shirley et al., 1992). The presence of numerous random mutations does not seem to adversely affect crops safety since 2543 varieties produced by radiation breeding have been recorded (Ahloowalia et al., 2004; <http://www-info-cris.iaea.org/MVD/>).

Crop plants display remarkable genetic fluidity. The high frequency of random variation allowed ancient crop breeders to turn wild ancestor plants into the crop varieties that are cultivated today (Darwin, 1859) and have been useful to crop breeders in recent years. More genetic diversity is observed between maize varieties than is found between humans and chimpanzees; maize likely has functional variation at every gene. In total, there could be 100,000s of functional single nucleotide polymorphisms (SNPs) or Quantitative Trait Nucleotides (Tenaillon et al., 2001; Zhao et al., 2000). A panel of repeated sequences were found to occur randomly throughout the genome in a variable number of copies and it has been found that massive differences in sequence observed between varieties of maize have most likely accumulated over only the last 200 years (Kato et al., 2004; Peterson, 2007). More remarkable is that genetic changes take place in a field during a single growing season. It has been demonstrated, for example, that flax plants change the amount of DNA in each cell in response to soil nutrient availability over the course of develop-

ment (Durrant, 1962; Evans et al., 1966). Germination temperature has been shown to directly affect the copy number of 4 repeated sequences in tall fescue (Ceccarelli et al., 1997, 2002).

Not only do crop plants display genomic plasticity in a field during a single growing season, plant breeders frequently accelerate the rates of spontaneous mutation in plant varieties through techniques such as mutagenesis. New crop varieties that are the result of germplasm mining and extensive out-crossing are also continually being introduced. All of these factors will contribute to a constantly changing transcriptomic profile for crops. It is thus highly unlikely that transcriptomic differences with a clear predictive cause and effect relationship with safety will be identified. It is concluded that transcriptomics cannot inform a safety assessment in any meaningful way. Technical improvements in the methodology and its application to building a robust body of baseline data are unlikely to improve the situation since a measurement of RNA transcription is not inherently a predictor of trait expression or safety. It is also noted that conventional breeding produces more numerous and larger changes in the genome than transgene insertion, facts that calls into question the need for transcriptomic analysis for unintended effects, and also question the need to single out of transgenic crops safety assessment for any more rigorous safety assessment than is applied to crops produced by other modalities of breeding (Parrott, 2005; Cellini et al., 2004).

3.2. Proteomics

Proteomic applications in the safety assessment of transgenic crops have been recently reviewed (Baginsky, 2010 this issue; Thelen, 2010 this issue; Sancho, 2010; Natarajan, 2010; Teshima, 2010 this issue; Rouquié, 2010 this issue). Research has focused principally on the use of modern protein analytical techniques—the techniques used in proteomics—for the analysis of changes in the abundance of a few well characterized proteins of interest in a particular crop (e.g. soybean allergens). These will be discussed in a following paragraph, however, it should be noted here that the application of modern protein chemistry techniques to measure the concentration of multiple allergens is not proteomic profiling, rather it is a very sophisticated and useful form of targeted analysis. As it is generally understood, proteomic profiling is an attempt to provide a very rough estimate of the concentration of all of the proteins that are expressed in an organism. Although the relative concentration of dozens of major proteins can be estimated using 2D-gel electrophoresis, and the presence or absence of hundreds more can be detected, proteomic technology is only able to detect about 1000 of potentially 10,000–30,000 proteins that could be encoded in the plant genome. Proteomic analysis is also not typically quantitative, is not rapid and inexpensive, and requires specialized equipment and software. The lack of laboratory-to-laboratory reproducibility and lack of standards for methodology, data handling and analysis all present challenges to an industry-standard approach.

