CENTER FOR
FOOD SAFETY

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Comments from Center for Food Safety on the Preliminary Plant Pest Risk Assessment and Draft Environmental Assessment of *Zea mays* Event MON 8741 Genetically Engineered by Monsanto for Protection Against Corn Rootworm and Resistance to Glyphosate

Monsanto has petitioned the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) to deregulate its genetically engineered (GE) corn, *Zea mays* event MON 87411 (Petition), which is designed to be resistant to the insect pest species of corn rootworm (CRW) and to the herbicide glyphosate.[1] APHIS has now made available its draft Environmental Assessment (dEA) and preliminary Plant Pest Risk Assessment (pPPRA) for public comment.[2]

CFS is a national nonprofit public interest and environmental advocacy organization working to protect human health and the environment by curbing the use of harmful food production technologies.[3] In furtherance of this mission, CFS uses legal actions, groundbreaking scientific and policy reports, books and other educational materials, and grassroots campaigns, on behalf of nearly 500,000 CFS members. CFS is a recognized national leader on the issue of GE organisms, and has worked on improving their regulation and addressing their impacts continuously since the organization's inception in 1997.

APHIS oversees GE crops pursuant to the Plant Protection Act (PPA),[4] which provides USDA broad authority to "prohibit or restrict . . . movement in interstate commerce of any plant" as necessary to prevent either "plant pest" or "noxious weed" harms.[5] The statute's multifaceted purpose is to protect not only agriculture, but also the "environment, and economy of the United States" through the "detection, control, eradication, suppression, prevention, or retardation" of these harms.[6] On March 6, 2014, CFS provided comments on the Petition stating that in

[1] Petition No. 13-290-01p, *Zea mays* event MON 87411.
[2] 80 Fed. Reg. 30997 – 30998 (Jun. 2, 2015).
[3] *See generally* www.centerforfoodsafety.org.
[4] 7 U.S.C. §§ 7701–7772.
[5] *Id.* § 7712(a); 7 C.F.R. §§ 2.22(a), 2.80(a)(36) (delegating to APHIS).
[6] 7 U.S.C. § 7701(1).

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evaluating Monsanto's MON 87411 corn APHIS needs to examine several specific issues under its PPA authority.[7]

Corn is grown on more acres than any other crop in the US, and any impacts from deregulation of MON 87411 corn are thus likely to be widespread. If approved, MON 87411 corn will be the first crop genetically engineered to express a specifically designed ribonucleic acid (RNA) in its tissues in order to "silence" - turn down expression - of essential genes in insects using their own RNA interference (RNAi) pathway, thus providing pest control. This host-induced gene silencing (HIGS) involving manipulation of fundamental gene expression pathways in another organism is a novel mechanism of pest control, and not enough is known about potential impacts of HIGS to assess risks to human health and the environment, although what is known indicates cause for concern. In addition, health and environmental impacts of the other engineered traits in MON 87411 – Cry-protein-based insect resistance and glyphosate resistance – are poorly addressed in the draft EA and preliminary PPRA. Cumulative impacts of combining these three GE traits of MON 87411 with other GE traits, as planned by Monsanto, are also inadequately assessed by APHIS.

APHIS does not disclose the full range of potential health and environmental risks associated with MON 87411 corn in the draft EA, and thus it does not support a finding of no significant impacts. Even if APHIS had adequately disclosed the risks of this particular product in the draft EA, it would not obviate the requirement for an EIS. Gene silencing via RNAi specifically for pest control is a novel technology that can be used in almost any crop and directed against many kinds of economically important pests (e.g. Kola et al. 2015).

APHIS's present assessment falls short of what is required under the National Environmental Policy Act (NEPA) in considering its proposed action of deregulating MON 87411 corn. NEPA requires a full Environmental Impact Statement (EIS) where an agency action may significantly impact the environment. APHIS must prepare an EIS in considering the deregulation of MON 87411 corn. Under NEPA, "significantly" is a defined to include both considerations of context and intensity, and includes considerations of the "degree to which the proposed action affects public health or safety" and the "degree to which the effects on the quality of the human environment are likely to be highly controversial."[8] Here, the effects of the proposed action (i.e., approving deregulation of the MON 87411 corn) on public health and the environment were inadequately reviewed in the EA. Thus, APHIS must generate an EIS that fully considers the potentially significant cumulative impacts of this proposed action. Further, this action is indeed highly controversial, because so little is known about the impacts the RNAi technology at use with MON 87411 corn on human health and the environment.
The inadequacy of APHIS's data is specifically egregious because MON 87411 present significant, novel issues for APHIS to analyze. The present assessment will set important precedents and must, at a minimum, be rigorously performed and analyzed in an EIS before any

[7] May 6, 2014, CFS Comments on "Monsanto Co.; Availability of Petition for Determination of Nonregulated Status of Maize Genetically Engineered For Protection Against Corn Rootworm and Resistance to Glyphosate", http://www.regulations.gov/#!documentDetail;D=APHIS-2014-0007-0338.
[8] 40 C.F.R. § 1508(b)(2), (4).

decision is made. In the EIS, APHIS should consider all "reasonably foreseeable" environmental impacts of the proposed deregulation of MON 84711, taking a programmatic approach to consider the use of RNAi technology on other crop and against other pests that will likely follow the deregulation of MON 84711.

APHIS must also comply with the mandates of the Endangered Species Act (ESA) by consulting with the US Fish and Wildlife Service. APHIS's claim that this proposed action would have no effects on threatened or endangered species and critical habitats is based on inadequate data and poorly supported assumptions.

Without adequate information about environmental and health impacts of MON 87411 corn, APHIS has failed to demonstrate that MON 87411 is unlikely to pose a plant pest risk, and APHIS must therefore deny Monsanto's petition for non-regulated status until a robust assessment is carried out.

The Center for Food Safety (CFS) submits the following comments[9], highlighting some of the important issues and potential significant impacts that APHIS did not adequately consider in its assessment of Monsanto's petition for deregulation of MON 87411 corn, and that lead to the conclusion that preparation of an EIS and an ESA consultation are required, and that Monsanto's petition to deregulate MON 87411 corn must be denied at this time. As demonstrated by our comments, APHIS simply does not have enough information to be able to adequately assess environmental and health impacts of approving MON87411 corn, and thus cannot make a responsible and lawful determination of nonregulated status. For the many reasons discussed in these comments, APHIS's draft EA is woefully inadequate: APHIS has failed to take the requisite "hard look at the environmental consequences" of its proposed decision to approve the petition,[10] and failed to provide a "convincing case" in support of its decision. Overall, APHIS's extremely deficient analyses and lack of basic data flouts NEPA's fundamental tenets of ensuring comprehensive, timely, and transparent environmental review of agency actions. APHIS must go back to the drawing board and prepare an EIS.

Respectfully submitted,

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[9] Cited references have been submitted to the docket as pdf files with titles that match the in-text citations.

[10] *See, e.g.*, *Friends of the Payette v. Horseshoe Bend Hydroelectric Co*., 988 F.2d 989, 993 (9th Cir. 1993); *see also Overton Park v. Volpe*, 401 U.S. 402, 416 (1971).

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I. Legal Background

National Environmental Policy Act

NEPA requires a federal agency to prepare a detailed EIS for all "major Federal actions significantly affecting the quality of the human environment."[11] NEPA "ensures that the agency . . . will have available, and will carefully consider, detailed information concerning significant environmental impacts; it also guarantees that the relevant information will be made available to the larger [public] audience."[12]

[11] 42 U.S.C. § 4332(2)(C).
[12] *Robertson v. Methow Valley Citizens Council,* 490 U.S. 332, 349 (1989).

