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Streamlining Data Requirements for the Environmental Risk Assessment of Genetically Modified (GM) Crops for Cultivation Approvals

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Abstract

Genetically modified (GM) crops have been cultivated safely for more than 25 years, and the framework for conducting an environmental risk assessment (ERA) is well-established. Currently, there is alignment of broadly-stated protection goals across global jurisdictions; however, there is a lack of consistency for the data that are required by regulators. Risk assessors have over 25 years of collective experience assessing the environmental safety of GM crops and have conducted hundreds of ERAs to evaluate GM crop safety. This experience provides a scientific basis to help determine which data informs the ERA, and which data does not inform the safety assessment. The goals of this paper are to: 1) define the process for identifying potential pathways to harm based on robust problem formulation; 2) provide an overview of data that inform the science-based ERA for cultivation approval; 3) provide examples of data that are routinely or occasionally required but do not inform the ERA; and 4) make recommendations for harmonization of global ERA data requirements. Refinement and harmonization of data requirements across global regulatory authorities will add transparency and predictability to the ERA of GM crops globally, while ensuring that each country's protection goals are respected.

Keywords: environmental risk assessment, genetically modified plant, problem formulation, data requirements, cultivation

1. Introduction

Genetically modified (GM) crops are cultivated on over 191.7 million hectares worldwide [30]. Prior to commercial approval, GM crops undergo thorough safety assessments to characterize food and feed safety in countries that cultivate the crops and those that import GM grain [13, 21, 59]. Additionally, in countries cultivating GM crops, environmental risk assessments (ERA) are conducted as part of the regulatory approval process to assess impacts on the agricultural and surrounding environments where the crop is intended to be grown. ERA investigates the potential types and magnitude of harm to valued elements of the environment that could arise from the crops' environmental release.

To date, the majority of commercialized GM crops has been limited to commodity row crops (e.g., corn, soybean, cotton, canola) containing herbicide tolerance and/or insect protection traits. As a result, there is a large body of knowledge (i.e., familiarity) surrounding the potential environmental risks associated with cultivation of these crops and traits. New insect-protection traits, tolerance to new herbicidal active ingredients, disease protection traits, and traits that improve agronomic performance, shelf-life and nutritional profiles are also being developed in corn, soybean, cotton, canola, sugar beet, as well as in new crops (apples, potatoes, banana, eggplant, etc.) [16, 15, 3, 48, 57, 52, 54]. As new crop and trait combinations are developed, additional considerations (potential pathways to environmental harm) may become relevant to consider as part of the ERA. However, a science-based ERA framework should be robust and flexible enough to be applied to any crop or trait combination to enable regulatory decision making (for example, [55]).

While most countries have similar broadly-stated protection goals (e.g., protection of biodiversity), there is a lack of global

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alignment and consistency in the data that are required for ERA of GM crops, and not all data that are required globally inform science-based decision making in the ERA. The goals of this paper are to: 1) define the process for identifying potential pathways to harm based on robust problem formulation; 2) provide an overview of data that informs the science-based ERA for cultivation approval; 3) provide examples of data that are routinely or occasionally required but do not inform the ERA; and 4) make recommendations for harmonization of global data requirements for ERA. Refinement of data requirements to those that inform the ERA and harmonization of data requirements across global regulatory authorities will add transparency and consistency to the ERA of GM crops globally while ensuring that countries' protection goals are respected.

1.1. Problem Formulation to Identify Potential Pathways to Harm

Protection goals are established by local legislation or by regulatory authorities to describe the species, habitats, and/or ecosystem services that are to be protected. These protection goals are typically broadly stated and are often translated into operational protection goals with clear relevance to the ERA [19, 24]. Understanding the operational protection goals of each regulatory authority is important for understanding what is to be protected and determining relevant risk assessment endpoints. In the context of a GM crop, a broad protection goal (e.g., protection of biodiversity) could be translated into an operational protection goal (e.g., protection of beneficial or charismatic species). While impacts on protection goals can be difficult or impossible to measure, information is available or can be developed on relevant assessment endpoints (e.g., non-target organism abundance or diversity), and studies may be performed to measure specific relevant effects (e.g., honeybee mortality). Protection goals must be accompanied by standards to judge adverse effects in the agricultural context (for example, 50 percent reduction in population abundance; [2]).

