Chapter 15
Regulatory Science, Research Science and Innovation in Agricultural Biotechnology

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Abstract Regulatory science produces data needed for risk assessments that help regulators make decisions about whether to allow certain activities such as the cultivation of transgenic crops. Research science, on the other hand, seeks to further objective knowledge for its own sake. Regulatory and research science have the same structure of erecting hypotheses as tentative answers to problems, and testing, that is attempting to falsify, those hypotheses by comparing their predictions with observations. In this paper, we discuss important differences between regulatory science and research, and in particular how they differ in the formulation and testing of hypotheses: regulatory science tests hypotheses that seek categorization of effects, whereas interesting research tends to test hypotheses that make precise quantitative predictions. When regulatory science is confused with research, many irrelevant data are produced, which confuse and delay decision-making, and increase the costs of regulation to the developer and regulator, ultimately harming innovation of new technology because business risks are too high. If research is confused with regulatory science, uninteresting hypotheses are tested, which slow the development of knowledge, again harming innovation. In some cases, particularly very early in the development of new technology, regulatory science and research may be

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indistinguishable; however, it is important for the effective development of new technology that regulatory data requirements are not laid down to answer research questions.

Keywords Biotechnology • Bucket theory • Hypothesis testing • Problem selection • Regulatory science • Risk assessment • Transgenic

15.1 Introduction

The cultivation of transgenic crops over the last 15 years has realized health, environmental and economic benefits in developed and developing countries (Brookes and Barfoot 2008, 2010; Qaim 2009; Raybould and Quemada 2010). The most widely documented benefits have come from transgenic crops with enhanced herbicide tolerance or insect resistance, but significant benefits have also resulted from virus-resistant crops (Fuchs and Gonsalves 2007). Experience with current transgenic crops suggests that agricultural biotechnology will help to solve some of the problems posed by the need to increase production of food, fuel and fiber under changing environmental conditions without worsening the loss of biodiversity (Federoff et al. 2010; Godfray et al. 2010); however, the innovation necessary for agricultural biotechnology to solve these problems may be constrained by high regulatory costs that limit research to products of interest to companies able to bear those costs (Chataway et al. 2006; Mittra et al. 2011).

Products of agricultural biotechnology that are regulated must be granted approvals by a competent authority before they can be used freely. High regulatory costs incurred by agricultural biotechnology arise from two main sources. First, large amounts of scientific data on the product and its intended use must be supplied to the competent authority for use in its deliberations on whether or not to approve the proposed use of the product. Secondly, decision-making on approvals may be lengthy and unpredictable; for example, in the European Union, some applications for commercial cultivation of transgenic crops are still awaiting decisions over 13 years after submission. Long and unpredictable regulatory decision-making complicates investment decisions and delays return on investment in the development of the product. The data and decision-making often interact to increase costs because large amounts of data complicate decision-making and data may be collected in a vain attempt to define decision-making criteria (Johnson et al. 2007; Raybould 2007).

Regulatory decision-making usually involves assessment of the risks posed by a proposed activity, such as cultivation of a particular transgenic crop, where risk is a function of the seriousness of the potential harms caused by the activity and the likelihood of those harms arising. Where significant risks are identified, evaluation of whether risk management suitably lowers risk may be considered by decision-makers. As well as risk, decision-making may include evaluation of the opportunities presented by the activity, where opportunity is a function of the size of potential benefits of the activity and the likelihood of those benefits arising. Assessing risk – and opportunity

– should be similar to fundamental research in that data are collected to test hypotheses. Risk assessment and fundamental research are different because in risk assessment hypotheses should be designed to help subjective decision-making, whereas in fundamental research, hypotheses are tested to increase objective knowledge.

In this article, we describe the importance of hypothesis testing in risk assessment and management, and why it is vital that risk assessment is not confused with fundamental research: testing no hypothesis, or unsuitable hypotheses, leads to the collection of large amounts of data that are irrelevant to risk assessment and unnecessarily constrain the invention of potentially beneficial products.