If the federal action *may* significantly affect the environment, APHIS *must* prepare an EIS.[13] As a preliminary step, an agency may prepare an EA to decide whether the environmental impact of a proposed action is significant enough to warrant preparation of an EIS.[14] If an agency decides not to prepare an EIS, it must supply a "convincing statement of reasons" to explain why a project's impacts are insignificant.[15] "The statement of reasons is crucial to determining whether the agency took a "hard look" at the potential environmental impact of a project."[16] An EA must "provide sufficient evidence and analysis for determining whether to prepare an EIS or a finding of no significant impact."[17] NEPA regulations require the analysis of direct and indirect, as well as cumulative, effects in NEPA documents, including EAs.[18] The assessment must be a "hard look" at the potential environmental impacts of its action.[19] APHIS's decisions in the EA must be "complete, reasoned, and adequately explained."[20]

Whether there may be a significant effect on the environment requires consideration of two broad factors: context and intensity. "Context" means that "the significance of an action must be analyzed in several contexts such as society as a whole (human, national), the affected region, the affected interests, and the locality . . . . Both short- and long-term effects are relevant."[21] In addition, a number of factors should be considered in evaluating intensity, including "[t]he degree to which the proposed action affects public health or safety," "[t]he degree to which the effects on the quality of the human environment are likely to be highly controversial," "[t]he degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks," "[w]hether the action is related to other actions with individually insignificant but cumulatively significant impacts," "[w]hether the action is related to other actions with individually insignificant but cumulatively significant impacts," and "[t]he degree to which the action may adversely affect an endangered or threatened species or its habitat."[22] An action may be "significant" if even one of these factors is met.[23]

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[13] *Idaho Sporting Cong. v. Thomas*, 137 F.3d 1146, 1150 (9th Cir. 1998) (citation omitted); *Steamboaters v. U.S. Fed. Energy Regulatory Comm.*, 759 F.2d 1382, 1392 (9th Cir. 1985).
[14] 40 C.F.R. § 1508.9.
[15] *Save the Yaak v. Block*, 840 F.2d 714, 717 (9th Cir. 1988).
[16] *Id.*
[17] *Id.*
[18] *See* 40 C.F.R. §§ 1508.8, 1508.9, 1508.13, 1508.18.
[19] *Nat'l Parks & Conservation Ass'n v. Babbitt*, 241 F.3d 722, 731 (9th Cir. 2001) (quoting 40 C.F.R. § 1508.27); *Blue Mountains Biodiversity v. Blackwood*, 161 F.3d 1208, 1211 (9th Cir. 1998).
[20] *Nw. Coal. for Alternatives to Pesticides v. U.S. Envtl. Prot. Agency*, 544 F.3d 1043, 1052 n.7 (9th Cir. 2008).
[21] 40 C.F.R. § 1508.27(a).
[22] *Id.* § 1508.27(b)(2), (4), (5), (6), (7), (9). "Human environment shall be interpreted comprehensively to include the natural and physical environment and the relationship of people with that environment." *Id.* § 1508.14.
[23] *Ocean Advocates v. U.S. Army Corps of Eng'rs*, 361 F.3d 1108, 1125 (9th Cir. 2004); *see Nat'l Parks & Conservation Ass'n*, 241 F.3d at 731 (either degree of uncertainty or controversy "may be sufficient to require preparation of an EIS in appropriate circumstances").

A thorough consideration of cumulative impacts is required in the preparation of an EA.[24] Specifically, an EA must provide a quantified assessment of project's environmental impacts when combined with other projects.[25] Notably, courts and the Council on Environmental Quality (CEQ) emphasize that a detailed cumulative impacts analysis is especially important in an EA, because there is a much higher risk of cumulative impacts resulting from many smaller decisions for which EAs are prepared.[26] The cumulative impact analysis must also include an assessment of potential aesthetic, historic, cultural, economic, social, and health impacts.[27]

Council on Environmental Quality

NEPA established CEQ and charged the agency with overseeing implementation of this law.[28] The regulations subsequently promulgated by CEQ[29] implement the directives and purpose of NEPA, and "[t]he provisions of [NEPA] and [CEQ] regulations must be read together as a whole in order to comply with the spirit and letter of the law."[30] CEQ's regulations are applicable to and binding on all federal agencies.[31] Among other requirements, CEQ's regulations mandate that federal agencies address all "reasonably foreseeable" environmental impacts of their proposed programs, projects, and regulations.[32] Direct effects are those that are caused by the action and occur at the same time and place.[33] Indirect effects are those that are caused by the action and are later in time or farther removed in distance, but are still reasonably foreseeable.[34] A cumulative impact constitutes the impact on the environment that results from the incremental impact of the action when added to past, present, and reasonably foreseeable future actions regardless of what agency or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time.[35]

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[24] *See, e.g.*, *Kern v. Bureau of Land Mgmt.*, 284 F.3d 1062, 1075 (9th Cir. 2002).
[25] *Great Basin Mine Watch v. Hankins*, 456 F.3d 955, 972 (9th Cir. 2006).
[26] *See, e.g.*, *Native Ecosystems Council v. Dombeck*, 304 F.3d 886 (9th Cir. 2002); *Kern*, 284 F.3d. at 1076, 1078 ("Given that so many more EAs are prepared than EISs, adequate consideration of cumulative effects requires that EAs address them fully. Without such individually minor, but cumulatively significant effects, it would be easy to underestimate the cumulative impacts of the action . . . and of other reasonably foreseeable future actions, on the [environment]."") (internal citation marks omitted).
[27] 40 C.F.R. § 1508.8; *see e.g.*, *id.* § 1508.14 (when "economic or social and natural or physical environmental are interrelated," then the NEPA analysis must discuss "all of these effects on the human environment); *Wyoming v. U.S. Dep't of Agric.*, 661 F.3d 1209, 1251 (10th Cir. 2011) (cumulative impacts analysis must consider all of the effects listed at 40 C.F.R. § 1508.8).
[28] *See* 42 U.S.C. §§ 4321, 4344.
[29] 40 C.F.R. §§ 1500–08.
[30] *Id.* § 1500.3.
[31] *Id.* §§ 1500.3, 1507.1; *see, e.g.*, *Hodges v. Abraham*, 300 F.3d 432, 438 (4th Cir. 2002).
[32] *See* 40 C.F.R. §§ 1502.4, 1508.8, 1508.18, 1508.25.
[33] *Id.* § 1508.8(a).
[34] *Id.* § 1508.8(b).
[35] *Id.* § 1508.7.

CEQ's regulations clearly lay out the purpose of an EIS: "The primary purpose of an environmental impact statement is to serve as action-forcing devices to insure that the policies and goals defined in the Act are infused into the ongoing programs and actions of the Federal Government."[36] An EIS shall provide "full and fair discussion of significant environmental impacts and shall inform decisionmakers of the reasonable alternatives which would avoid or minimize adverse impacts or enhance the quality of the human environment."[37] Agencies are to focus on "significant environmental issues and alternatives."[38]

Plant Protection Act

APHIS oversees transgenic crops pursuant to the Plant Protection Act (PPA),[39] which provides USDA broad authority to "prohibit or restrict . . . movement in interstate commerce of any plant" as necessary to prevent either "plant pest" or "noxious weed" harms.[40] The statute's multifaceted purpose is to protect not only agriculture, but the "environment, and economy of the United States" through the "detection, control, eradication, suppression, prevention, or retardation" of these harms.[41]

The PPA defines these harms expansively. A "noxious weed" is "any plant or plant product that can directly or indirectly injure or cause damage to crops . . . or other interests of agriculture, . . . the natural resources of the United States, the public health, or the environment."[42] "Plant pest" means "any living stage [of a list of organisms] that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product."[43]

Developers seeking to commercialize a transgenic plant must petition APHIS for deregulation,[44] which the agency can grant "in whole or in part."[45] The PPA mandates that all APHIS decisions "be based on sound science."[46]

Endangered Species Act

As recognized by the U.S. Supreme Court, the ESA is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation."[47] The ESA's statutory scheme "reveals a conscious decision by Congress to give endangered species priority over the

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[36] *Id.* § 1502.1.
[37] *Id.*
[38] *Id.*
[39] 7 U.S.C. §§ 7701–7772.
[40] *Id.* § 7712(a); 7 C.F.R. §§ 2.22(a), 2.80(a)(36) (delegating to APHIS).
[41] 7 U.S.C. § 7701(1).
[42] *Id.* § 7702(10).
[43] *Id*. § 7702(14).
[44] 7 C.F.R. § 340.6.
[45] *Id*. § 340.6(d)(3)(i).
[46] 7 U.S.C. § 7701(4); *see id.* § 7712(b).
[47] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).

'primary missions' of federal agencies."[48] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[49]

Section 7(a)(2) of the ESA requires every federal agency to consult the appropriate federal fish and wildlife agency—FWS, in the case of land and freshwater 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.[50] 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 FWS "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."[51] If FWS 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.[52]

If an agency determines that its proposed action may affect any listed species and/or their critical habitat, the agency generally must engage in formal consultation with FWS.[53] At the end of the formal consultation, FWS must provide the agency with a "biological opinion" detailing how the proposed action will affect the threatened or endangered species and/or critical habitats.[54]

Migratory Bird Treaty Act

The Migratory Bird Treaty Act (MBTA) implements the obligations of the U.S. under several international treaties and conventions for the protection of migratory birds.[55] The MBTA mandates that proposed projects must avoid the take of migratory birds entirely and must minimize the loss, destruction, and degradation of migratory bird habitat.[56] The vast majority of U.S. native birds are protected under the MBTA, even those that do not participate in international migrations.[57] Under the MBTA, "[n]o person may take, possess, import, export, transport, sell, purchase, barter, or offer for sale, purchase, or barter, any migratory bird, or the parts, nests, or eggs of such bird except as may be permitted under the terms of a valid permit."[58]

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[48] *Id.* at 185.
[49] *Id.*
[50] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).
[51] 16 U.S.C. § 1536(c)(1).
[52] *Id.*
[53] 50 C.F.R. § 402.14.
[54] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[55] 16 U.S.C. § 701.
[56] *Id.* § 701–712.
[57] *See* 50 C.F.R. § 10.13.
[58] *Id.* § 21.11.

