CENTER FOR FOOD SAFETY

Oct 30, 2023

US Environmental Protection Agency
EPA Docket Center, Mail Cod 2822IT
1200 Pennsylvania Ave., NW
Washington, DC 20460

Submitted electronically to www.regulations.gov

RE: Comments on EPA-HQ-OPP-2021-0271, Proposed Decision to Approve Section 3(c)(5) Registration for Ledprona (*Leptinotarsa decemlineata*-specific recombinant double-stranded interfering Oligonucleotide GS2)

Center for Food Safety (CFS) appreciates the opportunity to comment on EPA's proposed decisions to unconditionally register the new active ingredient, Ledprona (*Leptinotarsa decemlineata*-specific recombinant double-stranded interfering Oligonucleotide GS2), in one technical grade (Ledprona Technical) and one end-use product (Calantha). The requested registration would enable the application of Ledprona on potatoes nationwide.

CFS, on behalf of its members and undersigned twenty-two public interest organizations, submits the following comments opposing EPA's proposed decision to unconditionally register Ledprona under Section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), 7 U.S.C. § 136a(c)(5). As detailed below, EPA's proposed registration of Ledprona for use on potatoes is based on insufficient data, in contravention of EPA's own FIFRA regulations and FIFRA's registration standard for a section 3(c)(5) registration. EPA's proposed registration decision also violates the agency's duty under the Endangered Species Act (ESA). At a minimum, EPA must hold off on any registration decision until after completion of the experimental use trials under experimental use permits (EUP) EPA just granted back in May of this year, and that would expire by April 30, 2025.

RELEVANT LEGAL STANDARDS

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

FIFRA authorizes EPA to regulate the registration, use, sale, and distribution of pesticides in the United States. Pursuant to FIFIRA, EPA oversees both initial registration of an active ingredient as well as any new uses of the registered active ingredient.

Section 3(c) of FIFRA states that a manufacturer must submit an application to register the use of a pesticide.[1] Under Section 3(c)(5) of FIFRA, EPA shall register a pesticide only if the agency determines that the pesticide "will perform its intended function without unreasonable adverse effects on the environment" and that "when used in accordance with widespread and commonly recognized practice[,] it will not generally cause unreasonable adverse effects on the environment."[2] FIFRA defines "unreasonable adverse effects on the environment" as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide."[3] Alternatively and crucially here, where there are data gaps and missing information, EPA can register a pesticide with conditions (conditional registration) under Section 3(c)(7) of FIFRA "for a period reasonably sufficient for the generation and submission of required data," but only if EPA also determines that the conditional registration of the pesticide during that time period "will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest."[4]

The culmination of the registration process is EPA's approval of a label for the pesticide, including use directions and appropriate warnings on safety and environmental risks. It is a violation of the FIFRA for any person to sell or distribute a "misbranded" pesticide.[5] A pesticide is misbranded if the "labeling accompanying it does not contain directions for use which...if complied with …are adequate to protect health and the environment."[6]

The Endangered Species Act (ESA)

As recognized by the Supreme Court, the Endangered Species Act (ESA) is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation."[7] The ESA's statutory scheme "reveals a conscious decision by Congress to give endangered species priority over the 'primary missions' of federal agencies."[8] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[9] In all ESA analyses and decisions, agencies must "give the benefit of the doubt to the species,"[10] and use the best scientific and commercial data available.[11]

Section 7(a)(2) of the ESA requires every federal agency to consult the appropriate federal fish and wildlife agency—the U.S. Fish and Wildlife Service (FWS), in the case of land and freshwater species and the National Marine Fisheries Service (NMFS) in the case of marine

[1] 7 U.S.C. § 136a(c)(1); 40 C.F.R. § 152.42.
[2] 7 U.S.C. § 136a(c)(5).
[3] 7 U.S.C. §136(bb).
[4] 7 U.S.C. §136a(c)(7)(C).
[5] 7 U.S.C. § 136j(a)(1)(E).
[6] 7 U.S.C. § 136(q)(1)(F).
[7] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).
[8] *Id.* at 185.
[9] *Id.*
[10] *Conner v. Burford*, 848 F.2d 1441, 1454 (9th Cir. 1988).
[11] 16 U.S.C. § 1536(a)(2).

species—to "insure" that the agency's actions are not likely "to jeopardize the continued existence" of any listed species or "result in the destruction or adverse modification" of critical habitat.[12] The ESA's implementing regulations broadly define agency action to include "all activities or programs of any kind authorized, funded or carried out … by federal agencies," including the granting of permits and "actions directly or indirectly causing modifications to the land, water or air."[13] A species' "critical habitat" includes those areas identified as "essential to the conservation of the species" and "which may require special management considerations or protection."[14]

EPA is required to review its actions "at the earliest possible time" to determine whether the action may affect listed species or critical habitat.[15] To facilitate compliance with Section 7(a)(2)'s prohibitions on jeopardy and adverse modification, the ESA requires each federal agency that plans to undertake an action to request information from the expert agency "whether any species which is listed or proposed to be listed [as an endangered species or a threatened species] may be present in the area of such proposed action."[16] If FWS/NMFS advises the agency that listed species or species proposed to be listed may be present, the agency must then prepare a biological assessment for the purpose of identifying any such species that are likely to be affected by the proposed agency action.[17]

If an agency determines that its action "may affect" but is "not likely to adversely affect" a listed species or its critical habitat, the regulations permit "informal consultation," during which the wildlife agencies must concur in writing with the agency's determination.[18] If the agency determines that its action is "likely to adversely affect" a listed species or critical habitat, or if wildlife agencies do not concur with the agency's "not likely to adversely affect" determination, the agency must engage in "formal consultation," as outlined in 50 C.F.R. § 402.14 ("General Formal Consultation").[19]

At the end of the formal consultation, FWS/NMFS must provide the agency with a "biological opinion" detailing how the proposed action will affect the threatened and endangered species and/or critical habitats.[20] If FWS/NMFS concludes that the proposed action will jeopardize the continued existence of a listed species or result in the destruction or adverse modification of critical habitat, the biological opinion must outline reasonable and prudent alternatives" to the proposed action that would avoid violating ESA section 7(a)(2).[21]

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[12] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).
[13] 50 C.F.R. § 402.02 (emphasis added).
[14] 16 U.S.C. § 1532(5)(A).
[15] 50 C.F.R. § 402.14(a).
[16] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[17] *Id.*
[18] 50 C.F.R. § 402.14(a)-(b).
[19] 50 C.F.R. §§ 402.02, 402.14(a).
[20] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[21] 16 U.S.C. § 1536(b)(3)(A).

