CENTER FOR
FOOD SAFETY

Docket No. APHIS-2012-0025
Regulatory Analysis and Development
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Comments to USDA/APHIS on Plant Pest Risk Assessment and Environmental Assessment for Determination of Nonregulated Status of Apples Genetically Engineered to Resist Browning

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Docket No. APHIS-2012-0025

The Center for Food Safety (CFS) hereby submits these comments regarding the Animal and Plant Health Inspection Service (APHIS)'s draft Plant Pest Risk Assessment (PPRA) and draft Environmental Assessment (EA) prepared in regards to the Okanagan Specialty Fruits (OSF) Petition (10-161-01p) for Determination of Non-regulated Status of Arctic ™ Apple Events GD743 and GS784.[1]

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.[2] 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 its 360,000 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.

I. INTRODUCTION

USDA/APHIS is evaluating a petition to deregulate genetically engineered (GE) *Malus x domestica* (apple) events GD743 and GS784 (hereinafter, GE or Arctic apples), engineered to be resistant to browning. OSF petitioned APHIS for a determination of nonregulated status for the GE apples and APHIS has prepared a draft EA for public comment.

These GE apples have been genetically engineered with a transgene that produces specific RNAs to suppress the expression of at least four members of a family of genes coding for polyphenol oxidase (PPO) enzymes. These enzymes are normally made at higher levels

[1] *Okanagan Specialty Fruts Petition (10-161-01p) for Determination of Non-regulated Status of Artic™ Apple Events GD743 and GS784*, 78 Fed. Reg. 67,100 (Nov. 8, 2013).
[2] *See generally* www.centerforfoodsafety.org.

throughout the trees, including in fruit. In fruit, PPOs are responsible for browning when fruits are bruised or cut, so reducing PPO levels results in fruit that does not exhibit browning upon injury, a trait OSF says will be useful to fresh and processed apple industries.

If approved, GD743 and GS784 would be the first GE apples to reach the U.S. commercial market. This proposed action also would utilize new and novel technologies never before commercialized. Despite the unprecedented nature of this proposed action, APHIS inexpiably has not undertaken the legally required rigorous and overarching analysis of the GE apples, or of the foreseeable consequences of its approval. This EA is woefully inadequate under the National Environmental Policy Act (NEPA). It is based on incomplete and inadequate science and analyses, lacks critical data and vital risk assessments, and ignores potential consequences and uncertainties. The EA's scope is unlawfully narrow, thereby ignoring the plainly foreseeable environmental and socioeconomic impacts of introducing GE apples. In addition, the EA's alternatives section is unlawfully narrow and illegally predetermined. In sum, the EA fails to take the "hard look" at environmental impacts required by NEPA.

APHIS must prepare a full Environmental Impact Statement (EIS) in order to comply with NEPA's mandate to prepare an EIS where an agency action may significantly impact the environment. "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."[3] The effects of this proposed action on public health is a live question that was, as demonstrated later in these comments, given a wholly inadequate review in the EA. APHIS must generate an EIS that fully considers the potentially significant public health impacts of this proposed action. Further, this action is indeed highly controversial. A call-out for comments to CFS members resulted in an astonishing 71,123 members taking action to write to APHIS expressing their deep concerns regarding GE apples. These GE apples are highly controversial because so little is known about their impacts on human health and the environment, thus an EIS is called for.

Finally, APHIS must act expeditiously to comply with the mandates of the Endangered Species Act (ESA). The agency's failure to consult, both informally and formally, with U.S. Fish and Wildlife Services, is unlawful. APHIS's claim that this proposed action would have no effects on threatened or endangered species is premised on inadequate data and poorly supported assumptions.

The inadequacy of the agency's data is specifically egregious because GD743 and GS874 present significant, novel issues for APHIS to analyze. For example,

- This is the first GE crop assessed by APHIS to use RNA interference (RNAi) technology to suppress the expression of (i.e., silence) <u>most or all members of a multi-gene family</u> expressed throughout the plant. Thus far, commercialized GE crops using RNAi technology have been designed to silence one or two genes expressed in seeds only (e.g.,

[3] 40 C.F.R. § 1508(b)(2), (4).

high oleic acid soybeans), or a gene from an invading pathogen (e.g., virus resistant plum).

- The functions—the usefulness of the gene products to the trees themselves—of the genes being silenced in GD743 and GS784 trees are not known, although some are thought to be involved in defending plants against pests and pathogens. Other GE crops assessed by APHIS have either had genes of known function added to them (e.g., Cry proteins known to provide protection against certain insect herbivores, or EPSPS enzymes known to provide resistance against the herbicide glyphosate), or have used RNAi technology to silence genes of known function (e.g., viral coat protein genes necessary for the virus's lifecycle).

- The genes being targeted by RNAi technology in GD743 and GS784 have counterpart genes in other species that could be affected by the engineered RNAs. For example, pollinating insects that have PPO genes could be exposed to RNA products of the suppression transgene from GD743 and GS784 trees via ingesting pollen, nectar, or sap. In addition, RNAs from the transgene could also affect gene expression in exposed organisms independently of the specific RNA sequence. APHIS has no guidelines that we are aware of for assessing the unique impacts to other species of the RNAi technology used in GE crops.

- Suppression of PPO genes in GD743 and GS784 causes an organoletptic change that can affect consumer judgments about qualities of the GE apples. Other GE crops were changed in ways that could not be detected directly by human senses.

Given these new considerations, the assessments made by APHIS in response to OSF's petition will set important precedents and must, at a minimum, be rigorously performed and analyzed in an EIS before any decision is made.

CFS has analyzed the EA and PPRA and concluded that APHIS simply does not have enough basic information from OSF or from the scientific literature to be able assess environmental and health impacts of approving GD743 and GS784, 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,[4] 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's review also suffers from a fundamental problem of scope. Time and time again APHIS limits its review to just the apple fruit, when in fact the environmental and agronomic impacts of the genetic engineering, and this first GE apple tree, flow from the entire apple tree. In many ways, APHIS's approach underscores that the agency has not internalized the central

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

purposes of NEPA: to require detailed environmental analysis and to fully inform the public. Indeed, APHIS's highly restrictive approach is reminiscent of the failed effort by another federal agency to limit NEPA's effect in the first years following enactment of the Act.[5]

Further, OSF and APHIS have not provided an adequate or sufficient description of the phenotypes of the GE trees or the non-GE trees from which they were derived (i.e., the recipient trees) in relation to the levels and functions of the enzymes whose expression was altered. Knowing how the engineered trees differ from the non-engineered recipients as a result of the engineering process is a basic requirement for subsequent assessments of impacts,[6] so the missing, baseline, comparative information are critical omissions.

Nor is there a description of where and how much of the transgene products themselves—the novel RNA sequences responsible for lower enzyme levels—are made during development of trees, or the fate of the transgene products in the environment. Particularly given recent concerns about impacts to non-target organisms of double-stranded RNAs involved in RNAi, APHIS needs to know whether and how organisms that interact with apple trees will be exposed to these novel RNAs as an essential first step in determining potential risks.

More specifically, for the non-engineered recipient trees, it is necessary to know the expression patterns of PPO genes, including where in the trees and when during development different PPO genes are active. Since some PPO genes are induced by stress, pests, or pathogens, the characterization of phenotype should identify where inducible PPO gene family members are expressed in the trees. Also, the functions of PPOs from specific genes in different parts of the tree need to be described so that impacts of loss of these functions in GE trees can be assessed.

These same characteristics of PPO expression and function need to be described for GD743 and GS784 trees in order to determine the differences in phenotypes after the genetic engineering process. Not only will the GE trees differ because of the functioning of the introduced transgene, they also may differ because of unintended changes. Unintended, event-specific changes may include transgene insertion site effects (for example, insertion mutations to apple genes, or influence of the transgenic promoter on expression of nearby apple genes); mutations and epigenetic changes induced by tissue culture; and rearrangements of the transgenes that affect their functions unpredictably. Levels and locations of the transgene products themselves also need to be described to assess potential impacts to other species.

Based on all these concerns, APHIS should deny the petition to deregulate this GE apple. Alternatively the decision whether or not to deregulate this GE apple cannot be made until and unless, at a minimum, APHIS prepares an EIS to fully review the significant environmental, health, and socioeconomic effects of this possible deregulation, and complies with all other applicable statutory mandates.

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[5] *See Calvert Cliffs' Coordinating Comm. v. Atomic Energy Comm'n*, 449 F.2d 1109 (D.C. Cir. 1971) ("the agency's crabbed interpretation makes a mockery of the Act").

[6] *See* 7 C.F.R. § 340.6(c).

II. LEGAL BACKGROUND

National Environmental Policy Act

NEPA requires a federal agency such as APHIS to prepare a detailed EIS for all "major Federal actions significantly affecting the quality of the human environment."[7] 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."[8]

If the federal action may significantly affect the environment, APHIS must prepare an EIS.[9] 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.[10] 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.[11] "The statement of reasons is crucial to determining whether the agency took a "hard look" at the potential environmental impact of a project."[12] An EA must "provide sufficient evidence and analysis for determining whether to prepare an EIS or a finding of no significant impact."[13] NEPA regulations require the analysis of direct and indirect, as well as cumulative, effects in NEPA documents, including EAs.[14] The assessment must be a "hard look" at the potential environmental impacts of its action.[15] APHIS's decisions in the EA must be "complete, reasoned, and adequately explained."[16]

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."[17] 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

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[7] 42 U.S.C. § 4332(2)(C).
[8] *Robertson v. Methow Valley Citizens Council,* 490 U.S. 332, 349 (1989).
[9] *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).
[10] 40 C.F.R. § 1508.9.
[11] *Save the Yaak v. Block*, 840 F.2d 714, 717 (9th Cir. 1988).
[12] *Id.*
[13] *Id.*
[14] *See* 40 C.F.R. §§ 1508.8, 1508.9, 1508.13, 1508.18.
[15] *Blue Mountains Biodiversity v. Blackwood*, 161 F.3d 1208, 1211 (9th Cir. 1998); *Nat'l Parks & Conservation Ass'n v. Babbitt*, 241 F.3d 722, 731 (9th Cir. 2001) (quoting 40 C.F.R. § 1508.27).
[16] *Nw. Coalition for Alternatives to Pesticides v. U.S. Envtl. Ptor. Agency*, 544 F.3d 1043, 1052 n.7 (9th Cir. 2008).
[17] 40 C.F.R. § 1508.27(a).

"[t]he degree to which the action may adversely affect an endangered or threatened species or its habitat."[18] An action may be "significant" if even one of these factors is met.[19]

A thorough consideration of cumulative impacts is required in the preparation of an EA.[20] Specifically, an EA must provide a quantified assessment of project's environmental impacts when combined with other projects.[21] 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.[22] The cumulative impact analysis must also include an assessment of potential aesthetic, historic, cultural, economic, social, and health impacts.[23]

Council on Environmental Quality

NEPA established the CEQ and charged it with the duty of overseeing the implementation of this statute.[24] The regulations subsequently promulgated by CEQ[25] 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."[26] CEQ's regulations are applicable to and binding on all federal agencies.[27] Among other requirements, CEQ's regulations mandate that federal agencies address all "reasonably foreseeable" environmental impacts of their proposed programs, projects, and regulations.[28] Direct effects are those that are caused by the action and occur at the same time and place.[29] 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.[30] 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

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[18] *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.
[19] *Ocean Advocates v. U.S. Army Corps of Eng'rs*, 361 F.3d 1108, 1125 (9th Cir.2004); *see also 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").
[20] *See, e.g.*, *Kern v. Bureau of Land Mgmt.*, 284 F.3d 1062, 1075 (9th Cir. 2002).
[21] *Great Basin Mine Watch v. Hankins*, 456 F.3d 955, 972 (9th Cir. 2006).
[22] *See, e.g., Native Ecosystems Council v. Dombeck*, 304 F.3d 886 (9th Cir. 2002); *Kern*, 284 F.3d. at 1076, 1078 (emphasis in original) (quoting CEQ, Considering Cumulative Effects Under the National Environmental Policy Act at 4, January 1997) ("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].'").
[23] 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. Dept. of Agric.*, 661 F.3d 1209, 1251 (10th Cir. 2011) (explaining that a cumulative impacts analysis must consider all of the effects listed at 40 C.F.R. section 1508.8).
[24] *See* 42 U.S.C. §§ 4321, 4344.
[25] 40 C.F.R. §§ 1500–08.
[26] *Id.* § 1500.3.
[27] *Id.* §§ 1500.3, 1507.1; *see, e.g.*, *Hodges v. Abraham*, 300 F.3d 432, 438 (4th Cir. 2002).
[28] *See* 40 C.F.R. §§ 1502.4, 1508.8, 1508.18, 1508.25.
[29] *Id.* § 1508.8(a).
[30] *Id.* § 1508.8(b).

undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time.[31]

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."[32] 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."[33] Agencies are to focus on "significant environmental issues and alternatives."[34]

Plant Protection Act

APHIS oversees transgenic crops pursuant to the Plant Protection Act (PPA),[35] 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.[36] 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.[37]

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."[38] "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."[39]

Developers seeking to commercialize a transgenic plant must petition APHIS for deregulation,[40] which the agency can grant "in whole or in part."[41] The PPA mandates that all APHIS decisions "be based on sound science."[42]

Endangered Species Act

As recognized by the Supreme Court, the ESA is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation."[43] The ESA's statutory

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[31] *Id.* § 1508.7.
[32] *Id.* § 1502.1.
[33] *Id.*
[34] *Id.*
[35] 7 U.S.C. §§ 7701–7772.
[36] *Id.* § 7712(a); 7 C.F.R. §§ 2.22(a), 2.80(a)(36) (delegating to APHIS).
[37] 7 U.S.C. § 7701(1).
[38] *Id.* § 7702(10).
[39] *Id.* § 7702(14).
[40] 7 C.F.R. § 340.6.
[41] *Id.* § 340.6(d)(3)(i).
[42] 7 U.S.C. § 7701(4); *see id.* § 7712(b).
[43] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).

scheme "reveals a conscious decision by Congress to give endangered species priority over the 'primary missions' of federal agencies."[44] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[45]

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.[46] 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."[47] 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.[48]

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.[49] 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.[50]

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.[51] 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.[52] The vast majority of U.S. native birds are protected under the MBTA, even those that do not participate in international migrations.[53] 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."[54]

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[44] *Id.* at 185.
[45] *Id.*
[46] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).
[47] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[48] *Id.*
[49] 50 C.F.R. § 402.14.
[50] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[51] 16 U.S.C. § 701.
[52] *Id.* § 701–12.
[53] *See* 50 C.F.R. § 10.13.
[54] *Id.* § 21.11.