Administrative Procedure Act

The Administrative Procedures Act (APA) sets forth standards that govern judicial review of decisions made by federal agencies.[59] The APA provides that "[a] person suffering legal wrong because of agency action, or adversely affected or aggrieved within the meaning of a relevant statute, is entitled to judicial review thereof."[60] Under the APA, an agency decision is unlawful if it is arbitrary or capricious or fails to follow procedures required by law.[61] Agencies must "articulate a rational connection between the facts found and the choice made."[62] An agency's decision is unlawful if it, *inter alia*, "entirely fail[s] to consider an important aspect of the problem," "fail[s] to offer any explanation" about an important aspect of the problem, or "offer[s] an explanation for its decision that runs counter to the evidence before the agency."[63]

II. Overview

Corn is the most widely grown crop in the US, planted on over 90 million acres (dEA at 12, pPPRA at 3), and used mainly for animal feed, biofuels, and processed foods. Most of this corn is grown using intensive cultivation methods that have significant health and environmental impacts, including water and air pollution from fertilizers, pesticides, and soil erosion (CFS 2014a); loss of biodiversity when natural areas are converted to corn (Brooke et al. 2009, Wright and Wimberly 2013), and from aggressive pest control (CFS 2015b); emission of greenhouse gases from methods used in growing, transporting, and processing the crop; and reduced diversity of different plant foods in human and domesticated animal diets from over-reliance on corn-derived products.

Increasingly, corn planted in the US has been genetically engineered with traits that make it easier to grow (in the short run) in intensive monocultures. Corn varieties are genetically engineered with insecticidal proteins (Cry proteins) from strains of the soil bacterium *Bacillus thurigneinsis* (Bt) that target either moth and butterfly pests (Lepidoptera, such as European corn borer) or beetle pests (Coleoptera, such as corn rootworm). According to USDA, 76% of total US corn acres in 2013 were planted in varieties that contained at least one Bt trait (dEA at 19), reducing the field application of certain insecticides. However, if one accounts for the skyrocketing use of neonicotinoid insecticidal seed treatments (Douglas and Tooker 2015), the amount of insecticidal Cry proteins in Bt corn tissues (Benbrook 2012), and recent increases in use of insecticidal sprays to combat insect pests resistant to Cry proteins, overall insecticide use has likely increased substantially over the period of Bt corn adoption (CFS 2013a at 4-5).

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[59] 5 U.S.C. § 706.
[60] *Id.* § 702.
[61] *Id.* § 706(2)(A), (D).
[62] *Motor Vehicle Mfrs. Ass'n of U.S., Inc. v. State Farm Mutual Auto. Ins. Co.*, 463 U.S. 29, 43, 59 (1983).
[63] *Id.* at 43, 56.

In addition to Bt traits, most corn in the US also harbors one or more herbicide resistance (HR) traits, most often glyphosate resistance (GR), that allow growers to spray the paired herbicide over the top of the corn crop, killing interspersed weeds but not the corn plants. This has generally resulted in an increase in the number of herbicide applications, and in more herbicide used per acre, over a longer time frame during the growing season (Benbrook 2012, CFS 2013a at 7 – 13, CFS 2015b at 26 – 36).

MON 87411 corn has been developed by Monsanto in response to problems created by use of Monsanto's earlier GE corn varieties. In particular, corn rootworm in many regions have developed resistance to and are no longer effectively controlled by the Cry protein targeting rootworm in Monsanto's Bt corn (Cry3Bb1) (EPA 2013; Gassmann et al. 2014; CFS 2013b). Subsequently, CRW cross-resistant to both Cry3Bb1 and another Cry protein in other Bt corn varieties, mCry3A, has also been discovered (Gassman et al. 2014). These resistant corn rootworm have evolved especially in fields planted continuously (several years in a row) to Monsanto's Bt corn varieties.. Continuous corn is a practice that has increased in recent years (Plourde et al. 2013, CFS 2014a) partly in response to biofuels incentives, facilitated by Bt varieties (Fausti et al. 2015 at 44), and promoted by Monsanto (CFS 2013a at 31 – 32, CFS 2013b at 23). In MON 87411 corn, Monsanto has introduced a novel mechanism for protecting corn against CRW – a double-stranded RNA (dsRNA) made in corn that silences the CWR DvSnf7 gene after the insects eat the corn resulting in CRW death (Bolognesi et al. 2012) – described in detail below.

MON 87411 corn has also been genetically engineered with the rootworm-active Cry3Bb1 protein discussed above, to which a growing number of WCR (western corn rootworm) populations have already developed resistance (CFS 2013b, 2015a). Monsanto and APHIS hope that the DvSnf7 gene silencing mechanism will forestall more populations from evolving resistance to Cry proteins (Petition at 30, dEA at 93). In addition, Monsanto plans to breed (stack) other Cry proteins into the commercial lines (Petition at 5), again hoping that multiple modes of action will provide more durable resistance against rapidly evolving CWR.

Similarly, MON 87411 corn is engineered with a previously commercialized glyphosate resistance trait, cp4 epsps from *Agrobacterium* sp., even though many weeds of corn are now resistant to glyphosate (ISHRW 2015). Monsanto plans to stack GR with other HR traits, and will likely market pre-mixes of several herbicides that match those traits, resulting in an escalation in numbers and amounts of herbicides used on corn.

CFS has commented in detail on previous USDA and EPA actions related to Bt and HR crops, including particular GE corn events (CFS 2013a – comments to APHIS on deregulation of Pioneer 4414 Maize), impacts of Cry-protein-based CRW resistance (CFS 2013b – comments to EPA FIFRA Scientific Advisory Panel on CRW resistance monitoring for Bt corn, CFS 2015a – comments on EPA CRW resistance management plan), and environmental costs of increasing corn monocultures supported by Bt corn (CFS 2014a – comments to EPA on Renewable Fuel Standards). These comments are relevant to potential impacts and deficiencies in APHIS's assessment of MON 87411 deregulation, so are submitted to this docket, and specific pages will be referred to in these comments where appropriate.

CFS supports organic and other forms of sustainable agriculture that do not rely on genetically engineering crops to express insecticidal proteins or RNAs, or to facilitate intensive use of herbicides for weed control by making the crop herbicide-resistant. Insect pests and weeds can be managed by various methods, such as complex crop rotations and cover cropping, that provide multiple benefits for health and the environment (Plourde et al. 2013 at 50), without sacrificing agricultural productivity in the long run (Liebman et al. 2008). Therefore, risks associated with deregulation of MON 87411 corn – a product meant to prolong an inherently unsustainable system of corn production – should be weighed against the beneficial effects of sustainable systems that are less likely to be employed with deregulation of MON 87411..

In particular, APHIS must assess potential impacts of engineering plants to suppress genes in insects via gene silencing – host-induced gene silencing (HIGS). The DvSnf7 suppression cassette in MON 87411 corn is a novel trait. This is the first genetically engineered crop designed to produce an RNA that silences a gene in an insect eating the plant tissues. In fact, to our knowledge, no examples have been reported from nature where a plant itself uses RNAs via the RNA interference (RNAi) pathway to silence genes in insect pests, making this a completely novel defense mechanism. Additionally, the entire scientific field of gene silencing via RNAs is still in its infancy, with much to be learned, particularly about how these RNAs function between species and in the environment, including the human environment. As we describe below, there simply is not enough known about impacts of HIGS for APHIS to make a valid assessment.

III. Impacts of the DvSnf7 suppression cassette in MON 87411 corn on human beings and non-target species

APHIS must assess the unintended consequences on human beings and non-target species of expression of RNA designed to silence a gene in corn rootworm. RNAs involved in gene silencing are known to sometimes affect other genes besides the target gene, both through sequence-specific and non-sequence-specific mechanisms, thus making it possible that any species in contact with the genetically engineered RNA could be harmed by off-target effects, including human beings who consume corn products made from MON 87411 corn. Besides human beings, non-target organisms that could be affected include, but are not limited to, wildlife such as migratory birds and butterflies, threatened and endangered species, and species beneficial to agriculture, such as pollinators, mycorrhizal fungi, nitrogen-fixing bacteria, and predators of plant pests.

Genetic engineering that uses RNAi is a new technology based on complex cellular processes that are still being elucidated

The RNAi technology that was used to produce MON 87411 corn is based on an entirely new approach to pest control that can be applied to many pests (Scott 2013; Palli 2014). This new technology is emerging from still-evolving science, exploiting ancient natural pathways that arose early in the evolution of eukaryotes (Cerutti and Casas-Mollano 2006).

