Science-Based Risk Assessment for the Approval and Use of Plants in Agricultural and Other Environments

R. James Cook

The use of plants as crops to produce food, fiber, and other products has an amazing record of environmental safety. The greatest risk with any plant deliberately introduced into a new environment is its potential for invasiveness beyond the planted area—to become a weed—but even here crop plants have an outstanding record of safety. Crop plants typically must be managed as "weedy" volunteers after harvest, and some varieties of crop plants are more prone than others to carry over in the field after harvest. I am not aware, however, of a crop plant having become an invasive weed because of plant breeding. This remarkable record of safety for crop plants would indicate that either (1) the risks to the environment presented by crop plants are low; (2) the extensive field testing prior to commercial use and the institutional assessments and decisions on which plants or varieties to grow as crops have been sound; and/or (3) the management practices in place have been adequate to mitigate any risks inherent with plants.

Of the "risks" that have been associated with plant-based agriculture, virtually all are the consequence of the *management* practices needed to grow crop plants and keep them healthy. The environmental risks include soil erosion because of tillage used to form a seedbed and control weeds, nitrates left unused in the soil because of overfertilization (or underutilization because of disease), nontarget effects of pesticides on beneficial insects, and smoke from burning crop residue. These are just some of the more important environmental impacts associated with the growing of crops. Genetic modification of crop plants is unquestionably the best route to mitigation of these risks, but must be accomplished without introducing new risks.

In spite of the safety record, there is public concern worldwide that plants with genes introduced from outside their normal range of sexual compatibility—the so-called "genetically improved" plants (GIs)—might present new risks to the environment. Some of the more frequent claims expressed in the popular press or on the many websites established for the express purpose of raising concerns, include: virus-derived genes used as a source of virus resistance in crop plants will lead to new viruses with potential to kill native plants; the use of genes from *Bacillus thuringiensis* (Bt) as a source of resistance to insect pests will lead to super pests; the antibiotic-resistance genes used as selectable markers will transfer from plants to human pathogens, further exacerbating the medical dilemma of antibiotic resistance in these pathogens; or the use of crops with resistance to glyphosate (Round-up) will lead to greater use of herbicide in amounts that would "annihilate many life forms." Claims such as these are not supported by science. Nevertheless, governments, research organizations and companies must respond to these claims, and must have in place the means to scientifically assess and report on real risks presented by crop plants. This paper outlines an approach to a science-based risk assessment for plants intended for use in agricultural and other managed environments.

Focus on the Product Rather than Process

Numerous studies have been conducted over the past 20-25 years by governments and scientific societies on the safety of rDNA-modified organisms. One of the earliest studies was released by the National Academy of Sciences (NAS 1987). The four conclusions given in that landmark study still apply and are repeated here, with my notes of clarification or emphasis provided in parentheses.

- There is no evidence that unique hazards exist either in the use of rDNA techniques or in the transfer of genes between unrelated organisms.
- The risks associated with the introduction of rDNA-engineered organisms are the same in kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques.
- Assessment of the risks of introducing rDNA-engineered organisms into the environment should be based on the nature of the organism and the environment into which it will be introduced (product), not on the method (process) by which it was modified.
- There is an urgent (and ongoing) need for the scientific community to provide guidance to both investigators and regulators in evaluating planned introductions of modified organisms from an ecological perspective.

A follow-up study of the National Academy of Sciences on field testing of genetically improved organisms was released two years later (NRC 1989). A key conclusion in this report was that: "Crops modified by molecular and cellular methods should pose risks no different from those modified by classical genetic methods for similar traits."

Thousands of field trials have been conducted with transgenic crop plants during the past 10-12 years, with no evidence that the conclusions stated in the NAS and NRC reports were wrong. On the contrary, the evidence only continues to accumulate in support of the conclusions in these reports. Nevertheless, the rigor of tests for environmental and human health risks conducted with transgenic plants and food made from these plants has been taken far in excess of that done with plants genetically improved by more conventional methods or by induced mutations. Should the bar on safety standards be kept high only for transgenic crops, or should the bar be equally high for all new varieties of crops regardless of the method used to genetically modify them?

Accepting the principle that "the risks associated with the introduction of rDNA-engineered organisms are the same in kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques," and that "crops modified by molecular and cellular methods should pose risks no different from those modified by classical genetic methods for similar traits," then any risk assessment for the approval and use of plants in an agricultural or other managed environment should be the same regardless of whether that genotype or collection of related genotypes are unmodified genetically, genetically improved by a "traditional" method, or genetically improved by rDNA techniques.