15.2 Risk Assessment as Hypothesis Testing

The philosophy of scientific discovery deals with two different methods: induction and deduction. Induction makes generalizations from particular observations and is the basis of empiricism, which proposes that objective knowledge originates from observations made without preconceptions. In an essay published in German in 1949 and in English in 1972, Karl Popper (1979) describes empiricism as the "bucket theory" of scientific knowledge: observations are accumulated in a metaphorical bucket and accrete into knowledge. Eventually there may be sufficient observations supporting a generalization that it is regarded as true.

Popper (1959, 1979) proposed an alternative theory whereby knowledge increases through observations that test our preconceptions. In this theory, observations are made in response to preconceptions; that is, we always have expectations or hypotheses that guide our observations. Hypotheses are used to deduce particular expected facts, and when our observations differ from what we expected, we formulate new hypotheses in attempts to eliminate the flaws that led to the erroneous expectations. Knowledge thereby grows by repeated testing and correction of hypotheses.

Induction has proved problematic as a logical basis for science; for example, however many facts are added to the bucket, it is never possible to prove that no subsequent observation will contradict the generalizations drawn from those facts. A second problem is that infinite generalizations can be drawn from any set of observations, and simply adding similar observations to the bucket does not help to discriminate among those generalizations. Popper (1959) offered deduction as a solution to the problem of the logic of science. He proposed that we discriminate between hypotheses by searching for experimental conditions under which the hypotheses make different predictions. Hypotheses that make accurate predictions are corroborated and survive for further testing, whereas hypotheses that make inaccurate predictions are revised or discarded. Popper argued that it is active criticism of hypotheses, not the accumulation of facts in favor of hypotheses, which advances science.

The problems of the bucket theory of science also apply to risk assessment. First, it is not possible to prove that an activity is safe, because regardless of how many times the activity has been performed safely, there is no guarantee that harmful

effects will never be observed. Secondly, there is the problem of drawing different generalizations from the same collection of facts. Sarewitz (2004) has pointed out that science often makes environmental controversies worse because disagreements are not about science but about values. Trying to settle arguments by collecting more data increases controversy because opponents have a larger collection of data from which to select facts to support their argument. Finally, risk is a function of the seriousness of the harm that may arise from an activity and the likelihood of that harm arising as a result of the activity. What society regards as a harmful effect cannot be discovered by scientific research, it must be defined by policy objectives.

Problems with the bucket theory show that a risk assessment cannot be improved simply by collecting more data. To identify useful data, it is necessary to think of a risk assessment as hypothesis testing not data gathering, or as an exercise in deductive not inductive logic. Popper (1979) gives a simple scheme to show how objective knowledge grows by deductive logic:

$$\rightarrow \text{ initial problem } [P_1] \rightarrow \text{ tentative solution } [TS] \rightarrow \text{ error elimination } [EE] \rightarrow \text{ new knowledge and a new problem } [P_2] \rightarrow$$

The initial problem is a discrepancy between a tentative solution to a previous problem and observations made to test that solution.

The scheme may be adapted to give the structure of a risk assessment for cultivation of a transgenic crop (Raybould 2006, 2010):

$$\text{Decide what constitute harmful effects of cultivating the transgenic crop } [P_1] \rightarrow \text{ hypotheses that cultivation of the crop will not cause harm } [TS] \rightarrow \text{ test the hypotheses } [EE] \rightarrow \text{ increased knowledge of risk } [P_2] \rightarrow \text{ decision – making } [TS_2] \rightarrow$$

This simple scheme provides a conceptual framework for assessing the risks posed by the cultivation of transgenic crops (Raybould 2006; Wolt et al. 2010). The scheme could also be applied to risk management, where the hypotheses under test would be of the form "cultivation of the crop with the proposed risk management reduces the probability of harm below an acceptable threshold". The following sections discuss how the scheme may be implemented in practice.

15.3 Formulating and Testing Risk Hypotheses

Formulation of risk hypotheses begins with a conceptual model, scenario or pathway that describes how cultivation of the transgenic crop may cause harm. As far as practicable, this procedure should start by defining harmful effects from policy objectives, regulations or other guidance, and then analyze how cultivation of the crop could bring about those effects. Working out all possible effects

of cultivating a transgenic crop, and trying to deduce which are harmful is inefficient and ineffective.