An agency is relieved of the obligation to consult on its actions only where the action will have "no effect" on listed species or designated critical habitat. The "may affect" standard is extremely low: "[A]ctions that have any chance of affecting listed species or critical habitat—even if it is later determined that the actions are 'not likely' to do so—require at least some consultation under the ESA."[22] "'*Any possible effect,* whether beneficial, benign, adverse or of an undetermined character,'" triggers the consultation requirement.[23]

The status quo must be maintained until an agency has fulfilled its legal obligations under ESA section 7. Section 7(d) of the ESA provides that "[a]fter initiation of consultation . . . the Federal agency . . . shall not make any irreversible or irretrievable commitment of resources with respect to the agency action which has the effect of foreclosing the formulation or implementation of any reasonable and prudent alternative measures which would not violate subsection(a)(2) of this section." [24]

COMMENTS

EPA is proposing to unconditionally approve foliar application of a novel insecticide containing the new active ingredient Ledprona (*Leptinotarsa decemlineata*-specific recombinant double-stranded interfering Oligonucleotide GS2). Developed by GreenLight Biosciences (Greenlight), Ledprona is a double-stranded RNA molecule (dsRNA) of unidentified length that is the active ingredient in a pesticide formulation known as Calantha that is sprayed on potato plants for control of Colorado potato beetle (CPB). When ingested by CPB, Ledprona engages the CPB's RNA interference (RNAi) machinery, which cleaves it into 21-23 nucleotide-long small interfering RNAs (siRNAs) that are designed to silence CPB's *protesasome subunit beta type-5 (PSMB5)* gene. Ledprona sharply downregulates production of the PSMB5 protein, and in this way kills CPB, because PSMB5 is essential to CPB's survival.

The proposed end-use product, Calantha, contains 0.8% Ledprona, with the remaining 99.2% comprised of other unidentified, confidential ingredients that are in part designed to ensure the stability of the dsRNA oligonucleotide in storage, but which also lend Ledprona some degree of resistance to degradation in soil and water. Calantha is proposed for aerial and ground-based spraying of potato plants at 10 and 4 grams a.i./hectare, respectively. Up to four applications per year are permissible, with a minimum 7 days retreatment interval. Thus, up to 40 (aerial) and 16 (ground) grams/hectare of Ledprona could be sprayed within one month.

EPA acknowledges that "the gene silencing mechanism of Ledprona is a novel technology," and accordingly has proposed limiting the registration to 3 years.[25]

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[22] *Karuk Tribe of California v. U.S. Forest Serv.*, 681 F.3d 1006, 1027 (9th Cir. 2012) (en banc); *see also W. Watersheds Project v. Kraayenbrink*, 632 F.3d 472, 496 (9th Cir. 2011).
[23] *Cal. ex rel. Lockyer v. U.S. Dep't of Agric*., 575 F.3d 999, 1018-19 (9th Cir. 2009) (quoting 51 Fed. Reg. 19,926, 19,949 (June 3, 1986)) (emphasis in *Lockyer*).
[24] 16 U.S.C. § 1536(d); 50 C.F.R. § 402.09.
[25] EPA, Proposed Registration Decision, at 19.

I. EPA Fails to Provide Necessary Data for Public Input Required Under FIFRA.

EPA has failed to provide substantial information that is normally provided in proposed registrations and their underlying risk assessments. To our knowledge, EPA has even withheld the nucleotide length of the dsRNA that is the active ingredient at issue in the proposed registration. This is unacceptable. It would be as if EPA were to withhold the chemical formula and structure of a proposed conventional pesticide. Throughout these comments we note instances of information that has been withheld or omitted, but which is essential for a critical evaluation of the proposed registration of this novel technology, and which EPA should provide.

II. Studies on Formulated Product as well as the Active Ingredient

CFS notes approvingly that GreenLight provided some studies on the formulated product, Calantha, as well as on the technical active ingredient, Ledprona. It is not clear to what extent these were voluntarily submitted, or explicitly requested by EPA. In any case, we encourage EPA to (continue to) require submission of formulated product studies in the areas of human health, ecological impact and environmental fate for both biological and conventional pesticides in the future. That said, as discussed below, certain critical studies are still needed for Calantha, and the results of the Calantha studies that were conducted were not adequately considered in the overall risk assessment.

III. The Proposed Section 3(c)(5) Registration Violates FIFRA.

EPA's proposal to register Ledprona under section 3(c)(5) is improper. EPA admits that it is basing its proposed registration decision solely on data the registrant GreenLight submitted to satisfy its prior application for an experimental use permit (EUP), without any additional data. However, there are far fewer data requirements for approval of an EUP than are required for a new use approval under Section 3 of FIFRA.

