# Perspective

# The Problem with Nutritionally Enhanced Plants

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ABSTRACT   Among the next generation of genetically modified (GM) plants are those that are engineered to produce elevated levels of nutritional molecules such as vitamins, omega-3 fatty acids, and amino acids. Based upon the U.S. current regulatory scheme, the plants and their products may enter our food supply without any required safety testing. The potential risks of this type of GM plant are discussed in the context of human health, and it is argued that there should be very careful safety testing of plants designed to produce biologically active molecules before they are commercially grown and consumed. This will require a mandatory, scientifically rigorous review process.

KEY WORDS: • *bioactive compounds* • *cancer* • *diet* • *natural food* • *nutrigenomics* • *nutrition*

## INTRODUCTION

THE EVOLUTION OF genetically modified (GM) plant production is in a new phase that could have serious health consequences if the biology of these plants and their interaction with the consumer are not better understood. Currently the only widely planted GM crops are those engineered for insect and herbicide resistance, but there has been interest in marketing plant-based pharmaceuticals as well as nutritionally enhanced plants (NEPs), such as those producing vitamins and other food supplements. The best-known example of a NEP is golden rice, which is engineered for the overproduction of $\beta$-carotene, the precursor to retinol (vitamin A), but not yet commercialized.[1] Other examples include plants enriched in vitamin E[2,3] or omega-3 fatty acids.[4]

Protein-based pharmaceuticals meeting Food and Drug Administration (FDA) clinical standards have been difficult to produce in plants in their native form, in part because secondary modifications, such as glycosylation, are quite distinct from those made by mammalian cells and can contribute to the proteins' immunogenicity.[5–7] In contrast to protein-based pharmaceuticals, most NEPs only necessitate the manipulation of small molecule metabolism and will, based upon current GM crop regulation, likely be viewed by U.S. government agencies as generally recognized as safe (GRAS), thereby not requiring any mandatory safety testing.[8]

Substances produced in plants by GM technology are regulated by the food additives provision of the Federal Food, Drug and Cosmetic Act. Food additives are required to undergo extensive premarket safety testing, including long-term animal testing. However, testing is not required for foods that are generally recognized as safe by the FDA. Furthermore, it is largely up to the producer to decide whether or not the GM product and the plant that produces it are exempt from testing. To date, the FDA has not disallowed a single favorable biotech industry safety determination in over 100 completed applications.[9] Since a number of plants with altered small molecule metabolism, such as those producing high oleic acid, have already passed FDA's voluntary biotechnology review,[9] it is very likely that the FDA will accept this designation from other NEP producers. The FDA, however, has the authority to require the full testing protocol for food additives if there is evidence of possible harm.

While there has been an extensive discussion of the problems associated with aberrant secondary modifications of mammalian proteins expressed in plants, there has been no discussion about the potential harmful side effects of producing large amounts of biologically active compounds in plant hosts that have an enormous repertoire of enzymes capable of modifying small molecules in an unpredictable manner.[10] For example, with golden rice there has been concern about $\beta$-carotene absorption,[11] but none about the potential for teratogenesis. Indeed, in a recent article in *Science* on golden rice,[12] there was no discussion about safety, despite the fact that simple derivatives of $\beta$-carotene are known teratogens.[13–16]

NEPs are designed to make molecules that are biologically active in animals. Given that the transfection proce-

dures used to make GM plants cause random mutations that can alter the already unpredictable plant metabolism,[17–19] that there will be unforeseen pleiotropic interactions between overproduced metabolites of introduced enzymes and normal plant metabolism,[20] and that NEPs will likely have no required safety testing, there should be significant concern about allowing the introduction of this type of GM plant product into the marketplace.

To explain the reasoning behind these concerns, several examples illustrating how altering the human diet with biologically active compounds can have clinical consequences will be used. These examples include a tryptophan food supplement to demonstrate that an extremely small amount of a metabolite contaminant in a product can be lethal, glycolysis in GM yeast to show that changes in even the best understood metabolic pathway can produce unpredicated toxins, and golden rice to demonstrate how plant-derived small molecules based upon the $\beta$-carotene chemical scaffold may negatively influence human development.