In a conceptual model, the links in the chain of events from cultivation to harmful effects are logical: what are the necessary conditions for harm to arise, not what is the likelihood of those conditions occurring. There may be infinite ways by which harm *could* arise, it is necessary, therefore, to reduce the number of scenarios that will be used to generate hypotheses for testing in the risk assessment. Some logically possible scenarios may appear so implausible that it is almost inconceivable that they pose any risk, and therefore they are not evaluated in the risk assessment. It is important to recognize and explain that implausibility means that at least one step in the scenario is known to be highly unlikely; that is, if event A is necessary for harm to arise, existing data corroborate with extremely high confidence the hypothesis that event A does not occur (Raybould 2011).

The remaining plausible scenarios are the source of the risk hypotheses tested in the risk assessment. These scenarios may be examined in terms of discrete steps that must occur for the cultivation of the transgenic crop to result in harmful effects. From each step it is possible to formulate a hypothesis, which if corroborated or falsified by suitable testing, would characterize risk in a form that is useful to decision-makers. Hypotheses could take several forms: event A does not lead to event B; event A leads to event B at a frequency below that which would cause harm; or event A leads to event B, but event B is below the magnitude necessary for harm (Raybould 2006, 2010). In each case, the hypothesis can be regarded as a hypothesis of no harm from cultivation of the transgenic crop. Testing hypotheses of no harm, with new studies, with existing data collected for other purposes independently of the current risk assessment, or both, is the basis of risk characterization.

Initial tests of risk hypotheses are made under conservative conditions designed to minimize false negatives; in other words, if the hypothesis is that "event A will not occur", tests are made under conditions most likely to reveal the potential for A to occur. Two examples illustrate the point. First, if event A is adverse effects of an insecticidal protein on a group of non-target organisms, a conservative test is exposure, in the laboratory, of suitable representative test species to the protein at ten times the highest exposure likely to result from cultivation of the transgenic crop (Raybould et al. 2007, 2011a; Romeis et al. 2008; Raybould and Vlachos 2011). If no adverse effects are seen at this concentration, experiments using exposures to the protein at field concentrations add little to the risk assessment because the test is less likely to detect adverse effects (Raybould 2006). Should adverse effects of the protein be detected in the laboratory, further studies under more realistic conditions may be conducted to evaluate whether toxicity of the protein is likely to result in harmful effects in the field. Secondly, if event A is hybridization between a crop and a wild plant species, a conservative test is artificial cross-pollination of the species in the laboratory followed by embryo-rescue to detect any hybrid seed. If hybrids are not detected under these conditions, testing could stop; if hybridization is detected, the potential for hybridization in the field could be assessed, for example, by allowing the species to cross-pollinate spontaneously under glasshouse conditions (Raybould and Cooper 2005).

The concept of starting with conservative tests most likely to reveal the potential for harm and only moving to more realistic tests if that potential is detected is called tiered testing (Touart and Maciorowski 1997; Garcia-Alonso et al. 2006). It is an effective way efficiently to characterize activities into those that pose low risk and require little or no further evaluation, and those that may pose high risk and require further assessment to determine the level of risk. The criterion for deciding whether further testing is required is a judgment about the best balance between the costs of over-testing some activities that pose low risk and the costs of incorrectly determining that high risk activities pose low risk (Chapman et al. 1998; Caley et al. 2006).

Evaluation of risk management plans follows a similar conceptual framework to risk assessment in that hypotheses about the likelihood of harm following an action are tested. In risk assessment, the scenarios might start with unrestricted cultivation of the transgenic crop. In risk management, scenarios are developed from cultivation of the transgenic crop along with measures to limit the likelihood of harm arising. The evolution in pests of resistance to insecticidal proteins is regarded as a harmful effect of cultivating transgenic insect-resistant crops (e.g., McGaughey and Whalon 1992; McGaughey et al. 1998), and in many countries, suitable insect resistance management (IRM) plans are mandatory for regulatory approvals of such crops (MacIntosh 2010).

