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Leveraging experience to support contemporary risk assessments:

EPA's case-by-case considerations for ecological risk assessment of plant-incorporated protectants and the increased role of scientific rationale

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I. Introduction

Plant-incorporated protectants (PIPs) – biological substances produced and used in living plants for pesticidal purposes – by their very nature present different considerations than other types of pesticides and thus require a different lens when considering appropriate data needs for risk assessment. The U.S. Environmental Protection Agency (EPA or the Agency) does not have formal data requirements for PIPs. Accordingly, potential data needs for completing a risk assessment for a PIP are determined on a case-by-case basis depending on specific features of the PIP and the plant in which the PIP has been genetically engineered.[1] EPA's current approach for PIPs was developed from previous experience with *Bacillus thuringiensis* (*Bt*)-derived Cry (insecticidal crystal) and Vip (Vegetative insecticidal protein) proteins targeting lepidopteran and coleopteran pests and has been successfully applied to a diverse group of PIPs (e.g., dsRNA, proteins derived from non-*Bt* bacteria, proteins derived from ferns). Based on EPA's thirty years of experience in PIP ecological risk assessments, EPA builds on that approach by identifying instances where scientific rationale may be warranted in lieu of historical laboratory testing.

This document does not provide recommendations for formal data requirements. Instead, this document discusses EPA's case-by-case approach in considering ecological data needs for PIPs, which includes an outline of types of ecological data historically received for PIPs (with an emphasis on insect resistance traits), case-specific factors that may play a role in data needs, and the contributions that familiarity and scientific rationale can play in risk characterization. This information can be utilized to inform data needs for future PIP submissions; for case-by-case recommendations, the Agency welcomes pre-submission meetings to discuss a prospective registrant's specific PIP product.

II. Case-by-Case Approach to Ecological Risk Assessment for PIPs

In general, EPA evaluates risk to non-target species populations, communities, and the ecosystem by considering toxicity to terrestrial vertebrates (e.g., mammals, birds), aquatic vertebrates and invertebrates, terrestrial and aquatic plants, and terrestrial invertebrates, including selected beneficial insects (e.g., predators, pollinators). The overall risk determination is based on combining information on the expected exposure along with scientific rationale and toxicity testing from various functional groups.

[1] https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/plant-incorporated-protectants-data-symposium

Case-by-case attributes of the specific PIP, or application of the PIP, play a role in potential data needs, such as the FIFRA action type, the mechanism of action of the PIP, the specificity (i.e., activity spectrum), and exposure profile of the PIP.

A. Role of FIFRA Action Type

Federal pesticide law (the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)) authorizes EPA to issue permits for testing PIPs and to issue registrations for commercialization of PIPs. To issue a permit or register a pesticide, EPA evaluates the proposed pesticide to ensure that its use will not pose an unreasonable adverse effect to human health or the environment. Under FIFRA Section 5, EPA issues experimental use permits (EUPs) to allow prospective registrants to generate information or data necessary to register a pesticide via field testing. Under FIFRA Section 3, EPA issues two types of registrations for PIPs: a Seed Increase Registration, which is used for the purpose of producing seed for future commercial seed sales, and a Commercial Use Registration, which generally allows for use of the PIP throughout the United States for cultivation purposes. As the requisite purpose of an EUP is to generate data from additional testing to eventually obtain a Section 3 registration, the full suite of data typically submitted for a Commercial Use Registration is not expected to be available at the EUP stage. However, there is no requirement for EUP and Seed Increase Registration to obtain a full Commercial Use Registration.

In terms of ecological risk assessment, as risk is a consideration of both hazard and exposure, the FIFRA action type plays a significant role in the data needs for a PIP as these action types are directly tied to the scale of potential exposure to the PIP. An EUP is time-limited and has involved specification of both the acreage amount (historically ≤5,000 acres) and the county- or state-level location of the experimental field tests. A Seed Increase Registration has similarly involved specification of the acreage amount, although acreage for this registration may be significantly more than an EUP (≤250,000 acres). Finally, a Commercial Use Registration without geographic restrictions theoretically allows for planting of the PIP-containing crop across the United States.

