

Understanding the Unintended Effects of Genetic Manipulation

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This article provides background and explanatory information for The Nature Institute's project Unintended Effects of Genetic Manipulation (www.natureinstitute.org/nontarget).

Genetic engineering experiments are conducted with a specific objective in mind. The scientists aim to transfer foreign DNA into a host organism and effect specific changes within that organism. For example, the plant may be meant to become herbicide- or pest-resistant, to grow more quickly, to form compound instead of simple leaves, or to synthesize new substances. These are the intended (target) effects and the altered organism is called a transgenic organism. (Instead of this technical term, the expressions “genetically modified organism” (GMO) and “genetically engineered organism” are widely used and will be used interchangeably by us. We also use the expression “genetic manipulation” to indicate the process by which transgenic organisms are produced.)

Unintended (or, as I will sometimes say here, “nontarget”) effects are those experimental results that appear in addition to or, in some cases, instead of the intended results. Within the scientific literature a variety of virtually synonymous terms and expressions point to such unintended effects of genetic manipulation. Scientists speak of nontarget effects, unexpected effects, unintended consequences, or the pleiotropic effect of the gene. (Pleiotropy means that a gene affects more than one characteristic.) Sometimes a research report will signal unintended effects with a phrase such as,

“Surprisingly, we found . . .” or “Unexpectedly, the plants . . .” An instructive analogy is the common occurrence of drug “side effects.” Here, too, the intended effect of the drug is often accompanied by numerous, sometimes harmful, effects arising from the drug’s unanticipated action on nontarget tissues or processes.

Unintended Effects of Genetic Manipulation in the Scientific Literature

Of course, such nontarget effects are not always reported in research reports. As Dougherty and Parks (1995) write:

Organisms that do not perform as expected are discounted as defective or atypical in some way, are not the subject of study, and frequently are not reported in the literature. It is important, therefore, to recognize that most published works represent a selected subset of transgenic organisms that have been produced. These built-in biases have hindered our understanding of how transgene expression impacts the endogenous [host] gene.

I would add that we need to consider how the organism as a whole can be affected by the genetic manipulation. Reports in the scientific literature handle unintended effects in widely differing ways:

- In the kinds of cases Dougherty and Parks refer to, unintended effects are simply not reported, although some may have been

observed. There is no way to know how often this occurs.

- Sometimes no unintended effects are explicitly reported, although the research article gives evidence that they were present. We have found articles in which, for example, the morphology of plants was illustrated, but the visually evident abnormal development was not described as such (see Müller et al. 2006).
- The intended effect is the focus of the article, but unintended effects are also reported.
- Unintended effects are the primary subject of the research. Such investigations include risk assessment studies to establish whether there are unintended effects and, if so, whether they present safety or health concerns.

The articles we have used to report on unintended effects constitute a mix of the last three categories. We have selected research where either the authors have reported unintended effects or we have designated them as such in view of the evidence presented in the study, although the authors may not have drawn special attention to any unintended effects.

Because we are focusing on unintended effects related to transgenic organisms, we have not included examples of unintended effects that can arise in traditional breeding (selection and crossing) or in more invasive practices such as chemical or x-ray mutagenesis. Especially this latter can call forth extensive changes within the organism and many unintended effects have probably gone undetected, since — oddly enough — there is no regulation of crops produced using mutagenic methods. (For discussions of some of the kinds of unintended effects that can arise in traditional breeding, see Gepts 2002; Halsberger 2003; Kuiper et al. 2001; National Research Council and Institute of Medicine of the National Academies 2004.)

We have also not included articles that report the absence of unintended effects in transgenic organisms. Such studies may find, for example, that a genetically modified crop was, in terms of an analysis of selected nutrients and antinutrients, “substantially equivalent” to its unmanipulated parent variety. While such studies may find no significant differences in the levels of compounds they studied, they have not done a complete analysis of the plant’s composition.

