DRAFT GUIDELINES FOR ASSESSMENT OF ECOLOGICAL HAZARDS OF HERBICIDE AND INSECT RESISTANT CROPS

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INTRODUCTION

SCOPE/OBJECTIVES OF THE GUIDELINES

There are several concerns about the consequences of development and deployment of transgenic herbicide-resistant (HR) and insect-resistant (IR) crops. Objections to the use of these transgenic crops rest on several issues related to the associated risks, such as:

- the potential transfer of genes from herbicide resistant crops (HRC) to wild relatives thus creating super weeds;
- possibility of HRC volunteers to become weeds in subsequent crops;
- development of resistance by insect pests to crops with *Bacillus thuringiensis (*Bt) toxin;
- adverse effects on ecological processes and non-target organisms due to massive use of Bt toxin in crops.

All these concerns show the importance of assessment of possible hazards from the use of transgenic HR and IR crops. Assessment is required to decide whether these crops may be introduced and will not pose any hazard to the environment bringing expected benefits to the farmers.

In this context government's authorities of the Ministries of Agriculture or Rural Development need an appropriate tool to conduct the required assessment. To this end FAO decided to elaborate relevant guidelines on this subject.

Limited world experiences on this type of assessment clearly indicates that present draft guidelines are susceptible of further improvement with data and experiences to be gathered in the coming future.

Draft guidelines describe the process of analysis and assessment of ecological hazards associated with the introduction of herbicide resistant crops (HRC) or genetically modified insect resistant (e.g. with genes coding for endotoxins from *Bacillus thuringiensis*) crops (IRC). Furthermore, the guidelines list the responsibilities of governmental authorities, applicant or permit holders and farmers growing HRC and IRC. The main aim of the guidelines is to provide a framework, especially for countries that have not developed their own regulations, on assessing the ecological risks of HRC/IRCs.

Drs Kathrine H. Madsen, Bernal E. Valverde and Jens C. Streibig from Royal Veterinary and Agricultural University (KVL), Denmark, prepared the initial draft of the guidelines, which was then sent to more than 30 specialists from all over the world for their comments and suggestions. The first draft was revised, thoroughly discussed and improved at a workshop organized by FAO and Royal Veterinary and Agricultural University (KVL), Department of Agricultural Sciences (Weed Science), held in Copenhagen, Denmark, on 14 and 15 September 2000, with the participation of the specialists as follows:

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DEFINITIONS AND ABBREVIATIONS

Applicant/notifier	The party (e.g. seed producer or importer, agro-chemical company or farmers' organization) that requests permission to experimentally release or commercially introduce an HRC/IRC in a country.

Authority	A governmental institution, organization or entity officially designated by the government to deal with matters arising from the responsibilities set forth in the Guidelines.
Bacillus thuringiensis (Bt)	Bacterium species currently used as a microbiological agent to control larvae of *Lepidoptera*, *Diptera* or *Coleoptera*.
Competitiveness	A plant's ability to exploit essential elements such as light, water and plant nutrients at the expense of other plants.
Congeners	Refers to species belonging to the same genus.
Conspecific	Refers to individuals or populations of the same species.
Crop production system	A particular agricultural scheme, including monocultures, rotations and polycultures, and their associated practices such as tillage, plant protection and harvesting.
Ecosystem	A complex of organisms and their environment, interacting as a coherent unit (natural or modified by human activity, e.g. agro-ecosystem), irrespective of political boundaries, to maintain a flow of energy and to acquire, store and recycle nutrients.
Fitness	Reproductive success or the proportion of genes an individual leaves in the gene pool of a population.
Gene flow	The transfer of genes (specifically, alleles) from one population to another by way of interbreeding of individuals in the two populations.

Gene pool	All of the alleles available among the reproductive members of a population from which gametes can be drawn.
Genetic engineering	Altering the genetic material of cells or organisms to make them capable of making new substances or performing new functions.
Genetically modified (GM) plant	A plant whose genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.
Hazard	Intrinsic property of a physical situation (or dangerous substance) which can cause damage to human, animal and/or plant life or health and/or the environment.
Herbicide	A chemical substance or mixture of substances designed to control weeds.
Herbicide resistant crop (HRC)	A crop plant that by genetic modification(s) or breeding has acquired resistance towards a herbicide it would otherwise be sensitive to.
Insect resistant crop (IRC)	A crop that by genetic engineering has become protected from damage by one or more harmful insects.
Insecticide	A chemical substance or mixture of substances that controls insects that harm crop production or prevents their damage.
Introgression	The transfer of genes from one population to another by backcrossing.
Marketing	Supplying or making available to third parties.

