The Genetic Modification of NP Pathways—Possible Opportunities and Possible Pitfalls

Richard Firn

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Abstract and Keywords

Natural Products are such an important part of the world's economy that it was inevitable that academic and industrial scientists would cast their eyes over the organisms making NPs, and consider how they might usefully, profitably or interestingly modify those organisms by changing their genetic composition. However, because of the metabolic traits of pathways leading to NPs, it is predictable that the manipulation of these pathways will sometimes give unpredictable outcomes. This chapter argues that current methods of evaluating the safety of genetically modified (GM) crops are not well suited for judging the risks of intentionally or unintentionally manipulating NPs. Fortunately, because there is a low probability that any new substances being made by a manipulated organism will possess potent biomolecular activity, the risks will usually, but not automatically, be small.

Keywords: Natural Products, NPs, genetic manipulation, genetically modified crops
Summary
NPs are such an important part of the world's economy that it was inevitable that academic and industrial scientists would cast their eyes over the organisms making NPs and consider how they might usefully, profitably or interestingly modify those organisms by changing their genetic composition. However, because of the metabolic traits of pathways leading to NPs (see Chapters 5 and 9), it is predictable that the manipulation of these pathways will sometimes give unpredictable outcomes. Current methods of evaluating the safety of genetically modified (GM) crops are not well suited for judging the risks of intentionally or unintentionally manipulating NPs. Fortunately, because there is a low probability that any new substances being made by a manipulated organism will possess potent biomolecular activity, the risks will usually, but not automatically, be small.

What is genetic manipulation?
This is not an appropriate place to discuss exactly what the genetic manipulation of an organism is or how it is achieved. There are many books and websites that will explain the process more elegantly and authoritatively. Suffice to say that scientists now have the ability to change the genetic code of an organism, changing the sequence of nucleotide bases so that a new genetic variant is created. The extent of the manipulation varies very considerably, depending on the aim of the exercise and how well it has been carried out. The changes range from minor to major and could be one or more of the following:

- The organism can be genetically modified to make a minor variant of a protein that it normally makes.

(p.208)
- The organism can be genetically modified to make a protein it normally makes in larger or smaller quantities, or in different cells, tissues or organs or at different times or in response to different stimuli.
• The organism can be genetically modified so that it now makes one or more exotic proteins, those proteins were made previously only by other species.

The commonest manipulation is the insertion of one or more new, exotic genes into the DNA of an organism. Although the term genetic engineering is sometimes used to describe the process, the current state of the art is more akin to engineering as practiced by a nineteenth-century blacksmith than a twentieth-century aerospace engineer. The location of the inserted gene is usually random and often unknown. Genes that get inserted into sequences that are essential for short-term survivorship of the recipient kill the organisms, so the few organisms that survive the insertion technique must incorporate the new sequences into a less vital part of the genome. Most techniques used to do the insertion are very inefficient, hence it is common practice to insert at least two genes: one gene coding for the ability of the recipient to resist a toxic substance and the other gene coding for the important gene one wants to insert. If the manipulated organism survives when subsequently exposed to the toxin, it must have incorporated the gene coding for resistance to the toxin into its genome and hopefully the other important gene one seeks to insert will also be incorporated.

Why might one want to genetically modify an organism to change its NP composition?

There are two reasons why one might want to genetically modify the NP composition of a plant or a microbe. The first reason is an academic one. One might want to change the NP composition in order to judge the consequences of carrying out that manipulation when seeking to understand the synthesis or role of a particular NP or group of NPs. The second reason is a commercial one, driven by a desire to change the NP composition of a plant simply to gain increased value. It is this second type of work that is the focus of this chapter.

How might one increase the value of a plant by changing its NP composition?

