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HARMONIZATION OF PROTOCOLS FOR ASSESSING THE BIOEFFICACY AND BIOSAFETY OF GENETIC ENGINEERING AND CONVENTIONAL TECHNOLOGIES FOR PEST MANAGEMENT

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ABSTRACT

Several technologies are in use for the management of insect pests, wherein, different protocols and guidelines are being followed for testing their bioefficacy and biosafety. Therefore, it is important to have a comparative assessment of bioefficacy and biosafety of different pest management technologies viz. synthetic pesticides, biopesticides, natural plant products, natural enemies, and genetically modified organisms (GMOs) to the nontarget organisms in the environment. Toxicology and biosafety data should be generated on prescribed animals as per the national and international protocols recommended by the government agencies, FAO, WHO, OECD, and EPA. Natural plant products, natural enemies, and insect-resistant crops developed through conventional and genetic engineering approaches should be viewed differently and safety requirements simplified and relaxed as appropriate, as compared to the synthetic insecticides. Generation of data on bioefficacy should not only be done in micro-plots at the research stations, but also on the farmers' fields across a range of environments. Eco-safety data requirements and test protocols need a holistic review to ensure that priority risks are addressed and tests are focused on realistic exposure regimes.

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Table 1: Data requirements for registration/commercialization of pest control technologies.

Chemistry/Origin	Insecticides	Botanical pesticides	Biopesticides	Natural enemies	Insect resistant transgenic crops	Insect resistant cultivars
Source	√	√	√	Country of origin	√	√
Chemical composition, identity	√	Approximate	Species/strain	Species/strain	Transgene product	Parents
Physico-chemical properties	√	√	√	Biology	√	×
Technical details	√	√	√	×	√	×
Specifications	√	√	√	√	√	×
Method of analysis	√	√	Serotype	DNA barcoding	PCR/ELIZA based methods	DUS traits
Identification and quantification of impurities	√	√	×	×	×	×
Shelf-life	√	√	√	Storability	×	×
Nutritional equivalence	×	×	×	×	Nutritional equivalence	√
Process of manufacture	√	√	√	Rearing methods	Transformation protocols	Breeding method
Raw materials used, source	√	√	√	Origin	Vector/plant	Parents involved
Step-wise production process	√	√	√	√	√	√
Chemical formula	√	√	×	×	×	×
Effluent treatment method	√	√	×	×	×	×
Registration certificate / license/other approvals	√	√	√	√	√	√
Certificate from manufacturer	√	√	√	√	√	√
Methodology for residue estimation	√	√	×	×	×	×

Modified from data requirements by the Central Insecticide Board for synthetic pesticides (http://cibrc.nic.in). √ = required. × = Not applicable.

- Maximum permissible levels of chemical contaminants (synthetic pesticides/biochemical pesticides, mycotoxins, heavy metals, pathogens, etc.).
- Quality control for ensuring quality.
- Application of hazard analysis and critical control point (HACCP), and good hygienic practices during production and processing.
- Information on manufacture and process details.
- Information about raw materials, solvents used for extraction, and reagents entering the manufacturing process.
- Standardized criteria (chemical consumption).
- Stability of botanicals/botanical preparations (residual and shelf-life time).

Table 2: Information on phytotoxicity, metabolism and persistence in the environment.

Bioefficacy parameter	Insecti-cides	Botanical pesticides	Biopes-ticides
Effectiveness	√	√	√
Phytotoxicity	√	√	√
Effects on parasites and predators	√	√	√
Translocation in plants	√	√	×
Metabolism/degradation in plants, soil, and water	√	√	√
Persistence in soil/water/plant	√	√	√
Compatibility	√	√	√
Residues in plant/soil	√	√	√
Residue tolerance limits	√	√	√
Cost: benefit ratio	√	√	√
Registration status in other countries	√	√	√