From a food safety assessment prospective, the proteome is not likely a good predictor of the metabolome and, if it is a good predictor, we don't know yet how to decipher the prediction made by differences observed between two proteomic displays. An additional complication is that repeat analyses are highly variable, difficult to reproduce, and there is a total absence of accumulated knowledge about what would be a “safe” display and what should raise a red flag of caution (noting of course that food is inherently safe). Although proteomic analysis can reveal that differences exist between two or more samples, it is not yet clear that differences in the protein context are relevant to food safety risk since it is not likely that the proteome composition rigidly controls the metabolome. Recall that from a food safety perspective, it is the composition of a crop that determines the safety and nutritional value. Metabolic and cellular

regulatory circuits, differences in substrate availability and product utilization, as well as differential rates of protein turnover and differences in the external environment, all work to disconnect the proteome from a having a predictive value for the composition of the metabolome. Proteomic analysis does not appear to be useful for the detection of unintended effects either since conventional varieties with long histories of safe consumption appear to have larger proteomic differences compared with varieties developed through transgene insertion (see for example Lehesranta et al., 2005; Ruebelt et al., 2006; Lovegrove et al., 2008).

There is much ongoing research directed at developing a system with which the concentration of allergens that are present in specific sample of a food crop can be rapidly measured (Thelen, 2010 this issue; Sancho, 2010 this issue; Natarajan, 2010 this issue; Teshima, 2010 this issue; Rouquié, 2010 this issue). This targeted application of proteomic techniques is likely to be successful in the near future in defining a system for profiling and estimation of allergenic protein content. The problem here is not the technical approach but the relevance of the question. There is no published evidence that differences between varieties in allergen concentration materially change the degree of clinically relevant allergenicity of any crop. There is little quantitative information and even less agreement among allergists on the amount of any specific protein allergen that is required for sensitization and subsequent elicitation (Taylor et al., 2002; Taylor and Hourihane, 2008). Since food allergens are generally abundant proteins, it may well be that they are always present in ample amounts to participate in the allergy process (Sellge and Bischoff, 2008). Food allergy is an inappropriate response by an individual to a protein, or proteins present in a food, that are innocuous to more than 99% of human subjects under similar conditions of exposure. The adverse reaction appears to be caused by (1) host genetics, (2) the food matrix and environment of the exposure, (3) the physiological and nutritional state of the host, and (4) repeated exposure to an allergen (Sellge and Bischoff, 2008). A key observation is that patients who are allergic to various foods can have IgE molecules in their serum that react with different, and sometimes totally unrelated, proteins and combinations of proteins in the food. This formation of IgE antibodies points to a failure of the immune system to ignore or become tolerant to a protein more than it does to the allergic potential of specific proteins. Additional research is needed to explain the cellular basis for sensitization versus tolerance (Björkstén, 2008). Why do most people who are exposed repeatedly to a food allergen not become allergic? There are, in brief, many questions to be answered before the safety of varying concentrations of potentially allergenic proteins can be determined. At present no regulatory decision about safety could be justified on the basis of proteomic displays of foods as a way to categorize foods to which people already are or may become allergic.

3.3. Metabolomics

The potential for application of metabolomic analysis to food and agricultural research have been reviewed (Dixon et al., 2006; Harrigan et al., 2007; Ryals, 2010 this issue; Davies, 2010 this issue). It has been suggested that the primary value of metabolomic analysis is in support of plant breeding and selection (Harrigan et al., 2007); however, it has also been suggested that metabolomic analysis can be used in support of the food safety assessment of transgenic crops (see a discussion of this possibility in ILSI, 2004; Dixon et al., 2006). Although an extensive compositional data set is required, information derived directly from metabolomic analysis is not one of the tests currently required by food safety assessors in support of applications for the approval of new transgenic crops. The Substantial Equivalence (comparative assessment) paradigm equates changes in risk with changes in composition. Com-

parative compositional analysis provides a direct insight into any compositional changes that have occurred in the plant breeding process. Currently, in the process of targeted analysis approximately 50–100 or more macro- and micro-nutrients, anti-nutrients, potential toxicants, and bioactive compounds of special interest are evaluated. The following paragraphs describe the current potential of metabolomic analysis to add additional data of value in place of conventional analysis, or in supplemental support of a food safety evaluation.