For cultivation approval, a hypothesis-based approach should be used to determine potential environmental risks, and the data that are required should inform the ERA by providing reliable scientific evidence that addresses a plausible, testable hypothesis. Problem formulation is used to develop hypotheses of potential harm, based on knowledge of the receiving environment, the biology of the crop, and the characteristics of the introduced trait [47, 58, 43, 40]. Often, a testable hypothesis can be addressed with the use of existing knowledge/studies, and no additional data need to be generated. Because risk is a function of both hazard and exposure, if exposure can be shown to be low or negligible, additional hazard characterization may not be needed to inform the risk assessment. For example, if it can be demonstrated that there is low or negligible exposure of a non-targeted organism (NTO) to an insecticidal trait in a GM crop, additional hazard data are not needed to conclude low or no risk to that specific NTO. This is one of the fundamental reasons why hazard data on NTOs should not be necessary for import approvals [42]. Due to low-level exposure scenarios associated with import of GM grain (e.g., grain spillage at port or processing facility), there are rarely plausible hypotheses for harm to the protected elements of the environment [42, 29, 41]. Most potential harms would arise in the country of cultivation due to higher potential exposure, so the ERA conclusions in a cultivation country should be sufficient to inform potential risk in an importing country. In other instances, if the potential for harm cannot reasonably be ruled out based on existing information on the environment, crop, or trait, further examination of potential exposure or hazard may be warranted to assess risk. For instance, if the importing country has wild relatives of the crop that are not present in the cultivating country, there may be a potential for gene flow, which could trigger assessment of the environmental risk that could result from transgene introgression into populations of the wild relatives in that country. The problem formulation approach to ERA is a robust way to structure the risk assessment to consider plausible hypotheses of harm, assess available information that addresses those hypotheses, generate additional data that reduce uncertainty in the identified risks, and enable decision making that is relevant to the protection goals.

1.2. Overview of Data that Inform the Science-Based ERA for Cultivation Approval

ERA of a GM crop evaluates the likelihood of harm arising from the interaction of the GM crop with the environment, compared with non-GM counterparts. Therefore, the data that universally inform the ERA for all crops and traits include: 1) an understanding of the receiving environment and the basic biology of the unmodified plant; 2) an assessment of the agronomic similarity of the GM crop to its conventional counterparts; and 3) an understanding of the intended trait of the GM plant and assessment of how the intended trait may lead to environmental harm (Table 1).

1) Understanding of the receiving environment and the basic biology of the unmodified plant

By definition, the agroecosystem is ecologically disturbed, unstable, and dynamic, and the potential effects of cultivating a GM crop must be considered relative to the effects of cultivating the non-modified crop. Agricultural fields generally do not support high biodiversity, but instead are dominated by one or a few cultivated plant species and are managed to maximize yield (e.g., tillage, weed, insect, and pathogen management). Therefore, if a general protection goal to protect biodiversity is to be observed, the ERA should consider if the GM plant adversely affects biodiversity relative to the non-modified plants growing in the same agroecosystem and managed under standard agronomic practices.

An understanding of the basic biology of the crop is also a key component of problem formulation. For example, understanding if the unmodified crop has any weediness characteristics [5], if it survives outside of managed cultivation, or if it outcompetes other plants, are important considerations in the context of ERA. Most agricultural crops are highly domesticated, and the agronomic traits that make them efficient at meeting human needs under cultivation have been selected for by breeding over hundreds or thousands of years. Many of these characteristics selected for in the domestication process also make them

Table 1: Data that universally inform the environmental risk assessment for cultivation of a genetically modified (GM) crop

Data relevant for ERA of all crops and traits	**How data informs the ERA**
Understanding of the receiving environment and the basic biology of the unmodified plant	Understanding of the receiving environment allows for relevant pathways to harm related to the receiving environment to be considered. For example, does the receiving environment contain any wild or weedy relatives? Do wild relatives grow near or adjacent to the GM plant? Understanding the basic biology of the unmodified plant allows for relevant pathways to harm related to survival, weediness, reproduction, gene flow, etc., to be considered. For example, does the non-modified plant have weediness characteristics [6]? Can the non-modified plant survive outside of cultivation? Does the non-modified plant outcross with wild relatives?
Comparative assessment of the agronomic similarity of the GM crop to its conventional counterparts	Assessment of the agronomic similarity of the GM crop to its conventional counterparts allows for relevant pathways to harm related to survival, weediness, reproduction, gene flow, etc., to be considered. For example, is the GM crop similar to the non-modified crop in terms of the standard agronomic endpoints? Does the GM plant have traits that may increase weediness (seed shattering, dropped ears, etc.)?
Understanding of the intended trait of the GM plant and assessment of how the intended trait may lead to environmental harm	Understanding of the intended phenotype of the GM plant allows for relevant pathways to harm related to the trait to be considered. For example, does the intended trait confer insect protection? Herbicide tolerance? Drought tolerance? Understanding the intended trait(s) and a basic understanding of its mode of action will inform problem formulation and may indicate additional relevant data requirements for the ERA (as described in Table 2).

poor competitors with natural vegetation in the absence of human intervention. Maize, for example, is highly domesticated, and populations of maize do not survive outside of cultivation [36]. There is extensive information and knowledge about basic weediness and reproductive and survival characteristics for all major row crops like maize, soybean, cotton, and canola, which can be leveraged to inform the ERA [38, 37, 36, 35].