Bioefficacy parameter	Natural enemies	Insect resis-tant transge nic crops	Insect resis-tant cultivars
Effectiveness	√	√	√
Phytotoxicity	×	×	×
Effects on parasites and predators	Indigenous fauna	√	×
Translocation in plants	×	×	×
Metabolism/degradation in plants, soil, and water	×	√	×
Persistence in soil/water/plant	×	√	×
Compatibility	√	√	×
Residues in plant/soil	×	√	×
Residue tolerance limits	×	Transgene product	×
Cost: benefit ratio	√	√	√
Registration status in other countries	√	√	√

Modified from data requirements by the Central Insecticide Board for Synthetic Pesticides (http://cibrc.nic.in). √ = required. × = Not applicable.

Information on ecological testing is used by the Registration Committees to determine potential hazards, if any, to human health and nontarget organisms such as birds, livestock, fish, honeybees, etc. in the environment. Potential adverse ecological effects are assessed from the data generated in a tiered fashion, beginning with less expensive acute toxicity tests on a few representative organisms and progressing through more expensive chronic toxicity tests to a final tier of simulated and/or actual field tests. The minimum data on ecological-effects required to support the registration of an outdoor-use include toxicity tests on nontarget organisms such as: i) birds, ii) fish, iii) honeybees, iv) livestock, v) spray operators, and vi) industrial workers. Information on use pattern, environmental degradation, and mammalian toxicity is desirable to determine if additional testing is required to assess the risk further. Such information on risks and fate of the chemical/organism in the environment assists in deciding the toxicity status of the product.

The initial toxicity data are required to estimate acute toxicity (LC_{50}, EC_{50} and LD_{50}) of the active ingredient to aquatic and terrestrial organisms (rat/mice/fish). Further studies may be required for evaluation of toxicity to livestock, and the effects of a pesticide on parasites and predators. In case of botanical pesticides, information on acute toxicity, neuro-behavioral and reproductive effects, carcinogenicity, mutagenicity, and effects on spray operators is also necessary for sale and field application. The second tier data on toxicity to birds, honeybee, fish, livestock and spray operators are however not required in special cases due to proven safety of the natural molecule. Information on phytotoxicity is important for both natural and synthetic pesticides, as the effectiveness depends upon the inherent toxicity to the host plant. Normally, the effects of natural pesticides on parasites and predators are not essential, presuming that natural products are harmless. However, these products must be evaluated on a few representative species to determine their safety to the natural enemies and other nontarget arthropods. In the case of neem-based pesticides, long-term toxicity data on neuro-behavior toxicity, mutagenicity, carcinogenicity, effect on reproduction, and health records of workers are also required (Shetty 2004).

Studies on the fate of pesticides in the environment are a pre-requisite, whereas in the case of natural products (botanical pesticides and biopesticides), such studies may not be required as they degrade in the environment faster than the synthetic molecules. However, such decisions must be based on the known facts. Data related to fate in environment includes translocation, metabolism and persistence in soil, plant, and water bodies. Information on the primary/secondary metabolites, degradation/metabolic pathway(s), half-life, persistence in different environmental segments (soil, sediment, water, and air), dissipation, mobility and likely concentrations in the environment, and bioaccumulation is helpful to establish persistence of the pesticide. Aerobic and anaerobic metabolism

Table 3: Acute and chronic toxicity requirements for pest control technologies.