Such a complete analysis is impossible, but with the technique of metabolic profiling, scientists can at least gain a broader picture of how a genetic manipulation affects the organism’s physiology (Tretheway 2004). For example, a study was carried out to screen for potential unintended effects in transgenic potato lines in which the pathway for sugar (sucrose) breakdown was altered in different ways (Roessner et al. 2001). The researchers did a metabolic profile of 88 different substances (metabolites) that could be identified in the tubers. Surprisingly, the transgenic potatoes had altered levels of most of the 88 substances, even though the production of many of these substances, such as amino acids, was not known to be related to the sugar breakdown pathway targeted by the genetic manipulation. The various transgenic lines differed from each other as well as from the non-manipulated potatoes. The transgenic potatoes often produced more amino acids than the non-manipulated potatoes. Moreover, nine substances were found in the transgenic potatoes that could not be detected in the non-manipulated potatoes. Since a plant makes thousands of substances (the mustard plant *Arabidopsis* is known to make more than five thousand different compounds), such an example gives only an inkling of the extent of unintended changes that might occur in the organism’s physiology.

One other example: In transgenic potatoes that were altered to synthesize their own insecticidal substances, researchers investigated whether the production of particular substances in the leaves might have been altered as well (Birch 2002).

They found that this was the case and that the reduced levels of glycoalkaloids, which are toxic to mammals and some insects, may affect the potatoes' natural ability to ward off mammal and insect pests. In a screening for "substantial equivalence," such a change would never have been detected, because no one would have looked for it. While this particular unintended effect may have no bearing on farm animals or humans who eat potato tubers, it is in fact a change in the plant's physiological make-up and may have important implications for agricultural practices, such as pesticide use.

In relation to testing for unintended effects of transgenic organisms on the environment, Marvier (2001) points out that "the vast majority of the examined toxicity studies reported in USDA petitions for deregulation relied on appallingly few replicates, usually just three or four per treatment group." As a result, while such a study may conclude that there are no statistically significant effects, the lack of evidence may have been due to the small sample so that real effects may in fact have been overlooked.

These examples show that it is simply inaccurate to state that there are no nontarget effects in any given case. Rather, one must say: no nontarget effects were found within the boundaries of the analysis carried out in this case. If you fish with a wide-meshed net, you may not catch sardines. In scientific terms, the absence of evidence is not evidence of absence.

Another problem arises when studies report that the transgenic plants produced in the experiment were "phenotypically normal." This usually means that the researchers — who rarely are plant morphologists — detected no gross differences between the unmanipulated controls and the transgenic plants. But usually this judgment is not based on a rigorous comparative morphological study of the plants in question, much less a thorough compositional assessment of those aspects of phenotype that are not visible to the naked eye. Therefore such statements must be taken with rather large grains of salt.

Moreover, unintended effects often become apparent only under certain conditions — for example, when the plants are grown under different experimental conditions or when they encounter the vicissitudes of growing in a natural, rather than a laboratory, environment. Transgenic insecticide-producing (*Bt*) cotton does not, for example, always adequately protect the plants against insect pests. In China, farmers and agronomists have observed that *Bt* cotton is especially susceptible to the cotton bollworm later in the season when it is producing cotton bolls and when it has been hot. In experiments researchers found that *Bt* cotton subjected to a short period of high temperature while it is forming bolls produces significantly less *Bt* toxin in its leaves (Chen et al. 2005). In some unknown way, the high temperature suppresses *Bt* production at that particular stage of the plant's development. If a study remains within the narrow confines of particular experimental conditions and concludes there are no nontarget effects, then this conclusion, once again, must be taken with caution, for it means: no unintended effects were observed when the researchers applied their specific lenses of analysis under the specific conditions of this experiment.

All this indicates that in providing examples of unintended effects of genetic manipulations we are surely identifying only a portion of what actually occurs. Whether it is more than the merest tip of the iceberg, we cannot say. But the cases we have identified and summarized in our reports are documented within the scientific literature and form a good starting place to consider the implications of genetic modifications. One thing is already abundantly clear from the reports gathered to date: among the common types of genetic manipulation and among the main types of plants, agricultural and otherwise, used in experiments, there appear to be no notable exceptions to the fact that nontarget effects, often striking in nature, have been occurring.