Given that an EUP, and potentially a Seed Increase Registration, directly limit the scale of potential effects through reduced exposure, it is possible that "no unreasonable adverse effects" may be determined even if the available data does not rule out the potential for non-target organism hazard. For example, some activity spectrum characterization for an insecticidal PIP would typically be submitted at the EUP stage, but the full suite of insect Tier I studies may not be needed until the acreage (i.e., exposure) and thus the scale of potential effects increases (e.g., Section 3 Commercial Use Registration; see "*Non-target insect testing*" in III.A. for additional detail).

B. Role of Mechanism of Action

The mechanism of action (MoA) of the PIP plays a significant role in determining data needs. For example, in situations where a PIP has a MoA that modulates or mimics the plant's own internal processes (e.g., R-proteins that activate a plant's own immune response), EPA has not required ecological effects and environmental fate data.[2] The reasoning for not requiring these studies for PIPs with non-toxic MoAs is that there has been a history of safe prior exposure to the MoA, there is a specificity of the MoA, and there is no expected hazard to any non-target organism based on the MoA.

Conversely, a PIP with a toxic MoA targeting an insect, for example, has historically involved data generation via laboratory studies to characterize its pesticidal activity spectrum (i.e., the range of non-target organisms which may be sensitive). Although an exact understanding of the toxic MoA is not necessary, a general understanding of the MoA and its expected specificity can bolster rationale for more limited subsequent laboratory testing of surrogate species. For example, insecticidal PIPs have historically been designed to target specific insect orders by targeting insect-specific midgut receptors. In these instances, the value of testing more distantly related species (e.g., vertebrates) for assessing hazard to insect-specific active ingredients has been limited.

C. Role of Activity Spectrum and Exposure Profile for Insecticidal PIPs

To support the registration of insecticidal PIP active ingredients, registrants have typically performed initial spectrum of activity studies that survey multiple insect orders against the new active ingredient. Information from initial activity spectrum studies can then inform taxa selection for subsequent laboratory testing of beneficial non-target organisms that represent vital ecosystem functions to determine potentially sensitive taxa. As such, a PIP demonstrating a narrower activity spectrum (e.g., specificity to a single insect order) would likely need fewer subsequent non-target organism laboratory testing compared to a PIP with a wider activity spectrum. If a PIP were to have a wider activity spectrum (e.g., an insecticidal trait with activity across terrestrial invertebrates), then testing of organisms that are more phylogenetically distant from the target pest may be needed to confidently define the activity spectrum.

Further, EPA's approach to considering ecological data for PIPs considers the biological nature of PIPs (i.e., PIPs are composed of nucleic and amino acids), which limits their persistence in the environment. The intracellular location of PIPs in plant tissue further limits meaningful exposure to species that interact directly with the plant or plant parts. For PIPs, the source of exposure will mainly be ingestion of plant tissues that express the PIP (e.g., green tissue, pollen, roots, or

[2] https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated

seeds), and, to a lesser degree, through movement of the PIP into the soil or from consumption of exposed herbivorous prey (US EPA and USDA, 2007). This exposure profile limits the non-target organisms expected to be meaningfully exposed to the PIP and can also be used to inform taxa selection for subsequent laboratory testing.

III. General Considerations for Insecticidal PIPs in Terrestrial Crop Plants

The remainder of this document focuses on the historical data commonly received for insecticidal *Bt* PIPs in terrestrial crop plants, as these are the PIPs most reviewed by EPA. However, the logic discussed below of focusing laboratory testing on surrogate test species that are reasonably representative of non-target species with potential exposure (e.g., terrestrial invertebrates) and most likely to be sensitive (i.e., species more closely related to the target taxa) is also applicable to PIPs more broadly. For case-by-case recommendations, prospective registrants are encouraged to arrange a pre-submission meeting with the Agency to discuss the regulatory process concerning their specific PIP product.