There are several reasons to consider metabolomic analysis as a replacement for conventional compositional analysis. The first is that metabolomic analysis offers the possibility of analyzing a significantly greater number of metabolites in a short period of time than is possible with conventional analysis (Harrigan et al., 2007; Ryals, 2010 this issue). Metabolomic analysis also offers the prospect of reducing the cost of analysis for each analyte. It has also been suggested that analysis targeted at specific compounds might overlook an unintended and unexpected change in an unanalyzed component of the crop that could be of significance to health or nutrition. It is important to note that no evidence for these kinds of potentially adverse unintended changes in analytes (that are not currently measured) has appeared in the literature. Unintended changes can and do occur in all forms of plant breeding and it is for this reason that changes in nutrients and potentially deleterious compounds are evaluated in the safety assessment (Cellini et al., 2004). More importantly, since intracellular pathways and metabolic pools are tightly interconnected, targeted analysis of 50–100 or more analytes that are major components of the food (which can represent more than 95% of the composition) provides significant additional information about the metabolome. One potential outcome of research using metabolomic techniques, however, may be the identification of a small set of key indicator metabolites whose concentration can be used to predict the plant metabolome (Ryals, 2010 this issue).

Metabolite profiling techniques have been applied to potato (Roessner et al., 2000, 2001; Szopa et al., 2001; Defernez et al., 2004; Catchpole et al., 2005; Davies et al., 2005; Dobson et al., 2010), wheat (Baker et al., 2006; Ioset et al., 2007); rice (Wakasa et al., 2006; Dubouzet et al., 2007), tomato (Le Gall et al., 2003), Arabidopsis (Kristensen et al., 2005), and Gerbera (Ainasoja et al., 2008). These studies have demonstrated that profiling can provide a quick snapshot that is useful in comparing varieties and evaluating changes in metabolic pools. Taken together, these papers show that metabolomic profiling is a rapidly evolving technique; the number of components analyzed has grown from a few dozens to a few hundred and the quality of the analysis has improved as well. Most investigations focus on one or a very few pools of related metabolites and do not profile the entire plant metabolome. It has been observed in these studies that many metabolites are present at a considerable range in observed concentration (often for reasons that are not at all clear), and that the profiles of transgenic examples of a crop fall into the same ranges observed for conventionally bred plants. In a number of cases it was observed that the profile of transgenic plants more closely resembled that of parental strains than do the profiles of different varieties of that crop (see for example, Catchpole et al., 2005).

There exist some significant hurdles to be crossed before metabolomic profiling can be applied to food safety assessment. The cost of analytical equipment is high and few laboratories are prepared to perform the analysis. Owing to methodological differences, as well as differences in data analysis, statistical analysis and reporting, results obtained in different laboratories are difficult to compare thus limiting the ability to compare data over many years across the food industry. The data that are generated are often expressed as relative concentrations. Most metabolomic profiles include a significant number of unidentified metabolites. The

absolute concentrations of most metabolites are reported with poor run-to-run and sample-to-sample reproducibility, and unacceptably poor accuracy and precision. Databases with which to compare results with normal concentrations do not exist, and the reasons for the striking variability in plant metabolite concentrations are not well understood. The findings that transgenic plants composition closely resembles that of parental varieties, and that the range of concentrations observed in transgenic varieties falls within the normal ranges for those constituents found in conventional varieties, parallels the observations made using targeted analysis (for references see in Box 1, Herman et al., 2009).