We are quite aware that the studies we have selected can be criticized and questioned in various ways. That is simply the nature of experimental science. The theoretical framework, the experimental set-up, and the way conclusions are drawn are always limited and in one way or another biased, since no one can take everything into account in one study. If a study finds statistically significant unintended effects, this judgment is based on a certain kind of statistical operation and, in addition, does not necessarily indicate the role these effects may play under other conditions. Similarly, just because an unintended effect is not found to be statistically significant in a controlled experiment does not mean it will be insignificant in the wild.

Researchers also bring their personal biases — their specific background and context within the scientific community — to the work they do. In journal articles it is quite easy to recognize, by the tone of the writing and the type of literature cited, which data are given particular consideration, and which facts are brought together (or left out) in order to draw conclusions. This does not mean the results are merely subjective; it means they are informed by particular perspectives. There's no getting around this, much as some scientists would like the public or policy makers to believe they are reporting “just the facts.”

In our reports on this website, we provide brief compilations of the evidence for unintended effects that are contained in the articles cited. Of course, we too have been selective — our focus is on reporting unintended effects. Interested readers must consult the source articles that we cite if they want to form more nuanced judgments concerning a specific study. We believe that a primary contribution of our work is to bring together the diverse cases of unintended effects so that, out of a broad overview, we gain insights that are not available when we focus only on the results of this or that study.

Different Categories of Unintended Effects

In a review article, Cellini et al. (2004) make a distinction between “predictable unintended effects” and “unpredictable unintended effects.” Predictable unintended effects go beyond the target effect but “may be explicable in terms of our current knowledge of plant biology and metabolic pathway integration and interconnections.” For example, transgenic canola plants had increased levels of carotenes in their seeds — the target effect — but also had reduced levels of other substances (tocopherols and chlorophyll; see Shewmaker et al. 1999). While unintended, these results were not wholly surprising, since it was already known that the metabolic pathways of carotene production and those of tocopherols and chlorophyll are linked. So if one takes a slightly larger context into account, the reduction of the amounts of the latter substances might even be expected.

Unpredictable unintended effects, in contrast, fall outside present understanding — the scientists simply do not know how to interpret the effects. In the above experiment where the carotene content in transgenic canola was raised, the composition of fatty acids was also altered; there was more oleic acid and less linoleic and linolenic acid. As the authors note, this “alteration in fatty acid composition was unexpected” (p. 408), since there is no known connection between fatty acid synthesis and the carotenoid pathway.

Interesting in this context is how an unintended effect in one experiment may become an intended effect in the next one. In one study researchers investigated how genetic manipulation of starch metabolism in potatoes affected the production of other substances (Roessner et al. 2001). They found, to their surprise, that the levels of amino acids were also altered. This was surprising since there is no known link between the starch and amino acid metabolic pathways. In a study carried out subsequently, in part by the same researchers, an

increase in amino acid content was also found, but in this case the finding was not reported as unexpected, since it had been found in the previous research (Regierer 2002). However, there was no better understanding of connections, and the finding remained a riddle. In this case a nontarget effect became an expected effect, although it was not directly targeted. So we could add another category of unintended effects: those that are predictable, but not understood.

Regardless of how we group unintended effects, the main point is that the target effect of a manipulation does not arise in isolation from the rest of the organism. It is not simply added to the organism's suite of characters. The organism, by virtue of the genetic manipulation, develops differently and the whole organism is engaged in this development. The gene construct is not portioned off from the rest of the organism, and if it were, the new characteristic would never arise, because the production of new substances demands the physiological and metabolic activity of the whole organism. In this sense unintended effects can always be expected because they are — as is the intended effect — expressions (symptoms) of the way the organism changes and adapts to the genetic manipulation. That is, unintended effects arise because the organism is a tightly integrated whole; but because we have hardly begun to understand the complex web of interactions within this whole, the effects remain unpredictable.