For example and as discussed in further detail below, EPA's FIFRA regulations for biochemical pesticides require, for outdoor terrestrial use such as the proposed use of Ledprona, data on avian acute oral toxicity (EPA Guideline No. 850.2100), avian dietary toxicity (EPA Guideline No. 850.2200), data on acute toxicity for freshwater fish and invertebrates (EPA Guideline Nos. 850.1075 and 850.1010), and various data on plants and vegetative vigor. *See* 40 C.F.R. part 158, subpart U. Yet in issuing the EUP, which concerned a very limited scope, EPA waived any such data submission.[26] EPA's own regulations also conditionally require various soil, hydrolysis, and photodegradation studies, and terrestrial field testing. These testing guidelines are designed to ensure that EPA has sufficient data and information to conduct a detailed cost-benefit analysis before registering a pesticide. EPA's rash proposal to register Ledprona without the full data set of information violates FIFRA.

[26] EPA, Environmental Risk Assessment for a FIFRA Section 5 Experimental Use Permit for the New Product GS2 Formulation (Calantha) Containing Ledprona (Leptinotarsa decemlineata (Colorado Potato Beetle CPB)-specific recombinant double-stranded interfering Oligonucleotide GS2) at 0.8% (April 13, 2023). Henceforth, "EPA EUP Environmental Risk"

In light of the missing test data, EPA's proposed unconditional registration of Ledprona under FIFRA Section 3(c)(5) is unlawful. Instead, any registration of Ledprona without the necessary data is only lawful under FIFRA section 3(c)(7). FIFRA is clear: where there are data gaps and missing information, EPA can register a pesticide with conditions (conditional registration) under Section 3(c)(7) of FIFRA "for a period reasonably sufficient for the generation and submission of required data," but only if EPA also determines that the conditional registration of the pesticide during that time period "will not cause any unreasonable adverse effect on the environment, and *that use of the pesticide is in the public interest*."[27]

EPA entirely fails to articulate any reason why registering Ledprona now is in the public interest, other than to point out the fact that as an insecticide, Ledprona is intended to target Colorado potato beetles. But there are many ways to manage CPB,[28] and the agency's risk-benefit analysis is entirely lacking in any discussion of benefits specific to Ledprona. EPA has failed to articulate a public interest finding necessary for a FIFRA section 3(c)(7) registration.

IV. EPA's Human Health Risk Assessment is Deficient

Broadly speaking, Ledprona has the potential to impact human health in two ways, via sequence-specific or non-sequence specific effects. Sequence-specific effects could occur if siRNA's derived from Ledprona silence one or more human genes by matching a nucleotide sequence of the silenced gene(s). The nature of such effects would depend on the function(s) of the silenced gene(s). Non-sequence specific effects refer to the immune system's response to double-stranded DNA, irrespective of precise sequence, and generally involve some inflammatory process. It is also possible that the co-formulants that comprise 99.2% of Calantha could exacerbate any adverse effect of Ledprona (e.g. by increasing its penetration of tissue) or exert toxic effects in their own right.

Sequence-specific silencing

Bioinformatics analysis identified 3 potential siRNA's derived from Ledprona that match 2 long non-coding RNAs (lncRNAs) in humans. As EPA concedes, few lncRNAs have been characterized in humans, and there is no scientific consensus on what proportion of lncRNAs are functional. This in turn makes it difficult if not impossible to determine the effect of silencing a lncRNA.[29] The analysis suggested that the shorter, 539 lncRNA nucleotide sequence was expressed in the testis, but not in the other 26 tissues/organs that were analyzed. EPA considers it doubtful that Ledprona could suppress expression of this lncRNA in the testis due mainly to oral, dermal and respiratory barriers that hinder Ledprona's transport to this tissue via the blood stream, but if it did the effects would be uncertain because the function of this lncRNA is unknown.[30] The second transcript, which is much longer (9,248 base pairs) is

[27] 7 U.S.C. §136a(c)(7)(C).

[28] U. of Idaho Extension. Colorado Potato Beetle. https://www.uidaho.edu/extension/ipm/ag-pests/arthropods/colorado-potato-beetle.

[29] EPA Proposed Registration Decision at 7.

[30] Id. at 7.

regarded as a theoretical gene, and despite EPA's designation of it as non-coding, there is empirical mRNA evidence that it is in fact expressed, though no information on what its function might be. Neither did the analysis provide insight into which tissue(s) it may be expressed in, or its subcellular localization. Thus, this transcript could be expressed in lungs, skin, stomach or colon, tissues that EPA deems relevant for Ledprona exposure via inhalation, dermal contact or ingestion.[31] In short, the bioinformatics analysis provided no actionable information on Ledprona's hazard (potential to cause adverse effects), and EPA relies entirely on its presumption of negligible exposure to rule out human health risks.

Off-target effects

RNA sequences need not be perfect matches to trigger RNA interference, and in fact the scientific literature is replete with accounts of "off-target" effects,[32] in which partial sequence matches suffice to trigger RNAi.[33] Importantly, dsRNA oligonucleotides such as Ledprona have more opportunities to generate off-target effects than siRNA.[34] Off-target effects may extend beyond species that are closely related to the target pest, and afflict organisms that are genetically and taxonomically far removed from it.[35] The upshot is that the GreenLight bioinformatics analyses discussed in this paper will inevitably miss many off-target effects, and cannot be relied upon to provide anywhere close to a full account of the potential human health and environmental harms Ledprona/Calantha could cause.

Non-sequence specific immune effects

Longer double-stranded RNA oligonucleotides of the (unidentified) length of Ledprona often trigger an inflammatory response from the innate immune system, and it is uncertain how much would be required to elicit such adverse effects.[36] The inflammatory response to dsRNA of both viral and endogenous (from mitochondrial dsRNA released from damaged cells) origin has been linked to hypertension, autoimmune disease, neurological damage, and to preeclampsia in pregnant women, among other adverse effects.[37] EPA should require further studies to better characterize the potential non-sequence specific risks posed by exposure to Ledprona.

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[31] Id. at 7-8.

[32] A search conducted on PubMed (https://pubmed.ncbi.nlm.nih.gov on 10/20/23) using the search term "RNAi off-target effects" (without quotation marks) yielded 581 hits.