Administrative Procedure Act

The Administrative Procedures Act (APA) sets forth standards that govern judicial review of decisions made by federal agencies.[55] 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."[56] Under the APA, an agency decision is unlawful if it is arbitrary or capricious or fails to follow procedures required by law.[57] Agencies must "articulate a rational connection between the facts found and the choice made."[58] 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."[59]

III. COMMENTS

A. APHIS's NEPA Analysis Is Inadequate

NEPA is our national charter for protection of the environment.[60] It is designed to ensure that federal agencies take a hard look at the environmental consequences of their actions.[61] For the many reasons discussed in this section, APHIS's draft EA is woefully inadequate under NEPA: in short, the agency has failed to take the requisite "hard look at the environmental consequences" of the proposed action to approve GE apples.[62] Later sections of this comment letter will focus on the specific scientific inadequacies of the analysis; however, these scientific concerns all point to the inescapable conclusion that this EA is inadequate. NEPA's fundamental tenets include ensuring comprehensive, timely, and transparent environmental review of agency actions, and this EA fails to meet those obligations.

1. Process and Public Participation

NEPA "is a procedural statute intended to ensure environmentally informed decision-making by federal agencies."[63] In taking a "hard look" at the consequences of major decisions, agencies are required to "involve the public in preparing and implementing their NEPA procedures."[64] Further, agencies have an obligation to afford "interested persons an opportunity to participate in the rule making."[65]

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[55] 5 U.S.C. § 706.
[56] *Id.* § 702.
[57] *Id.* § 706(2)(A), (D).
[58] *Motor Vehicle Mfrs. Ass'n of U.S., Inc. v. State Farm Mutual Auto. Ins. Co.*, 463 U.S. 29, 43, 59 (1983).
[59] *Id.* at 43, 56.
[60] 40 C.F.R. § 1500.1(a).
[61] *See, e.g.*, *Sierra Club v. Bosworth*, 510 F.3d 1016, 1018 (9th Cir. 2007).
[62] *See, e.g.*, *Friends of the Payette v. Horseshoe Bend Hydroelectric Co.*, 989, 993 (9th Cir. 1993); *see Overton Park v. Volpe*, 401 U.S. 402, 416 (1971).
[63] *Tillamook Cnty. v. U.S. Army Corps of Eng'rs,* 288 F.3d 1140, 1142 (9th Cir.2002).
[64] 40 C.F.R. § 1506.6(a).
[65] 5 U.S.C. § 553(c).

The very purpose of NEPA is to "ensure that federal agencies are informed of environmental consequences before making decisions and that the information is available to the public."[66] Meaningful and effective public participation is one of the cornerstones of NEPA because it gives the public an opportunity to inform the agency of environmental consequences the agency may not have considered. For this reason, NEPA's implementing regulations require that agencies "make diligent efforts to involve the public in preparing and implementing their NEPA procedures."[67] Thus, the agency must "hold or sponsor public hearings or public meetings whenever appropriate"[68] and "provide public notice of NEPA-related hearings, public meetings, and the availability of environmental documents" so that interested persons can be informed.[69] Also, federal agencies must to the fullest extent possible "encourage and facilitate public involvement in decisions which affect the quality of the human environment."[70]

APHIS has failed to make an adequate effort to engage public participation in its review of this petition for the deregulation of GE apples. Millions of Americans grow apples and nearly all Americans regularly consume apples, but most do not check the Federal Register for actions that may impact apples. For an action that could potentially have incredibly far reaching impacts for apple growers and consumers, APHIS should have done significantly more to solicit public comment on GE apples. Appropriate actions to engage the public would include open houses throughout the nation, but especially in areas where apple growing constitutes an important segment of the economy. APHIS has taken similar public outreach actions in the past related to the agency's assessment of other GE crops. The lack of notice of this action outside of the Federal Register makes it very difficult for most people to provide meaningful input to APHIS. For this reason, APHIS should not proceed with any action until and unless it publishes an EIS and, concurrent with a new public comment period, provides the public with meaningful opportunities to give feedback by hosting open houses.

2. The EA Fails to State a Valid Purpose and Need for this Project

In preparing a NEPA document and determining the appropriate scope of analysis, the first thing an agency must define is the project's purpose.[71] The purpose and need statement is one of NEPA's threshold requirements, but in this EA, APHIS completely fails to articulate a purpose and need for this proposed action. APHIS simply states that when such a petition is submitted, it must "determine if the GE organism is unlikely to pose a plant pest risk."[72] APHIS offers no explanation for the purpose and need for the proposed action, it simply describes that it must consider this petition. The purpose and need of a proposed action is not just the agency is considering the action; rather, the purpose and need statement must actually describe the underlying purpose and need for the proposed action. APHIS briefly describes the purpose of the product, which is to resist enzymatic browning,[73] and the few sentences devoted to this in the

[66] *Citizens to Preserve Better Forestry v. U.S.D.A.*, 341 F.3d 961, 970-71 (9th Cir. 2003).
[67] 40 C.F.R. § 1506.6(a).
[68] *Id.* at § 1506.6(c).
[69] *Id.* at § 1506.6(b).
[70] *Id.* at § 1500.2(d).
[71] *See Citizens Against Burlington, Inc. v. Busey,* 938 F.2d 190, 195–96 (D.C. Cir.1991).
[72] EA at 4.
[73] EA at 1.

purpose and need section does not describe why this browning is a compelling enough problem to necessitate such a drastic measure as approving the first GE apple in the U.S. Thus, APHIS is contemplating a major action but provides very little insight into the purpose or need for the action. The agency cannot possibly take the requisite "hard look" where it has hardly articulated a purpose and need for the underlying action.

3. APHIS Fails to Consider a Reasonable Range of Alternatives

NEPA analysis "shall serve as the means of assessing the environmental impact of proposed agency actions, rather than justifying decisions already made."[74] APHIS appears to violate the statute's fundamental function by not even considering reasonable range of alternatives in its analysis because it does not evaluate alternatives that would minimize the environmental impacts of the proposed action. This type of resigned attitude calls into doubt whether it is undertaking this NEPA process to engage in informed decision making or whether this is simply a paper exercise. NEPA calls upon APHIS to fully consider the impacts revealed by its NEPA analysis. However, APHIS's alternatives analysis reveals a lackluster position toward the analysis in its entirety.

Section 102(2)(E) of NEPA requires agencies to "[s]tudy, develop, and describe appropriate alternatives to recommended courses of action in any proposal which involves unresolved conflicts concerning alternative uses of available resources."[75] Regardless of whether an EA or EIS is prepared, NEPA "requires that alternatives be given full and meaningful consideration."[76] In fact, the alternatives section is considered the heart of an environmental analysis.[77] "[I]t should present the environmental impacts of the proposal and the alternatives in comparative form, thus sharply defining the issues and providing a clear basis for choice among options by the decisionmaker and the public."[78] Agencies must therefore rigorously explore and objectively evaluate all reasonable alternatives, including the no action alternative.[79]

First, despite the rigor required by NEPA, APHIS's EA presents no serious analysis of potential alternatives. Instead, APHIS merely provides a review of just two options, either no action or approval of deregulated status. It is a classic NEPA violation to limit the consideration of alternatives simply to (1) action or (2) no action.[80]

Second, APHIS's alternatives analysis is fundamentally flawed because it is—like the rest of the EA—far too limited in scope. An agency's alternatives analysis should be a function

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[74] 40 C.F.R. § 1502.02(g); *see id.* § 1500.1(c) ("NEPA's purpose is not to generate paperwork—even excellent paperwork—but to foster excellent action").
[75] 42 U.S.C. § 4331(2)(E).
[76] *Bob Marshall Alliance v. Hodel*, 852 F.2d 1223, 1229 (9th Cir. 1988).
[77] 40 C.F.R. § 1502.14.
[78] *Id*.
[79] *Id*.
[80] *See, e.g.*, *Muckleshoot Indian Tribe v. U.S. Forest Serv.*, 177 F.3d 800, 813–14 (9th Cir. 1999) (consideration of only unqualified deregulation and the no action alternative is presumptively too limited to comply with NEPA); *Am. Oceans Campaign v. Daley*, 183 F. Supp. 2d 1, 17–21 (D.D.C. 2000).

of the "purpose and need" of the action under review,[81] and NEPA requires APHIS to consider and evaluate a wide range of alternatives capable of addressing the same problem.[82] However, in its EA, APHIS inexplicably limits its alternatives. NEPA also requires that the alternatives considered must include a "range of reasonable actions which might meet the goals of the agency by using different approaches which may reduce the environmental impacts of the agency's action."[83] This necessarily includes, among other things, the following examples:

- Identify alternate ways to stop browning in apples. OSF promotes GD743 and GS874 as solutions to the browning problem mainly for sliced apples, but also for all other apple products. Sliced apples are promoted as new market opportunity for a "flat" or declining sales of apples. However, there are various other ways to stop browning without use of genetic engineering. For example, apples can be dipped in vitamin C, which also preserves vitamin C levels, or lemon juice can be applied.

- Develop the non-GE apple varieties that are not as susceptible to browning.

- Consider alternatives to pre-sliced apples. All apple slices lose nutrients and are susceptible to diseases. There could be a public health campaign to get children to eat whole apples rather than slices, or to ensure that apples for children are smaller, of very high quality so taste good, etc.

- Focus on expanding other apple niche markets, instead of the pre-sliced apple market. For example, apple growers could expand niche markets by growing heirlooms for fresh market.

- Develop training programs for conversion to higher-value organic apple production.

As those unconsidered alternatives demonstrate, using genetic engineering, with its consequent potential for significant environmental and socioeconomic harms, to silence apple genes truly is not the only reasonable alternative to expanding a niche market for non-browning, pre-sliced apples. NEPA mandates that APHIS give consideration to those alternatives.

Third, as a consequence of the overly narrow design of APHIS's alternatives discussion, the commercialization of the GE apple may become a foregone conclusion. "An agency may not define the objectives of its actions in such unreasonably narrow terms as to make consideration of alternatives a mere formality."[84] Relatedly, such a tunnel-vision focus also impermissibly accepts OSF's own biased representation of its product, ignoring that "NEPA requires an agency to 'exercise a degree of skepticism in dealing with self-serving statements from a prime

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[81] *See* 40 C.F.R. § 1502.13 (agency must "specify the underlying purpose and need to which the agency is responding in proposing the alternatives"); *City of Carmel-By-The-Sea v. U.S. Dep't of Transp*., 123 F.3d 1142, 1155 (9th Cir. 1995) ("The stated goal of a project necessarily dictates the range of 'reasonable' alternatives and an agency cannot define its objectives in unreasonably narrow terms.") (citation omitted).
[82] 40 C.F.R. § 1502.13; *see, e.g.*, *City of Carmel-By-The-Sea*, 123 F.3d at 1155.
[83] *See, e.g.*, *Soda Mountain Wilderness Council v. Norton*, 424 F. Supp. 2d 1241, 1265 (E.D. Cal. 2006).
[84] *Citizens Against Burlington, Inc. v. Busey*, 938 F.2d 190, 196 (D.C. Cir. 1991).

beneficiary of the project and to look at the general goal of the project rather than only those alternatives by which a particular applicant can reach its own specific goals."[85]

Fourth, APHIS's purported reliance on a separate PPRA determination underscores that in APHIS's view the entire NEPA process is a predetermined façade, because the agency is making/has made a separate decision, pursuant to which the agency's hands are otherwise purportedly tied. Under this reasoning, presumably APHIS would then have no authority to restrict or deny approval of the GE apple, even if the agency's NEPA analysis concluded it would cause irreparable environmental harm or the collapse of the U.S. apple industry. Yet this would turn the NEPA review process into a charade, and subvert the requirement that "[e]nvironmental impact statements shall serve as the means of assessing the environmental impact of proposed agency actions, rather than justifying decisions already made."[86] APHIS would violate NEPA's fundamental goal if the agency erroneously concluded that it need not or could not take into account what its NEPA analysis reveals. APHIS has the NEPA analysis process precisely backwards: The NEPA analysis must inform the agency's decision-making process, not the other way around.[87] NEPA requires that environmental considerations be factored into government decision-making "early enough so that it can serve practically as an important contribution to the decisionmaking process and will not be used to rationalize or justify decisions already made."[88]

Here, however, APHIS summarily rejects alternatives without fully considering them on the basis that the PPA precludes those options. For example, the agency provides only cursory information about creating an isolation distance between GD743 and GS874 and non-GE apple varieties, or requiring testing for these GE varieties.[89] Doing so impermissibly eviscerates APHIS's NEPA responsibilities. Consequently, the agency fundamentally failed to consider reasonable alternatives in its EA.

4. APHIS Fails to Properly Consider Cumulative Impacts

Cumulative impacts are "the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (federal or non-federal) or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant

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[85] *Envtl. Law & Policy Ctr. v. U.S. Nuclear Regulatory Comm'n*, 470 F.3d 676, 683 (7th Cir. 2006); *see Forty Most Asked Questions Concerning CEQ's NEPA Regulations,* 48 Fed. Reg. 18,026 (Mar. 23, 1981) ("In determining the scope of alternatives to be considered, the emphasis is on what is 'reasonable' rather than on whether the proponent or applicant likes or is itself capable of carrying out the particular alternative. Reasonable alternatives include those that are practical or feasible from a technical and economic standpoint and using common sense, rather than simply desirable from the standpoint of the applicant.").

[86] 40 C.F.R. § 1502.02(g); *see id.* § 1500.1(c) ("NEPA's purpose is not to generate paperwork—even excellent paperwork—but to foster excellent action.").

[87] *W. Watersheds Project*, 632 F.3d at 491 ("The 'hard look' must be taken objectively and in good faith, not as an exercise in form over substance, and not as a subterfuge to rationalize a decision already made.") (internal citations and quotation marks omitted).

[88] *Metcalf v. Daley,* 214 F.3d 1135, 1142 (9th Cir. 2000).