To evaluate potential exposure to insecticidal PIPs, EPA has historically assessed PIP expression levels across multiple tissues and life stages of the plant as well as PIP degradation rates across representative agricultural soils. EPA has also assessed potential exposure to the PIP by considering the site of cultivation as well as basic information about the PIP-containing plant, including the biology, ecology, flower phenology, and taxonomy of the species and its relatives (Kough & Edelstein, 2013). Given the source of insecticidal PIP traits at the time of the technology's inception (i.e., the microorganism *Bacillus thuringiensis*), and in the absence of PIP-specific registration data requirements, EPA has historically used the 40 CFR Part 158 data requirements for microbial pesticides as a guide for non-target organism testing. To evaluate the potential hazard of a PIP, the Agency has historically received non-target insect toxicity studies, honeybee studies, a non-target soil invertebrate toxicity study, as well as studies from organisms more distantly related from the target pest such as an aquatic invertebrate study and an avian oral toxicity study.

Two separate FIFRA Scientific Advisory Panel (SAP) reports (October 2000 and August 2002) recognized the importance of considering plausible pathways to both potential hazard and potential exposure from a PIP as the first step in non-target organism risk identification. Given that the specific toxicity of *Bt* PIPs was already well established at this time, together with the inherent confined nature of PIPs, these SAPs recommended that non-target testing of insecticidal *Bt* PIPs should focus on invertebrate species exposed to the crop in which the PIP(s) will be expressed and on species with the potential to be adversely affected. Based on these recommendations, EPA previously determined that non-target organisms with the greatest risk

potential to PIPs in transgenic crop fields are beneficial insects related to the target pest that feed on plant tissues (e.g., pollen) (US EPA, 2010). While EPA's risk assessments of PIPs in crops have focused primarily on these taxa, EPA has historically still received testing on other representative species (e.g., *Daphnia*, bobwhite quail). However, nearly three decades of experience with PIP ecological risk assessments has confirmed that for insecticidal PIPs in crop plants, the determination to focus risk assessments on beneficial insects is justified as no biologically relevant treatment-related effects have been seen in toxicity testing outside of insects for PIPs registered to date (Appendix Table 1).[3]

As such, EPA generally believes that laboratory testing of non-target organisms more distantly related from the target pest is likely of limited value to support insecticidal PIP risk assessments. Consequently, EPA is supportive of a greater use of scientific rationale in assessing the potential risks to more distantly related non-target organisms (e.g., birds, fish, aquatic invertebrates). Such scientific rationale can include but is not limited to: a PIP activity spectrum limited to specific taxa, a MoA resulting in negligible likelihood of effects in distantly related taxa, knowledge of protein/nucleic acid degradation from the scientific literature supporting limited exposure, PIP expression levels, and/or knowledge of crop tissue movement in the environment supporting limited or negligible exposure.

The following summaries include greater detail of the types of data EPA has historically received when an individual applicant has sought registration of a new active ingredient insecticidal PIP (Appendix Figure 1). As stated before, in an ecological risk assessment EPA evaluates risk to non-target species populations, communities, and the ecosystem by considering toxicity to terrestrial vertebrates (e.g., mammals, birds), aquatic vertebrates and invertebrates, terrestrial and aquatic plants, and terrestrial invertebrates, including selected beneficial insects (e.g., predators, pollinators). However, that evaluation can use empirical data from laboratory testing of the individual active ingredient, or it can use scientific rationale. Based on its thirty years of experience evaluating PIP active ingredients, EPA also identifies those areas where laboratory testing has historically been performed, but an increased use of scientific rationale is likely to be warranted.