Maternal inheritance	The transmission of nuclear and extra-nuclear genes from the mother usually referred to extra-nuclear genes.
Pest	Organisms which are capable of transmitting disease or unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs.
Pesticide	Refers to any substance or mixture of substances intended to prevent, destroy or control any pest, including substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest or protect the commodity from deterioration during storage and transport.
Release	Introduction into the environment of a genetically modified organism (GMO) with or without provisions for containment. Release can be deliberate, experimental, accidental or commercial.
Resistance	In the case of plant populations, their inherited ability to grow and reproduce normally when exposed to high doses or levels of a specific agent (e.g. herbicide or insect attacks), which normally would harm plants.
Risk	The probability of occurrence of a hazard which can cause damage to human, animal and/or plant life or health and/or the environment and its potential economic implications.

Risk assessment	The qualitative or quantitative evaluation of risks resulting from the release of genetically modified plants or products containing GM plants.
Spread	Expansion of the geographical distribution of plants containing a genetically modified gene.
Tolerance	Referred to plants, it is an increased ability of a biotype to endure damage, survive and reproduce after a limited exposure to a specific stress factor (in this context, herbicide applications or insect attack) compared to other biotypes of the species. Tolerance is often a polygenetic inherited trait.
Transgene	A gene or DNA fragment from one organism that has been stably incorporated into the genome of a plant of interest.
Transgenic	See Genetically modified (GM) plant.
Vector	A plasmid that can be used to transfer DNA sequences from one organism to another.
Volunteer	A crop plant regenerated from seed or propagules left after a previous harvest and which can act as a weed in the present crop.
Weed	A plant that is growing where it is not wanted by humans.

DELIMITATIONS

The guidelines are confined to deal with ecological hazard assessment of HRC and IRCs based on a strictly scientific and technical approach. The hazard assessment must be performed on a case-by-case basis and adapted to the local conditions and agricultural production system. Other relevant aspects related to HRC/IRCs such as food safety, pleiotropic effects associated with transgenes, ethical concerns and socio-economic consequences are not considered in these guidelines.

1. RESPONSIBILITIES

1.1. Designation of authorities prior to release of HRC/IRC

Governments should have a competent authority empowered to regulate or otherwise control and, where appropriate, issue permits for the experimental release or commercial introduction of genetically modified HRCs or IRCs. The authority may exercise its powers by applying national legislation or by using an internationally accepted standard. Import or export of genetically modified HRC or IRC for any purpose should only be carried out with the consent of the proper authority.

1.2. Responsibilities of authorities

- Introduce and implement the necessary requirements to regulate the experimental release or marketing of HRC/IRC in their countries, and make provision of effective enforcement.
- Evaluate the petition prepared by the applicant, including the technical dossier. The information in the technical dossier could be derived from the list of 'information desirable for hazard evaluation', which should provide the authority with a basis for identifications of potential hazards involved.
- Issue permits stating conditions to be fulfilled by the applicant or stating the grounds for rejection.
- Keep records of notifications and ensure compliance with regulations for approval of experimental release/marketing.
- Develop procedures for the full documentation of the HRC/IRC and their use. These include experimental release (numbers/quantities, dates, localities) or commercial production, impact of each HRC/IRC in each country and any other data relevant to assessing the outcome, and make these records available to the public as may be appropriate while protecting any proprietary rights to data.

1.3. Responsibilities of applicant/permit holder

- To comply with all the regulations established by the country where the HRC/IRC will be introduced or grown.
- To prepare a dossier for submission to the authority with each application for experimental release or commercial production including all pertinent and required information on the HRC/IRC to be released.
- Ensure that persons involved in distribution of their HRC or IRC product are adequately trained, such that they are capable of providing a user with advice on efficient and safe use.
- Notify the authorities and voluntarily take corrective action and, when requested by authorities, help to find solutions to any problem related to the release and use of the HRC/IRCs.

1.4. Responsibilities of farmers growing HRC/IRC

The responsibilities of the farmer as the final user of a technology are those stated in the binding labels of HRC/IRC products and any contractual agreement signed with an importer, distributor or supplier of seed and by the regulations associated with the use of pesticides.