The natural RNAi silencing pathways, which are found in most plants, animals and other eukaryotic organisms, help defend cells against invading viruses or transposons by degrading messenger RNA or modifying chromatin (Cerutti and Casas-Mollano 2006). In addition to defense against genetic invaders, the silencing pathways are now also understood to be involved in the normal orchestration of gene expression (Ariel et al. 2015). In fact, new roles for RNAs are being reported regularly, and functions of some types of common RNA are still unknown:

> Classic central dogma indicates a flow of genetic information from DNA to RNA to protein. RNA molecules are the only messengers that pass information from DNA to protein, which ultimately decides the cellular function and phenotype. With the discovery of non-coding RNAs in the past decades, the classic central dogma has been greatly extended to encompass the developing roles of RNAs. A non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into protein. Transcriptomic analyses by whole genome tiling arrays or transcriptome sequencing have revealed that 70% - 90% of the mammalian genome is transcriptionally active, but only 1% - 2% code for proteins, suggesting that a large proportion of mammalian RNAs are ncRNAs [1-3]. In the model plant Arabidopsis (*Arabidopsis thaliana*), less than 50% of its genome is capable of coding proteins [4]. In addition to the structural ncRNA such as transfer RNAs, ribosomal RNAs, small nuclear RNAs and small nucleolar RNAs, some of the ncRNAs are believed to play regulatory roles in eukaryotes. Based on the length, the regulatory ncRNAs can be further divided into small ncRNAs (sncRNA, shorter than 200 nt) and long ncRNA (lncRNA, longer than 200 nt) [2,5,6]. Small regulatory RNAs [sRNAs], such as micro RNAs [miRNAs] and small interfering RNAs [siRNAs], have been extensively studied and are well-known for their important roles in post-transcriptional and transcriptional regulation. However, the regulatory function of lncRNA, which takes 80% of the ncRNAs, largely remains unknown. (Zhang et al. 2013 at 1038)

Of these naturally occurring regulatory non-coding RNA molecules, the best studied for their roles in specifying the messenger RNAs to be degraded are miRNA and siRNA (Buchon and Vaury 2006). SiRNAs are processed within the cells from longer pieces of RNA derived from organisms such as invading viruses. MiRNAs are generated by the organism's own DNA and regulate normal development and physiology, by they also they also "play a role in the genesis of obesity, diabetes, neurodegenerative diseases and cancer" (Wagner et al. 2015).

Much remains to be learned about these complex systems for regulating gene expression, including the role of RNAi in human disease. For example, according to a review in Nature Reviews Genetics, "[t]he relevance of the non-coding genome to human disease has mainly been studied in the context of the widespread disruption of microRNA (miRNA) expression and function that is seen in human cancer. However, we are only beginning to understand the nature and the extent of the involvement of non-coding RNAs (ncRNAs) in disease….Along with microRNAs, dysregulation of…ncRNAs is being found to have relevance not only to tumorigenesis, but also to neurological, cardiovascular, developmental and other diseases." (Esteller 2011, emphasis added).

Another recent area of active research on RNAi is the role of gene silencing between rather than within organisms, where exogenous or "environmental" RNA is taken up and impacts gene expression:

> The idea that small RNA molecules are transferred between organisms may once have seemed extraordinary, but it is now gaining wide acceptance (Knip et al., 2014). This is due to the increasing number of instances where RNA is known to be taken up by eukaryotic cells and thereby affect gene expression (Sarkies & Miska, 2014). (Spanu 2015 at 4)

Even as scientists are working out the basic details of how trans-species RNAi works, genetic engineers are exploiting it, as described in a recent review:

> This review focuses on the mobility of small RNA (sRNA) molecules from the perspective of transkingdom gene silencing. Mobility of sRNA molecules within organisms is a well-known phenomenon, facilitating gene silencing between cells and tissues. sRNA signals are also transmitted between organisms of the same species and of different species. Remarkably, in recent years many examples of RNA-signal exchange have been described to occur between organisms of different kingdoms. These examples are predominantly found in interactions between hosts and their pathogens, parasites, and symbionts. However, they may only represent the tip of the iceberg, since the emerging picture suggests that organisms in biological niches commonly exchange RNA-silencing signals. In this case, we need to take this into account fully to understand how a given biological equilibrium is obtained. Despite many observations of trans-kingdom RNA signal transfer, several mechanistic aspects of these signals remain unknown. Such RNA signal transfer is already being exploited for practical purposes, though. Pathogen genes can be silenced by plant-produced sRNAs designed to affect these genes. This is also known as Host-Induced Genes Silencing (HIGS), and it has the potential to become an important disease-control method in the future. (Knip et al. 2014 at 1, emphasis added)

The still-evolving nature of the understanding of trans-species RNAi is underscored by a recent publication from Monsanto scientists, investigating the role of environmental RNAi and HIGS in nature. The report showed, surprisingly, that some insects that feed on plants accumulate a substantial amount of plant dsRNA in their tissues. The puzzling finding suggests dsRNA taken up from the environment may play hitherto unknown role in insects, and the scientists recommended more research to better understand the ecological roles of plant-derived ncRNA in insects (Ivashuta et al. 2015).

Based on the state of the science, deregulation of MON 87411 corn is premature; at a minimum, APHIS must assess the impacts associated with this novel technology in an EIS.

The inescapable conclusion from surveying the literature on RNAi, particularly for pest control, is that it is much too early to apply HIGS technology in agriculture. Very basic research on how it works, and especially on its role in the environment and between species, is being carried out even as APHIS is proposing to deregulate the first HIGS application in agriculture, in the nation's most widely planted crop. For example, Monsanto's study, above, of how natural host

plant ncRNA might affect gene expression in insects (Ivashuta et al. 2015) is the first to directly address this important question required for assessing risks of manipulating the system with genetic engineering, and more research by independent scientists will be required before APHIS can have the confidence to determine the environmental impacts of MON 87411 corn.

APHIS states that genetically engineering crops to silence genes has been done in the past without adverse impacts. Although genetic engineers have used RNAi to silence genes in various crops in the past (until recently doing so without knowing exactly how it worked; e.g. Sanders and Hiatt 2005), so far these applications have involved silencing of virus genes within plants (GE papaya, summer squash, plum), or genes of the plant itself (GE potato, apple, altered oil soybean). Also, to date few of these "silenced" crops have been commercialized, and those that have been are grown on small acreages with no post-market analyses of off-target effects of the silencing mechanism or other careful studies of adverse effects now that some of the possible unintended consequences of RNAi-engineering are becoming known. APHIS cannot base claims of "no impact" for MON 87411 on these previous examples of gene silencing in GE crops.

A. Risks to humans of the genetically engineered RNAi pest control mechanism in MON 87411 corn are not adequately assessed by APHIS

Genetic engineers employing RNAi are exploiting complex processes used by most eukaryotic organisms, including humans, but not well understood. As noted above, dysfunction in these processes is associated with many diseases, which has spurred enormous interest in using RNAi to develop drugs. While the involvement of miRNA and other noncoding RNAs in the genesis of disease does not necessarily mean RNAi interventions will be harmful, it does counsel against rapid approvals in the face of large gaps in understanding.

Scientists have already identified important mechanisms leading to adverse impacts from RNAi, including off-target gene silencing, immune stimulation, and saturation of the RNAi machinery. (Lundgren and Duan 2013). As scientists gain a fuller understanding of the role of RNAi, the list may grow.

Currently, the most important health hazards associated with food appear to be 1) off-target silencing effects and 2) the production of bioactive small RNA molecules that might cross gastrointestinal (GI) barriers and modulate the expression of human genes, effects that are specific to RNAi processes.

Off-target silencing effects

Off target effects of gene silencing occurs when introduced dsRNAs direct the silencing machinery to messenger RNAs other than the target messenger RNAs, and inadvertently turn those genes down or off. Off-target effects can occur in two circumstances—when the target gene is a member of a cluster of genes derived from a single progenitor gene (a "gene family") and when the target shares gene sequences with unrelated genes. In either case, gene silencing can inadvertently affect the expression of genes, by increasing or decreasing the production of messenger RNA. It should be noted that down regulating gene expression, in some

circumstances, can lead to *increased* production of proteins or metabolites, as for example where the protein represses the activity of other genes.

Off-target effects of RNAi silencing are common – so common in fact that they constitute major obstacles to the use of gene silencing for human therapy (Haussecker and Kay 2015), the production of RNAi pesticides (Palli 2014)), and agronomic improvement of crops (Saurabh et al. 2014).

The off-target effects that interfere with therapeutic and other applications of RNAi are often obviously damaging and therefore relatively easily observed. Other similarly damaging effects may be subtle and pass unnoticed. Detection of such effects requires new modes of risk assessment, including creative application of genomics and other sophisticated techniques (Heinemann et al. 2013; Casacuberta et al. 2015).

Food consumption risks

For the RNAi trait in MON 87411 to work as a pesticide, dsRNA must survive ingestion by the rootworm pest and down regulate one or several of its gene. It is therefore reasonable to ask whether plant dsRNAs ingested by humans in plant foods could survive digestion and up or down regulate human genes.