2) Assessment of the agronomic similarity of the GM crop to its conventional counterparts

Like conventional breeding programs, GM plants are assessed and screened through many rounds of event selection to ensure the agronomic and phenotypic characteristics of the commercialized event meets farmer needs. If the agronomic and phenotypic characteristics of a GM plant have deleterious effects or are not desired by the developer or customer, the event is eliminated from further development. This basic process of selection that is used for both conventional breeding programs and GM plant development programs is important for developing robust, commercially viable products, while it also ensures that plants with undesirable phenotypes are not advanced [25].

Standard agronomic endpoints are collected throughout event selection as well as from large multi-site field trials, and these agronomic data can be used to assess the similarity of the GM plant to its conventional counterparts. Conventional crops can have an extensive range of agronomic properties, enabling them to be grown across diverse environments or to meet various societal needs. This range of agronomic properties is accepted by society because it does not present an unreasonable environmental risk and there is an established history of safe use of domesticated crops. If a GM plant is shown to be agronomically similar to non-modified plants with a history of safety, it would have no novel risks outside the range of the conventional crop other than the introduced GM trait. Therefore, the ERA can focus on the intended traits, and additional data would only be needed to inform the ERA if plausible risk hypotheses can be developed for potential environmental harm caused by that trait. For example, the basic biology of maize is well established and accepted [36]. As previously mentioned, maize does not survive outside managed agricultural environments [11], and its survival and reproduction is limited by environmental conditions (heat stress, frost, drought, excessive rainfall, etc.) [51]. If the GM plant is shown to be agronomically similar to non-modified maize, which has no weediness characteristics, and the intended phenotype is not related to a weediness characteristic, then there is no plausible hypothesis for how the GM plant could increase weediness potential. In this case, generating data that are related to plant weediness would not further inform the risk assessment. Risk can be assessed based on the biology of the unmodified plant, understanding of the intended phenotype, and similarity of agronomic characteristics.

3) Understanding of the intended trait of the GM plant and assessment of how the intended trait may lead to environmental harm

An understanding of the intended trait of the GM plant

helps inform problem formulation. A basic understanding of the mode of action of a newly expressed protein in a GM plant, which is often investigated as part of the food and feed risk assessment, can support the understanding of the intended trait. If plausible risk hypotheses can be developed for how a novel trait could lead to environmental harm, they can guide the risk assessment and help determine which data are relevant for assessing risk. For example, if the intended phenotype of the GM plant is to protect against insect pests, this information helps guide the ERA towards assessing hazards to non-target insects in the agroecosystem. In this example, a basic understanding of the mode of action of the insecticidal protein (e.g., receptor binding, pore forming, enzymatic catabolism, etc.), may support the understanding of the trait and problem formulation. A full understanding of how the insecticidal protein works at the molecular, cellular, or anatomical level should only be required if plausible hypotheses for harm could be developed and addressed with mode of action information. For a second example, if the intended phenotype of the GM plant is to confer drought tolerance, there may be no plausible hypothesis for hazard to non-target insects, but there may be a plausible hypothesis for increased survival of the GM plant. Understanding the intended phenotype is therefore important because it informs the science-based ERA for cultivation approval; however, the data requirements that are relevant for characterizing the intended phenotype and the need for extensive mode of action data should be assessed on a case-by-case basis, and driven by the development of relevant pathways to harm related to the intended trait (as described in Table 2).

1.3. Overview of Data that may be Relevant in the Science-Based ERA for Cultivation Approval and Should be Considered on a Case-by-Case Basis

Data that may be considered relevant for some crops and traits, in addition to the core data described above, are determined by problem formulation and the development of plausible hypotheses for harm (Table 2). The relevance of these data depends on the crop, trait and receiving environment, and therefore should be considered on a case-by-case basis. These data are related to characterization of the GM crop, which includes: 1) assessment of potential changes in agricultural practices; 2) generation of additional agronomic data based on relevant pathways to harm related to increased survival, weediness, reproduction, gene flow, etc.; and 3) generation of additional data based on relevant pathways to harm related to the intended trait. Depending on the intended trait (e.g., insect protection, herbicide tolerance, or stress tolerance), additional data that may be relevant to consider for ERA include: (a) characterization of potential hazard to NTOs; (b) characterization of trait expression; (c) characterization of environmental fate in soil, sediment or surface water; and (d) characterization of potential effects on soil microbial communities and other plants.

1) Characterization of the GM crop: Assessment of potential changes in agricultural practices

In some cases it may be relevant to consider if the introduced GM trait is likely or intended to alter the standard agricultural practices in ways that could cause adverse effects on the environment. Consider for example a GM plant that contains an herbicide tolerance trait (HT). In this case, there could be a change in management practices relative to the non-modified crop (herbicide application, tillage, etc.) that is associated with the HT trait. In most cases, changes in agricultural practices will remain within the normal accepted practices for that crop (for example, even with an HT trait, herbicides would still be applied per the labeled rates), and the potential for change may not result in harm. If there is a plausible hypothesis for how the GM crop could result in a change in agricultural practices (for example, if a GM trait allows the crop to be cultivated in new environments) that could lead to a new or heightened adverse effect on the environment, additional data may be required to assess that risk.