## A TRYPTOPHAN NUTRITIONAL SUPPLEMENT

An underappreciated fact of biology is that very small amounts of a compound can have profound effects in biological systems. Plant metabolism can produce toxic products, but while these have been selected against in our food supply during the 10,000 years of crop development or eliminated through food processing before consumption,[21] they may be unintentionally re-introduced by modern technology. A good example is the health disaster caused by tryptophan in the guise of a dietary supplement.

In the late 1980s, L-tryptophan was widely used as an over-the-counter supplement to combat insomnia and depression. In 1989, more than 1,500 people contracted a rare disease, eosinophilia myalgia syndrome (EMS), manifested by increased levels of a subset of blood cells (eosinophils) and severe muscle pain; at least 37 died from a complex of inflammatory conditions.[22] The epidemic was traced to the L-tryptophan producer, which had recently modified its production procedures.[22] The purity of the toxic preparations was greater than 99%. However, a comparison of high-performance liquid chromatography profiles between toxic and nontoxic lots revealed several case-associated minor contaminants. All of those that were identified were structurally related to tryptophan or biosynthetic intermediates.[23]

Although it was believed at the time that a tryptophan metabolite was the cause of the EMS outbreak, there was no explanation as to how a very minor contaminant (less than 0.01% by weight) could cause a fatal dysregulation of the immune system. It has since been shown that tryptophan metabolites control the immune response at steps where interference by competing tryptophan analogues could have clinical consequences. For example, the rate-limiting enzyme in tryptophan catabolism is up-regulated during some forms of inflammation.[24] The metabolic products of this enzyme, which are similar in structure to some of the compounds identified in the tryptophan preparations that likely caused EMS, are made by small numbers of cells, but derivatives are orally ac-

tive and can change cytokine profiles and suppress autoreactive T helper cell type 1 cells.[25] In addition, a defect in the synthesis of another tryptophan derivative, L-kynurenine, enhances the inflammatory response.[26]

These data show that minute amounts of a compound contaminating a dietary supplement can be lethal and that chemical modifications of common, small molecules such as amino acids can lead to biologically active derivatives.[25] GM maize with high tryptophan levels has recently been introduced in association with the introduced trait of high lysine content.[9,27] Although this product is intended for animal feed, the promiscuous nature of corn pollen and other routes of dispersal such as seed mixing will certainly lead to the contamination of corn destined for human consumption.

## GLUCOSE METABOLISM

An example of how the manipulation of a well-understood metabolic process by GM technology can have unexpected consequences is ethanol production in yeast. When three genes were introduced into yeast to enhance glycolysis a few-fold, there was a concomitant, unintended 30-fold increase in the synthesis of methylglyoxal (MG).[28] MG is a highly toxic 2-oxoaldehyde that reached concentrations of 1 m$M$ in the GM yeast strain.[28] MG is mutagenic and also causes protein glycation and oxidative stress, conditions associated with diabetes and neurodegenerative disease as well as a variety of autoimmune diseases.[29] The authors of the yeast study concluded that "careful thought should be given to the potential metabolic products and their safety when a genetically modified yeast is applied to food-related fermentation processes."[28] This advice has apparently not been heeded, for another GM yeast strain called ML01 is commercially available and can be used in the production of wines in the United States.[30] This yeast is modified to carry out a second fermentation step in the wine making process, the conversion of malic acid to the less acidic lactic acid, a step normally carried out by bacteria. The commercialization of this GM yeast was allowed via FDA GRAS status and required no safety testing, such as animal feeding studies. Since there are no food labeling requirements in the United States, the consumer has no way of knowing whether or not the wine they are drinking is made with the help of ML01. GM yeast is not allowed in the production of European wines.