[33] J. Chen et al. (2021). Off-target effects of RNAi correlate with the mismatch rate between dsRNA and non-target mRNA. RNA Biology 18(11): 1747-59.

[34] Id.

[35] E. Sirinathsinghji, K. Klein and D. Perls (2020). Gene-Silencing Pesticides: Risks and Concerns. Friends of the Earth, 2020. https://foe.org/resources/gene-silencing-pesticides-risks-and-concerns/.

[36] EPA Proposed Registration Decision at 3.

[37] V. Dela Justina et al. (2020). Double-stranded RNA and Toll-like receptor activation: a novel mechanism for blood pressure regulation. Clinical Science 134(2): 303-313.

Inhalation Risk

The bioinformatics analysis could not rule out expression in the lung of the second, longer of the two human dsRNA sequences that matched potential siRNAs derived from Ledprona. Sequence-specific silencing in the lung from inhalation is therefore a possibility, as is silencing of the first transcript discussed above that is expressed in the testis, if the corresponding Ledprona-derived siRNA were to accesses the bloodstream. In addition, the EPA is concerned that inhalation of Ledprona/Calantha could elicit an immune response in the lungs,[38] a potential risk that led it to impose the requirement that workers wear "a dust/mist filtering respirator" when using Calantha.[39] EPA presents no evidence that such respirators are up to the task of filtering out dsRNA oligonucleotides like Ledprona, and because a sprayable formulation of dsRNA is a novel development, testing should be conducted to determine whether they are effective, and also the extent to which applicators will in practice wear them.

Ocular risk

The bioinformatics analysis involved a search for expression of the two Ledprona-matching human dsRNA sequences in 27 organs/tissues, but the eye was not among them. Thus, one or both may be expressed in the eye, and if so silenced by Ledprona, with unknown effects. As EPA notes, there is not sufficient evidence to concluded that RNAases (RNA-degrading enzymes) in tears would break down naked Ledprona that drifts into the eye. Calantha co-formulants might well increase the potential for ocular absorption of Ledprona vs. the naked dsRNA. Hence, EPA is requiring protective eyewear.[40] EPA presents no evidence that such eyewear is up to the task of excluding dsRNA oligonucleotides like Ledprona, and because a sprayable formulation of dsRNA is a novel development, testing should be conducted to determine whether this eyewear is effective, and also the extent to which applicators will in practice wear them.

Dermal exposure

Calantha (but not Ledprona) was shown to be a skin sensitizer in mice,[41] which means it elicited an immunologically mediated cutaneous reaction, in an EPA Guideline Study (OCSPP

[38] EPA attempts to minimize the risk by comparing the anticipated level of lung exposure to Ledprona and the doses of experimental siRNAs being developed for inhalable therapeutics, stating that the former is several orders of magnitude lower than the latter. This comparison is highly speculative and uninformative because it assumes that adverse effects from Ledprona could occur only at dsRNA levels approaching those that are required for experimental drug siRNAs to exert strong therapeutic effects. Too little is known of Ledprona or experimental siRNA inhalable therapeutics to place any stock in this comparison.

[39] EPA Proposed Registration Decision at 8, 19.

[40] Id. at 9.

[41] EPA, Final human health risk assessment, review of product characterization and manufacturing process for the end-use product , Calantha, containing 0.8% of the new active ingredient Ledprona dsRNA…." EPA-HQ-OPP-2021-0271-0005, Sept. 27, 2023, at 14. Henceforth, EPA, Final human health risk assessment.

870.2600). The elicitation of an immune response in skin may indicate Calantha is more likely to elicit an immune response via other routes of exposure, including via inhalation.[42] While it is true that the skin hinders uptake of Ledprona upon dermal contact with Calantha, uptake would be rendered far easier via skin abrasions, cuts, etc. EPA is requiring that workers who mix, handle or spray Calantha wear personal protection equipment (PPE),[43] but presents no evidence that such PPE is up to the task of preventing dermal exposure to dsRNA oligonucleotides like Ledprona, and because a sprayable formulation of dsRNA is a novel development, testing should be conducted to determine whether this PPE is effective, and also the extent to which applicators will in practice wear the prescribed PPE.

EPA should not approve this first dsRNA pesticidal spray before a fuller assessment of potential human health risks. As part of this effort, field workers who mix, handle or spray Calantha should be closely monitored for any signs of adverse health effects throughout the duration of the ongoing Experimental Use Permit.

Environmental justice concerns

Moreover, given that EPA proposes to allow aerial spraying of this product, which is known to generate far more spray drift at greater distances than ground applications, the Agency should assess more fully the likelihood that children will be exposed. Given that potato growers typically employ significant numbers of People of Color, those workers and their children are likely to be the first and most highly exposed.

V. EPA's Ecological Risk Assessment Is Deficient.

Environmental Fate

EPA maintains that Ledprona degrades rapidly in the environment, resulting in negligible exposure to most organisms. Because this in turn becomes EPA's major rationale for waiving numerous guideline studies that would otherwise be required as the basis for a proposed registration, it becomes important to critically assess the environmental fate of Ledprona and Calantha.

Calantha contains co-formulants that are specifically designed to lend Ledprona resistance to degradation in storage.[44] EPA nevertheless maintains that Ledprona degrades rapidly in both soil and water.

[42] Tsui HC, Ronsmans S, De Sadeleer LJ, Hoet PHM, Nemery B, Vanoirbeek JAJ (2020). Skin Exposure Contributes to Chemical-Induced Asthma: What is the Evidence? A Systematic Review of Animal Models. Allergy Asthma Immunol Res. 12(4):579-598. doi: 10.4168/aair.2020.12.4.579.

[43] EPA, Final human health risk assessment at 22.