2) Characterization of the GM crop: generation of additional agronomic data based on relevant pathways to harm related to increased survival, weediness, reproduction, gene flow, etc.

As described above, standard agronomic endpoints are collected during event selection and from large multi-site field trials for any new crop variety (GM or conventional), and these agronomic data can be used to identify any phenotypic or agronomic differences from the conventional crop that could result in a relevant pathway to harm. If these agronomic data show that a GM crop is similar to its conventional counterpart, the ERA for the GM crop can focus on the intended traits, and additional data would only be needed to inform the ERA if plausible risk hypotheses can be developed for potential environmental harm. Therefore, the requirement for generating additional agronomic data related to survival, weediness, reproduction, gene flow, etc., should be based on the development of a plausible pathway to harm and testable hypothesis. For example, if the non-modified plant does not outcross to wild relatives or no wild relatives grow in the vicinity of pollen deposition, and the GM plant is agronomically similar to the non-modified plant, then there is no plausible hypothesis for harm arising from gene-flow, and no additional data are needed to assess the gene flow potential of the GM plant. Similarly, if the non-modified plant does not have weedy characteristics, and the GM plant is agronomically similar to the non-modified plant, there is no plausible hypothesis for the GM crop becoming more weedy or invasive than its non-modified counterpart. On the other hand, if the non-modified plant does have weediness or invasiveness characteristics and/or the GM plant is not agronomically similar to the non-modified plant for relevant endpoints (for example, if the GM plant has increased seed dormancy or dispersal compared to the non-modified plant), there may be a plausible hypothesis for increased weediness or invasiveness potential of the GM plant, and additional information may be needed to fully assess the likelihood and magnitude of this risk. Similarly, if the GM trait introduces increased weediness potential and there are sexually compatible wild relatives in the areas of intended cultivation, an assessment of the likelihood and consequences of trait introgression into the wild relative population may be warranted.

Table 2: Data that may be relevant in the science-based ERA for cultivation approval and should be considered on a case-by-case basis

Data that may be relevant for the ERA	**Cases when data may inform the ERA**
Characterization of the GM crop	
Assessment of potential changes in agricultural practices	The agricultural practices associated with the GM crop need to be considered within the context of the agricultural practices that are typical for the non-modified crop. If there is a plausible hypothesis for how the GM crop could change an agricultural practice, additional data may inform the ERA. For example, if a GM crop that confers tolerance to an herbicide could result in a change on herbicide application, tillage, etc., an assessment of the effects of this change in management practice should be considered. Alternatively, a GM crop that confers protection against an insect pest may not result in any relevant changes in management practices, and additional data may not inform the risk assessment.
Generation of additional agronomic data based on relevant pathways to harm related to increased survival, weediness, reproduction, gene flow, etc.	Standard agronomic data is collected as part of event selection and multi-location field trials. Generation of additional data related to weediness, invasiveness, survival, and gene flow should only be considered if a plausible hypothesis can be generated for environmental harm. If no plausible hypothesis can be generated, understanding of the basic biology of the non-modified crop, the intended phenotype of the plant, and agronomic similarity to non-modified plants should be sufficient to assess risk (Table 1). For weediness, invasiveness, survival: agronomic endpoints related to weediness, invasiveness, and survival from the standard agronomic assessment can be used to assess risk (for example, seed dormancy, dropped ears, etc.). In some cases, additional data beyond the standard agronomic endpoints may be required to inform the risk assessment (e.g., an overwintering study may be deemed appropriate for a GM plant where the intended phenotype is a cold tolerance trait). For gene flow: agronomic endpoints related to reproductive endpoints from the standard agronomic assessment can be used to assess risk (for example, days to flowering, time to silking). In some cases, additional data beyond the standard agronomic endpoints may be required to inform the risk assessment (e.g., if the intended phenotype is related to a reproductive trait). The occurrence of sexually compatible wild relatives (SCWR) in the cultivation area is also relevant to consider; if there are SCWR, additional data beyond the standard agronomic assessment may be required to inform the risk assessment (for example, an outcrossing study). In all cases, the trigger for generating additional agronomic data should be based on problem formulation and a generation of plausible risk hypothesis and pathways to harm.
Generation of additional data based on relevant pathways to harm related to the intended trait. Depending on the intended trait, additional data that may be relevant to consider for ERA include:	
Characterization of potential hazard to non-target organisms	Understanding the spectrum of activity (specificity) of the newly introduced trait is only relevant for traits with a toxic mode of action (e.g., insecticidal traits). Spectrum of activity studies provide a foundation for NTO testing strategy for a newly expressed trait that confers insect protection. Similarly, NTO insect bioassays are only relevant for traits with a toxic mode of action. For traits that do not have a toxic mode of action, or where there is no plausible hypothesis for harm, understanding the specificity of the protein and/or conducting insect bioassays to assess NTO hazard has limited value for ERA (for example, EPSPS protein that confers tolerance to glyphosate).