Current IRM plans originate from a high-dose – refuge strategy for the first commercial transgenic crops resistant to lepidopterous pests, which assumed, among other things, that resistance to the insecticidal protein is controlled by a single gene, and that alleles conferring resistance are recessive and rare, and therefore almost all resistance alleles are present in heterozygotes. High-dose refers to a requirement that the transgenic crop delivers a dose of insecticidal protein that is many times greater than the concentration required to kill heterozygotes carrying resistance alleles. The refuge part of the strategy is the requirement for farmers to grow a certain proportion of non-transgenic crop to act as a source of susceptible insects, so that any rare resistant homozygotes emerging from the transgenic crop will be highly likely to mate with the abundant susceptible homozygote from the refuge (Mendelsohn et al. 2003). The progeny of these individuals will, therefore, be heterozygotes and highly susceptible to the high dose of insecticidal protein in the transgenic crop, preventing the increase in the resistant allele frequency and outbreaks of resistant genotypes that could cause the *Bt* crop to fail (Bates et al. 2005).

Prior to the introduction of transgenic insecticidal crops, it was established that insects could become resistant to the insecticidal proteins being expressed (Tabashnick et al. 1990). Thus, the high-dose – refuge strategy is, in effect, a hypothesis that high doses of insecticidal protein and refuges of non-transgenic crops will delay the evolution of pest resistance to the protein for an acceptable period. Implicit in this hypothesis is the assumption that there is a high probability of an unacceptably rapid evolution of pest resistance should the IRM plan not be implemented. Unfortunately, this hypothesis cannot be directly tested in the field without creating the very harm one is trying to avoid. Small-scale glasshouse studies have shown that the high-dose refuge strategy can delay resistance in insect populations

(Zhao et al. 2003), leaving the current hypothesis that a particular transgenic insecticidal event or pyramid of events will delay resistance to the protein for an acceptable period. Given that resistance evolution in an insect population is driven by many uncontrollable external factors, efforts should be focused on testing hypotheses based on parameters we can measure or control. The most relevant hypotheses to test are that the plant produces protein at a high dose, the movement and mating behavior of the pest being controlled is compatible with the IRM strategy, and resistance alleles are sufficiently low. A negative or unexpected outcome from any one or all of these tests does not mean resistance cannot be sufficiently delayed, only that modifications to refuge size, configuration or proximity to the transgenic insecticidal trait fields may be required. These decisions are made with the aid of computer simulation models which help predict the relative impact of proposed IRM plans based on a given set of parameters.

15.4 Differences Between Regulatory and Research Science

Risk assessment is not scientific research and does not create scientific knowledge for its own sake (Hill and Sendashonga 2003). Instead, it organizes existing information, along with sufficient new observations, to help decision-making. It follows that while regulatory and research science both test hypotheses that are tentative solutions to problems, there are important differences between them, which if not recognized, will lead to inefficient and ineffective risk assessment and uninteresting scientific research (Raybould 2010).

Differences between regulatory and research science arise at all stages of knowledge production (Table 15.1). First, problem selection should be explicitly subjective in regulatory science because risk assessments estimate the likelihood and seriousness of harm, which is subjective. If harmful effects are not defined at the start of a risk assessment, regulatory science tends to become an effort to exhaustively characterize the effects of cultivating a transgenic crop instead of estimating the probability of harmful effects of cultivating the crop. Examples of the absence of a priori definitions of harm include the farm-scale evaluations (FSEs) of herbicide-tolerant crops in the UK (Firbank et al. 2003), many field studies that compare the abundance of non-target organisms in fields of transgenic and non-transgenic crops (e.g., Marvier et al. 2007), and the use of "omic" profiling to compare transgenic and non-transgenic crops (Ricroch et al. 2011). In each case, the research searched for differences, not potentially harmful differences. This approach is detrimental to risk assessment because differences between the transgenic and non-transgenic crops cannot be assigned a level of risk (as is the case with the meta-analysis non-target organism studies by Marvier et al. 2007), or because a subset of differences is selected after the experiment as being important (as in the FSEs), which means that resources were wasted measuring things that were irrelevant for risk assessment, that better experiments could have been designed to measure important endpoints, or both (Raybould 2007).