[44] EPA, Proposed Registration Decision, at 15-16

Aerobic soil metabolism studies of Ledprona, applied as Calantha, in three soils showed DT_{50} times[45] ranging from 0.5 to 2.92 days, and DT_{90} times of 4 days for all soils.[46] However, Ledprona residues remained at some unspecified level by the end of the study at day 12. According to EPA, "[t]he soils still maintained a low but detectable concentration of the test substance by the end of the study at day 12," which residue "could present a potential exposure to nontarget organisms until all of the added product is fully degraded or otherwise biologically unavailable."[47] EPA fails to note, however, that with the potential for up to four retreatments at 7 day intervals, Ledprona residues could accumulate in the soil, increasing exposure to organisms that dwell in or have contact with the soil. Further evaluation is hampered by EPA's failure to report information key to a critical evaluation of this study, including the amount of Calantha/Ledprona applied to the soil; the shape of the disappearance curve, which is not linear, the amount of Ledprona residue remaining at 12 days, etc.

As to its fate in water, EPA reports an aerobic aquatic metabolism study in which the DT_{50} values for Calantha were 1.86 and 1.27 days in two different river systems, and the DT_{90} values were 6.18 and 4.2 days, respectively. Unfortunately, EPA provides no other information needed for a critical evaluation of this study, including application rate, shape of the disappearance curve, the length of the study, any residues remaining at timepoints beyond DT_{90}, or any account of the factors (abiotic, biotic) responsible for the reduction in Ledprona levels. EPA also describes two "supporting literature" studies on the aquatic fate of uncharacterized dsRNA oligonucleotides other than Ledprona, whose relevance to Ledprona is therefore questionable.[48] In one, 3% of an unspecified amount of unidentified dsRNA in uncharacterized aquatic microcosms partitioned into sediment, a finding that triggers the need for testing Ledprona's ability to enter into and persist in sediment, where benthic invertebrates could come into contact with it.

Finally, EPA recounts a GreenLight study conducted for the human health risk assessment in which the resistance to degradation of Ledprona in water containing microbes is tested, both as a standalone dsRNA oligonucleotide and as part of the formulated product, Calantha. As expected, the dsRNA oligonucleotide active ingredient, Ledprona, has considerably greater resistance to degradation as part of Calantha. At an initial concentration of just 200 ng/liter, which is just 0.1% ($1/1000^{th}$) of the already diluted spray application rate of 0.2 g/l, 60 ng/l of formulated Ledprona (30%) remained after 70 hours, while naked dsRNA Ledprona was reported to degrade fully in just over 20 hours.[49] As noted, the concentration tested, 200 nanograms (billionths of a gram) active ingredient per liter of water, is just $1/1000^{th}$ the aerial application rate of 0.2 grams/liter (200 mg/liter). EPA notes that the capacity of co-

[45] DT refers to "disappearance time," and the subscript denotes the percent of the initial amount that disappears by the given time. Hence, DT_{50} = the time it takes for 50% of the initial amount to degrade.

[46] EPA EUP Environmental Risk, at 4.

[47] Id. at 4. In the paragraph in which these passages appear, EPA seems to err by equating DT_{90} with the time at which 90% of the initial concentration remains, rather than the time at which 90% has disappeared (with 10% remaining). EPA should correct or clarify this point.

[48] Id. at 5-6.

[49] Id. at 5.

formulants to lend Ledprona resistance to microbial degradation declines with dilution, which means that Ledprona's resistance increases with concentration. No rationale is given for the concentrations GreenLight chose to test, which is quite low, and its persistence would likely be still greater than reported at dilutions less than 1:1000.

Assessment of Ecological Exposure and Risk

EPA labels Calantha/Ledprona a "biochemical pesticide."[50] Presumably, this means EPA is making Ledprona subject to the regulations under 40 CFR, Part 158, Subpart U: Biochemical Pesticides. Yet it would appear that Calantha/Ledprona does not satisfy the tripartite definition of a biochemical pesticide, particularly the provision that a biochemical pesticide be one that "has a non-toxic mode of action to the target pest" (CFR 40, Part 158.2000(a)(1)(iii)). [51] Calantha/Ledprona, of course, have a toxic mode of action, in that they kill CPB, other beetles, and likely other non-target organisms.

In any case, EPA goes on to state:

> "To evaluate toxicity, EPA initially requires that a wide range of studies including Tier I testing be done on the following nontarget organisms: mammalian (acute, subchronic, prenatal developmental, and mutagenicity), birds (acute oral and dietary), fish (acute freshwater fish and aquatic invertebrates), plants, and insects. Testing is organized in a tiered structure, where Tier I studies test worst-case exposure scenarios and higher tiers (Tiers II and III) generally encompass definitive risk determinations and longer-term greenhouse or field testing. Higher tier testing is implemented only when unacceptable effects are seen at the Tier I screening level. All data requirements may be addressed with guideline studies or scientific rationales."[52]

This is an inaccurate and misleading description of what EPA did in the case of Ledprona, and must be changed. First, EPA did not require that Ledprona be tested in Tier 1 studies on birds (acute oral and dietary), fish (acute freshwater and aquatic invertebrates), or mammals (subchronic, prenatal developmental, and mutagenicity). Studies that are not conducted – which EPA waived – cannot produce data to satisfy FIFRA data requirements.

Neither are data requirements specified in 40 CFR, Part 158 satisfied by "scientific rationales." The major rationale for EPA's waiver of numerous, otherwise required studies is the assumption that the relevant organisms will experience "negligible exposure." The evidence of environmental persistence presented above casts doubt on this assumption. In any case, EPA should change the indicated paragraph to eliminate the misleading impression that

[50] EPA, Proposed Registration Decision, at 12.
[51] One example of such a non-toxic biochemical pesticide is the class of pheromones, chemicals released by insects and other organisms that can, for instance, attract others of the same species to congregate, facilitating their elimination. See CFR 40, Part 158.2000(a)(2).
[52] EPA, Proposed Registration Decision, at 12.