Toxicity	Insecti-cides	Botanical pesticides	Biopes-ticides	Natural enemies	Insect resistant transgenic crops	Insect resistant cultivars
Acute oral toxicity in rat and mice	√	√	√	×	√	×
Acute dermal	√	√	√	×	√	×
Acute inhalation	√	√	√	×	×	×
Primary skin irritation	√	√	√	×	√	×
Irritation to mucous membrane	√	√	√	×	√	×
Sub-acute oral rat	√	√	√	×	×	×
Sub-acute toxicity (oral/dermal/inhalation)	√	√	√	×	√	×
Neuro-toxicity	√	√	√	×	√	×
Teratogenicity	√	√	√	×	√	×
Effect on reproduction	√	√	√	×	√	×
Carcinogenicity	√	√	√	×	√	×
Allergenicity	√	√	√	×	√	×
Metabolism	√	√	√	×	√	×
Mutagenicity	√	√	√	×	√	×
Toxicity to birds	√	√	√	×	√	×
Toxicity to fish	√	√	√	×	√	×
Toxicity to honeybees	√	√	√	×	√	×
Toxicity to live stock	√	√	√	×	√	×
Toxicity to human beings	√	√	√	×	√	×
Effects on spray operators	√	√	√	×	×	×
Effects on industrial workers	√	√	√	×	×	×
Toxicity to livestock in field trials	√	√	√	×	√	×

Modified from data requirements by the Central Insecticide Board for synthetic pesticides (http://cibrc.nic.in). √ = required. × = Not applicable.

Table 4: Data requirements for storage, transport, application, symptoms of poisoning, and antidotes for pest control technologies.

Packaging/storage	Insecti-cides	Botanical pesticides	Biopes-ticides	Natural enemies	Insect resistant transgenic crops	Insect resistant cultivars
Chemical composition	√	√	×	×	×	×
Detailed directions concerning usages	√	√	√	√	√	×
Time of application	√	√	√	√	×	×
Application equipment	√	√	√	×	×	×
Waiting period	√	√	√	×	×	×
Symptoms of poisoning	√	√	√	×	×	×
First aid/ antidotes and treatment	√	√	√	×	×	×
Restrictions, if any	√	√	√	×	√	×
Instruction for storage	√	√	√	√	×	×
Disposal of used containers	√	√	√	×	×	×
Type of packaging	√	√	√	√	×	×
Manner of packaging	√	√	√	√	×	×
Secondary packaging/ transport	√	√	√	√	×	×
Labeling	√	√	√	√	√	√

Modified from data requirements by the Central Insecticide Board for synthetic pesticides (http://cibrc.nic.in). √ = required. × = Not applicable.

tests are performed in soil and water. Environmental transport is determined through leaching, adsorption/desorption and volatilization studies. Leaching and adsorption/desorption data are important as it depends on the chemistry of the active ingredient as well as the formulants used in the final product, use patterns, and other pertinent factors. These tests are considered basic to an understanding of likely field-dissipation routes under actual-use conditions at maximum label rates. Harvest time residue of a particular compound in the soil as well as in the produce needs to be estimated. Based on these data, maximum residual limit (MRL) and waiting period are estimated. Furthermore, for synthetic molecules, effluent treatment procedures need to be mentioned for environmental safety (Kapustka et al. 1996). For botanical pesticides, no such data are required for registration, as there are no reports of their persistence in the environment (Waage 2001). Test reports about the quality of the product should be obtained from a laboratory with ISO 9000. The applicant should also provide sample along-with standard technical sample from the principals/ authorized dealers for chemical verification. In case of technical grade pesticides, samples of standard impurities are also to be provided for chemical verification. Methodology for residue estimation should also be provided along with information on carcinogenicity and genotoxicity.

Biosafety of biopesticides

The World Health Organization (WHO) memorandum provides recommended safety tests for application to biological agents under consideration for widespread use in pest control (WHO 1979). The basic principles utilized in developing these recommendations are: i) the hazards presented by microbial pesticides are inherently different from those associated with the use of chemical pesticides, and the tests used to determine potential hazard to humans should be reflected, ii) a high proportion of negative results is likely, iii) tiered testing systems should be used, negative data obtained at any level would obviate the need for further testing, and iv) the primary tier testing protocols should be designed to expose test animals to the microbial agents under conditions that provide maximum opportunity for expression of any adverse effects (Burges et al. 1981).