It has also been proposed that metabolomic profiling could provide valuable insights into unintended changes that might take place when the composition of plants is deliberately altered using transgenic breeding. It should be noted that plant breeders have altered the macro- and micro- composition of crops for years with no apparent adverse effects resulting in spite of the black-box nature of the mutagenesis and breeding processes. Modifications in macromolecular components (protein, carbohydrate, lipid) and micronutrient vitamins and minerals have been reported (ILSI, 2004, 2008). While it is evident that intended changes in composition must be accompanied by compensatory changes in the content of other components (Davis, 2010 *this issue*), there is to date no evidence that large and unpredictable changes occur that cannot be detected by conventional compositional analysis. In fact, a high inulin potato did not reveal differences in metabolic profile beyond the intended changes (Catchpole et al., 2005), and similar results were observed with compositionally altered oilseeds (ILSI, 2004, 2008). Reasons for the apparent lack of major unintended changes may be attributed to selective screening during the plant breeding process as well as the fact that targeted analysis usually captures the composition of greater than 95% of the dry weight of a plant.

It is concluded that while metabolomic profiling is a powerful research tool that can be used effectively by plant breeders, it does not at present offer advantages for food safety assessment. It is not clear that metabolome profiling will ever meet currently applicable standards for compositional analysis. Safety assessment requires internationally validated and standardized measurement techniques and robust databases that establish norms and safety intervals.

3.4. The problem with –Omics

Omic approaches have generalized shortcomings that will need to be overcome before profiling techniques can contribute to a safety assessment. A paper that evaluated the use of the Omic strategy concluded that “False positives probably outnumber true positives. . .” (Lay et al., 2006). The reasons for this are briefly summarized below.

One of the shortcomings of Omic analysis is attributable to the rationale of the method itself. It is easy to measure many data points by profiling but few replicates are performed and few subjects are analyzed. The accuracy and precision associated with each data point is low. The methods employed are not standardized or validated. These factors are counter to the established paradigm of analytical chemistry that can be thought of as measuring a few compounds very well and very reproducibly.

A more fundamental problem with Omics is one of bias (Ransohoff, 2005). The lack of a hypothesis that underpins the omic approach leads to a bias toward false positive tests since the meaning of differences is not known. Ransohoff (2005) defined bias as “the systematic erroneous association of some characteristic with a group in a way that distorts a comparison with another group.” Appropriate statistical analysis must be applied as well; if $P=0.05$ many false positives will be observed. Lay and co-authors (2006) also pointed to fitness of use problems deriving

from poor experiment-to-experiment and laboratory-to-laboratory correlation. These authors point out that “It is important to demonstrate intra-laboratory and inter-laboratory reproducibility and standardization of new “Omic” tools as well as to maintain high quality standards using blind control and test samples.”

A difficulty that confronts traditional as well as Omic methods is that when two samples are *statistically different* in one or more aspects it is not the same as the two samples having a *biologically significant difference* between them. Omic technologies lend themselves to the observation of differences; however, the biological significance of those differences remains obscure. The differences observed between samples must be interpretable in terms of risk to be of value to the safety assessor.

4. Why diets are more important than crop varieties

As noted above, compositional analysis is a cornerstone of the comparative analysis of the safety of a food. Over the last decade, papers that document the composition of transgenic crops have appeared, and a database of common crops composition has been assembled (Ridley et al., 2004, 2010 *this issue*). One major conclusion that emerges is that transgenic crops composition falls within the range observed for conventional crops. Perhaps this should not be too surprising since acceptable agronomics is a requirement for selecting among numerous transgenic events (as it is for selections using traditional breeding methods) and plants with meaningfully altered compositions are likely to appear aberrant. After more than a decade of extensive compositional tests that have shown no differences, it has been asked if it is not time to stop requiring compositional studies on some kinds of transgenic crops (Herman et al., 2009). The argument has also been made that transgenic crops should be treated no differently than those produced by conventional breeding (Cellini et al., 2004). The reasoning underlying these suggestions is that: (1) somewhat contrary to the perceptions of non-experts, the process of transgenic insertion is generally less disruptive to the genome and more precise compared with traditional breeding methods, and that defined changes are introduced rather than the many undefined changes that accompany other breeding methods, (2) science draws conclusions based on evidence, and overwhelming evidence has emerged both from the laboratory and from years of extensive safe use in agricultural fields that transgenic breeding poses no new or different risks than conventional breeding. A conclusion that a food is reasonably safe to consume depends almost exclusively on a demonstration that the new components (usually proteins) introduced are safe to consume as described briefly above in “Assessing the Safety of Whole Foods.” Paradoxically, with the sole exception of Canada, nations regulate transgenic crops rather than new phenotypes.