[89] EA at 11.

actions taking place over a period time."[90] A thorough consideration of cumulative impacts is required in the preparation of an EA.[91] Specifically, an EA must provide a quantified assessment of project's environmental impacts when combined with other projects.[92] Notably, courts and the 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.[93]

It is well-established that "a cumulative impacts analysis must include 'some quantified or detailed information' since without such information it is not possible for the court or the public to be sure that the agency provided the hard look that is required of its review.'"[94] In a cumulative impact analysis, "general statements about possible effects and some risk do not constitute a hard look. . . . The cumulative impact analysis must be more than perfunctory; it must provide a 'useful analysis of the cumulative impacts of past, present, and future projects.'"[95] Moreover, a cumulative impact analysis must be timely; "it is not appropriate to defer consideration of cumulative impacts to a future date when meaningful consideration can be given now."[96] "If the agency did not present this detailed information and analysis it will be found to have violated NEPA unless it provides a convincing justification as to why more information could not be provided.'"[97]

In order to address the cumulative impact requirement, APHIS must examine and evaluate the cumulative impacts of reasonably foreseeable actions. Here, however, APHIS's brief, perfunctory three-page cumulative impacts analysis omits a number of reasonably foreseeable actions.[98] For example, the agency does not account for the fact that other varieties of GE apples are already in development, and OSF may introduce other types of GE fruit as well. Thus, if there are harms related to the RNAi process or to altered PPO levels, APHIS can assume that the harms will be similar in the additional GE varieties. As discussed above, harms from the RNAi process and altered levels of PPO may take a particularly significant toll on pollinators, including bees, which are already under significant environmental stress and therefore are especially vulnerable. But APHIS entirely failed to consider such cumulative impacts on pollinators.

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[90] 40 C.F.R. § 1508.7.
[91] *See, e.g.*, *Kern*, 284 F.3d at 1075.
[92] *Great Basin Mine Watch*, 456 F.3d at 972.
[93] *See, e.g.*, *Native Ecosystems Council v. Dombeck*, 304 F.3d 886 (9th Cir. 2002); *Kern*, 284 F.3d. at 1076, 1078 (emphasis in original) (quoting CEQ, Considering Cumulative Effects Under the National Environmental Policy Act at 4, January 1997) ("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].").
[94] *Soda Mountain Wilderness Council v. Norton*, 424 F. Supp. 2d 1241 (E.D. Cal. 2006).
[95] *Muckleshoot Indian Tribe v. U.S. Forest Serv*, 177 F.3d 800, 810 (9th Cir. 1999).
[96] *Neighbors of Cuddy Mountain*.
[97] *Id*. (citing *Ocean Advocates v. Army Corps of Eng'rs*, 402 F.3d 846, 868 (9th Cir. 1998)). The cumulative impact analysis is wholly distinct from the scope requirements and analysis discussed above. *See Earth Island Inst. v. U.S. Forest Serv.*, 351 F.3d 1291, 1306 (9th Cir. 2003) ("Even if a single, comprehensive EIS is not required, the agency must still adequately analyze the cumulative effects of the projects within each individual EIS.").
[98] EA at 52–54.

Similarly, the agency failed to adequately consider cumulative impacts on the apple market. The cumulative impact analysis must include an assessment of potential aesthetic, historic, cultural, economic, social, and health impacts.[99] APHIS concludes that deregulating GD743 and GS784 "will have no foreseeable adverse cumulative effects on domestic commerce."[100] However, the agency completely omits any discussion of the cumulative impacts deregulation of these GE apples will have on consumer preferences, and thus on apple growers. Markets for apples and apple products, which have decreased in recent years and thus are currently particularly susceptible to interference,[101] are likely to be very sensitive to transgenic contamination. Accordingly, APHIS must assess both scientific information on gene flow and also the likely significant adverse socioeconomic impacts of approval of GD743 and GS784. Further, APHIS must consider the effects that the very potential for such contamination may have on consumers, who may avoid apples to prevent unintended contact with the GE varieties.

In addition, APHIS must consider the cumulative effects of possible increased pesticide use as a result of the PPO effects in GD743 and GS784. However, as discussed above, APHIS failed to consider the potentially reduced pathogen resistance in these GE apples, instead evaluating them only under unrealistically limited and highly controlled circumstances that cannot predict actual resistance in a variety of commercial orchards with different management regimes. Thus, in order to assess the cumulative effects of increased pesticide use on GD743 and GS784, as it must, APHIS needs to perform the initial step of adequately investigating the pathogen-resistance effects of silencing PPOs, which it has not done in its EA and PPRA.

As indicated in the record and public comments, the potential significant socioeconomic, cultural and other foreseeable impacts are considerable. The cumulative socioeconomic analysis APHIS must perform should include an analysis of both the economic and cultural importance of apples, demographics of the communities that would be impacted, an analysis of potential impacts to commercial apple industries, and an analysis of the market impacts of this product's commercialization.

Thus, APHIS must prepare an EIS to evaluate the cumulative impacts related to the deregulation of the GD743 and GS784 GE apples.

5. APHIS Fails to Adequately Consider Socioeconomic Impacts

APHIS fails to adequately address potential adverse socio-economic effects from the deregulation of GD743 and GS874. Potentially significant adverse socio-economic impacts trigger the need for APHIS to prepare an EIS.

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[99] 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) (explaining that a cumulative impacts analysis must consider all of the effects listed at 40 C.F.R. section 1508.8).

[100] EA at 53.

[101] EA at 53 ("Current and historic economic evidence indicates that apple production in the United States has decreased since 2004 . . . .").

NEPA requires that economic effects are relevant and must be examined "when they are interrelated with natural or physical environmental effects." As the court explained in *Geertson Seed Farms*: "The economic effects on the organic and conventional farmers of the government's deregulation decision are interrelated with, and, indeed, a direct result of, the effect on the physical environment; namely, the alteration of a plant species' DNA through the transmission of the genetically engineered gene to organic and conventional alfalfa." The court continued, "APHIS was required to consider those effects in assessing whether the impact of its proposed action is 'significant.'"

Past contamination episodes from GE crops provide cautionary tales for why contamination is an important potential socioeconomic impact that must be considered here. For example, of particular interest is the recent contamination of rice by the unapproved GE LL601 "Liberty Link" rice. This type of GE rice was grown only in limited-acreage field tests, rather than on a commercial scale, and under the regulatory auspices of APHIS, which includes confinement recommendations. It had not been grown at all for several years, but contamination of the US rice supply was detected several years later at low levels that have nonetheless caused economic harm to the US rice industry. At least one identified source of contamination by LL601 occurred at Louisiana State University LSU, where one of the scientists in charge has claimed that they exceeded APHIS confinement recommendation considerably, but still experienced contamination.

By one estimate, rice farmers lost $150 million due to rejection of LL601-contaminated rice shipments by countries in Europe and elsewhere, and the consequent sharp drops in rice prices. Affected rice farmers were forced to sue Bayer CropScience, the developer of LL601, in an effort to recover their losses. In response to a petition from Bayer CropScience, APHIS subsequently deregulated LL601, but did nothing to redress the economic harms to rice farmers. Rather than accept responsibility for the episode, Bayer CropScience blamed farmers and an "Act of God" for the contamination episode. At least one identified source of contamination by LL601 occurred at Louisiana State University, where LL601 had been grown in small-scale field trials. One of the scientists in charge of the field-testing stated that LSU had grown LL601 under conditions that met and exceeded APHIS confinement recommendations considerably, but still experienced contamination. Just months later, still another unapproved GE rice variety developed by Bayer CropScience, LL604, was found contaminating a popular variety of conventional rice sold to farmers as seed rice (Clearfield 131). APHIS responded by issuing several emergency action notifications to distributors of Clearfield 131 to halt sales of the contaminated seed rice. As a result, rice farmers in the South experienced a severe shortage of seed rice for the 2007 season. APHIS conducted an investigation into the contamination episodes, but was unable to determine precisely how they occurred.

Here, the apple market potentially impacts by contamination from GD743 and GS874 is significant. As APHIS reports, apples are grown in all fifty states, constituting more than 330,000 acres.[102] In 2011, U.S. apples were valued at $2.7 billion, with fresh fruit amounting to $2.38 billion and processed fruit amounting to $338 million.[103] The U.S. currently produces

[102] EA at 18.
[103] *Id.* at 19.

about sixteen percent of the global apple export market.[104] Given that many other countries reject GE products, contamination from GD743 and GS874 could be catastrophic for U.S. apple growers. Further, consumers within the U.S. increasingly reject GE products, so a contamination event with GE apples would have substantial domestic effects as well.

Despite the potential for contamination, APHIS failed to address the socioeconomic effects such contamination would have. APHIS should have thoroughly considered the impacts of GE apples on communities where apple growing is a significant source of income, amongst other socioeconomic impacts. In this case, as in *Geertson*, "APHIS's reasons for concluding that the potential for the transmission of the genetically engineered gene is not significant are not 'convincing' and do not demonstrate the 'hard look' that NEPA requires." Thus, APHIS must prepare an EIS to disclose and analyze the potential for biological contamination prior to deregulating GD743 and GS874.

6. APHIS Fails to Adequately Consider Trans-Boundary Impacts

APHIS failed to adequately consider the impacts of approving GE apples on other nations. CEQ regulations explicitly state an agency must assess the cumulative impacts of the project when added to "all other past, present and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions."[105] A 1997 CEQ guidance clarifies that "NEPA law directs federal agencies to analyze the effects of proposed actions to the extent they are reasonably foreseeable consequences of the proposed action, *regardless of where those impacts might occur*."[106] CEQ concludes that "agencies must include analysis of *reasonably foreseeable transboundary effects* of proposed actions in their analysis of proposed actions in the United States."[107]

In this EA, APHIS only briefly considers the market impacts of GE apples to foreign trade,[108] but does not consider the full range of potential trans-boundary environmental impacts. APHIS also states that it considered international implications pursuant to Executive Order 12114,[109] but its analysis here is lacking in that it simply recites its obligations under various treaties without actually considering potential impacts. Many of the apples grown in the U.S. are grown in the northeastern states and Washington state, relatively close to the Canadian border. Much of the U.S. apple industry is in close proximity to Canada, thus APHIS should consider reasonably foreseeable trans-boundary impacts in accordance with CEQ's guidance.

7. APHIS Relies on Unenforceable OSF Assurances in Lieu of Actual Mitigation Measures

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[104] *Id.* at 21.
[105] 40 C.F.R. §1508.7.
[106] Council on Environmental Quality Guidance on NEPA Analyses for Transboundary Impacts, July 1, 1997, at ¶4, available at http://ceq.hss.doe.gov/nepa/regs/transguide.html
[107] *Id*. at ¶ 6 (emphasis added).
[108] EA at 38.
[109] EA at 63.

Under NEPA, mitigation must be enforceable, which includes the duty of on-going monitoring to ensure compliance.[110] "Monitoring is essential in those important cases where the mitigation is necessary to support a FONSI and thus is part of the justification for the agency's determination not to prepare an EIS."[111] APHIS fails to adequately explain or analyze how it will monitor compliance with the OSF mitigation measures upon which it depends. Mitigation measures cannot substitute for actually analyzing environmental impacts.[112] This is precisely what APHIS has improperly done here, relying solely on OSF's measures and failing to analyze the potential impacts.

CEQ defines "mitigation" to include

> (a) Avoiding the impact altogether by not taking a certain action or parts of an action.
> (b) Minimizing impacts by limiting the degree or magnitude of the action and its implementation.
> (c) Rectifying the impact by repairing, rehabilitating, or restoring the affected environment.
> (d) Reducing or eliminating the impact over time by preservation and maintenance operations during the life of the action.
> (e) Compensating for the impact by replacing or providing substitute resources or environments.[113]

Courts examine mitigated FONSIs to see whether such measures keep impacts below the EIS threshold, which is the "low standard" of whether a project "may have a significant effect."[114] APHIS's reliance here does not comply with NEPA.

In its EA, APHIS's discussion of mitigation measures is entirely inadequate. The agency recognizes that the GD743 and GS784 apples may contaminate organic growers but simply observes that such growers may impose their own isolation distances:

> Individuals might choose on their own to geographically isolate their non-GE apple production systems from GD743 and GS784 or to use isolation distances and other management practices to minimize gene movement between apple orchards.[115]

But such voluntary measures, which do not even involve OSF, are unreliable over the lifespan of apple trees, and APHIS cannot rely on such voluntary mitigation measures to avoid a finding of significance and the requirement to prepare an EIS.

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[110] CEQ, Appropriate Use of Mitigation and Monitoring and Clarifying the Appropriate Use of Mitigated Findings of No Significant Impact 7 n.18 (2011); *id.* at 2 (explaining that when agencies do not "monitor mitigation commitments to determine if mitigation was implemented or effective, the use of mitigation may fail to advance NEPA's purpose of ensuring informed and transparent environmental decisionmaking").
[111] CEQ at 10.
[112] *See, e.g.*, *N. Plains Res. Council, Inc. v. Surface Transp. Bd.*, 668 F.3d 1067, 1085–86 (9th Cir. 2011).
[113] 40 C.F.R. § 1508.20.
[114] *See, e.g.*, *Klamath Siskiyou Wildlands Ctr. v. Boody*, 468 F.3d 549, 562 (9th Cir. 2006).
[115] EA at 10.

As discussed, OSF does acknowledge the need to mitigate gene flow and states that traceability will be maintained for GD743 and GS874, and that it will provide "stewardship guidelines as part of their licensing requirements" that include measures designed to minimize pollen transfer of pollen from orchards of GD743 and GS784 to non-GE orchards or parts of orchards. In addition, OSF reassures APHIS that obligations will purportedly include providing "suitable isolation distances" between GD743 and GS784 trees and non-GE trees, with distances greater for organic apples.[116] However, these mitigation measures do not account for wild pollinators, and thus are unlikely to stop transgenic contamination. Further, voluntary measures as part of technology use agreements are not reliable, particularly over the life span of trees, changes in land use, and escape of trees into uncultivated areas. More importantly, APHIS cannot rely on such voluntary mitigation measures to avoid a finding of significance and the requirement to prepare an EIS.

Vague references to the mere concept that some hypothetical measures may prevent contamination is insufficient to absolve APHIS of its NEPA duties. CEQ has warned that "as a general rule . . . agencies should use a broad approach in defining significance and should not rely on the possibility of mitigation [of adverse environmental consequences] as an excuse to avoid the EIS requirement."[117] APHIS should heed this guidance and prepare an EIS analyzing, among other things, concrete stewardship measures such as quantitative isolations distances that actually prevent biological contamination.

That APHIS merely relies on the vague notion of stewardship measures is clearly insufficient. CEQ has indicated that "[m]itigation measures may be relied upon to make a finding of no significant impact only if they are imposed by statute or regulation, or submitted by an applicant or agency as part of the original proposal."[118] Here, no stewardship measure is required, never mind concretely explained. Nor has APHIS considered reasonable alternatives to the proposed action or propose any monitoring. The sufficiency of mitigation measures has been stated as whether they constitute "an adequate buffer against the negative impacts that may result from the authorized activity."[119] While APHIS admits that contamination might happen, the agency has not undertaken any of its own analysis regarding whether any stewardship measures might prevent such contamination.[120]

This, combined with all the other inadequacies described above, shows that this EA fails to comply with NEPA's mandates.