Farmers should:

- Maintain appropriate records of HRC/IRC varieties and area planted and pesticide use.
- Respect and obey indications and requirements related to refuges and other agronomic practices intended to prevent or delay the evolution of resistance in pests.
- Comply with any signed agreement regulating the production, saving and distribution of seed from HRC/IRCs.
- When growing HRC/IRCs, which involve the use of a pesticide, follow the regulatory rules for the particular pesticide and specific use.

2. HAZARD IDENTIFICATION

The assessment of potential hazards of growing HRC/IRC crops concerns both the crop itself and its impact on the wild flora. Consequently, understanding the interaction between the transgenic crops and all compatible relatives is crucial for a realistic hazard assessment. Consideration may also be necessary to the fauna associated with the crop, especially both insect pests and beneficial organisms.

- The HRC/IRC itself may establish beyond its agricultural boundaries and growing season and become a weed in the succeeding crops.

- The HRC/IRC may pollute the gene pool of non-transgenic relatives growing in the same or adjacent areas, depending on cross pollination characteristics and agents such as wind or by insects. In some instances where the population size of native relatives is low, genes from the transgenic crop may come to dominate the native population and lead to their extinction. The compatibility between the HRC/IRC and non-target species is of utmost importance in this regard.

- The HRC/IRC may have botanical identical or closely related species that can hybridise with the crop, either in the adjacent ecosystems or in the agro-ecosystem. Hybridization could lead to pollution of non-transgenic crops, gene stacking in volunteer plants and transfer of the resistance trait to weedy or wild species.
- The continuous use of HRCs with their associated herbicide over large areas for several years may unintentionally change the composition of the weed flora by selecting for naturally tolerant weeds. This is particularly important in monocultures or in cropping systems with limited crop rotation or minimum tillage.

- Intensive use of HRC/IRC may have a detrimental effect on the populations of non-target organisms (i.e. birds, beneficial insects)
- In case of IRC the engineered traits may increase fitness of volunteers or weedy hybrids, thus making a crop turn into a weed that can interfere with future crop production or aggravating the negative impact of existing weed species. The incorporation of resistance into a non-target species may also alter its competitive ability and displace other native species.
- Intensive use of IRC may select insect strains resistant to the toxins produced by the plant as a result of the genetic alteration.

2.1. The process of hazard assessment

The main objective of an ecological hazard assessment of HRC/IRCs is to identify possible adverse effects on the environment from growing these crops. Hazard identification is only the first step in a conventional risk assessment, the other steps being hazard characterization (magnitude of the hazard), exposure assessment (in this context an estimate of likelihood or frequency of identified hazards) and finally risk characterization. Risk characterization takes into account the results of the previous three steps to provide an estimation of the likelihood by which the adverse effects occur combined with their magnitude. This risk assessment may be quantitative or qualitative. The latter has prevailed in previous cases with approval of genetically modified organisms, because the complexity of biological systems makes it difficult to pursue a quantitative approach. Much of the needed information for a risk assessment can be obtained from practical experience with traditional crops growing in the same environment, but in some cases further experimentation is needed particularly regarding gene flow and fitness. In most established regulations of HRC/IRCs, the applicant is required to deliver the relevant information and the authorities may then base the evaluation upon this information combined with expert opinions and, sometimes, public hearings of scientific institutions, consumer organizations, NGO's and the general public. The objective of the following guideline is, however, only to identify potential adverse ecological hazards to the environment by using simple decision keys.

2.2. Information desirable for hazard assessment

Information related to the HRC/IRC:

- Taxonomic description and scientific name
- Cultivar's name
- Diagnostic phenotypic and genetic markers
- Description of geographic distribution and of the natural habitat of the plant
- Potential for gene flow and exchange with other plants
- Ecological and physiological traits:
  - Generation time in natural ecosystems, sexual and asexual reproductive cycle
  - Information on survival, including the incidence of volunteers and the ability to form perenniating structures (propagules)
- Information related to the genetic modification process
  - Methods used for the modification

  - Description of the inserted genetic material and vector construction
  - Sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question
- Information on the inserted genetic material in the HRC/IRC
  - Description of genetic trait(s) or phenotypic characteristics, particularly new traits and characteristics which may be expressed or no longer expressed
  - Characteristics of the vector
  - Stability of the genetic trait(s)
  - Rate and level of expression of the new genetic material
  - Description of identification and detection techniques
  - History of previous releases or uses of the HRC/IRC