EPA has required Tier 1 studies on the named organisms, and instead state clearly that many of these studies have been waived.

Potential for Adverse Effects to Nontarget Organisms

EPA reviewed registrant's non-target organism studies testing the effects of Ledprona and Calantha on a range of organisms, including daphnia, earthworm, honeybee (Ledprona only), green lacewing, ladybird beetle, parasitic wasp, predatory mite, and the soil-dwelling Collembola (aka springtail).[53] The tests conducted with Calantha revealed harm to at least three of these species at or near the aerial rate of application for Calantha, 10 grams a.i. Ledprona per hectare.[54]

- Parasitic wasp (*Aphidius rhopalosiphi*):
    - 48-hour Lethal Rate 50 (LR_{50}): 50% of a group of wasps were killed upon 48-hour contact exposure to Calantha when sprayed at the average rate of 15.801 g a.i./ha (95% confidence interval 7.548 to 27.506 g a.i./ha)
    - Reproductive harm: Effective Rate 50 (ER_{50}). The application rate of Calantha at which a group of wasps suffered a 50% decline in some unspecified parameter of reproductive performance exceeded 8.125 g a.s./ha, but could not be determined more precisely because the wasps were killed off at tested exposures greater than 8.125 a.s./ha.
- Predatory mite (*Typhlodromus pyri*):
    - Reproductive harm: Effective Rate 50 (ER_{50}). The average application rate of Calantha at which a group of mites suffered a 50% decline in some unspecified parameter of reproductive performance: 9.534 g a.i./ha (95% confidence interval 6.025 to 15.087 g a.s./ha).

These results suggest that Calantha poses potential risks to two important biocontrol organisms. Many parasitic wasps controls aphids, a significant pest of potatoes and many other crops, and some parasitize CPB eggs.[55] The predatory mite controls other species of mites that feed on the leaves of potatoes and other crops. Calantha may also be harmful to Collembola (springtail), a microarthropod that dwells on the surface of the soil and is an important decomposer of organic matter. The concentrations of Ledprona (as part of Calantha) in soil that suppress Collembola populations could well be reached by the recommended application rates of 10 and 4 g a.i./ha for aerial and ground applications, respectively, particularly with repeat applications at short intervals.[56] Collembola perform many vital functions in the soil, from breaking down organic matter, to consuming pathogenic soil fungi, to providing a favorable habitat for beneficial microorganisms by excretion of feces.[57]

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[53] EPA, EUP Environmental Risk, at 9-13 (Tables 2 and 3).
[54] Id. at 12 (Table 3) for following.
[55] JA Hough-Goldstein et al. Arthropod natural enemies of the Colorado potato beetle. Crop Protection 12(5): 324-334 (1993).
[56] EPA EUP Environmental Risk, at 13.
[57] S. Sandrine. Collembola: actors of soil life. Encyclopedia of the Environment, 2019. https://www.encyclopedie-environnement.org/en/life/collembola-actors-of-soil-life/.

EPA's Ecological Risk Characterization for the EUP entirely ignored these results for Calantha, focusing instead on the results for Ledprona, the unformulated active ingredient that is not sprayed in the real world and which exhibited lesser potency than Calantha in most tests.[58] EPA also discounted these results in the proposed registration decision.[59]

Unfortunately, there were apparently no studies of Calantha's impact on honeybees, even though corresponding tests were conducted on the active ingredient.[60] Given the fact that Calantha was far more potent than the active ingredient in most testing described above, Calantha should be tested for its effects on bees.

EPA reports a "statistically significant difference in mortality"[61] for ladybird beetles at a Ledprona treatment rate of 25 g/ha, not far above the 10 g/ha aerial application rate, but incorrectly dismisses the result; and EPA also gives an equivocal response on potential reproductive impacts to the ladybird beetle that suggests Ledprona might well have reduced reproduction vs. the control group, which would be an adverse impact potentially threatening populations rather than just individuals.[62] The adverse effect findings may or may not be related to the matching nucleotide sequences between CPB and this seven-spotted ladybird beetle that was the subject of the test.[63] Because ladybird beetles are predators of CPB eggs,[64] Ledprona applications could suppress the CPB control services they provide.

Until this point we have assumed that Calantha is more toxic to nontarget organisms because its co-formulants increase the persistence and/or toxicity of Ledprona, which may be the case. However, it is also possible that one or more of the unidentified co-formulants, which comprise 99.2% of Calantha by weight, are exerting toxicity of their own. EPA should require studies on the co-formulants sans Ledprona to clarify this issue.

Genetic / Taxonomic Relatedness Does Not Predict Risk

GreenLight conducted bioinformatics analysis of 12 pest coleopteran (beetle) species and nine nontarget species – analyses that sought to determine whether potential siRNA sequences derived from Ledprona matched nucleotide sequences in the pest and nontarget species.[65] The intended purpose of this study was to determine whether Ledprona posed risks to pest beetles other than CPB, or other nontarget species, based on the twin premises that

[58] EPA EUP Environmental Risk, at 16.
[59] EPA Proposed Registration Decision, at 13.
[60] Compare EPA EUP Environmental Risk, at 11-13 (Table 3) and 9 (Table 2).
[61] EPA should confirm that its meaning here is "increase in mortality," and make the corresponding correction.
[62] EPA EUP Environmental Risk, at 10 (Table 2).
[63] EPA Proposed Registration Decision, at 14.
[64] U. of Idaho Extension. Colorado Potato Beetle. https://www.uidaho.edu/extension/ipm/ag-pests/arthropods/colorado-potato-beetle.
[65] EPA, Proposed Registration Decision, at 14.

such matching sequences are more likely to be found in species closely related to CPB, and that matching nucleotide sequences are good predictors of risk.