A seminal, and controversial, article by Chinese scientists in 2012 suggests this may occur (Zhang et al. 2012). The paper reported that a bioactive small miRNA from rice was abundant in the sera and tissues of Chinese people, suggesting that small RNAs from plants had survived digestion and crossed GI barriers.

Further experiments suggested the plant miRNAs could regulate mammalian genes. In this case experiments in mice showed that plant miRNAs degraded messenger RNAs for the receptor for low density lipoprotein (LDL or "bad" cholesterol), thereby impeding the removal of the LDL from the mouse liver (Zhang et al. 2012).

The observation that plant miRNAs crossed human GI barriers was startling and generated vibrant discussion in two areas. First, it suggested that plant RNAs might routinely regulate human genes and as such may constitute a new kind of nutrient (Hirschi 2012, Wagner et al. 2015). Second, the observations suggested a mechanism by which the consumption of RNAi crops could be hazardous, i.e. ingested dsRNAs might cross the GI barrier and regulate genes by destroying mRNA dictated by the sequences of the synthetic or induced dsRNAs. Both ideas are plausible because of small bioactive RNAs have known roles as gene regulators.

The draft EA fails to demonstrate that the health risks of MON 87411 are not significant

The draft EA dismisses the possibility of adverse health impacts with the sweeping conclusion that "available information indicates that there are no adverse health effects of DvSnf7 in food products derived from MON 87411 corn" (dEA at 82). The conclusion is based on a flawed analysis as detailed below:

The draft EA confuses the lack of toxicity effects with discussions of RNAi -specific harms

The draft EA presents assertions that nucleic acids have a history of safe use as evidence that RNAi crops are safe (Petrick et al. 2013). But conclusions about the safety of bulk nucleic acids, for example that they are unlikely to be allergens or toxins, are irrelevant to an analysis of GE RNAi constructs designed to produce dsRNA that regulate the expression of a specific gene.

It is important to differentiate RNA-specific impacts like off-target effects from general concerns about toxicity. In the past, regulators have considered the possibility that bulk nucleic acids might be toxic or allergenic and dismissed it and as a result declared nucleic acids GRAS or generally regarded as safe (US-FDA 1992c as cited in dEA at 43). But these analyses were done without any awareness of, or reference to, the mechanisms of gene silencing, and are largely irrelevant to the analysis of the risks of new RNAi techniques.

Similarly, references to US Food and Drug Administration's (FDA) analysis of the FlavrSavr™ tomato 1992 or the US Environmental Protection Agency's (EPA) assessments of an engineered potato expressing the gene for potato leaf roll virus replicase in 1998 (US-EPA 2000a referenced in dEA at 165) add little to the analysis. Although some of these products were retrospectively understood to involve RNAi pathways (e.g. Sanders and Hiatt 2005), the referenced analyses were done prior to the emergence of science revealing the complicated roles dsRNAs play in cells. The failure of regulators to observe hazards in those products cannot be taken as evidence that no hazards occurred. Only the most egregious impacts would have been noticed when scientists had no reason to look for them.

The draft EA does not discuss all potentially adverse impacts of RNAi, including routes of exposure beyond ingestion, off-target gene expression effects, and saturation of the RNAi machinery

The draft EA analyzed only one kind of potential harm, the ingestion of bioactive RNA molecules. It ignores the possibility that off-target effects may have occurred from expression of the DvSnf7 suppression cassette in MON 87411 corn. There is no reason to believe that off-target effects, so common in attempts to use RNAi silencing, have not occurred in MON 87411 corn. Yet APHIS does not even mention, much less analyze, the possibility in the draft EA. APHIS should do such an analysis and in addition discuss the saturation of the RNAi machinery as a potential risk.

The draft EA presents evidence showing that ingestion of dsRNA is unlikely, but fails to include new studies suggesting that ingestion may occur.

The draft EA focused its health analysis on the scenario raised by the Zhang et al. (2012) paper – that plant dsRNA consumed by humans might have crossed the human GI barrier and affected the regulation of human genes.

The analysis correctly notes that several attempts to replicate the Zhang et al. experiments have failed, leading scientists to doubt that humans possess a mechanism by which plant RNAs

routinely modulate the expression of mammalian genes (Witwer and Hirschi 2014, Snow et al. 2013). But the EA fails to discuss two recent studies that support the idea that dsRNA can cross GI barriers. One, published in the Journal of Nutrition showed that miRNAs in cow's milk survived digestion and could alter gene expression in humans. The authors conclude that "miRNAs in milk are bioactive food compounds that regulate human genes" (Baier et al. 2014).

Another study (Lukasik and Zielenkiewicz 2014) reports that plant miRNAs found in exosomes (small vesicles that can protect RNA molecules) are abundant in both human and pig breast milk, supporting the idea that plant miRNAs can cross human GI barriers and are common constituents in breast milk.

Oral administration to mice of tumor suppressor miRNAs designed to mimic plant miRNAs was recently reported to be effective at reducing the tumor burden in intestines of these colon cancer-prone mice (Mlotshwa et al. 2015).

These papers suggest that small bioactive RNA molecules have roles and capabilities in humans that we do not yet understand and more research is needed before the possibility of plant dsRNAs crossing GI barriers can be dismissed (reviewed in Yang et al. 2015).

Even if dsRNAs do not *routinely* cross GI barriers, they may do so in special circumstances, like illness. As noted by Stephen Chan, MD, Ph.D. in comments to EPA on MON 87411 corn, "…theoretically, gastrointestinal disease, genetic conditions, or ingested substances could change gut permeability for diet-derived miRNAs (and other RNAi molecules), and such uptake could potentially, have regulatory consequences." (Chan 2014)

The draft EA, APHIS quotes selectively from a Scientific Advisory Panel (SAP) report on RNAi pesticides, ignoring material that supports concern about adverse effects

In 2013, the EPA grappled with the issue of the risks of engineering RNAi for pest control by preparing a white paper (EPA 2013) on the issue and seeking the advice of a Scientific Advisory Panel (SAP) on its work. It undertook the process, called a problem formulation, because it anticipated pesticidal products based on the RNAi process coming to EPA for approval under the Federal Fungicide, Insecticide and Rodenticide Act (FIFRA) and wanted to be in a position to prepare scientifically robust risk assessments. The SAP's report on the issue (EPA 2014) and the white paper to which it responds (EPA 2013) both contain richly detailed scientific background and analysis that should have been considered accurately in the draft EA.

Instead, in the draft EA APHIS quotes selectively from EPA's SAP report, ignoring its cautionary flags, acknowledgements of uncertainty and repeated calls for more research. APHIS also ignores many of the report's carefully written conclusions.

Among the important specific points made by the SAP panel but ignored by APHIS in the draft EA are the following:

1) The draft EA cited the SAP panel's conclusion that "ingested dsRNA is extensively degraded in the mammalian digestive system" (EPA 2014 at 81), but failed to discuss the possibility that

small RNAs can be protected from degradation by packaging. The SAP report noted that miRNAs resistant to low pH and RNases have been reported in milk and said that "[t]he extent to which dsRNA PIP products could be similarly protected within "plant specific packaging" is lacking and should be evaluated (EPA 2014 at 31). This recommendation has even greater relevancy in light of the paper by Lukasik and Zielenkiewicz (2014), discussed above, showing that plant miRNAs are packaged in exosomes in human and bovine milk.

2) The draft EA ignored the SAP report's discussion of multiple routes of ingestion. Although oral ingestion is the most likely route of exposure, it is not the only route. According to the SAP panel, "The question of the ability of different structural forms of dsRNAs to survive degradation in the gut should also be addressed for dermal and inhalation routes of exposure." (EPA 2014 at 17)

3) APHIS ignored the report's identification of data gaps. The report recommended that "additional data on dsRNA PIP abundance and tissue distribution is needed," and that factors that affect absorption and effects of dietary RNA should be investigated further. The reason the report recommended experimental testing of the mammalian blood and exposed tissues was telling. It was "to ensure that the siRNAs processed from the PIP dsRNAs are not present, since these could have off-target effects after human consumption." (EPA 2014 SAP Report at 14, emphasis added)

4) The SAP report also recommended that "[t]he stability of dsRNA should be tested in individuals that manifest specific diseases (e.g., Crohn's, colitis, irritable bowel syndrome, etc.), the immune compromised, elderly, as well as children. These individuals may have compromised digestion or increased sensitivity to dsRNA exposure." (EPA 2014 at 17)

The analyses of RNAi risks by EPA, FDA, NRC and EC do not buttress APHIS's dismissive analysis of RNAi risks in the draft EA

In the draft EA, APHIS mentions the actions of other agencies and groups addressing RNAi risks issues, perhaps assuming they bolster the draft EA's analysis. For the reasons discussed below, they do not.