Table 15.1 Differences between research and regulatory science

	Research science	Regulatory science
Problem selection	• Apparently objective – Arises from objective testing of prior problems	• Subjective – Arises from definitions of harm
Hypothesis formulation	• Seeks to be interesting – Makes precise predictions – Tests fundamental theory	• Seeks to help decision-making – Predicts no harm – GMOs are not inherently harmful
Testing	• Strong corroboration from presence of phenomena in field studies • Usually requires new data	• Strong corroboration from absence of phenomena in laboratory studies • Existing data are often sufficient

Problem selection may appear objective in research science because the knowledge it produces is not ascribed obviously subjective values such as whether or not it indicates potential harm or benefit. However, problem selection will always be subjective because it is influenced by the personal interests of scientists and by organizations funding research. Apparent objectivity is not a problem for research, but is a problem in regulatory science if it induces avoidance of definitions of harm at the beginning of a risk assessment.

Owing to the different types of problem to be solved, the hypotheses tested in regulatory and research science should often be very different. Research hypotheses seek to make interesting predictions. "Interesting" science is not easy to define. However, it is often associated with precise predictions, which means that the hypothesis is exposed to falsification (Popper 1979). It is easy to make accurate, but uninteresting, predictions; for example, that in southern England, the temperature is unlikely to fall below −10°C between June and August in any given year. Many observations would corroborate such a prediction and therefore the hypothesis behind the prediction is rather boring. Much more interesting is the precise value, time and place of the minimum temperature on any given day. There are many observations that would falsify the hypothesis behind the prediction, and therefore if the hypothesis is corroborated, something interesting has been discovered.

Regulatory science seeks to help decision-making by predicting the likelihood of harmful effects. For a given decision, perhaps whether to grow a certain type of plant in one's garden, it may be sufficient to know that the temperature is unlikely to fall below −10 °C over a given period, and if so, there is no need to develop and test hypotheses about the precise minimum temperature on a given day. The same applies to risk assessment; for example, if one has tested the hypothesis that an insecticidal protein is not toxic to a valued aquatic non-target organism at concentrations in excess of ten times the maximum mean concentration of the protein produced in a transgenic crop, it is probably unnecessary to develop and test hypotheses about the precise concentration of the insecticidal protein that will appear in water bodies following cultivation of the crop. Predicting the exact number of

hybrids between a transgenic crop and a wild species, instead of the likelihood that any hybrids will form is a similar example of over-quantification of an endpoint. Clear thresholds for decisions, and simple tests of the likelihood of being above or below the threshold, are more effective for decision-making than precise predictions without an indication of which values would indicate harm.

Another problem that arises in research using transgenic crops is that the motivation for studies is that the crop is transgenic, not that the transgenic crop is a useful tool for testing an interesting hypothesis. As pointed out above, exhaustive categorization of the effects of cultivating a transgenic crop often does not help risk assessment; neither does it help research unless there is an interesting hypothesis under test. In the FSEs, the hypothesis under test was that "GMHT [genetically modified herbicide-tolerant] crops had no effect on farmland biodiversity compared with a conventional cropping system" (Squire et al. 2003). This hypothesis was highly unlikely to be true given that different herbicide management was to be applied to the conventional and transgenic crops. However, because transgenic crops were involved, the study perhaps seemed to be interesting even though there was no attempt to develop and test hypothesis from existing knowledge. Similar problems face many studies that compare transgenic and non-transgenic plants using methods that sample multiple endpoints, ranging from metabolomics to faunistic analyses at the field- or landscape-scale (Raybould 2010).