Table 2: Continued

Characterization of trait expression	Characterization of trait expression in plant tissues is only relevant for traits with a toxic mode of action (e.g., insecticidal traits) or that otherwise directly harm valued entities. Risk is a function of both hazard and exposure. The concentration of a newly expressed trait in a GM plant is relevant for a trait that confers insect protection because this information is used to characterize the magnitude of an NTO potential exposure. If the newly expressed trait is not insecticidal or there is no toxic mode of action, trait expression in plant tissues does not inform the ERA, unless there is a plausible hypothesis (e.g., pathway to harm).
Characterization of environmental fate in soil, sediment, or surface water	Characterization of trait concentration in soil, sediment, or surface water is only relevant for traits with a toxic mode of action (e.g., insecticidal traits) and if there is a plausible hypothesis for why the newly introduced trait would persist in the environment. If the newly expressed trait does not have a toxic mode of action, environmental fate studies do not inform the ERA because there is no hazard. Generation of data on a new trait should only be needed if there is no existing data on closely-related traits to inform the risk assessment or if there is a plausible hypothesis for why the newly introduced trait would persist in soil, sediment, or surface water differently than other traits (i.e., proteins or dsRNA).
Characterization of potential effects on soil microbial communities and other plants	The potential effects of a newly expressed trait on soil microbial communities should only be considered if there is a specific hypothesis for how the trait could negatively affect the soil microbial community or specific microbes (for example, a trait that confers antimicrobial or antifungal properties). Consideration of the potential for allelopathic effects on other plants should be assessed if there is a specific hypothesis of changes in germination or growth inhibition based on biochemical properties of the introduced trait.

3) Characterization of the GM crop: generation of additional data based on relevant pathways to harm related to the intended trait

As discussed above, during problem formulation an understanding of the intended phenotype of the GM plant allows for relevant pathways to harm related to the trait to be considered. The ERA should focus on the known or expected effects of the trait on valued components of the biotic and abiotic environments. For instance, a trait conferring insect protection will generate different potential pathways to harm and require different data compared with a trait conferring drought tolerance. The data that are required for an ERA should be driven by problem formulation, assessment of the core data (Table 1), assessment of the intended trait, formulation of potential pathways to harm, and development of plausible hypotheses, which is why these data are considered on a case-by-case basis.

Assessment of the intended trait: (a) Characterization of potential hazard to non-target organisms (NTOs)

The need to understand and characterize the spectrum of activity (specificity) of a newly introduced trait and to assess potential hazard to NTOs is limited to traits that confer insect protection (insecticidal traits), have a toxic mode of action, or that otherwise could directly harm a valued entity. The spectrum of activity of the active ingredient will inform the risk assessment for insecticidal traits. For example, Cry1 protein activity is limited to the order Lepidoptera and Cry3 protein activity is limited to the order Coleoptera [56, 49]. Understanding the specificity of the insecticidal trait can be used to determine what non-target orders or species make sense to assess for potential hazard. Typically, for insecticidal traits, several non-target surrogate species from different orders are selected for testing (for example, honeybee, lady bird beetle, non-target lepidopteran [46, 44, 45]). Surrogate species are selected based on their relatedness to the target pest, relevance to beneficial NTOs of interest, and ability to be reared and tested in the laboratory using standardized methods. As described by Bachman et al. [4], laboratory hazard studies on surrogate species conducted in one country can be used in problem formulation for the ERA in countries. The spectrum of activity of the trait, as well as the potential for exposure and hazard to NTOs, can be used to develop potential pathways to harm and plausible hypotheses, which can direct if additional non-target organism laboratory assessment will inform the risk assessment. Likewise, the spectrum of activity of the trait, combined with information generated in laboratory assessments (Tier I and/or Tier

II testing), should be used to determine if additional non-target hazard assessment in a greenhouse (Tier III) or in the field (Tier IV) are required for risk assessment. A tiered testing approach to non-target hazard assessment should always be leveraged to avoid unnecessary higher-tier greenhouse or field studies when they are not informative for the risk assessment [7].