It is not clear why safety assessors have focused so intensively on a demonstration that the composition of a transgenic variety is virtually identical to its conventional counterpart when no such demand is made of newly developed conventional counterparts. Perhaps more importantly, the demand for equivalence of composition also ignores the role of the food in human and animal nutrition. Transgenic crops such as insect resistant sweet corn, and virus-resistant squash and papaya are designed to be consumed whole by humans; however, the proportion of the diet in industrialized countries represented by these crops is so small that it is difficult to conceive of any mechanism by which a change in their composition could adversely affect health or nutritional status. The major transgenic crops that are planted in the world (soy, cotton, maize, and canola) are for the most part consumed as highly purified ingredients such as starch or bleached distilled oils that provide food energy but are not major nutrient sources for those who consume them.

The world's five leading staple crops are maize, rice, wheat, potato and cassava (Chrispeels and Sadava, 2003). Developers of the transgenic maize varieties that are widely planted around the globe were required to demonstrate compositional equivalence with conventional maize in order to receive regulatory approval even though conventional maize varieties differ significantly in their composition and developers of new varieties are not asked to present composition data. Transgenic rice, potato, cassava and wheat have also been developed and will likely be required to demonstrate compositional equivalence prior to their approval. It is interesting to note, however, that none of these major crops could provide a nutritionally adequate diet that would sustain human growth, development, health and performance (Table 1). The percent of the Reference Daily Intake (RDI) for representative macro- and micro-nutrients that these crops could provide was calculated for a diet that was composed exclusively of that crop. Based on this partial analysis of nutrients, it is immediately apparent that a diet based on any one of these leading staple crops would be deficient in Ca⁺⁺, riboflavin, and vitamin B₁₂. Rice and cassava are essentially devoid of micro-nutrients, while consuming a diet of potatoes, wheat or maize would lead to multiple nutrient deficiencies.

The foregoing example was intended to show that dietary diversity is required to provide the combination of nutrients required by humans for good health. Evidence demonstrates that dietary diversity correlates well with nutritional status (Román-Viñas et al., 2009). The underlying reasons for the need for dietary diversity is that few foods are capable of providing 100% of the RDI for more than a few nutrients, and that most humans strongly prefer dietary diversity to monotony. Concerned about the effect changes in composition resulting from plant breeding might have on nutritional status, the US FDA some years ago considered the advisability mandating that changes in content of any nutrient of greater than 20% should require further study (Senti and Rizek, 1974). Analysis of food intake data in the US revealed that Americans derive about 75% of their protein from animal sources, with only grains proving more than 2.5% of the mean protein intake for Americans (Table 2). Animal sources figured prominently in the daily intake of vitamin A as well with only carrots, tomatoes, and potatoes supplying greater than 5% of the mean intake (Table 2). The plant-based sources of vitamin B₆ in the diet were primarily potatoes and grain products (Table 2). Vitamin C is largely supplied by plant foods in the diet, with oranges, potatoes, and tomatoes supplying more

Table 1
Nutritional Value of Leading Staple Crops Expressed as a %RDI¹.

Nutrient	%RDI for nutrient				
	Maize	Rice	Wheat	Potato	Cassava
Protein ²	101.4	75.6	158.6	103.0	33.4
Ca	4.2	16.8	22.0	34.3	22.0
Fe	203.8	60.0	285.0	278.8	46.3
Mg	191.3	37.5	233.5	164.3	72.3
Zn	120.9	60.0	245.5	75.5	42.5
Vit C	0.0	0.0	0.0	624.4	314.4
Thiamin	191.7	35.0	226.7	190.0	99.2
Riboflavin	92.3	22.3	60.0	70.0	50.8
Niacin	136.9	60.0	273.1	188.1	73.4
B ₆	292.3	76.2	209.2	648.5	93.1
Folate	28.5	12.0	69.8	114.3	92.8
B ₁₂	0.0	0.0	0.0	0.0	0.0

¹ The table assumes that a subject consumes a diet that is 100% of each staple crop with the amount consumed based on the recommended daily energy allowance.