There are so many different NPs, made by so many different organisms and used in so many ways that it is inevitable that many different goals will be identified but these will usually be based on some generic approaches such as

• enhancing the amount of an existing NP in a species that already make that product—for example, increasing the accumulation of a taxol precursor (see Chapter 7);
• changing where in an organism that an NP is made in order to reduce the cost of recovery;

(p.209)
• changing the relative amount of some NPs so that some increase and some decrease—diverting the flow of carbon between shared pathways to increase desirable NPs and reduce the synthesis of less useful NPs;
• changing the type of NP being made;
• enhancing or changing the flavour, for example, increasing the chemicals that give an apple a Cox's Orange Pippin flavour so that a poorly flavoured apple variety with a high yield becomes more valuable;
• enhancing or changing the odour, for example, giving a pretty but odourless rose the wonderful rich rose scent of the variety Fragrant Cloud;
• enhancing NP-linked disease resistance, for example, making all gooseberry varieties as resistant to mildew attack as the variety Careless;
• enhancing the NP-linked pest resistance, for example, making cotton plants resistant to cotton boll worms;
• improving the nutritional quality, as exampled by the attempts to make rice produce more carotenoids to enhance vitamin A in the diet of poor consumers in the Far East;
• enhancing or changing the colour of an organ, for example, producing carrots with shades of yellow to intense red, by changing their carotenoid composition;
• enhancing the NPs used by parasites to locate their prey, for example, enhancing the production of volatile chemicals that parasitic insects of common plant pests use to home in on their targets, hence promoting a more effective biological control of the insect pest.

How might an understanding of the Screening Hypothesis inform attempts to manipulate NP composition?
Predictably unpredictable

If an enzyme involved in NP synthesis is introduced into another organism, an organism with its own NP profile, there is a reasonably high probability that the introduced enzyme will act on more than one substrate to give more than one product (see Chapters 5 and 9). Consequently, there is an inherent unpredictability of the outcome of genetically modifying pathways that contribute to NP diversity. The organism making NPs has evolved to give uncertain outcomes—the unpredictability is built-in and nothing to do with the unpredictability of the actual genetic manipulation process. So it must be recognised that the experience gained by studying any one example of the genetic manipulation of a plant is inevitably specific to that specific example.¹
Adding a gene to supplement NP synthesis

If one combines the classical ‘One Gene—One Enzyme’ hypothesis, which won Beadle and Tatum the Nobel Prize in 1958, with the generally accepted view of most biochemists that every enzyme has evolved to convert one substrate to one product, it seems (p.210) logical to conclude that the addition of one gene will add one new product to a cell. However, as discussed in Chapters 5 and 9, this simple view of biochemical engineering, a view that prevailed at the time that genetic manipulation of organisms was being first attempted, is too simplistic.