Assessment of the intended trait: (b) Characterization of trait expression

Data characterizing the expression of the newly introduced trait in plant tissues should only be required for the ERA on a case-by-case basis. For example, for an insecticidal trait, understanding the concentration of the insect-active substance (e.g., protein or dsRNA) in appropriate plant tissues is relevant to consider, as it helps inform problem formulation (i.e., potential exposure to NTOs). In the case of a lepidopteran active trait that is expressed in maize pollen, there is a plausible hypothesis for risk to a non-target lepidopteran that could incidentally ingest maize pollen while feeding on leaves on which pollen has deposited. On the other hand, if the lepidopteran active trait is not expressed in maize pollen, there is no plausible hypothesis for exposure to non-target lepidopterans. A lepidopteran that feeds on other maize tissues is not considered in this scenario, as it would be viewed as a pest. The concentration of the insecticidal trait in pollen can also inform potential exposure to other non-target insects in agroecosystems that also may consume pollen (for example, ladybird beetles). Similarly, understanding the concentration of the insect-active trait in other crop tissues is also informative for potential exposure to other NTOs. For example, predatory insects that feed on herbivorous prey may be exposed to the insect active traits through prey feeding [7]. There is a large body of knowledge about the lack of bioaccumulation and persistence of *Bacillus thuringiensis* (Bt) Cry proteins in prey [45]. Therefore, a predator is unlikely to be exposed to a higher concentration of a Cry protein via prey, relative to the concentration of the Cry protein that is in the crop tissue. Understanding the insecticidal trait concentration in crop tissues can help inform the potential exposure to predatory insects and can be used to develop plausible hypotheses for harm, since risk is a function of both hazard and exposure.

However, for gene products that do not have a toxic mode of action, and gene products that do not otherwise directly harm valued entities in the environment, understanding the concentration of the newly-expressed trait in plant tissues has limited value for the ERA. In these cases, since there is no a plausible hypothesis for hazard to NTOs, it is not informative to characterize the concentration of the newly introduced trait. Therefore, trait expression should only be required to assess environmental risk on a case-by-case basis, which is limited to traits that confer insect protection or otherwise have a toxic mode of action.

Assessment of the intended trait: (c) Characterization of environmental fate in soil, sediment, or surface water

Similar to trait expression in plant tissues, data characterizing the persistence of a newly expressed trait in environmental compartments such as soil, sediment, or surface water should only be required on a case-by-case basis. As described above, risk is a function of both exposure and hazard. The duration of exposure of a newly expressed trait to an NTO is only relevant for insect protection traits or traits with a toxic mode of action or that otherwise could directly harm a valued entity. For example, for a non-insecticidal trait where there is no plausible hypothesis for hazard to an NTO, understanding the persistence of a newly-expressed protein in environmental compartments has limited value for the ERA. Therefore, soil or water dissipation data should only be required to assess environmental risk on a case-by-case basis, which is limited to traits that confer insect protection or have a toxic mode of action.

In cases where there are existing data about the persistence of an insect protection trait in soil, additional studies may not be necessary to characterize risk. For example, from over 20 years of commercial use and risk assessment, there is a large body of evidence that Bt Cry proteins do not accumulate or persist in soil [28, 12, 53]. Cry proteins, in general, dissipate rapidly in soil [50, 33, 27]. The lack of persistence in soil can be used to understand persistence of Bt Cry proteins in sediment, surface water or other environmental matrices. The ERA for a GM crop that expresses a Bt Cry protein may be able to use existing data, and additional soil dissipation data may not further inform the risk assessment. Similarly, the existing soil fate data on Bt proteins can inform the risk assessment of non-Bt proteins. Characterizing the soil dissipation of a non-Bt protein may not be needed to inform the risk assessment, unless there is a specific hypothesis for why the source of the non-Bt protein would alter a proteins dissipation and degradation in soil. Additionally, for GM plants using RNA interference, there is strong evidence that dsRNA does not persist in soil [39, 20], sediment, or surface water [1, 23]. The degradation kinetics and persistence of dsRNA is not sequence dependent [22], and additional soil dissipation studies for GM plants that contain different sequences of dsRNA may not be necessary to characterize exposure or risk.

Assessment of the intended trait: (d) characterization of potential effects on soil microbial communities and other plant

The potential effects of a newly expressed trait on soil microbial communities should only be considered if there is a specific hypothesis for how the trait could adversely affect the soil microbial community or specific microbes. For example, a plausible hypothesis could be developed for how an antimicrobial trait or an antifungal trait could affect soil microbial communities. In these cases, if the concentration and persistence of the trait in the environment is deemed meaningful (see *(c)*, above), assessing the soil microbial community to ensure there are no unreasonable adverse effects on microbial-mediated soil processes may inform the risk assessment. Similarly, consideration of the potential for allelopathic effects on other plants should be assessed if there is a specific hypothesis of changes in germination or growth inhibition based on biochemical properties of the introduced trait. However, for most GM traits commercialized to date (HT traits, insect protection traits, etc.),

there is no plausible hypothesis for harm to microbial communities in soil or allelopathy, and studies assessing the number, abundance, or community structure of soil microbial communities, or on production of neighboring or following crops, do not inform the risk assessment. To date, there are no indications that GM plants negatively affect soil microbial communities [28]. Soils are inherently dynamic, and soil microbial communities are known to be impacted to some degree by crop rotation, management practices, and other environmental variables. Changes in soil microbial communities does not necessarily indicate harm, and there is evidence to suggest that the magnitude of change in microbial abundance due to GM crops is small relative to the overall variability in the soil [28]. In any event, if evaluation of the soil microbial community is relevant on the basis of problem formulation, functionally-focused studies of relevant soil microbial processes will likely be more meaningful than community-wide studies.