Neither premise appears to be necessarily true, despite EPA's repeated appeals to them. First, EPA found matching sequences in earthworm, which is both distantly related to CPB and which did not show apparent adverse effects in bioassays described above, contradicting both premises.[66] Second, of the four coleopteran species (besides CPB) that did exhibit matching sequences, the two most closely related species of the four had diametrically opposed responses in subsequently conducted bioassays. There was no significantly increased mortality in Western corn rootworm exposed to Ledprona, while Southern corn rootworm was extremely sensitive, with significant mortality at just 20 ng/insect.[67] The southern and western corn rootworm reacted entirely differently to Ledprona, despite the fact that they are closely related members of the same genus (*Diabrotica*). Third, the red flour beetle (*Tribolium castaneum*), which belongs to an entirely different family (Tenebrionidae) of beetles than CPB and the rootworms (the latter three all members of the Chrysomelidae family), also exhibited increased mortality from exposure to Ledprona. Finally, several non-beetle arthropods (parasitic wasp, predatory mite, Collembola) showed susceptibility to Calantha (as discussed above), despite apparent lack of matching sequences.

In short, the data clearly show that the adverse effects of Calantha/Ledprona are not limited to the CPB and its close relatives, as EPA claims,[68] and they also show that taxonomic/genetic relatedness to CPB is not a good predictor of susceptibility to Calantha/Ledprona, again directly contrary to EPA:

> "Given the mode of action of Ledprona (i.e., relies on specificity at the nucleotide level) it is not unexpected that beetle species closely related to the target pest would have a higher likelihood of sharing significant sequence homology, and therefore have a higher likelihood of adverse effects from Ledprona."[69]

EPA's assumptions here about risk correlating with relatedness are contradicted not only by the data it mis-analyzes, as discussed above, but have also been disproven in other organisms. Mogren and Lundgren (2017) collated pesticidal dsRNAs into a database, determined their target gene sequences, and statistically evaluated the degree of similarity with sequences in the honey bee genome. They identified 101 insecticidal RNAs sharing high sequence similarity with genomic regions in honey bees, as the model nontarget organism. They concluded that "[t]he similarities of non-target genes to the pesticidal RNA was unaffected by taxonomic relatedness of the target insect to honey bees."[70] They also demonstrated that "[t]he likelihood that off-target sequences were similar increased with the number of nucleotides in the dsRNA molecule." The longer the dsRNA oligonucleotide, the greater the

[66] Id. at 14.
[67] EPA EUP Environmental Risk, at 16.
[68] EPA Proposed Registration Decision, at 14.
[69] EPA EUP Environmental Risk, at 16.
[70] CL Mogren and JG Lundgren (2017). *In silico* identification of off-target pesticidal dsRNA binding in honey bees (*Apis mellifera*). PeerJ 5:e4131; DOI 10.7717/peerj.4131.

probability of off-target sequences, and hence adverse effects occurring in species not genetically similar to the target pest. EPA should take this factor into consideration in its risk assessment, and also report the nucleotide length of Ledprona to the public.

To our knowledge, EPA even fails to identify 7 of the 12 pest beetle species that were subjected to bioinformatics analysis, but should do so to give the public further opportunity to critically evaluate its "relatedness" claim discussed above. EPA also needs to ensure that a range of non-pest beetles are tested in bioassays (beyond the single one that was tested, the seven-spotted ladybird beetle).

The order Coleoptera (beetles) is the most diverse of insect orders, and contains 40 families of beetles that include predatory species, many of which are important biocontrol species of utility in agriculture,[71] such as ground beetles that consume crop-damaging slugs.[72] EPA concedes risks to coleopteran species on-field, but nowhere weighs the clear potential for loss of the biocontrol services they provide.

Finally, EPA should also consult the 2014 Scientific Advisory Panel Report on risk assessments of RNAi technology deployed as a pesticide.[73]

VI. EPA Fails to Conduct a Risk-Benefit Assessment of the Proposed Ledprona Registration, in Violation of FIFRA.

EPA's one-page benefits assessment and conclusory one-sentence risk-benefit statement are woefully deficient.[74] EPA entirely fails to conduct any risk-benefit analysis specific to the proposed use of Ledprona/Calantha. Instead, EPA relies entirely on generalized potential benefits of biopesticides as compared to conventional pesticides, stating: "Biopesticides are …. usually [] inherently less harmful than conventional pesticides."[75] EPA is so far from an actual risk-benefit assessment of the products at issue that it even falsely attributes a general characteristic of *true* biochemical pesticides – "a nontoxic mode of action to the target pest(s)" – to Ledprona/Calantha[76] (see Section V above for discussion). To reiterate, Ledprona/Calantha indisputably have a toxic mode of action, in that they kill Colorado

[71] D. Brown-Rytlewski, Michigan State University Extension, A pocket IPM scouting guide for woody landscape plants, undated, pp. 1070110.
[72] MR Douglas, JR Rohr, JF Tooker (2015). Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. Journal of Applied Ecology 52(1): 250-260.
[73] Scientific Advisory Panel (2014). Scientific Issues Associated with the use of RNAi Technology as a Pesticide: Problem Formulation for Human Health and Ecological Risk Assessment. SAP Minutes No. 2014-02, May 1, 2014.
[74] See EPA Proposed Registration Decision at 18: Section 4, titled: "Benefits and Public Comments," and the single sentence statement in Section 5: "In considering the assessed risk to human health and the environment, EPA concludes that Calantha and Ledprona Technical meet the regulatory standard under FIFRA."
[75] Id.
[76] Id.

potato beetle, other beetles, and likely other non-target organisms. Neither does EPA even attempt to relate other generic attributes of biopesticides, for instance lower toxicity profile and faster degradation in the environment, to Ledprona/Calantha specifically.