When an organism is genetically altered, changes in its morphology, physiology, or metabolism will change the way it relates to its environment. Just as the gene is not isolated from the organism, the organism is not isolated from its environment. The expression “unintended (or nontarget) effect” is most widely used in the scientific literature in relation to effects that a transgenic organism may have on its environment. Can the implanted transgenes escape into the wild via spread of seeds or through cross-pollination with weedy relatives (Reichman et al. 2006; Warwick et al. 2007; Zapiola et al. 2008)? Can

residues of the *Bt*-toxin from genetically modified crops affect nontarget insects (Rosi-Marshall et al. 2007)? Can giving animals feed that contains GM plants have adverse effects (Prescott et al. 2005; Flachowsky et al. 2007)? Can the increased use of the herbicide glyphosate, which is sprayed on herbicide-resistant crops, have its own nontarget effects (Owen and Zelaya 2005)? Since such effects have been found, it is clear that, just as there are systemic ripple effects of genetic modification within the organism, so also there are ripple effects into the environment.

Explaining Unintended Effects

Unintended effects within the host organism are not necessarily due only to the gene directly related to the intended effect. There are numerous ways in which the genetic manipulation can affect changes in the host organism. Although the genetic intervention may seem simple, in reality one is dealing with a complex web of relations that can be altered in manifold ways. Here we mention just some of the possibilities to give an impression of why genetic manipulations are largely unpredictable.

- 1) Scientists discovered that it does no good to insert only the target gene into the host organism. This does not give the desired effect. What is needed is a gene construct consisting of DNA sequences from various sources (see Table 1). For example, in most genetically engineered plants this construct consists of plant, viral, and bacterial DNA in addition to the gene for the target effect. This means that each of these segments of DNA may elicit its own ripple effects in the organism. For example, DNA related to antibiotic resistance or herbicide resistance is often part of a gene construct and functions as a so-called marker. When hundreds or thousands of plants have been subjected to potential genetic alteration, researchers can treat them with the appropriate herbicide and

Table 1. The Gene Construct — *What A Genetically Engineered Plant Contains*

Plants are never modified by adding just one foreign gene. A whole gene construct made up of DNA from different sources is shot into the plant (see Table 2). Generally, such a gene construct in transgenic plants contains at least:

- A target gene derived from a different organism.
- A promoter, often derived from a virus, which ensures expression of the target gene in all the plant's tissues or, sometimes, mainly in specific tissues.
- One or more marker genes to help biotechnologists identify those plants that have been successfully transformed; these genes usually are derived from bacteria.
- A circular strand of DNA (called a plasmid) from a bacterium. All the other DNA sequences are biochemically inserted into the plasmid, which carries the foreign DNA into the plant's cells.

When the experiment goes according to plan, every cell of the organism contains, in the end, at least one copy of the complete construct. Through the gene construct, the metabolism of the cells is altered and the plant is obliged to produce novel substances. In "Roundup Ready" and "Bt" crops, the transgenic proteins are produced continually in every cell of the plant. By contrast, in normal protein metabolism, most proteins are specific to particular tissues and temporally restricted functions.

In the case of Monsanto's glyphosate-resistant Roundup Ready soybeans (line "40-3-2"), the gene construct was reported to have been integrated at one place (locus) in the plant's genome. It contained the following DNA sequences:

- One copy of the target gene (DNA sequence, originally derived from the bacterium *Agrobacterium*, for resistance to the herbicide glyphosate).
- Cauliflower mosaic virus promoter, so that the target gene would be expressed in all parts of the plant.
- DNA from the petunia to bring the gene product (an enzyme) to the chloroplasts so that it is present in adequate amounts to protect the leaves when the plant is sprayed with the herbicide.
- DNA from a bacterium (*Agrobacterium tumefaciens*) to regulate stable production of the enzyme needed for herbicide resistance.