As explained in Chapter 9, there will be pathways where evolution will be favouring the reduction of uncertainty and pathways where flexibility and uncertainty might be selected for, or certainly not selected against. Consequently, the addition of a gene coding for an exotic enzyme into an organism must inevitably carry with it a probability of an uncertain outcome.² A detailed knowledge of the properties of the enzyme in its native organism is only partly useful because it is the properties of the enzyme in its new biochemical environment that will determine which chemicals it transforms and at what rate. This problem is most acute when manipulating pathways involved in NP synthesis because it is already known that single gene mutations in enzymes involved in such pathways can result in multiple, sometimes unexpected, changes in chemical composition. Remember the example of the spearmint mutant that became similar to peppermint that was discussed in Chapter 5? This was an example of how a natural mutation of a single gene gave rise to a very dramatic and significant change in the NP composition of a plant. The gene coding for the one enzyme that switched the type of monoterpenes from spearmint type to peppermint type could be isolated. That gene could be added to another type of spearmint plant to give a plant that in theory would now make both spearmint oils and peppermint oils. But we know that the new gene added to the spearmint, the gene that codes for one enzyme, will have caused several new products to be made. It is also possible that by making a plant which expresses both these genes, some more new quite unexpected products will appear because there will be more substrates than ever in the new plant. Furthermore, the carbon flow into the new and existing pathways will be unpredictable so the relative composition of the NPs that will
be found will be unknown. So tinkering with NP pathways is inevitably going to be unpredictable—the types and the quantities of the NPs may or may not change after a new NP enzyme coding gene is introduced and expressed. This could be an iterative process. One gene added to a plant or microbe with a rich NP profile could in theory produce many new chemicals, each with unknown properties. This prediction has already been experimentally verified. A gene coding for (S)-linalool synthase, taken from *Clarkia breweri*, was expressed in three different plant species, tomato, petunia and carnation. Each of these different species made the expected S-linalool from their own endogenous geranyl diphosphate but tomato also made 8-hydroxylinalool, petunia also made linalool glycoside and carnation made two linalool oxides (Figure 10.1). In other words, the existing NP metabolic flexibility in these three species further elaborated the expected novel substances. So unlike the uncertainty associated with Bt gene insertion, where the uncertainty in outcome lay at the ecological level, when one alters the NP composition of an organism you have uncertainty at the biochemical level and even greater uncertainty at the ecological level because NPs are so important in determining the interactions between organisms. (p.211)
Evidence for certainty
Not everyone accepts that the genetic manipulation of plants is unpredictable. Kutchan\textsuperscript{4} concluded that plants can be tailored in a rational manner with marginal effects and hailed the work of Kristensen et al.,\textsuperscript{5} as being a milestone in the public acceptance of genetically modified plants. The elegant studies of Kristensen et al. showed that it was possible to add genes coding for enzymes responsible for the synthesis of an exotic NP (dhurrin) to a plant (\textit{Arabidopsis thaliana}) with no evident developmental or morphological consequences and only very minor changes in the chemical composition. This finding would seem to counter the argument advanced some years ago\textsuperscript{6} and summarised above. However, Kristensen et al. added a new functional metabolon (a group of enzymes spatially oriented in respect to each other) and this inevitably reduced the opportunity for inherently promiscuous enzymes to act on the exotic new intermediates. Such metabolic channelling of some stages in secondary product metabolism may well be the result of evolutionary selection tempering the inherent capacity of secondary metabolism to generate chemical diversity. However, there is evidence that such channelling is not universal.\textsuperscript{7} It is possible to speculate that the advantage of evolutionary selection favouring the metabolom strategy to reduce the impact of enzyme promiscuity, rather than the alternative strategy of tightening the substrate specificity of the individual enzymes, is that a greater capacity for promiscuity can be retained and released by subsequent mutations. Indeed, such ‘hidden pathways’ were predicted as part of the Screening Hypothesis (see Chapter 5).

Consequently, the fact that one part of an exotic pathway can

\textit{Figure 10.1.} The gene from \textit{Clarkia breweri} coding for (S)-linalool synthase (LIS) was added to three different plant species (tomato, petunia and carnation) and each species produced the expected product, S-linalool. However, the existing NP metabolic flexibility in each species allowed the novel substance, S-linalool, to be converted to other substances, those substances being different in each species due to the differences in NP metabolism in each plant.\textsuperscript{3}
be inserted into a plant with predictable results by no means provides a universal lesson.

In summary, both experimental evidence and the evolutionary model suggest that the manipulation of NP pathways will often produce unexpected changes in NP composition. Such manipulation will be predictably unpredictable. But can this unpredictability be compensated for by a more thorough study of the new NP composition?

Metabolomics—what it can and cannot tell us
The term metabolomics is a recent one, a term introduced after the terms genomics and proteomics became fashionable. Genomics was the generalised term used to encompass the knowledge that comes from identifying the genes that occur in an organism. Given that genes codes for proteins, the term proteomics was introduced to cover the methods, of identifying and quantifying the proteins that are made in an organism. While genomics were something quite new, proteomics was really a rebranding of a much older and well-established subject—the study of proteins and their contribution to cell functioning. However, by using the fashionable suffix ‘omics’, the subject could claim to be a part of the ‘new biology’ that attracted so much funding at the end of the twentieth century. However, the contribution of one class of proteins, the enzymes, to the current status of the cell, was not easy to judge simply by their presence or absence. It was known that the presence of an enzyme protein in a cell did not reliably predict whether it was currently active. A number of ways were known of regulating the activity of an enzyme, many of which were highly dynamic (e.g., feedback inhibition). Thus although proteomics could address some of the unknowns that genomics could not, uncertainties remained when judging the actual metabolic functioning of a cell. Recognising that the contribution of enzymes to the current status of a cell could possibly be best judged by measuring the products enzymes make, some analytical chemists and biochemists rebranded their subject and metabolomics was born. Metabolomics is the study of the metabolome; the metabolome is the complement of all the small molecules in an organism. While the term metabolome is fashionably recent, it is misleading to claim that that metabolomics is a new discipline. The concept of analysing the chemical composition of organisms stretches back at least (p.213) 200 years, as summarised in Chapter 1. Clearly, the renewed interest in the chemical composition of plants and microbes is to be welcomed but there needs to be a caution as to exactly what such an approach can deliver.