Finally, the testing of hypotheses may differ between regulatory and research science, particularly in the type of study that provides strong corroboration of a hypothesis and in the use of existing data. In regulatory science, a strong case can be made that if no potentially adverse effects are observed in controlled laboratory experiments, then field studies should not be required to demonstrate low risk from the cultivation of transgenic crops. Laboratory studies are designed to exaggerate hazards and controlled conditions mean that the effects of those hazards are more likely to be observed; this is the basis of tiered testing (Raybould 2006, 2007; Garcia-Alonso et al. 2006; Romeis et al. 2008; Raybould et al. 2011a). This does not mean that no field testing of transgenic crops is needed. A corollary of the argument for tiered testing for risk assessment is that laboratory experiments demonstrating efficacy only indicate the potential for efficacy in the field; therefore, extensive field trials are necessary to test the agronomic performance of the crop, even though laboratory tests may have shown that the crop is highly efficacious. Similarly, in ecological research laboratory testing is always likely to reveal an effect of a factor if conditions are sufficiently extreme, but this does not mean that the factor will produce that effect in the field or that the effect is ecologically important (Peters 1991). Field testing is required to demonstrate the ecological relevance of effects detected under laboratory conditions.

The important difference between regulatory science for risk assessment and efficacy trials and ecological research is that regulatory sciences usually test hypotheses that effects do not occur, whereas efficacy trials and ecological research usually test hypotheses that effects will occur. The most rigorous tests of hypotheses for the absence of effects tend to be laboratory studies, while the most rigorous tests of hypotheses for the presence of effects tend to be field studies. In both regulatory and

research science, if an effect is observed in the laboratory, its ecological importance should be evaluated in the field, and if no effect is observed in the laboratory field testing is unlikely to find an effect; therefore, while regulatory science tends to emphasize laboratory studies and research science tends to emphasize field studies, the reasoning is the same, only the hypotheses are different.

Finally, regulatory science and research science tend to differ in the use of existing data. In basic ecological research, existing data may provide good tests of new hypotheses. However, convincing corroboration of a hypothesis usually requires new experimental tests as well as re-interpretation of existing data. The data may not be in a form that provides the best test of a new hypothesis and may have been used in formulation of the hypothesis. In risk assessment, on the other hand, it is often possible and desirable to use only existing data to provide satisfactory corroboration of a risk hypothesis. In the case of a transgenic crop producing a non-pesticidal protein, for example, the risk hypothesis that the protein has no adverse effects on wildlife at concentrations in the crop can be tested using existing data on mode-of-action, amino acid sequence similarity to known toxins, and the taxonomic distribution of similar proteins (Craig et al. 2008; Raybould et al. 2010). And in the case of a transgenic crop newly developed to produce an insecticidal protein that has been extensively tested for non-target organism risk assessments for other transgenic crops, additional non-target organism studies should not be required, provided that the concentration of the insecticidal protein in the new crop is not greater than in the other crops, and provided that the species tested adequately cover the taxonomic and functional groups of non-target organisms likely to be exposed to the protein *via* cultivation of the new crop (Romeis et al. 2009).

15.5 Relevance to a Current Regulatory Problem: Combined Insect-Resistance Traits

New transgenic crops are continually being developed. Effective regulatory risk assessment and decision-making for new crops should apply experience of transgenic crops currently in commercial cultivation so that regulatory authorities are not overwhelmed reviewing studies that add little to our knowledge of the risks posed or likely benefits gained by cultivating the new transgenic crops. Transgenic crops with single insect-control traits were first commercialized over 15 years ago (Mendelsohn et al. 2003), and crops containing combinations of insecticidal traits (pyramids or stacks depending on whether the traits have overlapping or non-overlapping spectrums) are being produced by conventional breeding and are entering commercial cultivation (Halpin 2005; Gatehouse 2008). Combinations of traits may extend the range of insects controlled; for example, in maize, traits that control Lepidoptera are often combined with traits that control corn rootworm. Traits with different modes of action against the same pests may also be combined to reduce the probability of pests evolving resistance; this tactic is increasingly being used in transgenic maize and cotton resistant to Lepidoptera (Kurtz et al. 2007; Head and Dennehy 2010).