Information on identity, host range, mode of action in natural hosts, and optimum and maximum temperatures for growth of the organism should be elicited, especially in relation to hazard assessment early in the research phase. The suggested tests for bacteria and fungi are given in Fig. 1 (Chapter V). These tests are arranged in three tiers, any of which can lead directly to an assessment of the potential hazard. If all the tests in the first tier produce negative results, the organism can be considered safe for use as directed, without further experimentation. Information on the safety of material added subsequently to improve adherence and longevity need to be obtained

separately. The biosafety tests include: i) oral intraperitoneal, respiratory exposure, eye and dermal exposure, allergenicity, hypersensitivity and mutagenicity tests, and tier 2 and 3 tests. The emphasis should be on infectivity rather than toxicity, since spores of protozoa and viruses are not known to be associated with toxins. It is therefore necessary to use mammalian tissue cultures in Tier 1, as they provide a sensitive test for infectivity. In addition, they provide material with which the tests through incorporation of viral DNA into the host genome need to be conducted. Microsporidial insecticides may be related to a natural microsporidan pathogen of mammals, which is known to attack the nervous tissue. Therefore, the intracranial route should be included in the Tier 1 tests with insect microsporida.

Biosafety requirements for natural enemies

The Food and Agriculture Organization (FAO) code of conduct is being used to address the application of biocontrol measures prior to import and export of the natural enemies, which also provides internationally accepted procedures. The International Standards for Phytosanitary Measures (ISPM 3; Code of conduct for the import and release of exotic biological control agents) has been further revised and published by the Secretariat of the International Plant Protection Convention (IPCC in 2005 as "Guidelines for the export, shipment, import and release of biocontrol agents and other beneficial organisms"). The developing countries that have recently started the use of biocontrol agents or those with an opportunity to use biological control have benefited most from ISPM 3. This is expected to become the standard for all biocontrol introductions worldwide. ISPM 3 has ensured that environmental issues are addressed properly, and would provide a mechanism for formalizing good practice and setting standards for decision making within an internationally recognized framework.

The environmental impact of biocontrol agents can be defined as any measurable effect on a nontarget species resulting from the introduction of an exotic organism. The results could be complex, with effects on several species in the food-web spanning three trophic levels. An indirect effect could be increased competition for host or prey with the native natural enemies or habitat modification (van Lenteren 1997). Till now, very few problems have been reported concerning negative effects of releases of arthropods for controlling arthropods, despite more than 5,000 introductions in at least 196 countries or islands. Though ISPM 3 gives the regulations to be followed for release of biological control agents, it does not provide methods for assessment of environmental risks. van Lenteren et al. (2006) and Bigler et al. (2006) have tried to develop comprehensive and quick scan environmental risk assessment methods for biological control agents. van Lenteren et al. (2003) listed five risk factors: host range, establishment, dispersal, direct nontarget effects, and indirect nontarget effects. The risk assessment involves three phases:

- Risk identification and evaluation.
- Risk management, including risk mitigation or reduction.
- Risk/benefit analysis of the proposed release.

It has been recommended to avoid using generalists or adventive species, expand host-specificity testing, incorporate more ecological information, consider ecological risk in target selection, prioritize agents, and pursue genetic data on adaptation with which we can further increase the safety of biocontrol agents.

Biosafety assessment of entomopathogenic nematodes

Studies on the safety of entomopathogenic nematodes (EPN) to nontarget organisms have been reviewed by Akhurst and Smith (2002). Available evidence clearly indicates that EPN are safe biocontrol agents, which are more specific, and pose lower risk to the environment than the chemical insecticides. Since the first use of EPN for insect control as early as in 1935 (Glaser and Farrel 1935), no environmental or health hazard associated with their use has been reported, despite their widespread use particularly over the past two decades. In general, the protocols followed for the use of natural enemies can be adapted for the production and release of entomopathogenic nematodes.