A. Historically Received Hazard Data for Insecticidal PIPs

Activity Spectrum Bioassays

As previously discussed, EPA has historically received activity spectrum bioassays that survey multiple insect orders against the new active ingredient for both Section 5 and Section 3

[3] https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated

applications. Specificity of the PIP trait to a limited number of species can be initially shown by challenging several pest species across different insect orders against the PIP pesticidal substance, with a limited range of susceptible pests indicative of a narrow activity spectrum. This information is expected to be useful in identifying the additional non-target tests that may be needed for EPA to conduct a complete ecological risk assessment. For example, if a PIP shows activity restricted to Lepidoptera and Coleoptera, the focus of the risk assessment can then be geared towards beneficial/valued lepidopteran and coleopteran species that are phylogenetically related and exhibit a plausible pathway to harm. The degree of confidence surrounding this focus is increased with corroborating non-target terrestrial invertebrate studies. For RNAi-inducing PIPs, applicants have historically further bolstered initial activity spectrum testing with bioinformatics assessments evaluating sequence similarity of the pesticidal substance (e.g., dsRNA) with genomes/transcriptomes of various non-target organisms.

Non-target insect testing

For an insecticidal PIP, non-target insect testing has historically been the focus of laboratory testing for Section 3 applications as these are the non-target organisms most likely to be sensitive to the PIP.[4] Empirical studies to fulfill non-target insect testing have typically followed the standard tiered-based testing approach (US EPA and USDA, 2007). Under the standard approach, in Tier I testing, organisms are tested in the laboratory with no-choice bioassays using artificial diets with exposures typically more than ten times[5] the 95th percentile value of protein level seen in the relevant plant tissue. Tier I testing allows for tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results. If no adverse effects are seen in the "worst case scenario" of a Tier I study, then additional testing of that species is not needed. Conversely, an effect observed in a Tier I study may suggest a need to either more deeply analyze and characterize the existing data (e.g., consider how the level at which effects are seen relates to realistic environmental exposures), or to conduct higher-tier studies.

It is important to reiterate that there are no data requirements for PIPs. Although applicants have historically relied on EPA guideline 885.4340 for microbial pesticides which recommends testing three species of insects representing at least two of the following groups—parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites, predaceous neuropterans, or parasitic hymenopterans— this is not a requirement for PIPs. The groups

[4] Common test species for PIPs have included ladybird beetles, green lacewings, parasitic wasps, the insidious flower bug, and rove beetles.

[5] There may be instances in which reaching a 10x dose is difficult (e.g., intractable protein, significant negative effects on diet palatability). Prospective registrants are encouraged to discuss their specific product with the Agency in a pre-submission meeting.

listed in the 885.4340 guideline represent diverse taxa that provide beneficial functional services in an agroecosystem. However, unlike exogenously applied pesticides, exposure to PIPs is primarily driven by direct consumption of plant tissue and selecting three predatory species may result in redundant information of limited use for the risk assessment. Therefore, leveraging results from the spectrum of activity studies coupled with consideration of expected exposure based on crop biology and PIP expression levels allow applicants the flexibility to select more informative non-target insect species to test, and potentially reduce the number of non-target insect tests submitted.

For Section 5 EUP applications, higher-tier studies are not generally submitted given the limited time and acreage associated with the permits (i.e., limited exposure of the PIP to the environment). Depending on the experimental design in a Section 5 application (e.g., test sites in counties where threatened and endangered insects are not found), activity spectrum bioassays or other information indicating likely specificity may provide sufficient information on their own to allow EPA to evaluate a PIP's risk to non-target organisms.

Honeybee testing

Honeybee testing has historically been submitted for Section 3 applications and EPA has received testing of both larval and adult life stages. Although either life stage may be suitable depending on the case-specific application, the appropriate life stage is generally dependent on activity and exposure considerations of the PIP (e.g., honeybee larvae may be appropriate if the pesticidal substance is active on larval stages of target pests and exposure to honeybee larvae is expected). Given that PIPs are expressed within plant tissue, pollen consumption is the biologically relevant exposure route for honeybees.[6] Therefore, if PIP expression in pollen is below the limit of detection (<LOD), honeybees may not be a relevant non-target insect for testing as there would be no biologically plausible pathway to harm.