There are some questions that need to be answered concerning the ability to fully describe the metabolome of any organism:
• How easy will it be to complete a full analysis of the metabolome of any organism, let alone a genetically manipulated variant?

• How can that information inform us about the risk that the organism presents to organisms that interact with it (humans and other organisms if a plant or a microbe is grown in an open system)?

The challenge of conducting a complete chemical analysis

It is a remarkable fact that no complete chemical analysis of any plant has been published. The full genome analyses of several species of plant are available in publicly accessible databases and the protein composition of these plants can be partly predicted from these data. However, the chemical composition of even important crop plants has not been fully explored. Why?

The general public, and even many undergraduates studying science, often under estimate the difficulty of carrying out an analysis of chemicals in a sample. Given a sample to analyse for a ‘poison’, the chemist will normally ask several questions:

• Which chemical(s) do you want to measure? Every chemical needs its own method of analysis—it is the unique properties of an individual chemical which allows the chemist to find it among the thousands of chemicals also likely to be present in the sample. If the chemical being sought is a synthetic one, which of the 80,000 chemicals made by humans is to be sought?

• At what level of sensitivity do you want the analysis to be conducted? The difficulty, hence the cost, of carrying out an analysis rises as the sensitivity needed increases.

Thus the chemist given a sample to analyse, and with only a limited budget, will either have to analyse a few chemicals very sensitively or a wider range of chemicals with less sensitivity. The effort required to conduct a thorough analysis will depend to a large extent on the information already existing about the chemical composition of the sample.

The simplest case to consider would be one where a plant had been genetically modified to make a new protein, a protein without enzyme activity, such as the introduction of the Bt toxin.¹ In this case, the concept of equivalence ² is typically applied. The case is made that the chemistry of the unmodified
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Plant and modified plant are likely to be so similar that they can be considered to be equivalent.

Clearly the ‘primary metabolites’ (what were called basic integrated metabolites in Chapter 9) are the easiest types of chemicals to be analysed because they occur in highest concentration. Because so much of primary metabolism is shared by plants, it is not unrealistic to expect that methods will become available that can routinely, and largely automatically, report the concentration of the several hundreds of primary metabolites in a sample. In a genetically modified organism with an altered basic metabolism, it is also to be expected that many significant changes will have already revealed themselves by changes to the development, morphology or growth rate of the organism. However, even such dramatic changes might be hard to interpret in plants because the plasticity of plant development will enable a small localised change to be propagated into larger ones as a result of alternative developmental pathways opening up for the whole plant. Fortunately, there is a considerable body of knowledge available to judge the effect of changing the concentration of certain key metabolites on the well-being of an organism. However, because different organs, or indeed different cells, at different times, under different conditions will have very different metabolite concentrations, there can be no universal ‘metabolomic analysis’ for even a single organism. Thus, the tools that facilitate the analysis will need sensible and considered use with the limitations and uncertainty of the analysis given some prominence.