Accepting further that the focus should be on the product and not the process, then the steps used to conduct a risk assessment should be the same for all crops plants, regardless of the source of genes or method(s) used to transfer these genes. How else can the risk assessment be "science based"? Furthermore, whether the risk assessment is done by a government regulatory agency, an institutional variety release committee, or private organization, the assessment process as well as the conclusions on safety should be public information.

Risk Assessment for Plants

What possible hazards are inherent with plants *as plants* (not the management used to grow them) that should be considered when deciding whether to use a particular plant to produce food, fiber, fuel, or grow it for some other purpose? This question pertaining specifically to environmental safety has been addressed in a study released in 1993 by the Organization for Economic Cooperation and Development (OECD 1993). This study represents possibly the first attempt at identification of the environmental safety issues, including worker safety issues, presented by plants and how any risks identified can be managed. Six safety issues with brief explanations or comments follow:

- *Gene transfer*, meaning the movement of genes through outcrossing. This issue could also include gene transfer from plants to microorganisms. Although transfer from plants to microorganisms is possible based on laboratory studies (Gebhard and Smalla 1998), and obviously has happened in evolutionary time (Doolittle 1999), the probability of a functional and medically important antibiotic gene moving from a plant to a human pathogen is negligible.
- *Weediness*, meaning the tendency of the plant to spread beyond the field where first planted. This issue would seem particularly relevant to new crops or old crops introduced into new areas. The classic example is kudzu, introduced into the southeastern United States to control soil erosion, but which now has become a major invasive weed in this region. The tendency with plant breeding is to reduce rather than increase the weediness characteristics of crop plants.
- *Trait effects*, meaning effects of traits harmful to nontarget organisms. Plants with spines or thorns can present a hazard both to workers and wildlife, and many plants produce secondary metabolites that are toxic to animals, or possibly to beneficial insects. As with weediness, plant breeding has tended to reduce rather than increase trait effects on nontarget organisms, sometimes making the domesticated plant more susceptible to pest attack than its unmodified wild relatives.
- *Genetic and phenotypic variability*, meaning the tendency of the plant to exhibit unexpected (pleotropic) characteristics in addition to the expected characteristics. This trait is well known from conventional breeding, but becomes an identifiable hazard only if the variability leads to one of the other safety issues, such as greater weediness or greater tendency for outcrossing.
- *Expression of genetic material from pathogens*. An avirulence gene from a pathogen expressed as a transgene in a plant, for example, has been shown to trigger an uncontrolled hypersensitive response that is potentially lethal to those plants (de Wit and others 1998). Such "genetic disease" would be eliminated early in R&D. Another potential hazard would be the probability of recombination of a virus gene expressed by the plant with genes from another virus infecting that plant. This risk would be similar to the risk of genetic recombinations following mixed virus infections.
- *Worker (human) safety*, such as the effects of nicotine uptake through the skin of workers who handle tobacco. This effect is actually a variation on trial effects discussed above.

Two points regarding these safety issues should be made clear. First, the term "safety issue" is used in the OECD report as the first step to hazard identification; it does not mean that a hazard actually exists. A crop plant known for outcrossing, for example, would raise the issue of gene transfer, but unless there are sexually compatible plants within the range of movement of pollen, there is no hazard. The transfer of resistance to glyphosate from a Round-up Ready® soybean variety in not a hazard in North America because there are no sexually compatible relatives of soybean growing wild in North America. Second, these six safety issues apply regardless of whether the plant has been introduced into cultivation directly from the wild (without genetic modification) or modified genetically. Weediness would have been a safety issue when soybeans were first introduced into North America during the earlier part of this century, but is no longer an issue because (1) observations in small plots during the early years of work with these plants showed that weediness was not a likely problem, and (2) practices are in place to manage any propensity for weediness of this crop plant.

Since risk = hazard x exposure, a low hazard could be considered high risk if the exposure was high and, conversely, a low exposure could be considered high risk if the hazard was high.

Gene Transfer

Returning to the example of gene transfer, a trait that requires a chemical treatment to be expressed (genetic-use restriction technology or GURT) might, because of the phenotype, be identified as a potential hazard if the trait transferred to a wild relative from the crop plant. Without the necessary spray treatment of the fertile hybrid, however, there would be no exposure and therefore no known risk. Conversely, the trait itself may provide no competitive or reproductive advantage (or disadvantage) to a fertile offspring even if transferred by outcrossing, therefore representing a potentially high exposure but low or no risk because there is no identifiable hazard. Gene transfer with no consequence does not, of itself, present an environmental risk. Many traits intended to improve harvestability or other production characteristics, and most if not all traits intended to improve the nutritional quality of the harvested products of crop plants, could fall into this category.