Information on the receiving environment:
- Geographical location of the site
- Proximity to protected habitats or areas
- Proximity to compatible, related species
- Climatic characteristics and flora and fauna of the region
- Description of target and non-target ecosystems likely to be affected
- Any known planned developments or changes in land use in the region which could influence the environmental impact of the released crop
- Description of ecosystems to which the HRC/IRC could be disseminated

Information related to the interactions between the HRC/IRC and the environment:
- Characteristics affecting survival, multiplication and dissemination
- Studies of the behavior and characteristics of the HRC/IRC and their ecological impact
- Post release genetic transfer capability from the HRC/IRC into organisms in the affected ecosystems
- Likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the HRC or IRC
- Description of genetic traits, which may prevent or minimize dispersal of genetic material.
- Routes of biological dispersal and known or potential modes of interaction with the dissemination agent

Potential environmental impact:
- Potential for excessive population increase in the environment
- Competitive advantage of the HRC/IRC in relation to the unmodified recipient
- Anticipated mechanism and result of interaction between the released plant and wild and weedy relatives
- Known or predicted effects of non-target organisms on the environment, impact on population levels of all potential competitors

Information on the conditions of experimental release:

- Description of the proposed release including the purposes and foreseen products
- Foreseen dates of the release and time planning of experiment including frequency and duration of release
- Size of the site
- Method to be used for the release
- Quantities of HRC/IRC to be released
- Method of cultivation and description of general agricultural practices
- Post-release treatment of the site
- Techniques which will be applied for the elimination or inactivation of the HRC/IRC upon experiment completion
- Information on and results of previous releases of the HRC/IRC, especially at different scales in different ecosystems

Information required in the case of notification for placing in the market:

- Name of product and names of HRC/IRC contained therein
- Name and address of manufacturer in country of origin
- Specificity of the product including the appropriate environment and geographical area of the country for which the product is suited
- Estimated production or import to the country
- Proposed packaging (to prevent unintended release during storage or at a later stage)
- Proposed labeling in the official language(s) of the country including information on handling and agricultural use

Information on monitoring and control of release:

- Methods for tracing the HRC/IRC and monitoring its effects
- Specificity, sensitivity and reliability of monitoring techniques
- Techniques for detecting transgenes introgressed into non-target plants
- Methods and procedures to avoid and minimize the spread of the HRC/IRC beyond the site of release or the designated area for use
- Methods and procedures for controlling the HRC/IRC in case of unexpected spread

2.3. Hazard assessment

In assessing hazards associated with the introduction or planting HRC/IRCs in a particular area or country, a starting point will be to identify the scenarios (agro-ecosystems) under which the crop will be released and select the appropriate procedure to assess the specific hazards associated with it. Whatever approach is used to identify hazards, care should be taken to consider hazards to both agronomic and natural ecosystems.

As indicated before, any assessment of hazards and risk requires a case-by-case study and is location-specific. Specific local conditions would determine the relative importance of each type of hazard. For example, cropping patterns and landscape could have an important role in the possible escape of

transgenes, a process that involves hybridization followed by the subsequent establishment and persistence of the hybrid. The likelihood of GM crops and wild relatives forming hybrids is particularly pertinent in the centers of origin and diversity of crops, thus hazards derived from gene flow should be the priority in assessing the overall risk of release of GM crops in these areas. Another special case is that of a crop that has conspecific weeds, which increases the risk of gene movement from the GM-crop.

An important aspect, besides those mentioned above, that should be considered is the possible impact of HRC/IRCs on non-target organisms (e.g. pollinators, soil fauna or other organisms associated with the crop plant). Planting HRCs, especially over large areas, allowing the application of herbicides not previously used in the conventional crop could impose new selection pressure on weeds leading to the evolution of herbicide resistance. Similarly, exposure to insecticidal toxins from IRCs over long periods could also select for resistance in the target pests and affect predators, parasitoids and other non-target organisms. Side effects of IRCs producing insecticidal toxins are difficult to assess because of lack of knowledge thus scientific experts should be consulted regarding this on a case-by-case basis.