The difficulties in providing a full and understandable analysis of primary metabolites are small compared to the problems that face those seeking to show that the genetic manipulation of NPs may or may not be of consequence. NP chemicals will inevitably be much harder to analyse because every plant and microbial species will possess a unique spectrum of chemicals. Hence, unlike the methodologies being developed for the analysis of ‘primary metabolites’, the specific methodologies needed for a thorough analysis of the NP composition of one plant species might be only useful for that species and its close relatives. Furthermore, because NP metabolism is predictably unpredictable, an organism expressing an exotic gene coding for an enzyme involved in an NP biosynthetic pathway might be producing several unknown new structures. Looking for known chemicals, for which a methodology has been...
painsstakingly developed, is hard but seeking unknowns is a much bigger challenge. Determining the structures of these new, possibly rare chemicals might require larger quantities to be extracted and ultimately confirmation of the chemical structure might require chemical synthesis—a huge undertaking for many NPs. Thus whilst the metabolomic analysis of primary metabolites might be built on a database with 1000 known primary metabolites, a metabolomic analysis of NPs might need a database 100-fold to 1000-fold larger—with the majority of that data currently unavailable. Furthermore, at what level of sensitivity should the analysis be conducted? The common experience of those analysing NPs is that if one increases the sensitivity of the analysis, the number of compounds which reveal themselves increases significantly. Given that most NPs found in an organism would be expected to play no significant role in increasing fitness of the organisms making them (see Chapter 5), it is tempting to suppose that the chemicals made in the largest amounts must be most important to the maker (for good cost–benefit reasons) but that can only be an assumption (see also Figure 4.5). To further complicate the picture, it is known that the NP composition of an organism is very greatly influenced by the conditions under which the organism is grown.

What can we deduce from the analytical data?

As outlined above, it is predictable that any significant change to ‘primary metabolism’ will very often result in a deleterious effect on the plant or microbe, hence are unlikely to be of commercial value. Furthermore, the majority of ‘primary metabolites’ are unlikely to pose a threat to those who consume them. Most generalist organisms that consume plants or microbes have evolved the capacity to metabolise these chemicals, indeed their survival depends on the ingestion of these chemicals, and it is normal for such organisms to vary the mix of these primary metabolites on an hourly, daily or seasonal basis. These consumers are likely to have evolved methods to tolerate large changes in the concentration of primary metabolites in their diet. Hence, a metabolomic analysis of primary metabolites is not easy to justify on the grounds of human food safety but it could be more important in terms of judging any undesired effects on other consumers of the genetically manipulated product. For example, many insects are highly specialised herbivores and
will have evolved with a very consistent diet and hence may not have a capacity to tolerate changes in the primary metabolite composition of their diet without a loss of fitness.

What might a metabolomic analysis of NPs of a genetically manipulated plant tell us about the wisdom of adopting the widespread cultivation of such a crop? This question cannot be answered in general terms because there will be so many unknowns and/or assumptions involved in producing an answer. In contrast to the case of plants with changed ‘primary metabolite’ composition, where there are theoretical reasons to accept that the majority of consumers of the products will be preadapted to tolerate all but very large changes in ‘primary metabolite’ composition, in the case of changes in the composition of NP one cannot make any assumptions that the consumers will be preadapted.

Let us consider, as an example, a genetically manipulated plant that has been found by metabolomic analysis to produce three novel NPs in small amounts—say 5% of the mass of the major NP normally found in that species. What understanding does this new piece of information give us in respect of the safety of this crop for humans or for other members of the natural world? There is a very high probability that these novel chemicals will have completely unknown properties; consequently, it will be impossible to say whether these chemicals pose a risk to any organism that comes into contact with the plant. The Screening Hypothesis predicts that the probability of any one of these chemicals possessing potent, specific biological activity (or more accurately biomolecular activity) is very low. In other words, at this stage of the analysis, the actual identification of the new chemicals offers little more reassurance that the theoretical underpinning of the subject overall. For the evidence to surpass the theoretical logic, precise toxicological studies of the new chemicals would be needed. To undertake such studies would require larger quantities of the new chemicals to be made or extracted. This in itself would be a considerable task if these chemicals occur at low concentrations or if these chemicals are very difficult to make in the laboratory (which many NPs are). Even if such studies were undertaken, given that similar toxicological data will be unavailable for the great majority of NPs that occur in the same plant, there would be no appropriate
reference point to use to judge whether the risks to consumers (human or otherwise) of the genetically manipulated plant would be greater, or less, than the original plant.