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[116] Petition at 105.
[117] *Forty Most Asked Questions Concerning CEQ's National Environmental Policy Act Regulations*, 46 Fed. Reg. 18026, 18037 (1981).
[118] *Id.*
[119] *Nat'l Parks & Conservation Ass'n*, 241 F.3d 722.
[120] In *Geertson* APHIS similarly relied on "good stewardship" with regard to the development of weed resistance, without APHIS's own investigation and analysis of if that stewardship was effective or not, a reliance the court held arbitrary and capricious without APHIS own analysis, which it agreed to do in the alfalfa EIS. 2007 WL 5186624, at *10.

B. APHIS Fails to Consider Critical Issues, Rendering this EA Inadequate Because the Environmental Effects of GE Apples Remain Highly Uncertain

APHIS's decision to not complete a comprehensive EIS is arbitrary, capricious, and contrary to NEPA, in large part because it has violated the basic principle that NEPA—at its core—contemplates high-quality information and accurate scientific analysis.[121] Public scrutiny is essential to implementing NEPA.[122] The draft EA is inadequate because it does not contain actual analysis or real data supporting APHIS's decision; it primarily contains narratives of OSF's background information, much of which is quite dated.

"In the absence of such fundamental information, it would seem that any alleged 'finding' that the project will not significantly affect the species is the purest sophistry."[123] Accepting APHIS's failure to study the potential harms here "would turn NEPA on its head, making ignorance into a powerful factor in favor of immediate action where the agency lacks sufficient data to conclusively show not only that the proposed action would harm an endangered species, but that the harm would prove to be 'significant."[124] At the very least, APHIS is required to disclose uncertainties, explain their relevance, and has the burden to show why the necessary information could not be obtained.[125]

Underlying all discussion in the following section is one basic premise of NEPA. At its core, NEPA demands high-quality information and accurate scientific analysis.[126] As this section makes plain, this EA is severely lacking in both.

In sum, APHIS's failure to conduct the proper analyses and account for the many potential risks and uncertainties implicit in this petition is plain evidence that the agency did not take the requisite "hard look" at the environmental consequences of this application, and is overtly arbitrary, capricious, and contrary to law.

1. APHIS Lacks Critical Knowledge About the Normal, Pre-Engineering Expression of the Silenced Genes, So It Could Not Reliably Evaluate the Effects of Silencing those Genes

Preparation of an EIS is mandated where uncertainty may be resolved by further collection of data, or where the collection of such data may prevent "speculation on potential . . . effects." "The purpose of the EIS is to obviate the need for speculation by insuring that available data are gathered and analyzed prior to the implementation of the proposed action."[127] "Where

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[121] 40 C.F.R. § 1500.1(b).
[122] *Id.* §1500.1.
[123] *Sierra Club v. Norton*, 207 F.Supp.2d 1310, 1331 (S.D. Ala. 2002) (finding agency's FONSI arbitrary and capricious because it failed to address lack of certainty).
[124] *Id.* at 1335.
[125] 40 C.F.R. § 1502.22; *Envtl. Prot. Info. Ctr. v. Blackwell*, 389 F. Supp. 2d 1174, 1188 (N.D. Cal. 2004) (recognizing that 40 C.F.R. 1502.22 guides the court in determining "whether an agency can be charged with having failed to take a hard look" because information is incomplete or unavailable).
[126] 40 C.F.R. § 1500.1(b).
[127] *Nat'l Parks*, 241 F.3d at 732 (quoting *Sierra Club*, 843 F.2d at 1195).

an EA lacks certainty on one or more issues, it is the responsibility of the agency to provide a 'justification regarding why more definitive information could not be provided."[128] "Lack of knowledge does not excuse the preparation of an EIS; rather it requires the [agency] to do the necessary work to obtain it."[129] Here, in its EA, APHIS lacks crucial information about the phenotype of GD743 and GS874, so an EIS is required.

a. Background: PPO genes in Various Plants Are Diverse in Expression and Functions

Polyphenol oxidases ("PPOs") are copper-containing enzymes that catalyze two distinct reactions: hydroxylation of monophenols to *ortho*-diphenols and oxidation of *o*-diphenols to *o*-quinones (Fig. 1).

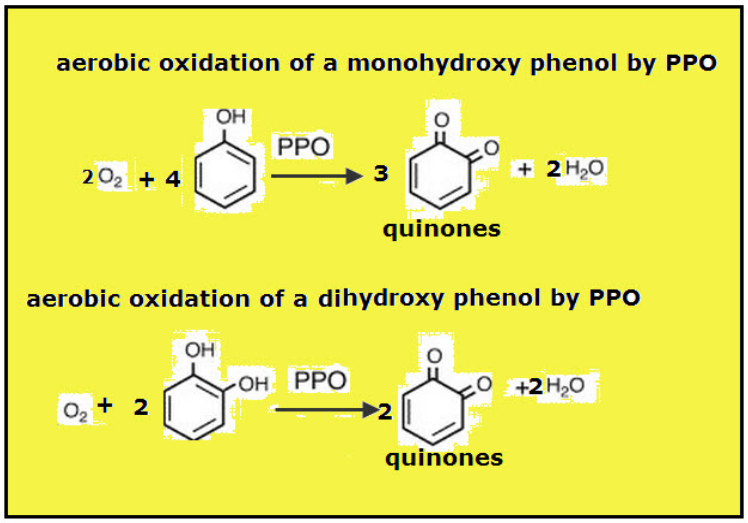

Fig. 1: Basic reactions catalyzed by PPO. http://scienceprojectideasforkids.com/2011/the-chemistry-of-fruit-browning/

These enzymes are responsible for most browning of fruits and vegetables that are damaged. For example, browning in apples that are cut or bruised occurs when PPOs in the damaged cells mix with mono- and di-phenols (e.g., catechin, epicatechin, and chlorogenic acid)[130] to form colorless quinones that then coalesce with amino acids to form dark-colored, lignin-like polymers.[131] Although browning is of interest to the food industry, the functions of PPOs for plants and other organisms themselves are generally poorly known.[132]

Some information about PPO functions is now coming from studies of gene evolution and expression. PPOs are found in bacteria, fungi, and animals, as well as most plants,[133] and

[128] *Blue Mountain*, 161 F.3d at 1213.
[129] *Id*.
[130] Kolodziejczyk et al. 2010)
[131] (Armstong & Lane 2009)
[132] (Tran et al. 2012)
[133] (Mayer 2006)

they often are encoded by multi-gene families where particular genes are likely to be differentially expressed during development in tissues and organs, or in response to stresses.[134] PPOs are presumed to play important and diverse roles in plants based on the results of experimental studies as well as their wide distribution and regulation in plants:

> Expression profiling of PPO transcripts in plants with multiple PPO genes such as tomato and poplar indicates that despite strong stress-induced regulation of some PPO genes, most PPOs are developmentally regulated. The diversity of tissues and conditions under which PPO is expressed suggests these enzymes can play roles in a variety of processes. In dandelion (*Taraxacum* spp.), a PPO has recently been implicated in latex coagulation, and the hydroxylase activity of some PPO-like proteins suggests they can function in the biosynthesis of phenylpropanoids. For example, aureusidin synthase (AmAS1) and larreatricin hydroxylase (LtLH) are PPOs that are involved in the biosynthesis of aurones and lignans, respectively. In the Caryophyllaceae [carnation family], PPOs function as hydroxylases in betalain biosynthesis.[135]

The pattern of evolution of gene families also suggests that PPOs have important, diverse functions:

> The features of the PPO gene family including variation in gene number, cellular localization, and lineage-specific diversification is consistent with the idea of PPOs as flexible enzymes that evolution has adapted to a variety of specific functions. Our data show that the PPO gene family is dynamic and greatly expanded in some lineages, but reduced in others. This pattern is reminiscent of the distribution of secondary plant metabolites, which is also very much lineage-dependent, varies tremendously among plant taxa, and appears to be the result of gene duplication and diversification. Secondary metabolites are known as important mediators of ecological interactions and environmental adaptation, and we speculate that the variable expansion of the PPO gene family also reflects such an adaptive function.[136]

By studying expression patterns of individual PPO genes, researchers are able to propose unique functions that can be tested in subsequent experiments. In European aspen trees (*Populus tremula*), comparison of the nucleotide sequences of two wound-inducible PPOs show that they have been subject to natural selection during their evolution, and the authors speculate that this could be a sign of coevolution with insect pests. These genes are also expressed differentially in parts of the tree: "*In Populus* [European aspen] both *PPO1* and *PPO2* have been shown to be wound-inducible, with *PPO1* being exclusively expressed in damaged leaves while *PPO2* is primarily expressed in stems, petioles and roots."[137]

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[134] (Tran et al. 2012)
[135] (Tran et al. 2012, internal citations ommitted)
[136] (Tran et al. 2012)
[137] (Bernhardsson & Ingvarsson 2011)

Now that many species have had their genomes sequenced, these genomes can be searched for PPO genes whose expression can then be determined using RNA localization or detection techniques with gene-specific probes (e.g., for *Populus*[138]). Tran and Constabel were the first to do a "genome-enabled analysis of a PPO gene family" from the genome sequence of *Populus trichocarpa,* black cottonwood. Of the nine complete PPO genes they found, seven were characterized further, and all were differentially expressed in tissues and organs of the tree. They examined RNA from immature fruit, female and male catkins, dormant buds, apical leaves, mature leaves, petioles, midveins of leaves, wood, periderm, and young and old roots. Female flowers, dormant buds, and young leaves expressed the most PPO genes, and roots expressed the fewest. Only one PPO gene was strongly induced by pathogens or wounding. Surprisingly, one of the PPO genes encoded an enzyme that is targeted to vacuoles via endoplasmic reticulum rather than the usual plastid location, suggesting it may have a unique function.

In red clover, different PPO genes are expressed in specific parts of the plant, including one gene that is found mainly in root nodules involved in nitrogen fixation, suggesting a specific role there, perhaps in interactions with symbiotic microbes.[139]

Experiments where PPO levels have been manipulated to be either higher or lower than normal show that some PPOs are likely to be involved in defense against pests and pathogens (e.g., review,[140] dandelion resistance to *Pseudomonas*,[141] tomato defense against caterpillars[142]). In some of these studies, leaves of transgenic plants with higher or lower levels of PPOs were fed to insect larvae to determine whether larval growth and survival was correlated with PPOs. For example, a recent study using GE tomato leaves concluded that cotton bollworm and beet armyworm larvae were negatively impacted by higher levels of PPO, in line with some but not all previous work on effects of PPO on insects:

> The results presented here are in general agreement with those reported in other studies that used plants with altered PPO activities. Wang and Constabel (2004) found that forest tent caterpillar larvae feeding on leaves of transgenic poplar overexpressing PPO had reduced average weight gains and higher mortality rates than those feeding on control leaves, but only when larvae from older egg masses were used. In tomato, feeding common cutworm larvae with leaves of transgenic tomato plants overexpressing PPO also reduced their growth rates and increased their mortality compared to those feeding on leaves of NT [non-transgenic] and transgenic plants with suppressed PPO activity (Thipyapong et al. 2006; Mahanil et al. 2008). This previous work on common cutworm also showed an instar-dependent PPO effect and a lack of effect on pupal weight (Mahanil et al. 2008). Recently, the effect of induced PPO and proteinase inhibitor (PI) accumulation on growth reduction of *H. armigera* also was found in transgenic tobacco overexpressing *Tobpre-proHypSys-A*, which encodes a hydroxyproline-rich glycopeptide systemin precursor protein (Ren and Lu, 2006). However, elevated

[138] (Tran & Constabel 2011).
[139] (Webb et al. 2013)
[140] Constabel & Barbehenn 2008
[141] Richter et al. 2012
[142] Bhonwong et al. 2008

> PPO levels do not always lead to reduction in the growth of insects; Barbehenn et al. (2007), for example, found only limited impact of elevated PPO activities on two lymantriid caterpillars, *Lymantria dispar* and *Orgyia leucostigma*, in transgenic poplar. Clearly, the effects of elevated PPO on insect growth and development vary according to both plant and herbivore species (Barbehenn et al. 2007; Mahanil et al. 2008).[143]

Within a plant, some PPO gene family members may code for enzymes that resist pests and pathogens, while other family members do not. Researchers in Germany showed that just one of five dandelion (*Taraxacum officiinale*) PPOs provided resistance against the bacterial pathogen *Pseudomonas syringae* pv. *tomato* but not the fungal pathogen *Botrytis cinerea*.[144]

Richter and colleagues first described the expression patterns for all 5 PPOs in dandelion using reverse transcription-polymerase chain reaction (RT-PCR) to detect gene-family-member specific expression of RNA in different tissues and organs of young dandelion plants (roots and leaves) and older plants (latex, stalks, and flowers). They also determined if any of the genes were induced when leaves were inoculated with pathogens, using both a fungal and a bacterial pathogen, or upon wounding.

Each PPO had a unique expression pattern in dandelion plants. RNA of *ppo-5* was not detected in any tissues or organs tested; ppo-4 was expressed at low levels only in stalks and flowers; *ppo-1* and *ppo-3* RNAs were detected in latex and roots (the authors say that *ppo-3* was only expressed in roots of some adult plants); and *ppo-2* was only expressed in roots. Only *ppo-2* was induced, transiently, when leaves were infected with pathogens. None of the genes was induced by wounding alone.[145]

Because *ppo-2* was the only pathogen-induced PPO gene, the researchers tested whether there was a relationship between PPO-2 enzyme activity and pathogen resistance. They specifically silenced the *ppo-2* gene with RNAi technology (gene knockdown):

> The specificity of the knockdown was tested using RT-PCR analysis on RNA isolated from different tissues (root, leaf, and latex) of noninfected wild-type and knockdown plants. The knockdown had no influence on *ppo-1* expression in root and latex, only the *ppo-2* expression in roots was reduced in the transformants compared with the wild type.[146]

They then inoculated three different *ppo-2*-silenced dandelion events with *P. syringae* pv. *tomato* or *B. cinerea*; all three PPO-2-deficient plants were much more susceptible to *P. syringae* but not to *B. cinerea*.