The final decision on releasing HRCs and IRCs is ultimately a balance between science, economics, ethics and values, local benefits and public interest. Consequently, the perceived hazard sometimes reflects conflicts of interests. The use of assessment keys should facilitate arriving to a decision based on scientific knowledge rather than on perceptions, although a quantitative approach is yet to be developed. The keys presented below were designed only as a guide in assessing the ecological hazards based on the most likely relevant scenarios. They have limitations and should be considered carefully according to local conditions and experience. It is important to take into account that cropping practices and local environmental conditions and characteristics can affect the hazards and how they are assessed or perceived. For example, interplanting an IRC with unmodified crop plants would affect the rate at which resistant individual could be selected in the target pest population. Also, the level of expression of the toxin in the crop plant can affect the likelihood of survival of slightly resistant individuals.

The keys are a useful method to begin the process of the hazard identification and assessment for IRC/HRCs, but do not in themselves provide the user with a conclusive description of the risks of planting IRC/HRCs. The questions in the keys have been arranged according to increasing magnitude of the hazards. Two main possible simplified scenarios are considered:

Scenario 1

The HRC/IRC is to be released in an agricultural system where there are compatible wild relatives or weed species.

When a HRC is to be released into an area where there are compatible wild or weedy relatives there is a possibility that the transgenes will escape and introgress into those compatible species. As a result, the wild or weedy relatives (congeneric or conspecific) could become resistant to the herbicide, making them a more noxious agricultural or environmental pest. There is also the possibility that the competitive ability of wild relatives might be altered especially if IR-genes become established in native populations. This possibility is of particular concern when the IRC is to be released into its wild progenitor's center of origin or diversity, which serve as a particularly valuable source of genes for plant breeding. Useful genes might be lost if introgression with transgenic crops results in the replacement of native genes. Under these conditions, assessment should consider all the corresponding keys below.

Scenario 2

The HRC/IRC is to be released in an agricultural system where there is no risk of gene flow to other species.

Under this scenario, there are three main concerns to consider. Weeds could evolve resistance to the herbicide that the HRC withstands because of the selection pressure imposed by its use. Secondly, management of HRC volunteers in succeeding plantings of the conventional crop or in rotation crops could become increasingly difficult. Third, there is the possibility that insects could evolve resistance to the insecticidal toxin produced by the IRC, due to increased selection pressure. Initiate the assessment by considering key no. 3 (assessing of volunteers' control) and 4 or 5 (build up of resistance).

When using a key, if you reach a point where you cannot continue any further or there is an indication of "stop", it means that you need to make a decision about a particular hazard.

Key 1: Likelihood that the competitive abilities of wild relatives occurring in undisturbed wild-lands will be altered by hybridization with transgenic crops

1. *Is the crop only self-pollinating?*
   If no: Go to No. 2 If yes: Stop, and go to key 3.

2. *Can viable hybrids form between the crop and wild relatives?*
   If yes: Go to No. 3 If no: Stop, and go to key 2.

3. *Do these wild relatives occur in the proximity of the crop?*
   If yes: Go to No. 4 If no: Stop, and go to key 2.

4. *Do the crop and the wild relatives overlap in flowering periods?*

If yes: Go to No. 5 If no: Stop, and go to key 2

5. *Do hybrids survive and reproduce in the native habitat*
   If yes: Go to No. 6 If no: Stop, and go to key 2

6. *Does HR/IR trait give hybrids or introgressants a fitness advantage in wild habitats?*
   If yes: Go to No. 7 If no: Stop, and go to key 2.

7. *Is the resistance trait maternally inherited?*
   If yes: Likelihood of producing new, more competitive native species. If no: Likelihood of producing new, more competitive native species rapidly.

Key 2: Likelihood that a new type of arable weed will be produced by gene flow between the transgenic crop and its relatives

1. *Do hybrids occur between the crop and any weedy/wild relative?*
   If yes: Go to No. 2 If no: Stop, and go to key 3.

2. *Do these weedy/wild relatives occur in the proximity of the crop?*
   If yes: Go to No. 3 If no: Stop, and go to key 3.

3. *Do the crop and the weedy/wild relatives overlap in flowering periods?*
   If yes: Go to No. 4 If no: Stop, and go to key 3.

4. *Are the hybrids and/or introgressants highly competitive in arable environments?*
   If yes: Go to No. 5 If no: Stop, and go to key 3.

5. *Are hybrids or introgressants herbicide resistant or insect resistant?*
   If HR: Go to No. 6 If IR: Go to No. 8

6. *Can HR hybrids or introgressants easily be controlled by other means besides the herbicides associated with the HRC?*
   If yes: Likelihood of losing one herbicide If no: Go to No. 7

7. *Is the same herbicide used in succeeding crops?*
   If yes: Likelihood of losing the only weed control option If no: Stop and go to key 3.