A further problem presents itself in that the NP composition of a plant varies significantly depending on the challenges that the plant has experienced or is experiencing. Temperature, water, insect infestation, fungus infection, vertebrate grazing and bacterial infection are some of the more common factors that can change the NP composition of a plant (see Chapters 5 and 8). Consequently, any analysis that is undertaken of the NP composition of a plant really only applies to the conditions used and a number of studies of the composition of plants grown under a range of conditions, with and without infestations and infections, would be required to provide more meaningful conclusions.

Thus, the value of metabolomics would currently appear to be greater as a research tool than as a universal tool to help assess the risks presented to humans or other organisms through the widespread cultivation of a plant with a changed NP composition.

Conclusion
The Screening Hypothesis was based on the simple idea that potent, specific biological activity is a very rare property for a chemical to possess. The hypothesis predicted that evolution would have favoured plants and microbes that possessed metabolic traits that enhanced the production and retention of NP diversity. Most of the traits predicted 15 years ago have been found; hence, the model has, so far, had a reasonable predictive value. The hypothesis predicted that these same traits would make the manipulation of pathways leading to NPs unpredictable. However, even if the genetic manipulation of an organism does cause it to produce some unexpected new products, the Screening Hypothesis suggests that these new chemicals have a very low probability of harming most consumers. Even if the new chemicals do possess some biomolecular activity that would be potentially harmful to the consumers, all consumers of NPs will have evolved generic methods of keeping the concentration of all ingested NPs low. In humans, this generic protection against NP accumulation must protect us efficiently from the thousands of NPs that a
human might encounter in a modern, very varied, and often highly spiced diet.

Thus the Screening Hypothesis predicts that the manipulation of the NP composition of plants will produce unknown outcomes but there is only a low probability of harm to human consumers. However, will the public be reassured by what in effect is a probability argument? I would suggest that there is a high probability that they will not.
What does this chapter tell us about the way science works?

When scientists first successfully inserted genes into microbes and plants, it was the potential to exploit this science commercially that was sold to, and excited, politicians (p.217) and investors. The governments of nearly all developed countries made sure that funds flowed into this new, exciting area of science, often at the expense of other areas of biology. This flow of funds automatically resulted in a bonanza for those with a training in molecular biology, with every university or research institute hiring new staff to work on this new, well-funded topic. Coincidentally or not, the early days of genetically modifying organisms corresponded with the acceptance that academic work should enrich society (and possibly academics). This was the first period of biological research when wealth generation and pure research became intertwined. It was also a time when the public became increasingly sceptical of science, a scepticism fed by the media. However, few of the public realised that the science they heard about in the media was science as revealed by press release from interested parties. The major science journals, very profitable ventures in themselves, feed journalists with predigested stories every week, in order to boost their own prestige. The science journal editors make sure that they hit the right buttons with the popular media; therefore, simplified, gross generalisations soon enter any debate in the popular media. Companies, universities, funding agencies and NGOs all learned the PR tricks with varying degrees of skill. Nowhere was this distortion of a proper scientific debate more evident than in the GM controversy. Press releases from the advocates of GM and from their opponents about the safety of GM foods, or the potential environmental harm that might result from growing weed-free or pest-free crops, polarised the debate. The public began to question the impartiality of scientists. Even the UK government found it difficult to identify GM scientists in the United Kingdom who could appear before the media without being vulnerable to claims that that person had a vested interest in supporting GM—many government institutes and universities had accepted money from GM companies. Scientists were slow to realise that once their work had become associated with wealth creation, their motives would inevitably be questioned. The GM food saga revealed, once
again, that the mechanisms that exist to discuss science policy, which were never perfect, were seriously deficient in the new era of the biased press release. (p.218)

Notes:

(1.) If one adds a gene coding for Bt toxin (a protein that is very toxic to insects made by some species of bacteria) to a plant, it is predictable that the plant will be more toxic to most insects that eat the plant (although mutants in the insect population will become increasingly resistant to Bt toxin). There is uncertainty as to the consequences of those insects being killed, if the genetically modified crop is grown in the field because ecological interactions are complex—that is why so much effort and energy has been spent arguing as to whether Bt corn or Bt oilseed rape should be grown in Europe. There is little or no uncertainty about the effect of humans or animals eating such crops. First, in the case of grain crops, the gene coding for the Bt toxin could be placed under the control of a promoter which ensures that the Bt gene is only expressed in the leaves or stem and not in the grains that are harvested for use. Furthermore, experiments could be conducted to determine precisely how much Bt toxin needs to be ingested to harm laboratory or farm animals (and by extrapolation humans). If the actual dose that the animals would receive after eating the crop is very much below that level, it could safely be predicted that the crop is ‘safe’ for farm animal or human consumption. Thus, with enough effort, a ‘channel’ of predictability can be constructed, linking the insertion of the gene(s) and the consumption of one product by a few populations of organisms. But the sea of uncertainty around that channel should be acknowledged, not ignored. With effort, a logical analysis could reduce the unknowns and confidence about the wider safety issues associated with the growing of the Bt crops could be increased. The past decade has seen the adoption of a number of different Bt crops by an increasing number of farmers in North America, parts of South America and China with no reported problems to consumers of the crops but the long-term effects on the agricultural ecology are still not fully documented. This has given confidence to the advocates of GM crops but the satisfactory experience (to date) of the Bt crops cannot provide a universal lesson. There can be no universal lesson about the wisdom of growing a GM crop because each gene added is unique. In the case of the
manipulation of the NP composition, it might be that establishing a ‘channel of predictability’ will be very difficult due to the inherent unpredictability of NP metabolism.

(2.) There might be a notorious early example of the unpredictability of manipulating pathways in microbes as evidenced by the presence of toxic contaminants in tryptophan, once sold widely as a ‘health supplement’. It has been argued that in the late 1980s, the main supplier of tryptophan, the Japanese company Showa Denko, had adopted a novel, genetically modified strain of bacteria to produce L-tryptophan and their purification failed to remove some new, unexpected minor contaminants which were highly toxic. See Crist WE. *Toxic L-tryptophan: shedding light on a mysterious epidemic*, http://www.seedsofdeception.com/Public/L-tryptophan/1Introduction/index.cfm


(8.) **Equivalence.** Crops grown for human consumption are usually judged on the basis of the equivalence of the processed product to existing products. So, flour made from Bt wheat could be regarded as equivalent to flour made from ordinary wheat. It is possible to aim for equivalence by choosing carefully and targeting where the added genes are expressed. For example, one can control in which tissues a gene is expressed so it would be possible to have the Bt gene silent in the wheat grains themselves so that there would be no Bt protein in the flour (proteins do not usually move between plant organs). One could check out the flour to demonstrate that the Bt toxin level was indeed as near to zero as the analytical method would allow. But what is the equivalence when dealing with NP composition? Are the two very different flavours of apples, Granny Smith and Cox's Orange Pippin, equivalent? Is spearmint equivalent to peppermint? So the concept of equivalence is hard to tie down and is difficult to apply universally.

(9.) The ‘primary metabolites’ include such compounds as simple sugars, amino acids, common lipids, the common nitrogen compounds and many phosphorylated compounds.

(10.) A fine example of this problem is the detection of an NP responsible for one species of insect finding its host plant. An analysis of the volatile chemicals made by the plant showed some chemicals made in large amounts, others in smaller amounts and some in minute amounts. By analysing the nerve output of the insect's olfactory cells to these chemicals, the insect was found to be responding to one of the very minor chemicals.

(11.) For example, many microbes make rather few NPs when growing rapidly and only start making large amounts of NPs when they enter a much slower growth phase. In plants, insect or fungal attack induces very large changes in NP composition and there is also evidence that plant nutrition, light and temperature can influence NP composition. In studies of the NP composition of a single species, wide variations of NP composition have been found between individuals in the population. In large perennial plants such as trees, the NP composition of leaves, for example, on different branches can reveal significant changes in NP composition.
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