Non-target soil invertebrate testing

Soil invertebrate testing has historically been submitted for Section 3 applications for PIPs. Commonly seen non-target soil invertebrate toxicity studies include testing Collembola using OECD Guideline 232 or earthworm using OECD Guideline 207/222. Given their closer phylogenetic proximity to insects, collembolan species are the preferred soil organism for testing an insecticidal PIP, but earthworm testing can also be useful in demonstrating specificity within invertebrates.

Synergy

Synergism testing has historically been submitted for Section 3 applications in instances in which a single plant contains multiple PIPs with toxic MoAs. The objective of this study is to

[6] Nectar primarily consists of sugars with low concentrations of proteins (Nicolson, 2022); thus, potential risk to honeybees from PIPs would most likely be from pollen feeding (Babendreier et al., 2004).

evaluate the potential for interactive effects (synergism, antagonism, or additive effects) between two or more PIPs. For the purposes of ecological risk assessment, EPA is primarily concerned about synergistic effects (i.e., greater than additive effects), as synergism between PIPs could increase hazard towards sensitive non-target organisms, as well as indicate that a modified testing regime may be appropriate (i.e., assessing the toxicity of the PIPs to non-target organisms jointly rather than separately). Typically, concentration-response studies challenging sensitive insect larvae with individual and combinations of two or more test substances are compared to characterize the potential for interactions in mixtures.

In general, synergistic activity between chemicals is rare (Cedergreen, 2014; Levine and Bogert, 2018) and previous EPA OPP guidance (US EPA, 2019) has emphasized the Agency's current approach to focusing on single active ingredients when evaluating chemical pesticide mixtures due to the rarity of toxicological interactions of consequence. Indeed, growing literature is also finding synergistic interactions among combined PIP stack products to be rare, with elevated levels of synergism (e.g., five-fold or greater) even more rare (Walters et al., 2018). In this vein, a previous EPA SAP (US EPA, 2009) identified that additional non-target organism testing would not be warranted unless PIP combinations triggered a ten-fold increase in potency against the target organism. Thus, it is expected that scientific rationale can be used in place of laboratory testing if no robust hypothesis for an elevated level of synergism exists. The logic underlying the use of scientific rationale for addressing synergism in a Section 3 registration for "new active ingredients" similarly applies for new PIP product registrations where previously registered PIPs are newly combined.

Avian oral toxicity

To date, submitted avian toxicity studies have not been found to be useful for assessing the ecological risks for insecticidal PIPs for Section 3 applications. Scientific rationale can be used to assess potential effects to avian species by leveraging results generated from the activity spectrum testing and terrestrial invertebrate toxicity testing to demonstrate that the PIP has a narrow activity spectrum (e.g., insect-specific) that would therefore not be expected to extend out to more distantly related taxa, like vertebrates. Such rationale could be bolstered by a description of the MoA (e.g., toxicity relies on interaction with insect-specific gut receptors) and how it relates to an expectation of lack of toxicity in vertebrates.

Aquatic species

In determining whether testing of aquatic organisms may be appropriate, EPA considers the potential for exposure to occur. For many row crops (e.g., corn, cotton, soy, potato), there is an expectation of minimal to negligible exposure to the PIP for freshwater fish or invertebrates (US EPA, 2001; US EPA, 2010; US EPA, 2012; US EPA, 2015) and, as such, for Section 3 applications of PIPs in row crops, aquatic organism studies are not expected to be needed. This expectation