Weediness

A weediness hazard might be identified for a trait for resistance to a pest if the plant population to which the trait might transfer is under selection pressure (biological control) from that pest. If, however, that pest plays no significant role in the ecology of the wild or weedy plant population, then transfer of resistance by outcrossing to an individual within this plant population would constitute no identifiable hazard regardless of exposure. In cases where a relative of the crop plant is both a weed intermingled with its domesticated counterpart, and a source of pests or pathogens of that crop plant, a fortuitous buildup of resistance to that pest within the weedy relative could constitute a component of IPM for that pest. In other words, gene transfer could be beneficial in some cases.

Trait Effects

One of the most significant breakthroughs, with practical implications for control of plant viruses through genetics, was the discovery that expression of a virus coat-protein gene as a transgene in the plant then makes that plant resistant to that virus in direct proportion to the amount of coat protein produced by the transformed plant (Beachy, Loesch-Fries, and Turner 1990). This discovery opened an entirely new genetic approach to control of diseases caused by plant viruses—diseases that heretofore have defied all attempts at their control by traditional plant breeding. One of the latest success stories is the control of papaya ringspot in Hawaii by coat-protein mediated resistance (Gonsalves 1998). This approach is an example of "pathogen-derived resistance," whereby a gene for production of a pathogen protein, when produced in the host plant, results in the expression of resistance if not immunity by that host plant to that pathogen.

The question has been raised as to whether virus coat protein produced in a transgenic plant could encapsidate the naked nucleic acid of another virus also present in that plant (mixed infections are common in plants), and thereby create a "new" pathogen (OECD 1993). Coat protein plays a role in the transmissibility of some plant viruses by insects; were this the case for a coat protein produced in a transgenic plant, then the nucleic acid of a virus not transmissible by a leaf-feeding insect might become insect-transmissible if encapsidated by that plant-produced coat protein. This would be an identifiable hazard. The exposure, on the other hand, would be limited to plants with ability to make that coat protein, since the new virus, being dependent on its host plant rather than its own genome for its coat protein, when moved to a plant lacking that coat-protein transgene, would essentially come to a dead end. Such viruses would then be limited to plant genotypes with the coat-protein transgene. This would be an example of a plant made resistant to one pathogen only to find that it is now susceptible to another pathogen.

The issue of "trait effects" led the U.S. Environmental Protection Agency to propose in November 1994 that traits intended for pest control would be subject to regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as "plant-pesticides." The regulation, which is still not final, would apply specifically to any substance(s) produced by the plant for its defense against pests and the gene(s) needed to produce the substance(s). A consortium of eleven scientific societies, led by the Institute of Food Technologists and the American Society of Agronomy, challenged the EPA concept that the traits used by plants for their defense against pests and diseases could be equated scientifically with pesticides applied to plants (Cook and Qualset 1996). Most plant defense responses involve the coordinated expression of many genes and a cascade of biochemicals no one of which could be singled out and subjected to meaningful toxicological tests used for pesticides. The consortium of scientific societies then produced a decision guide that categorized plant defense mechanisms into six groups, five of which included defense traits not of a character to be considered as pesticides. The sixth group included traits such as Bt, pyrethrum, nicotine, and other defense mechanisms where the substances involved could be subjected to toxicological tests and were of a character to be considered as pesticides.

Impact of Agriculture on the Environment

Agriculture, by its very nature, is disruptive to nontarget organisms in the environment. Tillage is highly destructive to populations of earthworms that return in numbers only after a few years of no-till management. Rotating maize with soybean—or changing any field from one crop to another—must be disruptive to populations of insects, soil microorganisms, even to macrofauna such as birds and other wildlife. Insecticidal sprays, used to protect crops, kill insect pests and beneficial insects alike. *Science-based* should mean that we know the baseline for non target environmental effects, and can then assess any additional nontarget effects of the new trait that would elevate the risk measurably above this standard.

Assembling a complete picture of this baseline as a defined standard would be costly in human and financial resources that could well be spent on higher priorities, such as developing better varieties. The unfortunate situation is that, having only a fragmentary or incomplete picture, the baseline is treated as virtually nonexistent. Every effort must be made to bring about a greater public and scientific understanding of (1) the environmental disruptions and perturbations that result from simply growing crops to feed and clothe people, and (2) the remarkable trends through genetics that are making agriculture not only more productive but also environmentally more benign.