1.4. Data Requirements that Can Add to the Weight of Evidence but Are Not Required to Inform the ERA for Cultivation

As part of the overall cultivation application, some data are collected for product characterization. While these data may not directly inform the ERA, it can add to the weight of evidence (WOE) of safety. This data should not be required specifically to conduct an ERA but could be used on a case-by-case basis to help add information and context to the risk assessment (Table 3). For example, understanding and characterizing the source of the donor genes is commonly included in cultivation dossiers as part of the molecular characterization of the event. The source of the gene is also considered in the context of the food and feed safety assessment for import approvals, because if the source of the gene can be shown to have a history of safe use (HOSU), it adds to the WOE that the gene products are safe for food and feed [18]. For ERA, genes that come from a donor that has a HOSU may also add to the WOE of safety (i.e., familiarity). For instance, the greater than 20 years of knowledge and experience gained during the cultivation of GM crops expressing Bt Cry proteins helps inform the ERA for new crops expressing Bt Cry proteins. In these cases, there may be less need for additional data to be generated, because published literature can be used to assess the risk of a new Cry protein based on familiarity with Cry proteins in general. Similarly, if a novel source of proteins is utilized, but the source is widely dispersed in agricultural or natural habitats, familiarity with the source can add to the WOE to support the ERA. For a GM crop that expresses a novel protein from a novel source, prior information about the environmental effects of that source may be useful as part of the problem formulation for the risk assessment. However, in the absence of such prior knowledge, establishing the safety of the inserted gene is more informative for the risk assessment than establishing the safety of the source of the gene.

Similarly, the mode of action of a newly expressed protein in a GM plant is often investigated as part of the food and feed risk assessment, and can also be leveraged in the ERA, but should not be required to assess risk. Some understanding of the mode of action of a protein informs problem formulation and helps determine and develop plausible hypotheses for harm. For example, for a GM crop expressing a Bt Cry protein, it is helpful to understand that this protein is insecticidal, binds to specific receptors in the midgut of certain insects, and is ingested in the diet: knowledge of the receptor specificity can be used to guide the NTO testing scheme. However, a detailed understanding of how the insecticidal protein works at the molecular, cellular, or anatomical level is not required to assess environmental risk if the effects of the GM trait, as expressed in the crop, on valued components of the environment are understood sufficiently well to address plausible risk hypotheses. Similarly, for an HT trait, understanding the basic mechanism for tolerance to the herbicidal active ingredient may add to the WOE, but is not needed to assess the environmental safety of the GM plant. In many cases, herbicide tolerance is conferred by an enzyme that can detoxify the herbicidal active ingredient when expressed in the plant. In the case of an enzyme, confirmation of substrate specificity and the affinity of the enzyme for the herbicide (inhibitor) may inform the safety assessment. In both of these examples, if the GM crop is shown to have substantially equivalent agronomics to the non-modified plant, and there is an understanding of the intended function of the trait, additional refinement of the specific mode of action of the trait would only be required if plausible hypotheses for harm could be developed and addressed with mode of action information.

1.5. Data Requirements that Do Not Inform the ERA for Cultivation

As part of the overall cultivation application, additional molecular, protein, and event characterization data are collected, but these data do not directly inform the ERA. For example, as part of product characterization, molecular studies are conducted to confirm that the insert is an intact single copy, stable across generations, and that there is no plasmid backbone DNA [8, 10, 26, 32]. Southern blots, and more recently, next generation sequencing (NGS) data are submitted as part of the cultivation application, but these data are not a requirement for an ERA (Table 4). Similarly, characterization studies are conducted to confirm that the surrogate test material (e.g., microbially produced protein) is equivalent to the plant-expressed trait. Because plant-expressed traits are typically difficult to extract in high enough amounts to support safety hazard testing, surrogate test materials are generated for laboratory safety testing. In the case of a proteinaceous trait, characterization of the microbial-produced protein (e.g., amino acid analysis, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot, N-terminal sequencing, mass spectrometry for intact mass determination and peptide mapping, glycosylation staining) are conducted to demonstrate equivalence, but these protein characterization data are not directly relevant for the ERA. It is the effects of the plant-expressed protein, rather than its sequence, that inform the risk assessment.