A number of DNA sequences that were originally part of the DNA construct were not integrated into the soybean's genome, including the primary marker (GUS) gene and a bacterial marker gene (nptII), both from the bacterium *Escherichia coli*. In 2000, four years after Roundup Ready soybeans had been commercially grown, Monsanto scientists reported the discovery that additional DNA sequences had been incorporated into soybeans: an additional segment of the target gene, located next to the construct described above, and a second insert comprising yet a different segment of the target gene. According to Monsanto, neither of these is expressed in the plant. This example indicates the unpredictability inherent in the actual integration of a gene construct into a plant's genome.

Sources

FDA/CFSAN Memorandum: <http://www.cfsan.fda.gov/~rdb/bnfm001.html>.

Health Canada: http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/ofb-096-100-d-rev_e.html.

the ones that survive possess the marker gene and, with some likelihood, the target gene. Insofar as this marker gene remains connected to the target gene, it and its effects will accompany the plant and its progeny through the generations.

2) Since the gene construct is inserted into the host organism in a haphazard fashion (see Table 2), the insertion process itself may be fraught with unintended effects (Day et al. 2000; Freese and Schubert 2004; Forsbach et al. 2003; Latham et al. 2006; Makarevitch et al. 2003; Wilson et al. 2004):

- Since multiple copies of the construct are shot into tissue cultures or inserted into bacteria (*Agrobacterium*) that are subsequently used as vehicles to get the construct into cells, multiple copies of the construct and/or multiple fragments thereof may be inserted into the plant's genome — in different places in the same chromosome or in different chromosomes.
- A single gene construct may be broken up and fragments inserted in different places in the host organism's genome. Such fragments may or may not have any direct effects.
- The insertion of the gene construct or its fragments may interrupt a functional gene at the place of insertion (“insertional mutagenesis”) and thereby cause a loss or change of function of that gene.
- There may be rearrangement of DNA in and around the site of insertion.

3) Assuming the target gene is functionally incorporated into the host organism's genome, the organism will produce new substances, most commonly enzymes, that lead to the intended effect. The host organism's metabolism can interact in unknown ways with the transgene and its products. For example, the organism may react to the genetic manipulation by shutting down the expression

of the transgene; this is called transgene silencing (Matzke et al. 2000). An organism may even overcompensate and stop production of similar substances it would normally produce. As a result, the effect of the genetic manipulation becomes the opposite of what was intended — less, rather than more, of the desired substance is produced (Tretheway et al. 1998).

4) Tissue culturing — the process of regenerating the manipulated plant tissue into full plants — may itself cause an increase in mutations in the plant, which can have their own effects and possibly interact with the foreign genes in unexpected ways (Jain 2001; Filipecki and Malepszy 2006).

5) Organisms belonging to the same species are not genetically identical. Each individual organism possesses a unique genome. This is called its genetic background, and it is into this background that the transgene is inserted. Therefore, the same gene construct can elicit subtly different effects in different specimens (Horvath et al. 2001). This plays a role, of course, in the selection of the specific variety of soybean, corn, or other crop one uses as the parent variety for genetic modification.

6) Unintended effects may arise when the transgenic organism is subjected to changing environmental conditions and its metabolism responds to these conditions by producing different substances and structures, which may affect, and be affected by, the transgene and its products (Gertz et al. 1999; Chen et al. 2005).

TABLE 2. Producing a Genetically Modified Plant

Here is an example of how scientists produce a genetically modified plant. Monsanto Company was interested in developing genetically modified plants that would be resistant to their best-selling herbicide, Roundup, which contains as an active ingredient glyphosate. Glyphosate inhibits the synthesis of an important enzyme (EPSPS) that is part of a primary metabolic pathway from which many essential substances are derived. When sprayed with glyphosate, a plant turns yellow and dies within a week or two.