The baseline also includes the tens of thousands of new varieties of crop plants with new traits added for improved performance or end-use quality during the past century or more using the range of technologies now grouped under "traditional breeding." For example, some 200 crops are grown in the United States, the great majority of which were, at one time, alien introductions to North America. Over the years they have been subjected to extensive genetic modifications to make them more adapted to local conditions, resistant to local or introduced pests, and acceptable to the preferences of U.S. consumers and export customers. Each of the crop plants introduced presented new exposures when first grown in American soil and encountered by American wildlife. Because of the detailed understanding of transgenes, and the fact that only the gene(s) of interest are introduced into the recipient plant, crop plants genetically improved to express transgenes are arguably safer to the environment than their traditionally bred counterparts.

Risk Management for Plants

Risk assessment cannot be discussed without also considering *risk management*. Many highly effective methods in place are in place for management of risks specifically associated with plants as plants, whether as new crops or old crops with new traits. Risk management for crop plants includes that combination of (1) cultural practices evolved over centuries of agriculture, and (2) the knowledge gained during the past century or more from research in agronomy, plant pathology, entomology, weed science, plant biology, soils, microbiology, and the many other disciplines that make up the agricultural sciences. These disciplines, along with engineering, provide the science and technology in place to mitigate any risks that might be associated with a new crop or new trait expressed in a familiar crop.

The risk, for example, of gene transfer by outcrossing from an herbicide-resistant crop plant to some relative that grows as a weed comingled with that crop plant (for example, from canola to weedy mustard), can be managed by the use of a different herbicide (many herbicides are available to manage weedy mustard) or through use of a crop rotation that allows the "rotation" of different weed-management practices. The risk of introducing seed of a fertile hybrid between the crop plant and its weedy relative into a cropped area can be managed by use of seed grown under stringent certification procedures designed to identify crop-weed hybrids when they appear in the seed-production field. The risk of gene transfer from a transgenic crop to a nontransgenic crop can be and is managed by maintaining a certain minimum distance between the crops, such as has long been done in areas that produce both oil-seed rape for industrial oil and canola for edible oil. Where there is an identifiable hazard of gene transfer between a crop plant and wild relatives of that crop plant (for example, in an area considered to be the geographic center of origin of the crop plant), serious consideration should be given to whether that crop plant should even be grown in such an area.

There is a great deal of experience and technology available for management of any risk of weediness that might be inherent with a crop plant. Crops such as canola are well known for their tendency to carry over after harvest and to become weeds in the next crop, unless managed by tillage or herbicide treatment. Small grains such as wheat and barley are notorious as volunteer in the field for several weeks or months after harvest and must be controlled. In Washington State, the spring barley variety 'Steptoe' eventually fell from favor among growers because of its unusual ability to survive as volunteer over the winter and become a grass weed in a following pulse crop or, worse, contaminate a subsequent wheat crop. This variety has since been replaced by varieties no less prone to volunteer but significantly less likely to survive an eastern Washington winter.

Replacing the variety with one that reduces or eliminates any identified hazard is another effective and well-established approach to risk management. When it was discovered that the 1970 southern leaf blight epidemic on maize was due to a race of the pathogen, *Bipolaris maydis* [=*Cochliobolus heterstrophus*], uniquely virulent on maize hybrids having the Texas male-sterile cytoplasm (used as a genetic alternative to detasseling in the production of hybrid seed), this method for producing the male-sterile inbred lines was replaced within the next one or two crop years.

Balancing Risks Against Benefits

The overall measure of any cropping system should be its sustainability. It might be useful, therefore, to establish a series of tests for each new crop variety—transgenic or conventional—aimed at helping to determine the contribution the new variety may make to the sustainability of the cropping system in which it will be used. Tests for contributions to sustainability may help identify the benefits of the new variety or new trait, which can then be weighed against any risk(s).

Sustainability includes consideration of economics, impact on environment/natural resource base, and social costs and acceptance. Of these three factors, agriculture needs to pay particular attention to the match between the cropping system and the environment/natural resource base as the test for long-term sustainability, because of the fundamental importance of the environment and natural resource base to both economic vulnerability and social acceptance. Examples of tests for potential to contribute to sustainability based on the need to protect the environment and natural resource base are posed below as questions. These questions can be answered with existing technology and knowledge of cropping systems and required inputs. Whether or not the variety is approved for commercial use could then depend on the answers to these questions.