Environmental risks associated with insect-resistant cultivars

Organization for Economic Cooperation and Development in 1993 made first attempt to consider environmental safety issues associated with growing or using a particular plant as food, fiber, fuel, or for any other purpose (OECD 1993). Gene transfer, weediness, trait effects, genetic and phenotypic variability, expression of genetic material from pathogens, and worker safety are the six important issues that need to be addressed for environmental safety. Environment Protection Agency (EPA) in 1994 proposed that traits intended for pest control be subjected to regulation under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as plant pesticides. The regulation has still not been approved, as a consortium of 11 scientific societies challenged the concept that the traits used in plants for defense against pests cannot be equated with pesticides applied to plants. Some plant defense chemicals also affect the food quality. Gossypol and related compounds are toxic to non-ruminant vertebrates (Lambou et al. 1966). Rutin, chloroginic acid, tomatine, and phenols may have toxic effects on humans, and some of these compounds may also have carcinogenic and mutagenic effects.

One of the major environmental impacts of deployment of insect-resistant genotypes has been development of virulent insect populations overcoming host resistance. Selection of inseect populations for virulence limits the utility of resistance genes. Nearly 86 biotypes in 22 insect species feeding on nine crops have been reported (Smith 2005). To cope with the problem of emerging biotypes, the foremost requirement is systematic surveillance and monitoring

of biotypes, which is helpful in breeding pest-resistant cultivars. There is a need for developing short- and long-term strategies to breed for insect resistance, with a focus to broaden the genetic basis of resistance, involving both major and minor genes. This includes identification of donors of new resistance genes, sequential release of varieties with major genes, pyramiding of major genes, development of multilines and synthetics through horizontal resistance, wide hybridization, and gene rotation.

Biosafety assessment of transgenic crops

No transgenic crop can be used commercially until its agronomic superiority is assessed vis-à-vis compared with traditional best checks in farmers' fields. A series of open field trials are mandatory to meet regulatory requirements for agronomic performance, once safety and efficacy has been established. The open field trials are divided into: confined field trials, multi-location research trials, and large-scale field trials. These trials are spread over a period of 5 to 6 years. The initial information is generated under laboratory and greenhouse conditions with the permission of the Institutional Biosafety Committee (IBSC). The environmental impact studies are undertaken as per the norms stipulated by the Genetic Engineering Approval Committee (GEAC) of the Department of Biotechnology (DBT) by different public sector institutions and accredited private laboratories (DBT 2007). These include pollen flow, germination, aggressiveness and weediness, soil analysis covering effect on soil micro-biota and the presence of transgene protein in soil, effects on nontarget and beneficial insects, and baseline susceptibility studies (Table 5).

Table 5: Assessment of environmental biosafety/impact of genetically engineered crops.

Test	Remarks
Greenhouse evaluation	The greenhouse and confined field trials are conducted for selection of elite events and to assess preliminary biosafety, food safety and environmental effects.
Pollen flow and gene transfer	The pollen flow studies are carried out to estimate outcrossing frequencies, determine possibility of gene transfer into closely related species, and assess weediness characteristics of GM crops.
Germination, aggressiveness, weediness	These studies are undertaken to confirm that the introduced gene will not confer any fitness disadvantage to the wild species.
Effects of rhizoshpere	These studies include effects of expressed protein on soil microflora and invertebrates. These studies cover different agro-climatic zones as per protocols approved by the regulatory authorities.
Impact on nontarget organisms	Given the importance of beneficial insects, it is mandatory to conduct studies on the effect of GM crops on nontarget organisms.
Baseline susceptibility	Data on baseline susceptibility should be generated as a part of post-release requirements to assess development of resistance following large-scale release of the transgenic crop.

Source: ISAAA (2009).

The regulatory authorities have specified a number of defined safety tests to determine potential impact of genetically engineered crops on human and animal health based on the modification of the proteins in the plant or based on the use of transgene (*e.g. Bt* protein). The transgenic crop under evaluation is subjected to toxicity, allergenicity and feeding tests, which includes acute, sub-chronic and chronic testing. More precisely, the human/animal health safety testing is done on fish, chicken, rabbit, rats, goats and cows, and on other model animals as recommended on a case-by-case basis. The transgenic crops are also tested for toxicity to ascertain that it contains no new allergenic compounds and is non-allergenic. The foliage and fruit feeding studies are undertaken in goat and cow. Other studies include protein expression and quantification, substantial equivalence, and protein estimation in cooked food. The toxicity, allergenicity, and food and feed safety evaluation tests are listed below.