² The percent of the RDI of each nutrient recommended for a 75 kg adult male provided by 9210 kJ (2200 kcal) of that crop is presented. Food composition data were taken from the USDA National Nutrient Database for Standard Reference (<http://www.ars.usda.gov/Services/docs.htm?docid=8964>; accessed May 17, 2010) and RDIs are based on those published by the Institute of Medicine (NAS USA; http://fnic.usda.gov/nal_display/index.php?info_center=4&tax_level=3&tax_subject=256&topic_id=1342&level3_id=5140; accessed May 17, 2010).

Table 2
Sources of nutrients in the US Diet ca. 1972¹.

Nutrient	Source	Percentage of RDA
Protein	Grains Products (Wheat)	17.7 (14.2)
	Nuts	2.3
	White Potatoes	2.3
	Dried Beans	1.7
Vitamin B ₆	White Potatoes	11.3
	Grains Products (Wheat)	6.8 (5.4)
	Tomatoes	3.5
	Bananas	3.3
Vitamin A	Carrots	13.9
	Tomatoes	9.5
	Sweet potatoes	5.6
	Cantaloupe melon	2.6
Vitamin C	Oranges	20.4
	White Potatoes	19.7
	Tomatoes	12.2
	Cabbage	5.1

¹ Data taken from Senti and Rizek (1974).

than 50% of the needed daily intake (Table 2). The analysis showed (1) that a large proportion of nutrients in the American diet are derived from animal sources, (2) and that for most nutrients one or a very few foods were a significant dietary sources. No evidence could be found that plant breeding had an adverse effect on nutritional intakes. For these and other reasons, the FDA tabled the idea of regulating nutrient composition of new crops varieties. The ramifications of these early studies speak directly to concerns about effects of transgenic breeding on nutrient intakes since if a crop is not a significant source of that nutrient in the local diet, changes in composition with respect to that nutrient will be without biological effect and are meaningless (ILSI, 2004). Only a few crops or foods make a major contribution to supplying each required micro-nutrient. The OECD consensus documents identify which nutrients are important in each crop (OECD, 2001–2009).

There are many factors that control dietary diversity in a population; important among them are food availability, ability to pay, and consumer preference. More than one billion people in the world suffer from malnutrition that results from inadequate supply of food and lack of dietary diversity largely as a result of inability to produce the necessary food and/or poverty (Chassy, 2010). In affluent countries, consumer preferences and eating decisions play a much larger role than they do for the poor in determining nutritional status of most consumers (ILSI, 2004). The mean intake of each individual nutrient in the American diet appears more than adequate for proper nutrition and a balanced diet; however, there are large numbers of consumers who fail to consume the Recommended Dietary Allowance (RDA) for one or more nutrient on average each day (ILSI, 2004).

An example of the large variation in dietary intakes of nutrients in a normal population appeared in a study of the effects of antioxidants on human male fertility (Eskenazi et al., 2005). In this study of male adults in California it was observed that a large percentage of the subject population consumed 10 to 1000-fold lower intakes than recommended of Zn⁺⁺, vitamin E, vitamin C, and folic acid (Table 3). Presumably these large deficits in intake of vital nutrients are representative of the entire diet. The inadequate intake is most likely attributable to poor diet choices. It is concluded that if these results are representative of the population as a whole, nutrition education should be an important focus of public health policy, and that the impact of diet choices far outweigh the risks of transgenic crops in public health significance.

Table 3Distribution of Micro-nutrient intake in adult men¹.