- Will the variety help reduce the dependency of this crop or cropping system on pesticides?
- Is the pest resistance expressed by this variety of the "durable" type, that is, not likely to be defeated by the pest during the first 10 years and preferably 15-20 years of its use?
- Will the variety help to save soil by making it possible to grow this crop with less or no tillage?
- Will the variety help to improve soil quality, prevent runoff of water from the planted field, or provide wildlife habitat by producing at least as much if not more crop residue than other varieties of this crop likely to be grown?
- Will this variety produce to its full potential without the aid of field burning?
- Will this variety capture as much if not more of the nitrogen added as fertilizer for its production than other varieties of this crop likely to be grown?
- Will this variety assure that no more water and possibly less water will be required to grow the crop than other varieties of this crop likely to be grown?

As an example, Washington State University is working to develop a variety of barley with resistance to Rhizoctonia root rot caused by *Rhizoctonia solani* AG8. Resistance to this disease is needed to reduce the risk and achieve the full yield potential of barley seeded directly (no-till) into standing stubble of cereals. Research in Australia and the U.S. Pacific Northwest has shown that this disease is of only minor importance on barley and wheat seeded into conventionally prepared seedbeds, but is potentially devastating, especially on barley seeded directly into stubble as needed to save soil, fuel, and water (Rovira and Venn 1985; Weller and others 1986). The pathogen has a wide host range and can survive through at least two years of breaks to broadleaf crops. Varieties of wheat and barley differ slightly in tolerance, but these differences are inadequate to reduce the risk of this disease.

Rhizoctonia species are naturally parasitized in soil and on plant roots by *Trichoderma* species, of which the best studied is *T. harzianum* (Kubicek and Harmon 1998). One of the several mechanisms by which this natural enemy of *Rhizoctonia* can inhibit or kill its prey includes a gene with potential, when transferred to plants, to provide a natural defense within the plant. The gene is for production of endochitinase, an enzyme produced by *Trichoderma* to soften and penetrate the chitin-containing walls of *Rhizoctonia* hyphae. Chitinase genes from plants also provide some level of resistance to *Rhizoctonia* species, but the endochitinase genes from *Trichoderma* have proved highly effective in potato and tobacco (Lorito and others 1998).

What are the possible risks of barley varieties transformed to express a *Trichoderma* endochitinase gene for defense against *Rhizoctonia*? Gene transfer is not an issue since there are no wild relatives with ability to hybridize with domestic barley in the area where the varieties will be grown. There would also be no plant pest risk because of the transformation and no risk to workers that would handle the straw or grain. The production of the *Trichoderma* endochitinase enzyme could affect nontarget fungi present within the root or other plant tissues, most or all of which will be endophytic fungi, but some of which may be other fungal pathogens of barley with chitinous cell walls. Such effects would not likely affect soil ecology any more than rotating crops. One identifiable hazard might be ability of transgenic plants to volunteer and survive the winter since the volunteer plants, like the crop produced from these varieties, would be healthier and therefore more robust and more likely to withstand freezing.

Although the risks are small and manageable, the benefits following the proposed agricultural sustainability protocol above would be enormous. There are no fungicides used at present on barley for control of Rhizoctonia root rot, other than traditional seed treatments, but plants with resistance to Rhizoctonia root rot, being more robust, would be more competitive with weeds and therefore would require less use of herbicides in some fields some years. There is no evidence of the emergence of *Rhizoctonia* strains insensitive to endochitinase, and therefore the resistance expressed by barley transformed with this gene should be of the "durable" type. Furthermore, these varieties, by making it possible to grow barley with less or no tillage, would allow for practices that save soil and improve soil quality, prevent runoff of water, provide wildlife habitat as standing stubble, and encourage the return of earthworm populations. Since stubble burning is largely to reduce pressure from root diseases (Cook and Haglund 1991), varieties of barley with resistance to Rhizoctonia root rot would reduce if not eliminate the need for some field burning. Because of healthy roots, these varieties would also then be more efficient in outreach and absorption of nutrients such as nitrogen that otherwise could move below the rooting zone. Crops produced from these varieties of barley would probably need more water, because of their ability to produce more grain, but it would be water that would otherwise be left unused in the soil. Clearly the benefits of these transgenic barley varieties would be enormous, especially when balanced against the risks which would be negligible to the environment and nonexistent to consumers.

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