[143] (Bhonwong et al. 2008)
[144] (Richter et al. 2012)
[145] (Richter et al. 2012, Fig. 1)
[146] (Richter et al. 2012, p. 203)

Richter and colleagues also added the *ppo-2* gene to the plant *Arabidopsis thaliana* that does not have any PPO of its own, showing that extracts of the transformed plants that only had one PPO—the dandelion PPO-2—were active against *P. syrigae* pv. *tomato* in culture, whereas extracts from control plants were not.

These experiments with dandelion are an example of a well-designed analysis of PPO expression and function in a whole plant, using information about when and where specific genes in a PPO family are expressed:

> We have applied RNAi technologies and heterologous expression as powerful tools to conclusively establish an active role for an individual dandelion PPO isoenzyme in defense against pathogens. Since the different PPO isoenzymes appear to have different functions (Wahler et al. 2009), similarly detailed studies of other or all PPO isoenzymes will be needed to dissect and fully understand the actual roles of PPO in a given plant species.[147]

In sum, until more studies like this are performed, including research on both developmentally regulated PPO genes as well as inducible genes, knowledge of PPO functions in plants remains substantially incomplete. More specifically here, without fuller knowledge of the diverse functions of PPO enzymes in apple trees—and in particular the recipient lines engineered by OSF—there is absolutely no baseline for assessing the full impacts of PPO suppression in GD743 and GS784 beyond the intended anti-browning effect. Accordingly, APHIS's EA entirely lacks highly relevant data.

b. PPO Genes and Expression Patterns in Recipient Apple Trees: Information Provided by OSF and APHIS Is Inadequate for Assessment of Risks

OSF aspires to silence all members of the PPO gene family in apples.[148] They have attempted to identify the number of PPO genes and to determine DNA sequences that will silence them all.[149]

In the Petition, OSF says that "[f]our gene fragments have been cloned from apple, although more may remain undiscovered", citing Boss et al. (1995). They then present a synthesis of information from a variety of studies on apple PPO genes[150] based on sequences and other information in their patent[151] and from other researchers.

[147] (Richter et al. 2012, p. 206)
[148] Petition at 36.
[149] Petition at 32.
[150] Petition at 32, Table 1.
[151] (Armstong & Lane 2009)

Table 2: Overview of the Apple PPO Gene Family

Group	Members[1]
PPO2	PPO2, PPOJ, pSR8
GPO3	GPO3, AP14[2], APO9, APO3
APO5	APO5, PPO3, PPO7
pSR7	pSR7

[1] Apple PPO gene sequences were sorted into four groups and the groups were named for the PPO sequence type. The members of a group are either the same gene, or are expected to be equivalent from an antisense point of view.
[2] AP14 is a pseudogene that is highly related to GPO3.

OSF sorts ten differently named PPO gene family members into four groups in their overview of the apple PPO gene family (Petition, p. 33, Table 2; excluding the pseudogene AP14). They state that "members within a given PPO group are either the same gene, or are closely related enough at the nucleotide sequence level that they are expected to be equivalent from a gene silencing point of view." Based on this assessment, OSF suggests that there are between four and ten PPO genes in apple, "although more may remain undiscovered."

If all apple PPO genes were silenced, what effects would the lowered PPO levels have on tissues in which they are normally expressed? Without understanding the normal expression pattern of PPO genes in recipient apple trees, and the various roles the gene products play in development, stress response, predator defense, disease resistance and perhaps other areas, we have no phenotypic point of comparison for engineered trees. OSF has provided very little information on the expression profile for specific PPO genes in the apple tree.

In its Petition, OSF does not directly identify or localize PPO gene transcripts (messenger RNAs) in any parts of the apple tree, including in fruits. For example, there are no northern or *in situ* hybridizations of RNAs or RT-PCR results with PPO gene-specific probes. Boss et al. (1995) show a northern analysis of RNA from immature and mature fruit using an APO5 probe, and mRNA for APO5 was only present in the immature stage. APO5 PPO mRNA was also detected in apple leaves and fruit peels. No other tissues or organs were tested.

OSF did identify specific gene products in cDNA libraries from apple fruits and leaves. They state that they found "GPO3, APO5, PPO2 and PPOJ in apple fruit and apple leaf cDNA; and GPO3 and pSR7 immature apple fruit cDNA library (Eugentech). HortResearch (now Plant & Food Research) found PPO2, GPO3, APO5 and pSR7 in its apple EST [expressed sequence tag] library (personal communication)."[152] However, the apple tissues and organs represented in Plant & Food Research' EST library are not disclosed. The presence of these sequences in cDNA libraries indicates that the corresponding genes are expressed in leaves and fruits, but this does not exclude expression in other parts of the tree. Also, without more detailed information on the differences between the putative ten genes, it is not possible to completely describe expression patterns.

[152] Petition at 32, legend to Table 1.

At the protein level, OSF looks at PPO enzyme activity in young and mature fruits, and in leaves from trees grown under different conditions. They do not distinguish specific isozymes to show expression from individual genes. They do not look at PPO levels in most of the tissues and organs of apple trees. For instance, they do not report expression in flowers or parts of flowers, stems, vegetative buds, bark, vascular tissue, roots, or other tissues.

Nor does OSF identify PPO genes that may be expressed in response to stress, wounding, or to pests and pathogens. OSF cites the 1995 study by Boss et al. where a cDNA clone of an APO5 PPO gene from apple fruit peel was used to show that the corresponding mRNA was induced in apple fruit and leaves upon wounding.[153] Induction of APO5 by wounding, but not PPO2, was confirmed in 2001,[154] as noted by APHIS in the PPRA. Apparently, no other gene family members were tested for their ability to be induced by wounding, and none were tested for induction by pests or pathogens.

In sum, this description of PPO gene family expression in recipient apple trees by OSF in its Petition, and APHIS in its draft analysis, is much too limited in scope to provide a sound scientific basis for phenotypic comparison of GD743 and GS784 apples trees and non-transgenic recipient lines. This problem of inadequate, overly narrow scope is repeated by APHIS many times. Further, APHIS's reliance on the lack of data and its expectations for no impacts is improper because NEPA requires it to take a hard look at environmental impacts itself, not assume that if any impacts were to exist they would be disclosed by the applicant.

c. Recipient Apple PPO Genes and Expression Patterns: Information Not Provided by OSF and APHIS, but in Scientific Literature, Adds to Need for Phenotypic Analysis

The OSF Petition was submitted February 21, 2012, so it should cite relevant studies published through 2011. APHIS says that it used "data and information submitted by the applicant, in addition to current literature," to assess if GD743 and GS784,[155] and dated their assessment documents "August 2013." Therefore, APHIS should have included relevant studies published through early 2013.

i. Information on Apple PPO Gene Family

Instead, in discussing PPO gene families, OSF does not cite any literature after 1997, more than fifteen years ago, well before studies of PPO gene families based on whole genome sequences were published, for example.[156]

Of particular concern for the APHIS assessments, though, is the fact that neither OSF nor APHIS cite or discuss the publication of a "high quality draft genome sequence of the

[153] (Boss et al. 1995)
[154] (Kim et al. 2001)
[155] PPRA at 1.
[156] (see references in Tran et al. 2012 for examples of relevant studies, and our discussion of PPO gene families, above)

domesticated apple," based on DNA from "Golden Delicious,"[157] the recipient variety for GD743. Thousands of researchers have now cited the main paper, and some have used the apple genome sequence to elucidate gene families and functions.[158] Presumably, OSF could have used this apple genome sequence as Tran and Constabel (2011) used the *Populus trichocapa* genome sequence to do a "genome-enabled analysis of a PPO gene family", confirming and extending information about the numbers and kinds of PPO genes and expression patterns in apple trees. They also could have checked the specificity of their gene silencing strategy (as discussed below).

For example, Di Guardo et al. (2013) used the apple genome sequence reported by Velasco et al. (2010) to find and map ten PPO genes onto chromosomes, using the inducible PPO sequence from Boss et al. (1995) as a guide to identify PPO genes.[159] Later, they found an eleventh gene that was more highly diverged. They examined expression of just one of the genes in apple fruit in response to wounding—the same gene identified by Boss et al. (1995).

These results based on the whole apple genome that show there are indeed eleven PPO genes confirms the importance of knowing where and when each PPO gene is expressed in the tree, and of determining how many will be subject to silencing with the suppression construct that OSF developed based on just four PPO genes.

ii. Information on Expression of Particular PPO Genes in Apple Trees

OSF describes literature on PPO synthesis in various plants, but focuses on expression in various stages of fruit development—both PPO made as the fruits develop and PPO induced by wounding of fruits—and does not describe studies of PPO in different tissues and organs of whole plants.[160] For apples, OSF describes expression in leaves and tissue culture callus in addition to PPO in young and mature fruit, but there are no descriptions of PPO in stems, flowers, bark, vascular tissue, roots, or other parts of apple trees where they may have different functions and interactions.

CFS knows of only one study where researchers looked for expression of PPO genes in parts of apple trees other than leaves and fruit: Kim et al. (2001). Although the paper was cited by APHIS in the dPPRA (p. 6), the fact that PPO expression was found in flowers was not mentioned.

Kim and colleagues extracted RNA from flowers at four stages of development, when tiny buds had just emerged to flowers that were fully opened. They also extracted RNA from fruits and leaves of various stages. When probed with APO5 or PPO2 gene-specific sequences, northern blots showed differential expression of the two genes. APO5 RNA was found in early fruits but not later in fruit development, in young- to mid-stage leaves, and in the oldest flowers.

[157] (Giovannoni 2010; Velasco et al. 2010)
[158] (e.g. Kumar et al. 2013; Malnoy et al. 2012)
[159] (Di Guardo et al. 2013).
[160] (Petition, p. 29–30).

PPO2, on the other hand, was expressed in the three earliest stages of flower development but not the oldest flowers, a little later in fruit development than APO5, and also a little later in leaves. The fact that PPO2 is differentially expressed in flowers may bear on its function, and also on what kinds of organisms may be affected by PPOs, such as pollinators (discussed below).

These limited data fit with what is known about expression of different PPO gene family members of other plants—that they are differentially expressed—and confirm that it is important to determine expression patterns of each PPO gene in many different tissues and organs in order to describe the relevant phenotype of recipient apple trees.

Studies of specific PPOs in other plants (as we discuss in the Background section, above) have shown that particular PPOs can have unique functions, such as defense against bacterial pathogens or insect herbivores, biosynthesis of other molecules, and coagulation of latex. Functions of particular PPOs in different parts of apple trees have not been studied, so are unknown. In the absence of actual data from OSF or the scientific literature, in its assessments APHIS must assume that PPO genes are expressed in all tissues and organs of apple trees throughout development, and that some PPO genes may also be induced by wounding, or by pests and pathogens. APHIS must also assume that PPOs have diverse functions throughout the apple tree, and that other species that interact with the trees may be affected by PPOs in parts of apple trees that they come in contact with.

2. APHIS Failed to Describe or Assess Significant Phenotypic Factors for GD743 and GS784, and Thus APHIS Cannot Adequately Predict How these GE Apples Will Interact with the Environment

In order to properly assess impacts of GD743 and GS784 apple trees, any analysis requires the proper baseline for analysis: the first step is to describe how these trees are different from recipient trees, as a result of the engineering. However, APHIS failed to describe critical components of the GE apples' phenotype—a highly relevant factor—such as the novel RNAs these apples produce, which PPO genes are silenced and where, and how silencing the PPO genes might have affected other non-target genes. APHIS's failure to properly acknowledge and address the many gaps in its environmental risk analysis is in itself yet another NEPA violation. 40 C.F.R. section 1502.22 requires agencies to "always make clear" when there is "incomplete and unavailable information."[161] APHIS's substantial omissions render the agency's analysis inadequate to fulfill the agency's statutory mandates, and would render decision based on such analysis as arbitrary and capricious.

a. Expression of the Gene Product: Novel RNAs Produced but Not Described or Assessed

OSF has failed at the most basic level to describe let alone analyze the fundamental change in GD743 and GS784 apple trees that resulted from genetic engineering: production of RNA from the engineered transgene. Before discussing the transgene product in GD743 and

[161] *See, e.g.*, *Lands Council v. Powell*, 395 F.3d 1019, 1033 (9th Cir. 2005) (citing 40 C.F.R. section 1502.22 to hold that NEPA "requires up-front disclosures of relevant shortcomings in the data or models.").

GS748, the basic terminology and acronyms of the RNAi process are summarized in this "What is RNAi" paragraph from a paper by Lundgren and Duan:

> RNA interference (RNAi) is a posttranscriptional technique for the sequence-selective silencing of genes. Fragments of small RNAs (small interfering RNAs [siRNA] or microRNAs) bind to messenger RNAs (mRNAs) and promote cleavage by a complex of enzymes, thereby reducing the expression of specific genes. For decades, RNAi was known to occur in plants (as *posttranscriptional gene silencing*) and fungi (as *quelling*) but was only first reported in animals (the nematode *Caenorhabditis elegans*) in 1998. A cell produces double-stranded RNAs (dsRNAs) or microRNAs that target mRNAs from a specific gene, depending on nucleotide sequence, or dsRNAs are taken into a cell from the exterior environment (environmental RNAi). The dsRNA (generally fewer than 1000 nucleotides long) is then cleaved into much smaller siRNAs (almost always 21–23 nt long), which are sometimes amplified intracellularly. It is noteworthy that this amplification has not been widely found in insects (a primary target of RNAi based GM crops; an exception is embryonic *Drosophila melanogaster* [fruit flies]) or mammals. The siRNAs are incorporated into an RNA induced silencing complex (RISC), where mRNAs are cleaved with an enzyme in the Argonaute family, and their translation is silenced. Silencing in the absence of cleavage may result if the RISC unit simply binds to an mRNA, thereby restricting its translation. RNAi is not a way to knock out gene expression, only a way to suppress it, and sometimes only temporarily.[162]

The transgene introduced by OSF into Golden Delicious and Granny Smith recipient trees is designed to silence at least four of the eleven apple PPO genes by RNAi technology (discussed more below). Partial sequences of coding regions of each of the four chosen PPO genes are arranged in a "sense" orientation, one after another, behind a CaMV viral promoter that usually causes high levels of expression of transgenes in most tissues and organs of plants. The initial transgene product, then, is an RNA transcribed from the transgene that contains regions of homology to four PPO genes, and has regions of sequence identity with the mRNAs produced from these genes, but does not itself code for any PPO proteins. This specific RNA is the initial gene product:

> GD743 AND GS784 are genetically engineered with a silencing construct designed to reduce the expression of four apple PPO genes: PPO2, GPO3, APO5, and pSR7 (OSF, 2012); therefore, the gene product is a chimeric, sense-silencing RNA rather than a functional protein or new enzyme.[163]

The RNA product of the transgene is novel. Although the RNA product of the transgene contains sections of nucleic acid sequences identical to sequences in mRNAs normally found in apple tissues and organs that express those PPO genes, the new RNA is not found naturally in apple trees. No naturally occurring RNA in apple trees has fragments of four different mRNAs

[162] (2013, Box 1, internal citations omitted, emphases added)
[163] (PPRA, p. 6).

arranged in that fashion. The normal mRNAs from these four genes would be full length, one type from each gene, and would participate in synthesis of PPO proteins.