FDA's consultation does not mention specific risks of RNAi

In 2014, the FDA conducted a voluntary consultation safety and nutritional issues associated with MON 87411 corn and concluded that it did "not identify any safety or regulatory issues… that would require further evaluation at this time." (FDA 2014b). But FDA did not even mention the possible health impacts associated with RNAi, much less assess them. Most of its analysis focused on compositional analyses of standard nutrients. As such, the FDA consultation does little to bolster APHIS's analysis of the novel risks of RNAi.

FDA's voluntary consultations on three other foods produced through genetically engineered RNAi, non-browning apples (FDA 2015a) and two types of potatoes with altered properties (FDA 2014a, 2015b) also fail to mention RNAi-specific risks. It is not evident in the consultations that FDA is aware of the possible health impacts of the emerging RNAi

technology. If it is not, this may be a result of FDA's dependence on product developers to provide the agency information for risk assessments.

EPA's analysis of the MON 87411 corn is still underway and the scope of its analysis is unknown

The DvSnf7 suppression cassette of MON 87411 corn meets the definition of a plant-incorporated protectant (PIP)—a pesticidal substance produced by a plant and the genetic material necessary for the plant to produce the substance (US-EPA 2014e as cited in dEA at 168). EPA is charged with evaluating the human health risks associated with direct contact and dietary exposure routes of PIPs before registering them for commercial use.

EPA granted MON 87411 corn an experimental use permit in 2013 (EPA 2013), but the Agency has not yet issued a PIP registration for the variety and so the content of the analysis is unknown, although it can be hoped EPA will take seriously the recommendations of its SAP report (EPA 2014). Although an EPA PIP analysis would not relieve USDA of its obligations under NEPA, it could inform the USDA analysis. Here it cannot do even that because it has yet to be published.

The National Research Council (NRC) and European Commission (EC) studies cited by USDA were done before genetically engineered RNAi technology was intentionally applied to crops and food

The draft EA cites a 2004 National Research Council (NRC) report's conclusion that no adverse human health effects attributed to genetic engineering have been documented (NRC 2004 as cited in dEA), and a European Union-funded research commission (EC) that concluded foods derived from GE crops were as safe as those derived from conventional non-GE counterparts European (European Commission 2010). Both of the studies were completed before RNAi technology was intentionally applied to widely grown crops and food, and it is unlikely that RNAi-caused adverse effects would have been noticed in these crops even if they were present, as discussed above.

APHIS failed, however, to cite the recent European workshop devoted to the assessment of the specific risks of RNAi (EFSA 2014, Ramon et al. 2014) that highlighted some of the challenges of analyzing these crops.

In sum, APHIS failed to adequately assess the possibility of human health risks from the MON 87411 corn in the draft EA

APHIS did not provide important background in the draft EA on the issues with RNAi technology as a context for the risk discussion, as was done by the EPA through its SAP process or by the European Food Safety Agency through its workshop. It focused narrowly on the possible uptake of dsRNAs by humans, ignoring the possibility of other adverse impacts, especially off-target effects. On the dsRNA uptake issue itself, APHIS provided an incomplete analysis that omitted new evidence suggesting that dsRNA does cross the human GI barrier.

APHIS also failed to acknowledge how incomplete the current understanding of the RNAi process and roles of non-coding RNA is. Against that background, it is important to identify and fill data gaps before taking action. In the draft EA, APHIS failed to mention important data gaps, particularly on multiple routes of exposure and impacts on individuals with intestinal diseases.

APHIS's analysis of MON 87411 corn in the draft EA does not support its unqualified conclusion that "available information indicates that there are no adverse health effects on human health associated with consumption of DvSnf7 RNA in food products derived from MON 87411 Maize" (dEA at 82).

B. Risks to the environment of the genetically engineered RNAi pest control mechanism in MON 87411 corn are not adequately assessed by APHIS

APHIS has not gathered and evaluated all relevant information on environmental impacts of deregulating MON 87411 corn, and has not made a convincing case that impacts are insignificant. As with health impacts, the science on how genetically engineered host-induced gene silencing (HIGS) targeted at the DvSnf7 gene in pest CRW species will affect non-target organisms is simply not well enough established to make an informed assessment.

APHIS goes into the most detail on risks to non-target organisms in the "Cumulative Impacts" section (e.g. dEA at 102), whereas off-target and other effects discussed as cumulative impacts are actually straightforward impacts of deregulation. Even in this more detailed analysis, APHIS depends almost entirely on Monsanto's own, very recently published studies that are preliminary and too limited in scope to make the sweeping conclusion that there are unlikely to be impacts on non-target organisms (e.g. dEA at 76-78).

Studies and reviews from independent scientists are full of statements expressing how much is still unknown about potential impacts of RNAi-based pest control on the environment and the resulting challenges for regulation, but APHIS does not gather and evaluate these relevant sources of information. For example, the following quotes from the scientific literature show significant uncertainty about the ability to adequately determine environmental impacts:

- The unintended effects caused by dsRNA include off-target silencing of genes in the target as well as in non-target insects, silencing of target gene homologs in non-target organisms, stimulation of immune response and saturation of RNAi machinery. All these effects could influence performance of non-target organisms including, parasites, predators and pollinators resulting in adverse effects on crop performance. Therefore, the persistence of dsRNA in the field as well as the effect of dsRNA on organisms present in the pest and crop ecosystem need to be investigated thoroughly before using dsRNA in the field. (Palli 2014 at 4)

- … the established framework used to assess environment risks of GM plants is not likely to work well for RNAi-based technologies that express dsRNA *in planta* or that use exogenously applied dsRNA (US EPA 2014). Because of the potential mode of action in non-target species and insufficient understanding of the uptake mechanisms in a wide

range of species from a variety of taxa, more information is needed to reduce the uncertainties in ecological risk assessments. (Zotti and Smagghe 2015 at 13)

- Because of the large diversity (phylogenetic and molecular) of non-target organisms associated with agroecosystems that may be exposed, it is difficult with the current state of knowledge to draw conclusions about the potential risks, some of which are not unique to RNAi-based technologies. (Zotti and Smagghe 2015 at 14)

- In spite of the value of maize production and the economic costs associated with insect pests in this crop, the arthropod community of maize arguably remains poorly understood. (Lundgren et al. 2015 at 447)

- Non-target and off-target effects. Although the binding of siRNA is considered to be highly specific, nonspecific binding also often occurs. The binding of siRNA elsewhere within the target genome is not a problem, but concerns increase if offtarget binding occurs in non-target organisms. Most of these effects would most likely be sublethal with delayed consequences rather than lethal, which can be difficult to predict. Because of the lack of knowledge on the persistence of dsRNA in the field (crop stubble and soil), the RNAi spectrum, the physical and physiological exposure of non-target organisms, and the trophic movement of siRNA, the weight of the risks posed by RNAi-based insecticides versus the benefits for the protection of crops are difficult to determine. (Zotti and Smagghe 2015 at 16)

- Although some insects, such as coleopterans, are very sensitive, the efficiency is much lower for other insects, such as hemipterans and lepidopterans. The knockdown of genes mediated by dsRNA was demonstrated in many insect species, but several important questions remain to be answered, particularly with respect to the specificity and the efficiency. These remaining gaps in the RNAi puzzle make our predictions on the fitness of exposed non-target organisms difficult. (Zotti and Smagghe 2015 at 4)

- The amplification of the RNAi signal by RdRPs [RNA-dependent RNA polymerases] was characterized in worms, fungi and plants as part of the RNAi machinery; however, whether similar mechanisms occur in other organisms, such as insects or mammals, has not been determined. Indeed, although RNAi acts according to a general conserved strategy, some components can radically change depending on the taxonomic kingdom or group. (Zotti and Smagghe 2015 at 6)

- Since there are no internationally agreed and validated procedures for excluding either exposure routes or potential adverse effects of particular dsRNA molecules that may be produced as a result of genetic engineering, whether intended or otherwise, for the foreseeable future all GMOs intended for release (as a field trial or to unregulated status) or food should be submitted to a battery of testing for unknown dsRNAs and unintended effects of dsRNAs. The testing should provide empirical evidence capable of delivering confidence for any claims of the absence of any unintended dsRNAs or of an unintended effects of any dsRNAs. (Heinemann et al. 2013 at 50)

- For the environmental risk assessment, bioinformatic analyses could guide the selection of non-target species which harbour genes that share a certain level of homology with the gene targeted in the pest and which should be the focus of further assessment. However, bioinformatic data cannot be reliably used as a standalone to predict the presence of RNAi activity at present. More research is needed on the exact rules for small RNA-target matches, to design more efficient algorithms and make more reliable predictions. There is also a necessity to expand knowledge on genomes and their expression, especially in non-model lines and other species. (Ramon et al. 2014 at 1272 – 1273)

APHIS defers to EPA's general authority when summarizing its own conclusion that deregulation of MON 87411 corn poses no environmental risks: "EPA regulates PIPs in IR [insect resistant] corn and herbicides applied to HR corn, and determines whether they, including the RNAi PIP that is the subject of this draft EA, pose an unacceptable risk or impact on non-target organisms" (dEA at 60). However, EPA has not registered the dsRNA in MON 87411 as a PIP, and the SAP report on risk assessment of RNAi-based pest control concluded that there were many serious data gaps.