## RETINOIDS AND PLANT SECONDARY METABOLISM

Of perhaps even greater concern than the modification of amino acid or carbohydrate metabolism in plants are the attempts to alter plant secondary metabolism to create NEPs. Examples include increased synthesis and accumulation of lycopenes, vitamin E, and $\beta$-carotene, the precursor to vitamin A.[31] Unlike primary metabolism, which is similar in plants and animals, plants possess the ability to synthesize between 90,000 and 200,000 nonessential, small molecules, with up to 5,000 in one species.[32,33] These molecules have

adaptive functions to counteract various forms of predation and infection,[32,33] but the regulation of their synthesis and, indeed, many of their structures largely remain mysteries. For example, potatoes engineered to accumulate zeaxanthin have an unexplained threefold increase in vitamin E.[34] This enormous repertoire of phytochemicals is due in part to the fact that they are synthesized by enzymes with very low substrate specificity whose amounts and specificities are unpredictably altered by the types of mutations and pleiotropic effects associated with GM technology.[10,18–20] A National Academy of Science advisory panel on GM food safety concluded that the genetic engineering of a biosynthetic pathway "raises the potential for unintended changes in the chemical composition of the resulting food" and "could lead to an increased concentration of catabolic products" (pp. 78–79).[35] The well-publicized example of GM-enhanced $\beta$-carotene production in golden rice will be used to illustrate potential health risks resulting from this type of genetic manipulation of plant secondary metabolism.

Retinoids are a family of compounds derived from plant carotenoids that are required for many aspects of human health and development. The best-studied retinoids are retinol (vitamin A), retinal, and retinoic acid (RA). Deficiencies in retinol are found in many developing countries where insufficient dietary vitamin A is a leading cause of blindness and other maladies.[36] Dietary vitamin A can be derived from $\beta$-carotene in plants, but also indirectly from many animal tissues, in particular the liver, where retinol esters are stored. In countries such as the United States most dietary retinol is obtained from animal products; only 30% is provided by the metabolism of $\beta$-carotene from plants. However, in some cultures up to 60% of their calories come from plants such as rice,[37] which contains relatively little $\beta$-carotene. Therefore there has been an effort toward making rice that is augmented with $\beta$-carotene. It is called golden rice.

$\beta$-Carotene consists of an 18-carbon polyene chain with a six-carbon $\beta$-ionone ring on either end. Upon ingestion by animals, $\beta$-carotene is cleaved in half by a dioxygenase to generate retinal for use in the visual cycle. Retinal is also reduced to retinol, or oxidized to RA, which interacts with highly specific nuclear receptors.[38] Essentially all of the biological activity of retinoids, except for vision, involves RA. While high concentrations of retinol are toxic,[13] RA is biologically active at concentrations several orders of magnitude lower than retinol, and for this reason excess RA or RA derivatives are exceedingly dangerous, particularly to infants and during pregnancy.[14] RA is required for the development of the nervous system, both by directly controlling nerve differentiation and by generating concentration gradients that direct cell migration, embryonic segmentation, and development.[38] Therefore, RA and synthetic derivatives of RA are teratogenic. Furthermore, they can accumulate in fat and plasma, becoming a risk factor for pregnancy for up to 2 years following ingestion,[13–16,38] and multiple low doses of retinoids have greater toxicity than a single high dose.[39]

Although toxicity from carrots has been reported,[40] it is difficult to ingest sufficient plant $\beta$-carotene to cause toxicity because the enzyme in the gut required to cleave it to retinal is rate limiting. In contrast, RA and derivatives are directly assimilated and are not subject to the same physiological safety net as $\beta$-carotene following ingestion.[14,39] Because of the type of biological functions controlled by low levels of RA, any perturbation of its signaling pathways by plant-derived RA receptor agonists or antagonists will have clinical consequences. Could the GM modifications used to enhance $\beta$-carotene synthesis create such compounds?