EPA states that "products formulated with Ledprona are expected to align with some of the potential benefits above and could fill specific pest control needs in areas where potatoes are grown,"[77] yet does not explain how Ledprona "fill[s] specific pest control needs," nor provide any analysis comparing Ledprona's toxicity and effects on nontarget organisms to currently-used insecticides on potatoes. Nor does EPA assess Calantha/Ledprona in comparison to the multitude of other tactics for management of Colorado potato beetle, which range from cultural practices such as crop rotation, elimination of alternative CPB host plants, adjustment of planting dates or application of mulches; to physical techniques such as trench traps and flamers; to effective organic biopesticides such as spinosad and neem products; to biological control methods such as the pathogenic fungus *Beauveria bassiana* (which reduces CPB populations by up to 75%), and fostering natural enemies of CPB such as ladybird beetles, ground beetles, predatory stink bugs, harvestmen, spiders, parasitoid wasps and tachinid flies.[78] This assessment of alternative CPB management tactics would have to include a better analysis of the extent to which use of Calantha/Ledprona would suppress natural enemy populations, particularly of beetles and parasitoid wasps. EPA should also assess the potential for CPB to evolve resistance to Ledprona, given published research demonstrating the Colorado potato beetle's rapid evolution (after just nine selection episodes) of high-level (> 11,100-fold) resistance to another insecticidal dsRNA intended for foliar application.[79] In the end, EPA's risk-benefit analysis amount to nothing more than a generalized recitation of the potential benefits of a particular class of pesticides. This lack of a real cost-benefit analysis of Ledprona's proposed registration violates FIFRA.

EPA's failure to assess the risks and benefits of Ledprona's proposed registration is alarming, particularly because the limited set of data before the EPA indicates potential risks to human health, the environment, and federally endangered and threatened species. EPA must conduct an actual assessment of the toxicity of this novel insecticide, and its risks, cost and benefits in light of the full range of alternatives, in order to meet the FIFRA standard for registration.

VII. EPA Fails to Comply with Its Duty under the ESA.

EPA restricted its Endangered Species Act analysis in two ways: 1) To the potential effects of Ledprona; and 2) To threatened and endangered beetles (Coleoptera order), of which

[77] Id.

[78] U. of Idaho Extension. Colorado Potato Beetle. https://www.uidaho.edu/extension/ipm/ag-pests/arthropods/colorado-potato-beetle. On natural enemies of CPB, see also: JA Hough-Goldstein et al. Arthropod natural enemies of the Colorado potato beetle. Crop Protection 12(5): 324-334 (1993).

[79] S. Mishra et al. Selection for high levels of resistance to double-stranded RNA (dsRNA) in Colorado potato beetle (*Leptinotarsa decemlineata* SAY) using non-transgenic foliar delivery (2021). Scientific Reports 11: 6523.

19 species are listed.[80] As discussed above, there is evidence that Calantha in particular poses risks to organisms other than beetles, for instance parasitoid wasps, predatory mites and Collembola. Thus, EPA should broaden its assessment to encompass the effects of the formulated product, Calantha, and the broader range of species that it appears to impact based on the limited studies carried out thus far.

Of the 19 listed coleopteran species/critical habitats, EPA issued "no effect" determinations for 15 based on lack of overlap between the species' ranges and areas of commercial potato production since 2008. Of the remaining four species whose ranges do overlap with potato production areas, EPA anticipates negligible exposure for three due to non-agricultural habitats, the presumed efficacy of label language intended to mitigate spray drift, and data on Ledprona's degradation in soils and in water. EPA does not appear to provide any support for the efficacy of its label language to mitigate spray drift, and degradation, particularly of Ledprona as part of Calantha, is not so rapid as to preclude harmful effects to susceptible species off-field as well as on-field.

EPA likewise concludes Ledprona has "no effect" on the fourth species, American burying beetle, based on two "negligible exposure" arguments: that the beetle burrows underground during the day when the product is applied, and feeds on underground carrion rather than fresh vegetation. But closer examination reveals that exposure may not be negligible at all. As discussed above under Environmental Fate, EPA itself concedes that residues of Ledprona persist in the soil for at least 12 days, indicating that the American burying beetle could be exposed for a week or more after application, with increased exposure when multiple (up to 4) applications are made in as little as one month. This persistence and possible accumulation means that American burying beetles could encounter substantial amounts while on the soil surface during the day, or potentially in their burrows. Moroever, EPA has no idea whether American burying beetle is sensitive to Ledprona/Calantha, and if so the degree of sensitivity.

With regard to other listed species, and more generally for all species, EPA's assumption of negligible exposure from spray drift is mistaken, particularly for the proposed aerial application, which is known to produce far more drift at much greater distances than spray drift from ground-based application; and is also proposed at over twice the application rate as ground applications (0.2 g a.i./l aerial, vs 0.08 g a.i./l ground).

EPA must complete the entire process of consultation under the Endangered Species Act to ensure the proposed registration does not jeopardize the existence of species protected as threatened or endangered under the ESA prior to finalizing its registration decision. Without having fulfilled this duty under the ESA, in consultation with the expert wildlife agencies, EPA cannot determine the full impacts of Ledprona or Calantha on ESA-listed species and their critical habitats and ensure that they will not jeopardize any of those species.

Sincerely,

[80] EPA Proposed Registration Decision, at 16-17.

Center for Food Safety
Alliance for Humane Biotechnology
Beyond Pesticides
Ecoropa
Farmworker Justice
Friends of the Earth
Florida Keys Environmental Coalition
GE Free NZ in Food and Environment
GE Free Tai Tokerau (Northland)
GeneWatch UK
GMWatch
Hawai'i Alliance for Progressive Action-HAPA
Institute for Responsible Technology
Institute on Agriculture and Trade Policy
International Center for Technology Assessment
Maine Organic Farmers and Gardeners Association
Massachusetts Pollinator Network
Maryland Pesticides Education Network
Non-GMO Project
Pesticide Action Network
Pollinator Stewardship Council
Regeneration Massachusetts
Vigilance OGM