Nutrient	Median Intake	DRI Intake	Intake Range	% Below DRI (Diet)	% Below DRI (Diet + supplements)
Folate (mg)	484	400	115–1150	65%	40%
Zinc (mg)	12	11	3.8–74	78%	48%
Vitamin C (mg)	165	90	26–3394	39%	20%
Vitamin E (mg)	25	15	2.2–833	84%	42%
β-Carotene (μg)	2579	na	376–33,444	na	na

¹ The data were taken from the California AGES study 1997–1999 as reported in Eskenazi et al. 2005.

Crops produced using transgenic technology, but not those produced using conventional breeding technologies, are considered to be novel foods. From a scientific risk perspective, foods and feeds derived from transgenic crops pose no new, different, or greater risks than those posed by conventional crops (Cellini et al., 2004; Bradford et al. 2005; Parrott, 2005; Herman et al. 2009). Human risk perception is not purely rational; novelty is a cause for precaution (Winter and Francis, 1997). Nonetheless, human societies have a long history of accepting novel foods; many foods that are eaten today around the world were unknown over most of the globe prior to the last 200–400 year (Chassy, 2010; Dubock, 2009). Figure 1 shows an incomplete map of the history of the migration of novel foods around the world (Dubock, 2009). Each of the crops shown on the map was a novel food when it was adopted in countries around the world. It is noteworthy that if international protocols such as the Cartagena Protocol on Biosafety, that seeks to tightly regulate the perceived damages caused by the trans-border movement of Living Modified Organisms, were in force during the epoch of globalization of the world's crops, the great majority of these crops would not have been approved for movement across borders and the world's regional cuisines would not be nearly so interesting, nourishing, and diverse. Crop plants produce a variety of toxic compounds (Chassy, 2010) and some can produce food allergy (Sellge and Bischoff, 2008). Although most crops were distributed around the world without regulatory control, today the presence of potential toxins or allergens would block adoption if the crops were assessed by the standards applied to transgenic crops in the world today.

5. Conclusions: The price of precaution

The emerging omic technologies have proven to be powerful tools for discovery research. The methods are useful for detection

of transcriptional, proteomic and metabolomic differences between two or more samples. Based on the foregoing discussion, it should be clear that broadly applied non-quantitative transcriptomic and proteomic profiling are unlikely to become useful tools for food safety assessment in the near future. Not only do profiling technologies have inherent methodological shortcomings when applied to quantitative analysis of risk factors, since the transcriptome or proteome is not predictive of the metabolome, the analysis also does not predict composition upon which safety ultimately depends. Proteomic techniques may prove useful in rapidly assessing the distribution of allergenic proteins in a crop variety, however, the value of such information is unclear since the quantity of a protein allergen required to sensitize is not well-established for each allergen, and it is also unknown if the quantity required is unique to each individual (for a recent review of sensitization and avoidance see: NAIAD, 2009; <http://www.niaid.nih.gov/topics/foodAllergy/clinical/Documents/guidelines.pdf>). The practice of intentional food avoidance to avert sensitization to certain foods is not recommended, however, allergists do advise that total avoidance of an allergen is the only certain means of avoiding elicitation of a response in a sensitized subject. It is thus unlikely that differences in allergen content between varieties will be of practical value.

At present, metabolomic profiling does not produce data that could be useful in a safety assessment. If future developments lead to a technology that can determine the content of numerous plant components with accuracy, precision and reproducibility more efficiently and more rapidly than conventional analysis, metabolomic screening could be an attractive tool for compositional analysis. Metabolomic profiling may someday also be useful as an adjunct to conventional analysis.