In the 1980s Monsanto scientists discovered a mutant strain of a soil bacterium (*Agrobacterium* spp. strain CP4) that was not killed by the herbicide. This bacterium forms its own variety of EPSPS enzyme, which is slightly different from plant EPSPS and is not affected by glyphosate. They succeeded in isolating the DNA (gene) connected with the synthesis of this enzyme. They fashioned a gene construct consisting of a variety of DNA from various sources (virus, plant, and bacteria; see Table 1) that they hoped would allow the uptake of the gene and then the expression of the bacterial EPSPS enzyme in plants. This construct was multiplied in bacteria and then re-isolated. The DNA construct was precipitated onto microscopic gold particles and these microprojectiles were shot into embryonic tissue from immature seeds of soybeans. The tissue was cultured in a nutrient medium. Thousands of shoot tips needed to be generated from the tissue cultures to find a few that actually had taken up the gene construct and were herbicide-resistant. From these shoots whole plants were regenerated, which became the first herbicide-resistant, genetically modified soybean plants. Field testing ensued and one particular line of transgenic soybean was highly resistant to the herbicide and also had good agricultural qualities. This “40-3-2” line was selected and cultivated further, finally becoming the parent line of subsequent Roundup Ready soybeans marketed by Monsanto Company.

Sources

McCabe et al. 1988; Padgett et al. 1995.

In our reports of unintended effects, the particular effects may be related to any one of the above factors or several of them. For the scientific investigator, discovering the “mechanism” behind an unintended effect is an intriguing challenge. But so far as the organism and the environment are concerned, this is of secondary importance. The effect itself is what matters.

What Unintended Effects Can Teach Us

In a sense it is paradoxical that genetic manipulation, which aims to effect discrete, clearly demarcated alterations in organisms, can make us more aware of the dynamic, context-dependent nature of life. Unintended effects do that. The manipulated organism is a dynamic, active context for the inserted genes and therefore

does not simply take in genetic instructions passively and do as it is told. That is why, among the many plants and animals that may have been modified by a genetic experiment, only very few are found that fit the researchers’ expectations. In order to be successful, the genetic engineer must circumvent as far as possible the active, adaptive, and changing organism.

The manipulated organism does not exist in a vacuum. It is critically influenced by its environment and influences it in turn. In this dynamic, changing relation between organism and environment, unintended effects have further opportunity to make themselves known. So while the genetic engineer wants control, stability, regularity, and constancy, life plays itself out in dynamism, unpredictability, and change. This has become evident even at the level of genes

themselves. Although the gene figures in the popular view of genetic engineering as a kind of stable unmoved mover, within genetic research of the past decades the gene concept has undergone radical transformation — and has come to be viewed in more dynamic and contextual terms (Beurton et al. 2000; Holdrege 2005; Holdrege and Wirz 2001; McClearn 2006; Pearson 2006).

So we learn that at all levels of life — from gene to organism to environment — we need to take into account dynamic, changing relations. The ideal to control life through genetic engineering rather as we control a manmade machine begins to appear sadly one-sided.

To ask, “Do we understand what we are doing?” is not merely to ask whether, in some narrow sense, we understand the genetic “mechanisms” involved. Rather: Do we truly fathom the consequences of our actions? We are producing hereditary alterations in organisms, with all their consequences for the life of the organism itself and its environment, and the array

of unintended effects can alert us to the far-reaching impact we are having on life and to how little we actually know about what we are doing. I could also say: genetic engineering experiments — when we take into account all the unintended effects and not just what succeeds from a particular scientific, agronomic, or economic standpoint — could make us keenly aware of our ignorance. (See Vitek and Jackson 2008.) Awareness of ignorance brings with it circumspection and careful consideration. It wisely counterbalances a gung-ho “let’s get the job done” attitude. We do well to cultivate such caution when we are dealing with a powerful technology that is changing organisms and environments around the globe — organisms and environments that cannot simply be restored to their previous state when we discover the unpredicted results of our transgenic experiments.

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