Also, the initial RNA product of the transgene in GD743 and GS784 apple trees is presumably processed into small RNAs of various sizes as part of the RNAi pathway for gene silencing—the whole point of OSF's engineering project. These specific small RNAs can be biologically active throughout the plant and in other species that are exposed to them (as we describe below). The small RNAs can also be amplified and move throughout the plant, unlike most mRNAs,[164] and thus may occur in novel locations. The CaMV promoter may result in presence of the novel RNA in tissues and organs of the apple tree that normally do not have PPO mRNAs, or not in those amounts. In other words, although the transgene products are not new proteins, they are novel, bioactive molecules that can have specific impacts; hence, those impacts need to be analyzed, and cannot be analyzed without a description of the expression of the transgene product.

OSF does not identify, measure, localize or in any other way characterize any of the novel RNAs that are produced from the PGAS transgene in GD743 and GS784 as it goes through the RNAi process. Methods are available for detecting and characterizing such RNAs.[165]

In its review, APHIS does not mention the crucial lack of information from OSF on the products of the transgene, and does not consider potential impacts of novel RNAs produced by the transgene. In fact, APHIS appears to conclude that there are no novel products that require assessment, if no new proteins are made.[166] APHIS dismisses concerns about possible effects of RNAs from the transgene in a single sentence,[167] without any discussion or analysis of OSF's lack of data or of current literature related to risks from ingestion small RNAs (see our discussion below). This was fundamentally flawed and contrary to sound science.

b. Functioning of the Transgene: APHIS Does Not Know which PPO Genes Are Silenced, or Where in the Trees

OSF does not provide information about which PPO genes were actually silenced in GD743 and GS784 apples trees. As discussed, they expected all PPO genes to be silenced:

> The PPO suppression transgene (PGAS) consists of 394, 457, 457 and 453 bp regions of apple PPO genes (PPO2, GPO3, APO5, pSR7, respectively), placed in the sense orientation under control of the cauliflower mosaic virus 35s promoter (PCAMV35s) and nopaline synthase terminator (TNOS). The use of a constitutive promoter such as the PCAMV35s is indicated here, as PPO is expressed both in early fruit development and in response to wounding. The transgene is designed to reduce overall expression of the entire apple PPO gene family, and to induce a reduced browning or nonbrowning phenotype in apple.[168]

[164] (Molnar et al. 2010; Dunoyer et al. 2010; Martienssen 2010)
[165] (see, for example, Fig. 3 in Baum et al. 2007)
[166] (EA, p. 63).
[167] (dPPRA p. 11),
[168] (Petition, p. 36)

OSF did not use specific probes that would detect silencing of particular genes to monitor mRNA levels in any GD743 and GS784 tissues or organs, including fruits. In fact, PPO mRNA levels were not monitored at all. Nor were PPO proteins from specific genes measured directly (with antibodies or by sizes on gels, for example) in GD743 and GS784 tissues and organs, including fruits.

The only description provided by OSF showing that silencing of PPO genes occurred in GD743 and GS784 apple trees is a study of browning in wounded apple fruits and leaves. They find lower browning and assume it is the result of gene silencing of at least the four PPO genes that were directly targeted. This low- or non-browning phenotype was only examined in apple fruits and in leaves, and was not examined in flowers or flower parts, stems, buds, roots, vascular tissue, or any other parts of GD743 and GS748 apple trees.

Because of this fundamental failing, APHIS thus does not have enough information about phenotypes related to intended changes in expression of PPO genes as a result of genetic engineering to meaningfully assess risks of deregulating GD743 and GS748 apple trees, rendering any approval decision arbitrary and capricious.

c. Unintended Effects of the Genetic Engineering Process: Disruption of Non-PPO Apple Genes from Transgene Insertions and Changes due to Tissue Culture Not Described or Assessed

Many unintended changes from genetic engineering result from the DNA transformation process.[169] The introduced genes insert unpredictably into the host plant's chromosomes, and changes at the insertion sites can lead to unpredictable changes in the expression patterns of both the inserted genes and some host genes. After this DNA transformation process, recipient plant cells must be selected and then regenerated into functional plants. The tissue culture process itself often leads to extensive genome-wide disruptions, including mutations, transposon activation, and epigenetic changes—collectively referred to as somaclonal variation.[170]

Here, the likelihood of transgene insertion site changes are increased by the incorporation of two copies of the transgene at unique sites in the genome of GD743 and 4 copies of the transgene at unique sites in the genome of GS784. Each insertion has the potential to disrupt expression of local genes.

Also, GD743 and GD784 are vegetatively propagated, so will carry whatever unintended changes occurred during the tissue culture process with them, even if those changes are not closely linked to the transgenes. In most GE crops, such unlinked changes are lost during backcrossing or other breeding techniques that transfer the transgenes to agronomically desirable cultivars from the cultivar used in the engineering process.

[169] (Latham et al. 2006; Wilson et al. 2006)
[170] (Miguel & Marum 2011; Neelakandan & Wang 2012)

As a result of insertion-site and genome-wide changes from DNA transformation and tissue culture, the phenotype of engineered lines needs to be examined carefully for consequences. OSF did not provide any information on genome-wide somaclonal variation, or discuss any methods they may (or may not) have used to detect unintended changes associated with tissue culture of GD743 and GS784.

d. Unintended Effects of Transgene Expression: Silencing of Genes Other than PPO Not Examined

It is now known that small RNAs targeted to suppress expression of specific genes often also silence genes that were not targeted, because some other genes are likely to share small regions of nucleotide homology,[171] as we discuss in more detail in relation to impacts on pollinators. APHIS did not take these off-target effects into account in their assessments:

> Off-target gene silencing. One conclusion from the recent advances in functional genomics that has important implications for risk assessment of RNAi-based GM crops is that siRNAs commonly have off-target effects within a targeted cell or organism (Davidson and McCray 2011).[172]

There are methods for assessing likelihood of off-target effects, and also for detecting changes in expression of non-targeted genes, and protocols for when to use these methods in assessing RNAi impacts.[173]

e. Changes in Disease and Pest Susceptibilities: Tests in Managed Orchards Not Adequate for Determining Phenotypes

Given that specific PPOs in some plant species are known to be involved with resistance to pests and pathogens, determining whether GD743 and GS784 apple trees show changes in susceptibility is an important part of describing their phenotypes. In addition, from the standpoint of basic biology, GD743 and GS784 apple trees with silenced PPO genes provide a good experimental system for determining functions of PPOs in apple trees—something that has not yet been done.[174] However, OSF does not perform the kinds of experiments that would show whether or not GD743 and GS784 are changed in susceptibilities to particular pests and pathogens. Possible impacts of increases in susceptibility of GD743 and GS784 include increased use of pesticides, use of different pesticides, and spread of pests and disease organisms to other trees.

OSF performs all of their tests and phenotypic descriptions on trees growing in a "highly-managed agricultural production environment."[175] In these orchards, pests and pathogens are

[171] (Miguel & Marum 2011; Neelakandan & Wang 2012)
[172] (Lundgren and Duan 2013, p. 659)
[173] (US EPA 2013)
[174] ("Transgenic apple, modified in PPO expression, has not been assessed for changes in resistance or susceptibility to pests and diseases outside of the data provided in this petition." (Petition, p. 31).)
[175] (see Petition, p. 66, Fig. 9 for photos; reproduced below in "Weediness" section).

controlled by frequent applications of fungicides, and insecticides used as needed, and all comparisons are done on trees younger than five years of age:

> It is essential to manage apple field trials in a manner consistent with commercial apple cultivation methods, adhering to integrated pest management (IPM) and Good Agricultural Practices (GAP) approaches. By assuring this type of management, the data collected from the trials is both reproducible and can be extrapolated with confidence to a commercial setting. OSF field trial Standard Operating Procedures (SOPs) include, but are not limited to: soil preparation and testing, tree planting, tree fertility, pest management, disease management, irrigation scheduling, crop load management (i.e., pruning and thinning), insect monitoring and data collection, crop spraying and reporting, harvest, postharvest fertility, rodent and wildlife control, and disposal of transgenic trees.
>
> A commercial apple orchard is a highly-managed agricultural production environment. This is particularly the case in modern high-density orchards (which average more than 1,000 trees/acre) using dwarf rootstock. Careful monitoring of tree vigor, insect pressure and disease allows for timely optimization of tree growth, production and yield. For this reason, little pest and disease pressure is tolerated or observed in commercial apple orchards.[176]

However, tests performed by observing levels of pests and pathogens in commercially managed orchards with no or low pest and disease pressure are simply not able to provide information about changes in susceptibility to most pests and pathogens. For example, if management involves spraying fungicides frequently so that no fungal diseases are observed, neither resistant nor susceptible apple trees will be exposed to fungal pathogens, so both will be free of these diseases. In fact, that appears to be what happened for some diseases in OSF's tests:

> 6.3.1 Scab (*Venturia inaequalis*)
> This fungal disease infects both apple fruit and leaves. It is a serious pest of concern to commercial apple growers particularly in wet and humid areas, less so in drier areas such as Washington State.
>
> This disease was monitored in both the Washington and New York field trials in each year of the trials. No incidences of this disease were detected in GD743, GS784 or controls, nor were any infection sites observed. This result was probably the consequence of a normal commercial scab spray-control program. Controlled inoculations without chemical control were not carried out.
>
> Typically, apple cultivars that are sensitive to Powdery Mildew, such as Golden Delicious and Granny Smith are sprayed with fungicide every two weeks. As a consequence, scab is controlled on these cultivars. Consistent with this, very few instances of scab have been reported from either field trial.

[176] (Petition, p. 63).

> In 2006, scab lesions in the fruit were reported in the NY field trial, but there were very few trees or fruit affected. None of the trees were the subject of this petition. In NY there were no other reported instances of scab between 2007 and 2011. The WA field trial has never reported an instance of scab.[177]

In order to determine adequately whether GD743 and GS784 apple trees are fundamentally more or less resistant to fungal diseases such as scab, leaves or appropriate parts of trees should be challenged with inoculum of purified strains of the diseases under controlled conditions, and then monitored for disease presence and severity. For example, Richter and colleagues were able to show that dandelions with lower PPO-2 levels were more susceptible to a bacterial pathogen but not a fungal pathogen by challenging with innocula.[178]

Similarly, to test whether GD743 and GS784 apples trees are fundamentally more or less resistant to particular insect pests, leaves or the appropriate part of the trees should be fed to the insect larvae, and growth and survival of the pest compared with non-engineered recipient trees. For example, these kinds of studies were done with GE tomatoes and showed PPO enhanced resistance to specific lepidopteran pests.[179]

Fire blight is a particularly devastating bacterial disease, caused by *Erwinia amylovora*. Impacts of the disease are influenced by environmental conditions, including weather, cultivars grown, and tree age.[180] OSF did not find more fire-blighted GD743 and GS784 trees than diseased controls in their field tests with young trees, over three growing seasons. No fire blight was found at all in one of the two test sites, though.

On whether published studies support the role of PPOs in defense against fire blight in apple, APHIS states: "To date, steady state levels of PPO have not been correlated with scab (Kolodziejczyk et al., 2010) or fire blight resistance (Korba et al., 2008; Sobiczewski et al., 2006)."[181] A new study published in March 2013, though, does find a correlation between higher PPO activity and less susceptibility to fire blight, using leaf disc assays from susceptible and resistant apples infected with the pathogen.[182] The authors conclude that PPO activity is linked to fire blight resistance, and that PPOs from specific genes may have different roles in resistance, and propose other differences in PPO behavior as well:

> Our present data strongly support a potential role of apple PPO in the resistance to fire blight. It would be interesting to compare *ppo* genes expression in the leaves of both apple genotypes and to compare the protein sequence (functional domains) of different PPO isoforms in order to explain the difference in PPO activity measured in apple genotypes displaying contrasted susceptibilities to the disease. These enzymes have also the characteristic to exist in an inactive state

[177] (Petition, p. 68–69)
[178] (Richter et al. 2012)
[179] (Bhonwong et al. 2008)
[180] (EA, p. 26).
[181] (dPPRA, p. 6).
[182] (Gaucher et al. 2013)

> [46]. An alternative hypothesis could be the differential activation process of constitutive latent enzymes between genotypes after infection. It is interesting to note that this activation process could be regulated by methyl-jasmonate [46] and that such treatment was recently reported to decrease the susceptibility of the MM106 apple genotype to fire blight [47]. (Gaucher et al. 2013, p. 186)

This experiment by Gaucher and colleagues provides strong suggestive evidence that GD743 and GS784 may be more susceptible to this devastating disease. Increased susceptibility could lead to more frequent applications of antibiotics used to control fire blight, possibly resulting in antibiotic residues on apples[183] and risk of antibiotic resistance in soil bacteria.[184]

Other deficiencies in the tests of pest and pathogen resistance done by OSF is that very few trees were tested; they were tested in only one or two locations; and the testing period was only one to two, or occasionally three, years.[185] This test methodology is incapable of delivering meaningful results, in view of the variety of conditions apple trees encounter in the US over their life spans. In fact, given how long trees live, testing should occur over more years and locations than for annual crops, not fewer. With more testing over more years and locations, and with older as well as young trees, differences in susceptibility to pests and pathogens may become apparent, even in highly managed orchards.