To produce $\beta$-carotene in rice endosperm, genes for enzymes that convert geranylgeranyl diphosphate to $\beta$-carotene with high efficiency were transfected into plants. Six hundred naturally occurring compounds exist in the carotene family, and at least 60 can be precursors to retinoids.[13] Plant enzymes involved in carotenoid metabolism have homologies to human enzymes, including the oxygenase required for the cleavage of carotenoids to retinoids in the gut. Therefore, plants have the potential to make many potentially harmful retinoid-like compounds when there are increased levels of synthetic intermediates to $\beta$-carotene as in golden rice.[31,41–43] It is well known that the accumulation of a biosynthetic intermediate will lead to the synthesis of new compounds by broad-specificity plant enzymes.[44] While all retinoids and derivatives are likely to be teratogenic, good assays and information regarding the behavioral and teratologic activity are available for only three: retinol, RA, and retinal.[13] Therefore, extensive safety testing should be required before the introduction of golden rice as a food.

## CONCLUSIONS

The above paragraphs summarize published data that clearly show the following: (1) Compounds structurally related to a common small molecule can have a lethal effect when present as even a minor contaminant in a food supplement. (2) The GM enhancement of a metabolic pathway by the overexpression of genes for that pathway can have unpredictable consequences in the form of synthesizing a toxin. (3) Finally, in the case of golden rice, it is argued that biologically active compounds derived from aberrant plant carotenoid synthesis could have profound effects on human development. Similar arguments can be made for NEP-derived fatty acids that are directly incorporated into brain lipids and about NEPs overproducing vitamin E. Aberrant fatty acid composition of brain lipids is implicated in Alzheimer's disease,[45,46] and vitamin E has a role similar to RA in mammalian development.[47]

The excess consumption of a nutrient can also have negative effects. For example, a clinical trial with vitamin E supplementation showed that a relatively small dose increased the risk of heart failure,[48] and smokers who supplemented their diet with $\beta$-carotene had an increased risk of lung cancer.[49] Therefore, there is a potential for nutrient toxicity in NEPs because upper tolerable levels of many nutrients are not well established (p. 107)[35] and are likely to vary between individuals and lifestyles.

The information presented here shows that not only the

potential harm of the product should be considered for risk assessment, but the GM process itself. The data clearly invalidate the argument that "the regulatory trigger for risk assessment should be based upon the physical features of the product rather than the process by which the product was generated."[50] While it is true that traditional breeding methods can give rise to potentially hazardous products, the most recent assessment of GM food safety by the National Research Council[35] stated that GM "has a higher probability of producing unanticipated changes than some genetic modification methods" (p. 118), but it curiously concludes by stating that the risk of GM technology is no greater than conventional breeding methods. There are, in fact, no data comparing the food safety profiles of GM versus conventional breeding, and the ubiquitous argument that since there is no evidence that GM products make people sick, they are safe (see, for example, McHughen and Smyth,[50] Bradford et al.,[51] and Miller et al.[52]) is both illogical and false. There are, again, simply no data or even valid assays to support this contention.[53] Without proper epidemiological studies, most types of harm will not be detected, and no such studies have been conducted. The necessity of labeling all GM products and particularly NEPs is therefore critical if there is any hope of monitoring adverse health consequences due to their consumption. For example, it would have been impossible to identify the source of the toxic tryptophan supplement if the product were not traceable through labeling.

It follows that before NEPs producing biologically active molecules such as $\beta$-carotene, omega-3 fatty acids, or vitamin E are introduced into the food chain, great care must be taken to do rigorous, multigenerational animal safety assessments with the hope of identifying risks to health (for methods, see, for example, the 2007 publication by the National Toxicology Program[54] and Pusztai and Bardocz[55]). In addition, the products must be labeled and traceable, and the unpredictable and unintended metabolic changes that may occur in NEPs require the thorough testing of the entire edible portion of the plant, not just the designated product as is almost always done by biotech companies.[56] To date there is essentially no multigenerational animal safety testing published for GM plants[55,57] and no required labeling in the United States for any GM product. In an excellent review of our current GM regulatory process, Mandel[58] concluded that for second-generation GM products, like NEPs, "it is necessary to establish a comprehensive, efficient and scientifically rigorous regulatory system." As discussed herein there are very valid scientific concerns to support this conclusion.

## AUTHOR DISCLOSURE STATEMENT

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