- Acute oral toxicity studies in Sprague dawley rats.
- Mucous membrane irritation test in female rabbit.
- Primary skin irritation test in rabbit.
- Substantial equivalence (compositional analysis) studies.
- Sub-chronic oral toxicity study in Sprague dawley rats.
- Assessment of allergenicity of protein extracts using brown Norway rats.
- Dietary feed tests on common carp for evaluating growth performances.
- Food cooking and protein estimation in cooked food.
- Sub-chronic feeding studies using New Zealand white rabbit.
- Effect on performance and health of broiler chickens.
- Sub-chronic feeding studies in goats.
- Feeding studies in lactating crossbred dairy cows.
- Protein expression studies.

Effects of Various Crop Protection Technologies on Human Health

Human health effects are caused through: i) skin contact, ii) inhalation, and 3) ingestion through food or in water. Industry and farm workers are exposed to inhalation and skin contact during preparation and application. However, for the majority of population, the principal source of contamination is through food and water. The harmful effects of pest control technologies on human beings are:

- Death.
- Cancers, tumors, and lesions.
- Reproductive inhibition.

- Disruption of immune and endocrine system.
- Cellular and DNA damage.
- Teratogenic effects (physical deformities).
- Poor health marked by low red to white blood corpuscles ratio.
- Intergenerational effects that are more pronounced in subsequent generations.

Many of these may not be due to direct effects, but associated with a combination of environmental stresses such as eutrophication and pathogenesis (Baker and Wilkinson 1990; Margni et al. 2002). Although several documents on safety assessment of pesticides as ingredients in food and food supplements are available, the relevant legislative framework and guidance for risk assessment have not yet been established. For transgenic crops, the standard tests for biosafety of transgenic crops for human health include: i) mammalian toxicity, ii) digestibility, iii) allergenicity and homology with known food allergens and toxins, iv) compositional analysis, v) nutritional assessment (concentrations and effects on bioavailability), and vi) unexpected or unanticipated effects. A comprehensive evaluation of the new technology in terms of its environmental impact that contributes towards sustainable agriculture forms an integral part of India's regulatory system for the approval of transgenic crops.

Conclusions

There is an urgent need for standardization and harmonization of protocols for assessment of bioefficacy and biosafety of synthetic pesticides, biopesticides, natural plant products, natural enemies, and GMOs to the nontarget organisms in the environment. Toxicology and biosafety data should be generated on prescribed animals as per the national and international protocols recommended by the government agencies, FAO, WHO, OECD, and EPA. Regulatory measures need to be reinforced to prevent use of spurious pesticides, and check adulteration of botanical and microbial products with undesirable materials. Plant products intended as pesticides should be devoid of undesirable substances toxic to humans and domestic animals. Unintended pathogenicity and long-term impact of microbials towards non-pathogens and other nontarget organisms should also be studied properly. Natural plant products, natural enemies, and insect-resistant crops developed through conventional and genetic engineering approaches should be viewed differently and safety requirements simplified, and relaxed as appropriate as compared to the synthetic insecticides.

Expert groups of researchers, academia, practitioners, and regulatory bodies should be established at the national and international levels to prevent injudicious use of pest control technologies and monitor nontarget effects on the environment. Generation of data on bioefficacy should not only be done in micro-plots at the research stations, but also on the farmers'

fields across a range of environments. There is a need to develop a database on protocols and effects of pest management technologies on the environment to make informed decisions about the possible and safer interventions for pest management. Eco-safety data requirements and test protocols need a holistic review to ensure that priority risks are addressed and tests are focused on realistic exposure regimes.

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