For food and feed safety assessment, the composition of the grain and forage of the GM plant has historically been compared to the composition of the non-modified plant. However, there is strong evidence, based on more than two decades of experience, that compositional assessment beyond assessment of the intended change(s) is unwarranted for food and feed safety

Table 3: Data that can add to the weight of evidence but are not required to inform the ERA for cultivation

Data that are not necessary for ERA	Adds to weight of evidence
Characterizing the source of the donor gene	Prior knowledge of the safety of the source of the inserted gene in a GM plant may add to the WOE (for instance, if the source can be shown to have a history of safe use). However, in the absence of such prior knowledge, establishing the safety of the inserted gene is more informative for the risk assessment than establishing the safety of the source of the gene.
Characterizing the mode of action/mechanism of action	An understanding of the mode of action (MOA) of an introduced protein may inform problem formulation and be used to help develop pathways to harm. However, a full understanding of how the insecticidal trait works at the molecular, cellular, or anatomical level is not required to assess environmental risk if the effects of the GM trait, as expressed in the crop, on valued components of the environment are understood sufficiently well to address plausible risk hypotheses.

Table 4: Data requirements that do not inform the ERA for cultivation

Data that are not necessary for ERA	Not required for ERA for cultivation
Molecular characterization	Southern blots, and more recently, next generation sequencing (NGS) data, are submitted as part of the cultivation application. This information is needed to support the molecular characterization of the inserted gene, but it does not directly inform the ERA.
Trait characterization	If a surrogate test material is used in acute toxicology studies or NTO laboratory hazard studies, characterization data are typically generated to demonstrate equivalence to the plant-expressed protein. This information is needed to support use of surrogate test material for use in hazard studies, and is therefore relevant in the context of protein safety, but it does not directly inform the ERA.
Composition	The composition of the grain and forage of the GM plant is compared to the composition of the non-modified plant. These data have historically been required for food and feed safety assessment. For ERA, compositional assessment should only be considered if there can be a plausible pathway to harm to the environment.
Product efficacy	The overall efficacy of the trait is relevant when considering the commercial value and benefits of the product, but this information is not directly relevant to the ERA. In the context of the overall assessment of the product, benefits should be factored into the decision-making process, because in some cases the benefits of a product or trait may outweigh the risks. Nevertheless, assessment of benefits does not directly inform the ERA.
Horizontal gene transfer (HGT)	There is no evidence that HGT occurs under natural conditions at rates that have an environmental impact. Therefore, while HGT may be considered as part of the ERA, generating data specific to the inserted gene is not needed to assess risk.

assessment. There is, in fact, more natural variability in composition among conventional varieties, which all have a history of safe use, than there is between a GM crop and its genetically-close conventional comparator [14]. For food and feed safety, composition of a new GM plant should only be assessed if the data, as determined using a hypothesis-driven, stepwise approach, will inform the safety assessment [9]. Likewise, for ERA, compositional assessment should only be considered if there is a plausible pathway to harm to the environment. For GM products, crops, and traits commercialized to date, composition data has not been scientifically relevant for the ERA. Likewise, while the efficacy of the product is considered in the overall product submission, data on product efficacy is not a relevant consideration for ERA. Finally, the requirement to assess the potential for horizontal gene transfer (HGT) of the inserted DNA into microbes in soil or digestive tracts does not inform the ERA because there is extensive evidence that HGT does not occur under natural conditions [31, 17, 34].

1.6. Recommendations for Harmonization of Global Data Requirements for ERA

The ERA framework is robust and flexible. This framework uses problem formulation to generate plausible risk hypotheses and allows risk to be assessed using a science-based approach. There are only a few key pieces of data that should be universally required to inform the ERA for all crop and trait combinations. These include an understanding of the receiving environment and the basic biology of the unmodified plant; an understanding of the intended phenotype of the GM plant and assessment of how the intended phenotype may lead to environmental harm; and an assessment of the agronomic similarity of the GM crop to its conventional counterparts. These key pieces of information serve as the foundation of any ERA, and most global regulatory data requirements assess these key pieces of information for cultivation approvals; however, there remains a lack of global alignment and scientific consistency for when additional data are required for the ERA of GM crops.

Problem formulation and the development of plausible risk hypotheses are both important steps in the ERA, which help identify additional data that are needed to inform the ERA. The concepts of familiarity (i.e., using the existing body of knowledge for GM crops and traits with a history of safety) and data transportability (for example, using an agronomic study conducted in one country can be used to assess the need for additional agronomic data to be generated in another cultivation country) are both key components of problem formulation that are used to structure the risk assessment. These key pieces of data serve as a foundation for the risk assessment, and the need for additional data is assessed on a case-by-case basis, depending on the crop, trait, receiving environment, and protection goals. Refinement of data requirements to those that inform the ERA and harmonization of data requirements across global regulatory authorities would add transparency and consistency to the ERA of GM crops globally.

2. Declaration of Conflicting Interest

All the authors of this paper are currently employed by, or have been employed by, the agricultural biotechnology industry.

3. Disclaimer

The findings and conclusions in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. government determination or policy.

4. Article Information

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