Transgenic crops containing combinations of approved traits often require additional regulatory approvals before they may be cultivated (De Schrijver et al. 2007; Taverniers et al. 2008). As the number of products with unique combinations of insect-control traits is likely to be high, an important question is whether data are required to assess the risks from cultivating a crop with two or more insecticidal proteins in addition to those used to assess the risks from cultivation of crops containing the single traits. Below we consider approaches to assessing ecological risk and developing insect-resistance management plans for crops with combinations of insect-resistant traits.

15.5.1 Ecological Risks

One way to approach the problem of assessing the ecological risks from combined insect-resistance traits is to consider the hypothesis that the ecological risk of the insecticidal traits in combination is no greater than the combined ecological risk posed by the traits separately; *i.e.*, there is no synergism between the insecticidal proteins, and the concentrations of the insecticidal proteins in the separate events are no greater than in the pyramid or stack (Raybould et al. 2011b). If these conditions hold, then if the insecticidal proteins separately have no adverse effect on non-target organisms at high concentrations relative to the concentration in the single events, the mixture of proteins is also likely to have no adverse effects on non-target organisms exposed *via* cultivation of the pyramid or stack. If the proteins separately have adverse effects on non-target organisms at concentrations likely to result from cultivation of the crop, there are methods to predict the effects of the mixture of proteins from their separate effects (Wolt 2011).

The key question for this approach is which data are needed to test the hypothesis of no synergism between the insecticidal proteins. For proteins that have no adverse effects at high concentrations relative to the crop, a mixture of proteins at the concentration in the crop is unlikely to show synergism because synergism is rarely, if ever, detected in mixtures of chemicals below their no observed adverse effect concentrations (Syberg et al. 2009); therefore, one could argue that no test of the hypothesis of no synergism should be required to assess the ecological risks from cultivating plants containing those proteins.

If additional data are required to assess the risk to non-target organisms, species that are sensitive to at least one of the proteins provide the most rigorous tests of the hypothesis of no synergism. These species are likely to be pests; however, it is their sensitivity to the proteins that is important, not whether they are non-target organisms. Several methods are available for testing the hypothesis of no synergism depending on whether the proteins have overlapping (e.g., Colby 1967; Herman et al. 2002; Fernández-Luna et al. 2010) or non-overlapping spectra (Raybould et al. 2011b), and if overlapping spectra, whether the proteins have similar or different modes-of-action (Bliss 1939; Tabashnik 1992). If no synergism is detected using sensitive species – pests or non-pests – then there is highly unlikely to be synergism

Table 15.2 Criteria for choosing testing strategies for ecological risk assessments for the cultivation of crops with combined insect-resistance traits

	Existing effects data	Number of active ingredients	Number of target groups	Combination strategy
Synergism tests	Yes	2	1	Breeding of separate events
Effects tests with mixture	No	Many	Several	Single transformation event

in non-target organisms that are insensitive to all of the proteins (Syberg et al. 2009; Raybould et al. 2011b). Such a result would provide strong corroboration of the hypothesis that two or more traits that separately pose minimal ecological risk would also pose minimal ecological risk when combined in a stack.

Finally, it should be emphasized that the ecological risk assessment does not necessarily need information on whether there is synergism among the proteins in a pyramid or stack. Tests for synergism are a means to establish whether existing data on the effects of the insecticidal proteins separately are applicable to the proteins when combined. It is perfectly possible to treat the mixture of proteins as a new active ingredient and test its effect in a series of representative surrogate organisms as is normal for single protein active ingredients (e.g., Romeis et al. 2008). This approach might be the most effective for products in which the active ingredient is a so-called binary toxin consisting of two proteins that separately have low pesticidal activity, but have high activity when combined, and when the two proteins are expressed from genes on a single DNA insert in the transgenic crop. Transgenic maize producing a toxin comprising a 14 kDa protein Cry34Ab1 and a 44 kDa protein Cry35Ab1 is an example of such a product (Moellenbeck et al. 2001; Herman et al. 2002). Table 15.2 lists some criteria that may be used to decide on testing strategies for ecological risk assessments for pyramids and stacks.