is largely independent of the specific PIP and is based on the biological nature of PIPs more broadly as well as how the crop tissue is expected to move and be processed in the environment. There is an expectation of minimal to negligible aquatic exposure for the aforementioned crops given: 1) that the actual percentage of PIP-containing crop tissue entering a waterway will only be a small percentage of the total field, 2) crop tissues that are most likely to enter waterways (i.e., pollen, senescing tissue post-harvest) often have less of the PIP than the most concentrated tissue(s), 3) the PIP will not be immediately bioavailable as it is contained within plant tissue that must first be conditioned or degraded, and 4) there is an expectation of rapid environmental degradation of proteins and nucleic acids of which PIPs to date have been composed. Indeed, reviews on the persistence of both protein and dsRNA PIPs support this expectation, as little evidence for the accumulation of these substances in soils or sediments has been found (Christiaens et al., 2018; Icoz & Stotzky, 2008). Furthermore, the determination of minimal to negligible aquatic exposure has not required consideration of the physical or chemical qualities of freshwater versus brackish or salt water and therefore have applied to freshwater, marine, and estuarine environments.

For other PIPs contained in different plants where exposure is more likely or unknown, or if the PIP is a substance in which EPA does not have experience (i.e., a substance other than a protein or nucleic acid), further consideration may be warranted regarding aquatic organism testing. This consideration could include whether the insecticidal PIP's MoA (e.g., a protein binding to insect midgut receptors) would be expected to change upon entering an aquatic environment, and whether the PIP's activity spectrum could still be reliably inferred from the terrestrial invertebrate toxicity tests.

B. Historically Received Exposure/Fate Data for Insecticidal PIPs[7]

Soil degradation

Crop tissue may remain on the field after harvest and be tilled into the soil resulting in the expectation that soil is the ultimate destination of PIPs in crop plants in the terrestrial environment. Accordingly, the degradation pattern of a PIP in soil is typically considered when characterizing potential exposure in the risk assessment. As such, soil degradation studies have historically been submitted for Section 3 applications. In EPA's data requirements regulations for microbial pesticides, which EPA often looks to when considering what data would be appropriate and necessary for assessing risks from PIPs, soil degradation studies are not

[7] PIP expression level data is used in the exposure assessment, but it is not discussed in detail here as it is formally reviewed as part of molecular characterization. Tissues typically tested include leaf tissue across multiple life stages (e.g., young vs. senescing leaves), root, grain, and pollen. EPA typically uses the 95th percentile values from fresh weight concentration in relevant tissues to determine worst-case estimated environmental concentrations.

required for Section 5 EUPs (40 CFR § 158.2174) and are only required for Section 3 applications when effects are seen in Tier I studies.

In further determining whether a soil degradation study is warranted for a Section 3 application for a PIP, or whether scientific rationale can provide sufficient information for the ecological risk assessment, the PIP substance can be considered. For example, while there are reports of proteins and dsRNA binding to soil particles (Sander et al., 2010), thus potentially increasing persistence, the biological nature of proteins and RNAs make them readily susceptible to metabolic, microbial, and abiotic degradation, particularly within agricultural systems (Icoz & Stotzky, 2008). For instance, the near neutral pH of most soils utilized for crop production likely promotes microbial activity (and thus, degradation rates), as near neutral soils have been shown to greatly increase the degradation rate of Cry proteins (Tapp & Stotzky, 1998). It is therefore expected that functional proteins and plant-produced RNAs (i.e., "naked" RNA) have a limited lifetime in the environment due to such degradation and the ubiquitous nature of proteases and RNases. Indeed, in the context of PIPs, a large volume of data exists in the scientific literature for PIP degradation for various active ingredients and crops spanning multiple decades. These studies have quantified the persistence of Cry proteins and dsRNAs in agricultural soils and have found strong support for their rapid degradation in both laboratory and long-term field settings, indicating that these biologically-derived PIPs degrade rapidly in soil and are not expected to experience long-term persistence (Head et al., 2002; Ahmad et al., 2005; Dubelman et al., 2005; Li et al., 2007; Shan et al., 2008; Gruber et al., 2012; Dubelman et al., 2014; Joaquim et al., 2019).