APHIS, after studying the data from OSF, made the following conclusion about susceptibility to pests and pathogens:

> OSF's pest and disease field data and post-harvest rot data (OSF 2012, Appendix 3, p. 141-163, Tables 53-65) indicate that in a highly managed orchard environment GD743 and GS784's non-browning phenotype did not increase the pest and disease incidences on GD743 and GS784, with the exception of the slight increase in incidence of Tentiform Leaf Miner in GS784 compared to GS; therefore, GD743 and GS784 are expected to be no more susceptible to the same plant pathogens and insect pests as their conventional apple cultivars GD and GS. It therefore follows that there should be no indirect plant pest effects on other agricultural products that are grown or stored in proximity to GD743 and GS784.[186]

Although commercial growers expect to keep GD743 and GS784 under intensive management, in fact apple trees are not always managed so intensively, so the phenotype of GD743 and GS784 must be described in less managed situations, as well. Seedling apple trees sometimes grow outside of orchards from seeds spread as animals or people move fruits away from the trees.[187] Orchards are abandoned with some regularity, depending on economic conditions for apple growers,[188] or other factors (described below in gene flow section). For

[183] (Mayerhofer et al. 2009)
[184] (McManus et al. 2002)
[185] (Petition, p. 67–79).
[186] (PPRA, p. 10, emphasis added)
[187] (EA, p. 25; Petition, p. 104).
[188] (Murray 2000)

example, there has been a steady decline in apple acreage in the US since 2002 of about 65,000 acres (EA, p. 39, Fig2), some of which are no doubt abandoned rather than razed. Abandoned trees are often minimally managed or not managed at all, for decades, or even for a century or more.[189] Such trees can harbor pests and pathogens that potentially spread to managed orchards, so their degree of resistance is an important phenotypic characteristic. OSF has not provided any phenotypic information on any characteristics of GD743 and GS784 apple trees outside of intensive management. These failings of scope and depth of disease and pest susceptibilities risk assessment render APHIS's review fundamentally flawed. Further, APHIS's reliance on the lack of data and its expectations for no impacts is improper because NEPA requires it to take a hard look at environmental impacts itself, not assume that if any impacts were to exist they would be disclosed by the applicant.

f. Weediness: No Relevant Tests Reported

Apple trees are found outside of cultivation.[190] Seeds from cultivated trees can and do disperse, germinate, and grow into saplings and mature trees in various habitats, and diverse locations in the U.S.[191] APHIS acknowledges this in its assessment. APHIS also cites some studies that predict whether trees will become weedy, and apples are recognized as having some weediness potential:

> Hancock et al. (2003) describe apple as having compatible wild relatives, an intermediate number of weediness traits and capable of escaping and persisting in the environment.[192]

APHIS concludes that the non-browning trait is unlikely to contribute to weediness:

> In the context of the genetically engineered trait introduced, non-browning, GD743 and GS784 are not likely to become weedier than their non-GE counterparts GD and GS.[193]

This statement belies a simplistic view of the introduced trait, evident throughout APHIS's assessment, that the only phenotypic alteration produced by the PGAS transgene is lack of browning in apple fruits. Rather, four (and perhaps eleven) PPO genes with unknown but likely diverse functions have been silenced, probably throughout the tree, using methods known to be able to generate pleiotropic, unintended changes. The only way to determine if any of these intended and unintended changes will result in increased weediness in GD743 and GS784 is to look for relevant differences.

Instead, APHIS examined the phenotypic and agronomic characteristics, including pest and disease resistance data, from the field trials done by OSF at two locations (New York and Washington) over a two- to five-year period with young trees on dwarf rootstocks in highly

[189] (Routson 2007)
[190] (PTES 2011; Routson 2007; U of ME n.d.; USDA Forest Service 2009)
[191] (Petition, Biology of Cultivated Apple, p. 9–10).
[192] (PPRA, p. 12)
[193] (PPRA, p. 12)

managed orchards. Most measurements and observations were done on trees less than five years old, and for a period of two years at most. Here is a photo of what the trees that were described looked like in their field locations:

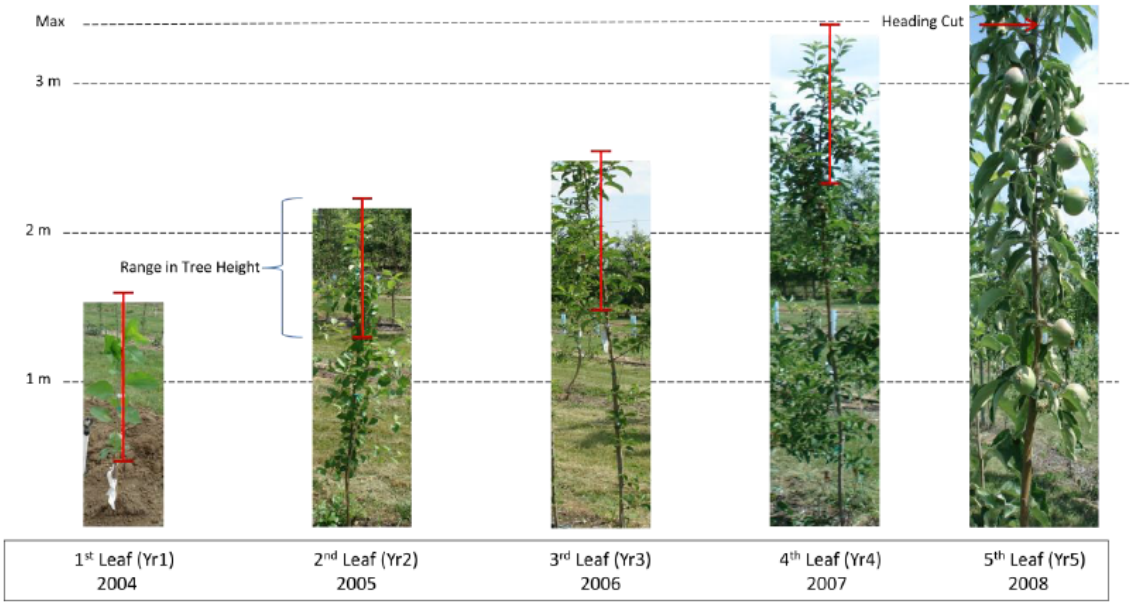

Figure 9: Agronomic Performance Overview
This figure pictorially represents the establishment of the WA2004 field block…[194]

OSF measured tree height (New York only), trunk cross sectional area, flower clusters (New York only) and fruit number at harvest. No differences were found between GD743 and GS784 and their controls, although sample sizes were too small to give meaningful results. OSF also collected the pest and pathogen data from these same deficient field trials.

It is hard to imagine how data from these field trials can lead to any non-arbitrary conclusions about weediness. Trees that become "weeds" will grow from seeds and will not be managed. No seeds were collected to determine whether the transgenic manipulation had altered their size, dormancy, germination rates, vigor, disease resistance or other standard weediness characteristics that might increase their chances of survival outside of cultivation. No seedling trees with the engineered trait were examined for growth characteristics or other qualities that would lead to greater fitness in unmanaged situations. In other words, no data were analyzed that are relevant to the question of weediness, so no conclusions based on evidence can be drawn. APHIS' cursory assessment of weediness is contrary to sound science and fails to consider critical aspects.

[194] (Petition, p. 66, copy of part of the figure)

C. The EA Is Inadequate Because the RNAi Technology Used to Create GE Apples Involves Unique and Unknown Ecological Risks, Many of which APHIS Entirely Failed to Consider

An EIS "must be prepared if substantial questions are raised as to whether a project may cause significant degradation of some human environmental factor."[195] "The plaintiff need not show that significant effects will in fact occur, but if the plaintiff raises substantial questions whether a project may have a significant effect, an EIS must be prepared."[196] "This is a low standard."[197]

A wide array of species is known to interact with different parts of apple trees at various times in tree development, and over different timespans. For example, soil microbes interact with apple tree roots; fungi grow inside and upon roots,[198] wood, bark, leaves, flowers and fruits; insects burrow into different plant parts, eat various tissues, and visit flowers for pollen and nectar[199]; birds use trees for nesting, drink sap, eat fruit, and consume insects that reside in and on the trees[200]; mammals eat twigs, bark, flowers and fruits[201]; beneficial and pathogenic microorganisms live on every surface and inside some organs[202]; and so on. Given the long lifespan of apple trees and the wide range of environments in which they grow, their interactions with other organisms are difficult to predict, but are certain to be numerous and complex.

In addition to the use of commercial apple orchards by wild animals and other organisms, apple trees are often grown intentionally for the benefit of wildlife.[203] In some areas, wild apple trees from old homesteads and abandoned orchards, now within public forests, are intentionally maintained for wildlife.[204]

APHIS does acknowledge that there are many interactions between apple trees and other species, but does not adequately consider possible impacts to those species of exposure to the RNA transgene product in GD743 and GS784. And, except for possible impacts to pests and pathogens of lower PPO levels in fruits and leaves, APHIS does not consider impacts to other species of altered PPO levels in any other parts of the trees.

These are critical omissions in environmental impacts analyses, particularly for assessing impacts to pollinators and species listed under the ESA, and they render any approval contrary to APHIS's statutory mandates under, *inter alia*, the PPA, NEPA and the APA.

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[195] *Klamath Siskiyou Wildlands Ctr. v. Boody*, 468 F.3d 549 (9th Cir. 2006) (emphases added).
[196] *Id.* (emphases added).
[197] *Id.* (emphasis added).
[198] (Kristin & Miranda 2013)
[199] (Garibaldi et al. 2013; Šťastná & Psota 2013; Altieri & Schmidt 1986)
[200] (U of ME n.d.)
[201] (PTES 2011; U of ME n.d.)
[202] (Shade et al. 2013)
[203] (U of ME n.d.)
[204] (USDA Forest Service 2009; Routson 2007)

1. RNAi Technology Can Result in Silencing of Genes and Physiological Changes in Organisms Exposed to Small RNAs

APHIS completely ignores the potential impacts of RNAi technology on other organisms. There is no mention of any kind of RNA in the EA. APHIS does briefly address one aspect of potential RNAi impacts in one paragraph of the PPRA:

> As discussed earlier, GD743 and GS784 [apple fruits] are similar in nutritional and compositional analysis to their untransformed counter parts GD and GS except for the changes in the total phenolics and vitamin C. GD743 and GD784 apples are engineered to silence PPO gene expression and therefore do not express a PPO protein. The four apple PPO genes targeted for suppression lack significant sequence similarity to each other (with the exception of APO5 and GPO3) to design a single RNA sense silencing transgene capable of silencing all four genes. The PGAS transgene contains sequences unique to each individual transgene indicating that sense silencing of apple PPO genes requires a specific level of sequence similarity. RNAi mediated gene suppression generally requires sequence homology of at least 90% between the silencing construct and the target sequence to be successful and even higher degrees of homology over 21–23 nucleotide stretches (Sharp 2001). <u>It is not likely that the PGAS transgene would contribute to PPO silencing in other non-target organisms such as pollinators or herbivores whose PPO sequences are expected to be even more divergent than those in apple.</u>

This cursory, conclusory treatment by APHIS of the potentially significant environmental impacts of RNAi technology, as used in GD743 and GS784 apple trees, is wholly inadequate. APHIS cites one out-of-date review—the only literature cited at all by APHIS regarding possible impacts of RNAi. APHIS ignores a plethora of recent studies showing that small RNAs derived from transgenes frequently have effects within the transformed organism itself and in organisms exposed to resultant small RNAs, that are independent of the exact nucleotide sequence, or that do not require such high sequence homology.[205] The fact that APHIS ignores the potential for RNAi impacts to non-target species is even more perplexing given that USDA funds research in this very area. For example, USDA is currently funding research on RNAi risks to insects in the food chain in cornfields, with the goal of determining how to incorporate such risks into assessments.[206] The grant is based on concerns that small RNAs move up trophic levels and can silence genes unpredictably in non-target insects. The funded "research will establish crucial infrastructure that can be used to establish risk of both existing RNAi-based GM crops as well as future constructs", filling vital knowledge gaps about how RNAi in GE crops impacts herbivores.

In a recent review of potential effects on nontarget speicies of RNAi-based insecticidal crops, Lundgren and Duan (2013) list the “hazards posed by RNAi technology to nontarget organisms”:

[205] (Lundgren & Duan 2013; Heinemann et al. 2013; US EPA 2013)
[206] (USDA REEIS 2013).

> Although small interfering RNAs (siRNAs) were originally believed to be extremely specific (Dillin 2003), recent experience with RNAi in functional genomics has revealed that siRNAs often silence unintended genes (Davidson and McCray 2011). Moreover, the process of RNAi can affect organisms in ways that transcend the effects of gene silencing. The hazards of siRNAs within nontargets can be categorized as off-target gene silencing, silencing the target gene in nontarget organisms, immune stimulation, and saturation of the RNAi machinery (this list is adapted from Jackson and Linsley 2010). Knowledge gaps in the genomics and physiologies of highly exposed nontarget organisms currently preclude our ability to assess the activity spectrum of RNAi, determine whether toxicity assays will be sufficient in predicting the risks of RNAi-based crops, and explain how these risks may affect food webs associated with agroecosystems. This last knowledge gap is not unique to RNAi-based technologies.[207]

Although this and some of the other treatments of RNAi risks to non-target organisms focus on RNAi technologies designed as pesticides, most of the concerns raised are equally applicable to the RNAi technology used in GD743 and GS784 apple trees, and in fact have been studied primarily in non-pesticidal applications. The suppression transgene in GD743 and GS784 is not *designed* to affect other organisms that are exposed to the RNA products (herbivores and pollinators, for example). However, other organisms will in fact ingest, inhale, absorb or otherwise come into contact with these novel RNAs. The suppression transgene in GD743 and GS748 apples is engineered for expression at high levels throughout the trees, potentially resulting in exposure to small RNAs in excess of normal exposure to naturally occurring small RNAs in these tissues and organs. Also, the PGAS transgene has 4 different nucleotide sequences that are not likely to be found in the population of naturally occurring small RNAs (miRNAs) of apple. The transgene is targeted at four different PPO genes that could have homology with gene sequences in nontarget species, including but not limited to the PPO genes that are found in most organisms and that may have similar sequences. And non-sequence-specific RNAi effects such as immune stimulation and saturation of RNAi machinery are independent of sequence homology.

We use the example of apple pollinators, below, to show more specifically how RNAi technology in GD743 and GS748 has the potential to impact non-target species, which APHIS has failed to assess.

2. PPOs Are Involved in Many Interspecies Interactions

APHIS stresses the fact than no novel proteins are intentionally produced in GD743 and GS784. However, lower levels of specific PPOs in some tissues and organs of apple trees could affect nontarget organisms, and this risk was not addressed by APHIS in their assessments. As already discussed, the functions of PPOs in plants are not well known, but evidence points to diverse functions, many related to ecological adaptations and interactions. The potential for altered PPO levels to affect non-target species is also modeled using apple pollinators.