15.5.2 Insect Resistance Management for Combined Insect-Resistance Traits

In many countries, IRM plans are a compulsory part of regulatory submissions for insect-resistant transgenic crops. Such plans synthesize information about the sensitivity of the pest to the insecticidal protein. In a recent article commissioned by the Insect Resistance Action Committee, MacIntosh (2010) outlines the types of data needed to develop an effective IRM plan adapted to local environments. The author states that in regard to IRM plans and regulation, “The goal should be to enable growers to have access to the technology while providing stewardship that will provide an acceptable level of protection against resistance”. The key areas where data are needed to fulfill that goal are outlined in the article, and include understanding primary pest biology and ecology, potential trait use patterns, local cropping and patterns systems, dose (level of target pest control) and number of insecticidal

proteins expressed by the plant, and the potential for cross resistance between insecticidal proteins.

Of particular importance to an IRM plan is the dose of insecticidal protein delivered by the product. When combining insect-control traits, it is important to predict whether stacking or pyramiding single events will have an impact on the dose of each individual insecticidal protein expressed by the product. In some regions, data may not exist for the single traits and should be generated; however, in regions where single traits have previously been commercialized, the existing data can be used to inform regulatory risk assessment and decision-making for combined insect-resistance traits (stacks or pyramids) rather than generating new data on the dose of protein expressed by single traits.

If the dose of insecticidal protein delivered by each commercial single trait against key pests has previously been determined, it should not be necessary to repeat additional dose studies for the combined insect-resistance traits. A quantitative assay comparing protein concentrations in products with the single trait events to those expressing the combined insect-resistance traits should be sufficient. If expression of the proteins in the stacked product is comparable to expression of each protein in the single-trait events, insect pests will receive the same dose of insecticidal protein given that no synergism or antagonism was observed in the studies described in the section above. In the case of IRM plans for stacked products with non-overlapping spectra, the IRM plans developed for the single traits can apply directly to the stacked product. For pyramided products with overlapping spectra, the dose of the single trait events can be used to determine what IRM plan would be most appropriate.

Though the existing data on single traits can be used to inform regulatory risk assessment and decision-making for combined insect-resistance traits, ensuring that the combined stacks or pyramids are performing as expected is also important. The results of the quantitative protein assays are often supplemented with standard field efficacy trials comparing the performance of the stack or pyramid to the known performance of each single trait component.

15.6 Conclusion

When regulatory science is confused with research, many irrelevant data may be produced, which confuse and delay decision-making, and increase the costs of regulation to the developer and regulator, ultimately harming innovation because business risks are too high. High costs are particularly problematic for public sector institutions and small companies that cannot afford the regulatory costs even if they wished to run the business risks (Kalaitzandonakes et al. 2007). On the other hand, if research is confused with regulatory science, uninteresting hypotheses are tested, which slow the development of knowledge, again harming innovation. Good regulatory and research science should be directed by the formulation and testing of suitable hypotheses, but it is important that the objectives of research to test scientifically interesting hypotheses are not confused with the objectives of regulatory

science to test hypotheses that help decision-making. In some cases, particularly very early in the development of new technology, regulatory science and research may be indistinguishable; however, it is important for the effective development of new technology that regulatory data requirements are not laid down to answer research questions.

We are now beginning to see clarification of the concepts that allow identification of data essential for risk assessment of transgenic crops ("need to know information") and data that may appear useful for a risk assessment, but at best are irrelevant, and at worst create delay and confusion in decision-making ("nice to know information"). As new technologies such as synthetic biology and nanotechnology are applied to agriculture, it is essential that regulation of resulting products learns from the experience with transgenic crops. Science can help to assess the risks from new classes of product, but thinking that objective scientific knowledge is all that is needed to make good decisions is mistaken; subjective elements are needed to decide what to regard as harmful effects and to set decision-making criteria. Often difficult decisions require clear thinking about the nature of the problem: what do we want and how should we decide whether we are likely to get it? If we are unsure what we want, more information is likely to confuse us rather than clarify the choices. We have found this to be the case with transgenic crops, and we must not forget it when deciding whether or how new agricultural technologies should be regulated.

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