Methods are changed and improved continuously over time. Classical microbial identification methods, for example, are giving way to modern molecular methods. In the future, metabolomic technologies may well contribute to determination of composition of crops. Further research on metabolite variability and the assembly of databases that document safe ranges of metabolite profiles are also necessary. While the Omic technologies as they now exist would not be informative today as safety assessment tool, the more important question is whether the safety assessment needs more or better data. There is no evidence that the composition studies currently employed in food safety evaluation are inadequate to the task. In fact, the scientific need for compositional studies of transgenic crops has been questioned (Herman et al., 2009), and it has been argued that the safety assessment system applied to transgenic crops is not commensurate with real risks (Bradford et al., 2005). From a

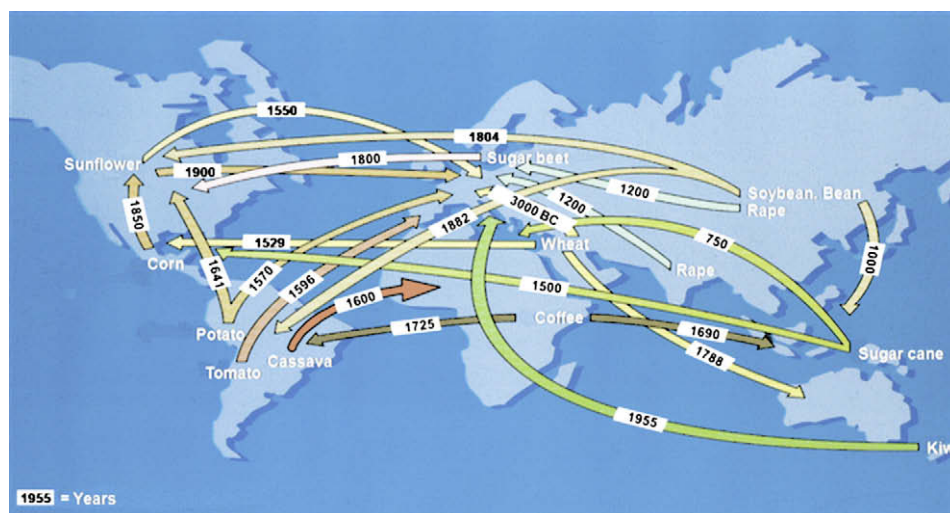


Fig. 1. Crops have been introduced as novel food to new populations for thousands of years (Dubock, 2009).

purely scientific perspective, transgenic crops pose no greater risk of unintended effects than those produced through alternative methods of breeding (Cellini et al., 2004).

The real risks in the food system are not having sufficient food, consuming a diet deficient in needed nutrients and, for a fortunate few, overconsumption. Foodborne illness is a major killer in the world. Mycotoxins and other natural toxicants in foods cause cancer, birth defects, reproductive failure and a host of other ills. Allergy poses serious health consequences to the 6–8% of children and 2–4% of adult sufferers who must have constant vigilance over the foods they eat. These factors cause morbidity and mortality in the uncounth millions.

Considerable resources are devoted around the world to the safety assessment and regulation of transgenic crops, although not a single illness has been traced to the transgenic nature of a crop. There is no scientific reason to believe these crops inherently pose risks as long as the genes inserted into them, and the products they produce, are safe. It is notable in this regard, that since the changes introduced into transgenic crops are clearly defined, the safety of inserted genes and their products can be determined more easily than can be done for a conventional variety produced by a technology such as irradiation. Ames and Gold (2000) have coined the phrase “damage by distraction” to describe over-regulation of a technology: “Putting huge amounts of money into minuscule hypothetical risks damages public health by diverting resources and distracting the public from major risks.”

The consequences of scientifically unjustified regulation of transgenic crops are numerous. Not only are resources squandered on the regulation and testing of transgenic crops, researchers divert their attention to less controversial methods, government and industry fund alternative strategies, trade barriers are created, and newly developed crops are not deployed or are under-utilized. These and other factors cause potential benefits to be lost. Two excellent examples of adverse consequences of over-regulation of transgenic crops are the failure to deploy transgenic crops to improve agriculture in sub-Saharan Africa (Paarlberg, 2008), and the fact that ten years after development, Golden Rice which has the potential to save millions of lives every year, has yet to be deployed to poor farmers (Chassy, 2010).

The question should not be whether Omics can inform a safety assessment, but why is the safety assessment so disproportionate to risk?

Conflict of interest

I declare no conflict of interest.

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