8. *Does the IR trait confer an increased fitness in the wild/weedy relative compared to non-IR relative?*

If yes: Likelihood of increased weed problems

If no: Stop and go to key 3.

Key 3: Likelihood that the transgenic crop will become a volunteer problem on arable land or wild areas

1. *Is the crop known to leave volunteers in succeeding crops?*
   If yes: Go to No. 2
   If no: Stop. There should not be a volunteer problem. Assess hazard of evolution of herbicide or insecticide resistance (keys 4 and 5).

2. *Does the crop have weedy traits?*
   If yes: Go to No. 3
   If no: Stop, and go to key 4

3. *Is the volunteer plant expected to be herbicide resistant or insect resistant?*
   If HR: Go to No. 4
   If IR: Go to No. 6

4. *Can the HR-volunteer easily be controlled by other means but the herbicides associated with HRC?*
   If yes: likelihood of losing use of a herbicide
   If no: Go to No. 5

5. *Is the herbicide used for control of non-transgenic volunteers in succeeding crops?*
   If yes: likelihood of losing the weed control option (herbicide)
   If no: Stop, and go to key 4

6. *Is the IR-volunteer crop able to establish itself in the wild?*
   If yes: likelihood of escapes into wild habitats
   If no: Go to No. 7

7. *Can the IR volunteer easily be controlled in succeeding crops?*
   If no: Go to No. 8
   If yes: Stop, and go to key 5

8. *Does the IR trait confer an increased fitness in the volunteer compared to non-transgenic volunteers?*
   If yes: Likelihood of increased weed problems
   If no: Stop, and go to key 5

Key 4: Likelihood of build-up of HR-resistant weeds

1. *Are resistance cases to the herbicide that the HRC withstands or herbicides belonging to the same chemical family or having the same mode of action (MOA) or degradation known to occur, or is gene flow possible from HRC to related weedy species, or is the herbicide a new chemical?*

If yes: Go to No. 2

If no: Stop. There should be a low hazard of evolution of herbicide resistant weeds, especially if integrated weed management is used

2. *Is the cropping system primarily a monoculture or the HRC is or will be fully rotated with other crops?*
   If monoculture: Go to No. 5
   If fully rotated: Go to No. 3

3. *Is weed management primarily based on an integrated strategy or on chemical control?*
   If chemical control: Go to No. 4.
   If integrated strategy: Stop. Very limited hazard of Herbicide resistance evolution.

4. *Is the MOA of the herbicide used in HRC crop similar or different to that used in the other rotational crops?*
   If same: consider likelihood of selection for resistant weeds
   If other: Stop. Very limited hazard of herbicide resistance evolution.
5. *Is weed management under the monoculture system primarily dependent on herbicides?*
   If yes: Go to No. 6
   If no: Stop. Very limited hazard of herbicide resistance evolution.

6. *Is the herbicide to be used in the HRC a new persistent compound or a chemical to be used twice or more in cropping cycle?*
   If yes: consider the likelihood of selecting new resistant weeds
   If no: Go to No. 7

7. *Does the herbicide used in HRC share MOA with others in use?*
   If yes: Risk of aggravating or speeding resistance problems
   If no: Stop. Limited hazard of herbicide resistance evolution.

Key 5: Likelihood of build-up of resistant insects

1. *Does the IRC comprise a major proportion of the local area planted with non-transgenic varieties of that crop?*
   If yes: Go to No. 2
   If no: Stop. Limited hazard of insecticide resistance evolution.

2. *Does the IRC express only a single or few insecticidal-toxin(s) active against the harmful insect?*
   If yes: Go to No. 3
   If no: Stop. Limited hazard of insecticide resistance evolution.

3. *Is expression of the IR trait confined to a short lasting selected growth stage of the crop?*

If no: Go to No. 4

If yes: Stop. Limited hazard of insecticide resistance evolution.

4. *If resistance in insects occurs, is expression of the IR trait associated with a significant fitness penalty for the resistant insect?*

If no: Go to No. 5

If yes: Stop.

5. *Are resistant insects easily controlled by other control measures?*

If yes: likelihood of losing effect of the IR trait

If no: likelihood of losing the IR trait and specific-toxin based biological pesticides.

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