Aquatic degradation

As all the insecticidal PIPs registered to date have been in terrestrial crops with an expectation of minimal to negligible aquatic exposure (US EPA, 2001; US EPA, 2010; US EPA, 2012; US EPA, 2015), EPA has not historically received many aquatic degradation studies. An aquatic degradation study may be appropriate for a PIP if significant aquatic exposure is expected.

Gene flow

EPA has not historically received laboratory studies related to gene flow, but the topic is routinely a consideration in EPA's ecological risk assessments for PIPs. The potential for movement of the PIP trait into wild plant populations by introgression (i.e., gene flow) and the possible effects of the PIP's pesticidal trait in wild populations is considered the biological fate of the trait. While EPA does not consider gene flow in and of itself to be a negative occurrence, an assessment of the potential for a gene flow event is considered during the ecological risk assessment because movement of the PIP into wild relatives would affect the scale of exposure (Wozniak & Martinez, 2011). The possible environmental risk is contingent on the

verification that the PIP expressing plant and the wild relative are capable of forming fertile progeny and that the PIP trait could introgress into the wild population. For introgression into the wild population to occur at biologically meaningful levels on a plant population-scale, there would have to be selection pressure from a PIP controlled pest present that was significantly affecting the wild relative population (Kough & Edelstein 2013).

Based on guidance from the FIFRA SAP (October 2000), the gene flow risk for certain crop plants is already known and is based on crop plant biology and either the absence or restricted ranges of wild relatives. As these determinations are for the crop, and did not require consideration of the PIP itself, they are expected to apply regardless of the insecticidal PIP trait. As such, for insecticidal PIPs in corn, cotton, soy, and potato, PIP-specific information on the potential for gene flow is not expected to be needed (US EPA, 2001; US EPA, 2010). For PIPs in crops other than corn, cotton, soy, and potato, information from the scientific literature considering the points described above (e.g., presence or absence of wild relatives) can be used in determining the potential for gene flow.

IV. Summary

The registration of the first PIP product ushered in a new era of biological pest control, one that has not only provided new pest control tools to farmers but also environmental benefits (Carpenter, 2011; Dively et al., 2018; Naranjo, 2009; Wolfenbarger et al., 2008). As the Agency's understanding of these once novel products has evolved over the years, so too has our understanding of the data most valuable in characterizing their risk(s) to the environment. PIP products have generally been found to pose little to no risk to the greater non-target organism community (Appendix Table 1); therefore, in this document, EPA has outlined the types of ecological data historically received for insecticidal PIPs, and highlighted instances where data has previously been considered effective or potentially superfluous to PIP risk characterization based on its thirty years of experience in PIP ecological risk assessments. As this technology is poised to grow, both in its continued adoption and in the diversity of PIPs developed, streamlined application packages containing only the most pertinent data would allow risk assessors to focus on the toxicity studies where non-target effects have the most potential to occur. Therefore, EPA encourages prospective registrants to consider whether scientific rationales may be sufficient in lieu of testing and, for case-by-case recommendations, prospective registrants may consult with the Agency concerning their specific PIP product as needed.

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Appendix

Table 1. Tier I non-target organism guideline toxicity studies submitted to support Section 3 registrations of PIPs over the last 30 years. Updated July 2025.

Study Type	Number of Studies	Number of Studies with Non-Target Effects
Bt-derived protein PIPs		
Avian oral toxicity (acute and dietary)	36	0
Freshwater fish toxicity	13	0
Freshwater invertebrate	15	0
Non-target soil invertebrate	39	0
Honeybee testing (adult and larvae)	41	0
Non-target insect	91	6
Non *Bt*-derived protein PIPs		
Avian oral toxicity (acute and dietary)	3	0
Freshwater fish toxicity	2	0
Freshwater invertebrate	2	0
Non-target soil invertebrate	5	0
Honeybee testing (adult and larvae)	6	1
Non-target insect	17	5
RNA-based PIPs		
Avian oral toxicity (acute and dietary)	1	0
Freshwater fish toxicity	2	0
Freshwater invertebrate	1	0
Non-target soil invertebrate	3	0
Honeybee testing (adult and larvae)	4	0
Non-target insect	8	0