Although APHIS does occasionally cite EPA SAP's report in the draft EA (e.g. dEA at 102), APHIS ignores the main conclusions of the report that stress how many data gaps there are in every environmental parameter, from soil and water quality to impacts on non-target organisms. Throughout the EPA SAP's report, its wording conveys the high degree of uncertainty in assessing environmental risks of RNAi-based pest control:

> "…additional data are critical" (at 19, re. stability of dsRNA in soil and water);
> "…deficiencies in the testing regime" (at 19, re. fate of dsRNA in soil and water);
> "…additional information is needed" (at 20, re. uptake of dsRNA in organisms);
> "…there is insufficient understanding" (at 20, re. dsRNA uptake);
> "…insufficient data…to comment on the importance of this route in non-target risk assessment" (at 21, re. direct uptake of dsRNA in nontarget organisms);
> "…there is uncertainty in defining the spectrum of insecticidal activity", "…knowledge gaps make it difficult to predict with any certainty whether unintended effects will occur in non-target species" (at 22);
> "…data are needed in several areas" (at 22, re. determining non-target impacts);
> "…uncertainties in the potential modes of action in non-target species, potential for chronic and sublethal effect, and potential unintended consequences in the various life stages of non-target organisms are sufficient justification to question whether the current Agency framework for environmental fate and ecological effects testing is applicable to dsRNA PIPs …" (at 23).

APHIS, on the other hand, ignores these many caveats, calls for additional data, and warnings of potential adverse impacts, in coming to definitive conclusions about potential environmental risks, stating that:

> "[t]here is no evidence that that DvSnf7 dsRNA will persist or function any differently in soil than naturally occurring dsRNA" (dEA at 71);

> "…it is not anticipated that DvSnf7 RNA will persist in water, or impact water use, so no differences between the Preferred Alternative and the No Action Alternative are likely" (dEA at 72);
> "…because of its high specificity, it is highly unlikely DvSnf7 dsRNA will impact individual animals or animal communities in a manner that will result in a plant pest risk, nor will it cause any other substantial impacts. The Agency determined that the high level of sequence specificity attributable to western CRW single nucleotide polymorphism is also highly unlikely to promote the development of resistance in this pest (Bachman et al., 2013b)" (dEA at 76, citing a publication by Monsanto scientists);
> "[b]ased on the best available information, APHIS concludes that there is no difference between impacts associated with the No-Action Alternative and those of the Preferred Alternative [deregulating MON 87411 corn] with regard to biodiversity." (dEA at 78)

In other words, APHIS ignores the substantial uncertainties and data gaps identified by independent scientists in research studies, reviews, and advisory panels, and comes to strong but unsupported conclusions regarding the supposed lack of impacts of deregulating Monsanto's MON 87411 corn based mainly on a few, very recent studies by Monsanto itself that have not yet been corroborated and extended by the scientific community.

APHIS does not adequately assess the likelihood and consequences of CRW resistance to the genetically engineered pest control traits in MON 87411

Because RNAi is a novel means to confer crop protection to an insect pest there is little known about how targeted insects will respond. CRW species have demonstrated a capacity to rapidly evolve resistance to numerous classes of insecticide. Insect resistance to gene silencing by specific RNAs would be, to the best of our knowledge, a new phenomenon.

In addition to the DvSnf7 suppression cassette, MON 87411 corn contains a previously commercialized Bt gene targeted at corn rootworm, the cry3Bb1 gene. However, many populations of corn rootworm in a growing number of states have already developed resistance to the product of this gene, and recently to another Cry protein (mCry3A) (Gassman et al. 2014) that may be stacked in commercial corn varieties of MON 87411 corn. These insects, already resistant to Cry3Bb1 toxin, would be exposed to just one effective mode of action if MON 87411 corn is deregulated, and thus would likely develop resistance to MON 87411 corn's RNAi-based DvSnf7 gene silencing mechanism as well.

Implications of insect resistance to the novel mechanism of gene silencing by specific RNAs, alone or in addition to resistance to Cry proteins, must be fully examined by APHIS in an EIS. If there is insufficient information available to conduct a meaningful assessment of resistance, a decision on the petition must be postponed until such information has been collected and assessed.

Corn rootworm likely to develop resistance to the RNA-interference-based mechanism of MON 87411 corn, but more data needs to be collected to assess the severity of the threat

One important reason that corn rootworm populations have rapidly evolved resistance to Cry proteins is that the corresponding Bt corn varieties produce insufficient levels of Cry toxins to kill a high percentage of rootworm pests – so-called "low-dose" events – fostering evolution of resistance in those that survive. Accordingly, "high-dose" varieties that kill a very high percentage of insect pests is far superior in terms of forestalling resistance. As APHIS concedes, the gene silencing mechanism in MON 87411 is regarded, like Bt corn with Cry proteins, as "low-dose" (dEA at 93: "However, both traits [Cry3Bb1 and DvSnf7 dsRNA] confer similar levels of mortality" and "[a] high dose strategy for insect control is more optimal" for preventing resistance). The "low-dose" nature of the DvSnf7 ds RNA gene-silencing mechanism makes evolution of resistance to it more likely, particularly in rootworm that are already resistant to Cry3Bb1 and/or mCry3A. Although scientists are generally confident that target insects will develop resistance to RNAi-based pest control strategies, they can only speculate about when and how such resistance will occur because no experiments have been done to test various theories.

A variety of mechanisms by which insects such as CRW could become resistant have been proposed by independent scientists, including modification of the basic RNAi "machinery", changes in uptake of exogenous RNA, and tolerance of persistent virus infections:

> Insects such as diamondback moth and CPB [Colorado potato beetle] developed resistance to almost all insecticides introduced for their control. Therefore, there is no reason to believe that dsRNA is immune to resistance development by these and other insects. Mutations to genes coding for proteins involved in dsRNA transport, processing, Risk Complex formation and other processes involved in RNAi pathway as well as mutations to dsRNA target genes are potential mechanisms of resistance development. (Palli 2014 at 5)

> As for every method for insect control, however, the rise of insecticide resistance is always a major issue. It has been argued that resistance against dsRNA or RNA hairpins might be difficult to occur because long dsRNAs can still function effectively, even when multiple mutations have accumulated in the target sequence (Swevers and Smagghe, 2012). However, the RNAi machinery that is dedicated to defense against exogenous dsRNA is considered to be dispensable (Shabalina and Koonin, 2008) and could therefore mutate quickly if maintenance costs are too high and alternative mechanisms evolve against RNA virus infections. Persistent virus infection may appear as a major mechanism that inactivates RNAi (a correlation between persistent virus infection and RNAi deficiency in the soma is observed in natural isolates of *C. elegans*; Félix, 2008; Félix et al., 2011; Nuez and Félix, 2012). Thus, the rise of resistance in insect pest populations that are managed by RNAi-based techniques will give the opportunity to evaluate the importance of the acquisition of persistent viral infections as a resistance mechanism. (Swevers et al. 2013 at 10 – 11)

There is already evidence that natural populations of WCR are variable in their responses to RNAi-based pest control in experimental situations, and thus are likely to become resistant to the mechanism fairly quickly (Chu et al. 2014). If resistance involves changes in how the pest populations respond to RNAi generally, future RNAi-based pest control strategies could be compromised (Chu et al. 2014 at 4). In addition, if CRW populations develop resistance in ways that change their susceptibility to or "load" of viruses or other pathogens (Swevers et al. 2013), their population dynamics could be affected with ripple effects on other pests and pathogens.

APHIS concludes that the likelihood of CRW pests developing resistance to the novel RNAi-based component of MON 97411 corn is reduced by the presence of a CRW-targeted Cry protein. However, APHIS fails to adequately consider the full extent of current and future resistance to such Cry proteins in some populations of CRW, and how that increases the likelihood of resistance to the RNAi (Gassmann et al. 2014 at 4), as discussed in CFS comments (CFS 2013b, 2015a)

APHIS did not consider mechanisms by which CRW would become resistant the RNAi-based pest control in MON 87411 corn in the draft EA, or possible environmental impacts. APHIS did not consider impacts of CRW resistance to RNAi in the preliminary PPRA, either.

APHIS did not propose alternatives to full deregulation that would possibly mitigate development of resistance in CRW populations to the RNAi-based pest control in MON 87411 corn, such as only allowing it to be grown in areas that do not currently have CRW populations that are resistant to Cry proteins.

IV. APHIS did not evaluate all relevant information when assessing impacts on monarch butterflies of deregulating MON 87411 corn

APHIS failed to adequately assess the impact of approving MON 87411 corn on monarch butterflies, a species whose population has plummeted in a large part due to the use of glyphosate on GR corn and soybeans (CFS 2015b). Glyphosate use has all but eliminated milkweed from corn and soybean fields in the Midwest, and with it a major portion of the breeding habitat for monarchs, whose larvae can only eat milkweed. Continued use of glyphosate on MON 87411 corn and other herbicides that will accompany stacked HR genes, will further degrade monarch butterfly habitat by preventing reestablishment of milkweed, and also reducing populations of nectar plants because of injury from herbicide drift.

Nor did APHIS assess the toxicity of MON 87411 corn to monarchs that may ingest pollen, anther tissues, or other plant parts.

APHIS did not evaluate the relevant literature on risks to monarchs (e.g. Pleasants and Oberhauser 2012) citing only two studies (dEA at 108), where dozens of relevant studies have been published (CFS 2015b).

V. Environmental impacts of Cry protein genes and herbicide resistance traits that are present in MON 87411 corn or will be stacked with MON 87411 corn in commercial varieties are not adequately assessed by APHIS

APHIS hides behind its previous assessments of Bt and HR corn events, and EPA's role in registering previous Cry protein PIPs and herbicides for use on HR crops, claiming that no further assessment is need for MON 87411 for these traits. However, there is new information about health and environmental impacts of herbicides used with HR crops, and new information about Cry protein PIPs that APHIS must consider in the dEA and pPPRA, independently of any such assessments by EPA. For instance, the World Health Organization's International Agency for Research on Cancer (IARC) recently determined that glyphosate is "probably carcinogenic to humans" (Guyton et al. 2015). Given the massive and growing use of glyphosate in U.S. agriculture (USGS 2015), and its frequent detection in the air, rainfall, surface water, food (e.g. bread) and human urine, it may well pose a previously unrecognized risk to human health that APHIS did not assess (CFS 2015c).

In addition, APHIS continues to commit factual errors, misinterpret data and rely on poor-quality studies with respect to pesticide use with GE crops, as CFS has commented before (CFS 2013a at 3 – 13, e.g. explaining factual errors in APHIS's pesticide use assessment, repeated in this draft EA, and critiquing APHIS's inappropriate reliance on studies by industry contractors, Brooks and Barfoot). APHIS in particular fails to appreciate and assess the many impacts of glyphosate-resistant weeds driven by cultivation of prior GE crops with glyphosate resistance. APHIS selectively cites a 2010 report from the National Research Council (NRC 2010 as cited in dEA) to the effect that herbicide-resistant crops have had a positive impact (dEA at 41), but ignores other passages in the same report which warn that glyphosate-resistant weeds triggered by glyphosate-resistant crop systems have increased the use of herbicides and soil-eroding tillage (NRC 2010 at 2-15: "For controlling problematic weeds, they [farmers] prefer increasing the magnitude and frequency of glyphosate applications, using other herbicides in addition to glyphosate, or increasing their use of tillage."). Moreover, glyphosate-resistant weeds have increased dramatically in extent, with correspondingly more serious adverse impacts on the environment and agriculture, since this report was written (ISHRW 2015), developments that APHIS fails to assess. An EA written in 2015 should not rely on science from 2010. APHIS should update its analysis with regard to both herbicide and insecticide use in US agriculture, making it clear that use of both categories of chemical on GE crops is higher not lower than in the past, and that that further increases in pesticide use are highly likely given increasing pest and weed resistance (CFS 2013a at 12 - 28, 2015a, 2015b).

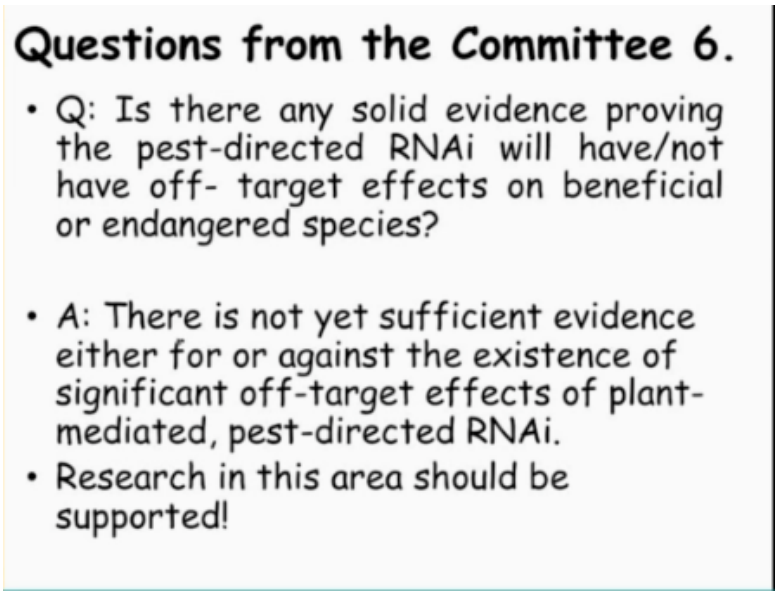
VI. APHIS did not adequately consider impacts of deregulation of MON 87411 corn on species listed as threatened or endangered under the ESA, as well as those protected under the Migratory Bird Treaty Act

Given the uncertainties and data gaps regarding impacts of the RNAi-based pest control in MON 87411 on the environment identified by independent scientists in studies, reviews and panels, as discussed above, APHIS cannot conclude that deregulation of MON 87411 will have no effect

on federally listed threatened and endangered species under the ESA, or migratory birds under the MBTA.

The RNAi-based pest control in MON 87411 may significantly affect threatened and endangered species, but APHIS failed to consider those effects or consult with the expert wildlife agencies regarding these risks, as the ESA requires. The ESA requires APHIS to consult with FWS and/or NMFS to determine "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."[64] If APHIS learns from FWS or NMFS that threatened or endangered species may be present, a biological assessment must be prepared to identify any endangered species or threatened species that are likely to be affected by such action.[65] The initial request for information from FWS and/or NMFS is a predicate to further agency action and cannot be ignored.[66]

In fact, the question of whether listed species or beneficial species could be impacted by pest-directed RNAi was recently put to a presenter during an NRC information-gathering webinar, David Heckel, Professor, Max Planck Institute for Chemical Ecology, who does research in this area. Dr. Heckel concluded that there isn't enough evidence to make a conclusion[67]:



[64] 16 U.S.C. § 1536(c)(1); 50 C.F.R. § 402.12(c) (requiring federal agencies to request information regarding listed species and critical habitat from the Department of the Interior).
[65] 16 U.S.C. § 1536(c)(1).
[66] *Thomas v. Peterson*, 753 F.2d 754, 764 (9th Cir. 1985).
[67] National Research Council, 2015, gathering information for upcoming report: A Science-Based Look at Genetically Engineered Crops. http://nas-sites.org/ge-crops/category/pastevents/, scroll to the webinar on RNAi Technology, advance to 1:29:32 hr)

In addition, APHIS must assess the impacts of all three GE traits incorporated into MON 87411 and other GE traits that are likely to be combined in final commercial varieties on listed species and migratory birds. For example, the short-term increase in effectiveness against CRW insect pests purportedly conferred by the combination of Cry proteins and DvSnf7 dsRNA in MON 87411 corn will facilitate continued expansion of corn at the expense of grasslands and other natural areas, putting listed species and migratory birds at risk. The combination of herbicides that can be used over a greater span of the growing season will facilitate greater use of herbicides on more land area with effects on listed species and migratory birds.

Accordingly, prior to a completion of the deregulation, APHIS must demonstrate that, at the very least, it has consulted with FWS and/or NMFS and taken the first step in considering the impacts of an APHIS deregulation of MON 87411 on threatened or endangered species. However, APHIS failed to take even the first step of consultation.[68] APHIS has already once been previously found to have violated the ESA when it skipped this initial, mandatory step of obtaining information about listed species and critical habitats from FWS and/or NMFS.[69] The court emphasized that regardless of whether there is any evidence that species or habitat may be harmed in any way, "an agency violates the ESA when it fails to follow the procedures mandated by Congress, and an agency will not escape scrutiny based on the fortunate outcome that no listed plant, animal, or habitat was harmed."[70]

Similarly, APHIS fails to analyze the potential impacts of MON 87411 on migratory birds. This constitutes a failure to take the required hard look at impacts to migratory birds and could potentially lead to take under the MBTA.

VII. Conclusion

APHIS must deny Monsanto's petition for nonregulated status of MON 87411 corn based on serious data gaps that preclude a meaningful assessment of health and environmental impacts, and prepare an EIS. APHIS must also consult with FWS on impacts to threatened and endangered species and their critical habitats. Approval of MON 87411 corn based on the current inadequate DEA and PPRA would violate the mandates of NEPA, the PPA, the ESA, the MBTA, and the APA.

[68] *Ctr. for Food Safety v. Johanns*, 451 F. Supp. 2d 1165, 1182 (D. Haw. 2006).
[69] *Id.*
[70] *Id.*

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