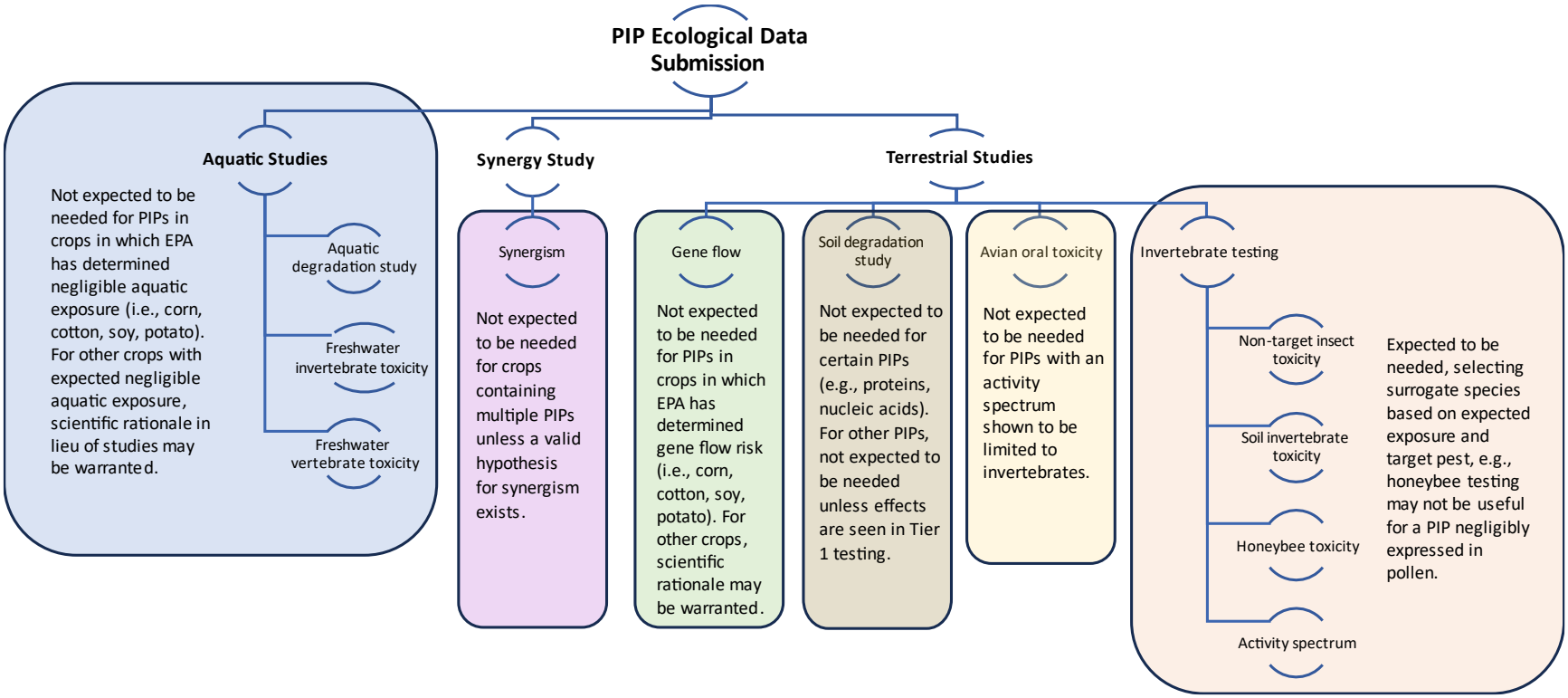

Figure 1. Graphical abstract of EPA's case-by-case considerations for ecological risk assessment of insect resistant plant-incorporated protectants and the increased role of scientific rationale