[207] (Lundgren and Duan 2013, p. 658–59)

3. Pollinators May Be at Particular Risk from Transgene Products and Changes in PPOs

a. Exposure

Apple flowers are visited by many different kinds of insect pollinators[208] (as we discuss in more detail in the gene flow section, below). Apple growers often house hives of domesticated honey bees within their orchards, although wild insects may do most of the pollination, depending on the surrounding landscape.[209] These insects collect nectar and pollen from apple flowers, and some also collect sap and resins, and drink from guttation droplets. Nectar and pollen may be eaten directly, or taken back to hives or nests to feed immature stages. Exposure of insect pollinators to RNA products of the PGAS transgene in GD743 and GS784 would thus occur primarily through ingestion of nectar, sap, and pollen. It is also possible that immature stages may absorb small RNAs from these plant sources.

A first step in assessing risks to pollinators would be to determine whether any transgene-specific RNAs are found in pollen, nectar and sap of GD743 and GS784, and if so, at what levels. However OSF did not provide this information.

The similar question of whether dsRNA from RNAi-based "plant incorporated pesticides" (PIPs) would be found in nectar, sap or pollen was recently addressed by the US EPA, in their white paper on RNAi impacts. They did not know whether such dsRNAs would occur in these plant parts, but concluded that it needed to be considered in risk assessments:

> It is unclear at this point whether a dsRNA PIP also would be incidentally present in root exudates, guttation droplets, or nectar, providing additional on-field sources of nontarget exposure. This may be affected by the characteristics of the dsRNA or the plant. Most PIPs target insect pests that chew plant tissue, and exposure considerations thus have been focused on nontarget organisms that eat solid plant tissues. However, some current work is focused on using dsRNA expressed *in planta* to control plant hoppers and other sucking insects (Pitino et al., 2011; Zha et al., 2011), and Mlotshwa et al. (2002) indicate that systemic gene silencing may occur as a result of movement of the silencing signal in phloem, although the exact mechanism is not confirmed. These studies suggest that these sources of exposure may also need to be considered in a risk assessment.
>
> Off-site movement of plant tissue is also possible. Depending on whether the dsRNA is expressed in the pollen, significant consideration may be given to the role pollen may play in off-field nontarget exposure. Some pollen is expected to move off site, and the amount and the distance moved will depend on the characteristics of the pollen (e.g., morphology, weight) and the mechanism relied upon for pollination (e.g., wind, pollinators, self-pollination). Animal-pollinated

[208] (Park 2012)
[209] (Park 2012; Watson et al. 2011; Holzschuh et al. 2012; Garibaldi et al. 2013; Adamson 2011)

> crops would be expected to play a smaller role in off-field nontarget exposure, and concerns in these cases would be more focused on the exposure of the pollinators themselves.
>
> …
>
> Nontarget organisms, particularly insects, may consume pollen produced by the crop plant, either while it is on the plant or once it has fallen within the crop field or outside it. Pollen may be consumed directly as food (or, in the case of bees, may be carried back to the hive or nest to feed to larvae), or may be consumed incidentally on food plants upon which the pollen falls. While pollen consumption may be more likely to be a means of exposure for insects, it is also possible for other animals (e.g., grazing vertebrates) to consume plants upon which pollen has fallen. Plant material that becomes dust, as described above, could also incidentally expose nontarget organisms to dsRNAs.[210]

In fact, recent work on small RNA movement in plants has shown that small RNAs move in phloem tissue along with assimilates, from "sources" to "sinks." Sources would be photosynthesizing leaves, sinks would be rapidly growing tissues and organs such as root tips, shoot tips, and developing flowers and seeds. In this way, gene silencing from small RNAs can become systemic in plants. Martienssen (2010) specifically addressed the movement of dsRNA into flowers, including pollen. Although he discusses movement of small RNAs normally produced during plant development, the principal applies to small RNAs derived from transgenes as well:

> Flowers are also well-known sink tissues. Translocation of small RNA into flowers could affect the inheritance of epigenetic alleles. Sperm cells are loaded with mobile 21-nt transposon-targeting siRNA [small interfering RNA] from the surrounding pollen grain, whereas the ovule and embryo sac have predominantly maternal 24-nt siRNA, which are required to silence transposons (13, 14) and inhibit germ cell fate in adjacent cells (13). Those small RNA derived from the plant body could find their way into ovules and pollen grains, which are physiological sinks, just like meristems and roots.

OSF did not report whether small RNAs derived from the transgene in GD743 and GS784 become systemic in phloem and throughout the plant, or not. If they do, then sap and guttation liquid would also be likely to contain these RNAs.

To determine systemic movement of small RNAs in apple trees, OSF should have looked for transgene specific RNAs in phloem. They also should have determined whether PPO genes were silenced in non-transgenic rootstocks of GD743 and GS784, indicating systemic silencing, assuming PPOs are expressed in apple roots.[211] The transgene in GD743 and GS784 may be expressed directly in nectar-secreting cells, or in pollen grains or tapetal cells that deposit

[210] (EPA RNAi white paper, boldface added)
[211] (Dunoyer et al. 2010; Martienssen 2010; Molnar et al. 2010)

materials onto pollen. OSF did not provide information on localized expression of the PGAS transgene in these cell types.

Assuming that pollinator insects such as honey bees are exposed to the novel small RNAs produced in GD743 and GS784 apple trees, there are several possible consequences. Genes in the insects could be silenced, their immune systems could be stimulated, RNAi machinery could be saturated and become unavailable for regulation of the insect's own genes, or there could be no effects. APHIS could not assess these impacts and possible consequences, because the petition lacked this necessary data.

b. Gene Silencing in Pollinators from Exposure to PGAS Transgene Products

It is possible that there are genes in pollinating insects that are related to the PPO genes silenced by the small RNAs from the PGAS transgene. Insects do have PPOs and other phenol oxidases (POs).[212] POs are important components of insect immune systems, among other functions,[213] so silencing might have sublethal and chronic effects. Recent studies are showing that identical sequences are not always required for this kind of silencing—some lesser degree of homology, particularly in certain regions of the dsRNA, can be enough.[214]

It is also possible that unrelated, off-target genes in pollinating insects could be affected. However,

> little information is available with which to estimate the likelihood of these effects resulting from unpredicted interactions of dsRNA with genes not intended for silencing and they may be significant factors affecting nontarget risk (Auer and Frederick 2009, Lundgren and Duan 2013).[215]

The short dsRNA sequences that silence genes are often identical or similar in sequence to regions of genomic DNA that are not related to the intended "target" of silencing, so might silence genes not PPO-related. This "off-target" gene silencing is thought to be quite common within the transgenic organisms themselves, but there have not been many studies of off-target silencing between species. It is likely to be species specific and RNA sequence specific, so will require case-by-case analysis.[216]

As a first step, the presence of sequences within the genome of the pollinating insect that match the small RNAs involved in silencing in GD743 and GS784 should be determined. The honey bee genome sequence is published, for example.[217] If there are homologous regions, these can be studied further to see if they are within expressed "target" or "off-target" genes, and then

[212] (González-Santoyo & Córdoba-Aguilar 2012)
[213] (González-Santoyo & Córdoba-Aguilar 2012; Moret & Schmid-Hempel 2001; Schmid et al. 2008; Zufelato et al. 2004)
[214] (see studies cited in: US EPA 2013)
[215] (US EPA 2013)
[216] (Lundgren & Duan 2013; US EPA 2013; Heinemann et al. 2013)
[217] (Weinstock et al. 2006)

experiments could be conducted to determine whether those genes are silenced in exposed honey bees. OSF failed to do these kinds of analyses or experiments.

Although searching for homologies in a pollinator's genome can be helpful, it is not a conclusive way to assess off-target silencing. Honey bees are subject to off-target effects from small RNAs generated during RNAi experiments, even when no homologous sequences are present in their genome, as recently reported in a study of a dsRNA derived from a green fluorescent protein (GFP) gene:

> Off-target effects are non-specific and caused by undesired base-pairing of non-target genes with small interfering RNA (siRNA) derived from double-stranded RNA (dsRNA). Off-target effects can be widespread and can alter expression of large numbers of genes, as previously reported in RNAi experiments involving plants, invertebrates, vertebrates, as well as honey bees.
>
> A green fluorescent protein (GFP)-derived dsRNA (dsRNA-GFP) has been used as an exogenous control for RNAi assays in several arthropod species, including . . . *Apis mellifera* [honey bee]. Its gene sequence is not found in the honey bee genome. Although dsRNA-GFP is not expected to trigger an RNAi response in treated bees, undesirable effects on gene expression, pupal pigmentation or developmental timing have been routinely observed. To better understand the molecular and phenotypic effects of dsRNA-GFP in honey bees and to evaluate its use as a control for RNAi studies, we examined the impact of dsRNA-GFP on global gene expression patterns in developing workers. The dsRNA-GFP was introduced using a non-invasive feeding protocol. We found that dsRNA-GFP causes large-scale changes in gene expression associated with multiple biological processes. Furthermore, dsRNA-GFP exposure tended to preferentially decrease, rather than increase, expression of genes compared to controls.[218]

In all, there were 1,461 genes that changed in expression in response to feeding dsRNA-GFP to honey bees, around ten percent of all honey bee genes. These genes control a wide range of important functions.

Nunes and colleagues were able to identify some of the down-regulated genes, and found that a few of them did have regions of homology with the dsRNA-GFP that were not found in the computer search of the whole genome sequence, but most did not. These genes only aligned perfectly for 8 to 11 nucleotides, and there were other regions of less complementarity. They cite other studies that showed off-target gene silencing caused by as little as 7 nucleotides of perfect matches between the siRNA and its targets.

While most observed effects involved down-regulation, up-regulation also occurred. Up-regulated genes, with no homology to the introduced RNA, were identified as having a variety of functions, including response to infection or stress, indicating an indirect response:

[218] (Nunes et al. 2013, p. 91–92, internal citations omitted)

> RNAi machinery plays an important role in the insect immune system by triggering antiviral responses in response to exogenous dsRNA viruses. Thus, it is plausible that dsRNA-GFP molecules are recognized as a viral infection, culminating in the activation of immune genes, RNAi systems, siRNA production and consequent off-target effects.[219]

They did not see many dsRNA-GFP effects in adult bees, showing that RNAi effects can be different in the same species based on stage of development.

Nunes and colleagues concluded that honey bee researchers should use a different control than dsRNA-GFP for their RNAi studies of gene function. Of course, the implications are much wider than that. This particular dsRNA had "substantial direct and indirect effects on transcript levels of genes associated with a variety of biological processes in developing honey bee workers." But these effects were not predicted ahead of time, even though they searched the honey bee genome for homologies that would indicate off-target silencing and found none. If there is no way to predict off-target effects with our current knowledge, then every dsRNA is a candidate for off-target and indirect effects in honey bees via diet, until proven otherwise by experiment. And the experiments need to be done over a fairly long period of time with immature as well as mature stages.

c. Immune System Stimulation

A new study the effects of dsRNA injected into bees has shown that dsRNA, regardless of sequence, ramps up the antiviral response in honey bees.[220] In invertebrates, as well as in plants, RNAi is an important part of defense against viruses. However, most studies of viral immunity in various insects have shown that virus-specific dsRNAs are required to trigger immune responses. In this case, all dsRNAs were able to stimulate immunity to the test virus.

Whether similar immune stimulation could occur from dsRNA in the honey bee diet is not known. Nor is it known whether immune stimulation in the absence of pathogens is a good or bad for honey bees in the long run, although such immune stimulation could represent a metabolic cost that decreases their fitness.[221]

d. Saturation of RNAi "Machinery"

Within organisms, RNAi is used to regulate some aspects of normal growth and development by turning down expression of particular genes in certain tissues at particular stages of development.[222] There is some evidence that the various proteins involved in these natural developmental RNAi processes may become "occupied" or saturated by exogenously introduced dsRNA.[223] Whether this would happen from honey bees consuming nectar, pollen or sap from GD743 and GS784 needs to be determined experimentally.

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[219] (Nunes et al. 2013, p. 93)
[220] (Flenniken & Andino 2013)
[221] (US EPA 2013)
[222] (Eldem et al. 2013)
[223] (Lundgren and Duan 2013)

e. Changes in PPOs

In addition to being exposed to the PGAS transgene product—the RNAs involved in RNAi—honey bees may consume PPO proteins. Kim et al. (2001) showed that apple flowers do contain mRNA from specific PPO genes, so probably also contain PPO protein, although where, when and how much is unknown.

Many kinds of proteins are deposited onto the surface of pollen grains as they mature, synthesized by tapetal cells that surround the developing pollen.[224] This could be a source of PPOs in mature pollen. (Synthesis of PPOs inside pollen itself is unlikely since pollen lacks plastids, and most PPOs are targeted to plastids.) Proteins are also secreted into nectar and sap, including enzymes,[225] and could include PPOs, if any are targeted to the secretory pathway as a *Populus* PPO is.[226] OSF did not provide information about where PPOs in general, or specific PPOs, are found in flowers or sap.

PPO proteins may affect honey bees indirectly, for example by inhibiting specific microbes that inhabit their guts. This would have to be determined by feeding studies. Lower PPOs in GD743 and GS784 flowers could thus potentially alter the composition of bee gut flora.

Or PPOs in flowers could influence the properties of flowers to make them more or less attractive to honey bees or other pollinators, as has been shown for nicotine levels,[227] and for other constituents of nectar.[228] Nectar is a very complex substance with many functions,[229] and often contains microbes that interact with pollinators, as well.[230] These microbes may be influenced by components of nectar,[231] such as antimicrobial molecules.[232]

Recently the microbiome of apple flowers as they develop has been described,[233] and it shows a remarkable succession of diverse microorganisms as flowers mature. Changes in PPO levels could influence the population structure of these microorganisms, with impacts on pollinators, as well as pests and pathogens. Without knowing whether, where or how much PPO is present in GD743 and GS784 flowers and sap compared to recipient apple trees, or the roles of PPOs in flowers, APHIS cannot begin to assess possible impacts to pollinators such as honey bees. That omission is contrary to APHIS's responsibilities under NEPA.

f. Unanticipated Changes to Flowers that Could Affect Pollinators

[224] (Lundgren 2009a; Lundgren 2009b)
[225] (Brandenburg et al. 2009; Heil 2011)
[226] (Tran & Constabel 2011)
[227] (Kessler et al. 2012)
[228] (Klatt et al. 2013)
[229] (Brandenburg et al. 2009; Heil 2011)
[230] (Aizenberg-Gershtein et al. 2013)
[231] (Fridman et al. 2012)
[232] (Sasu et al. 2010)
[233] (Shade et al. 2013)

In addition to changes in PPO levels, constituents of flowers could be altered by any of the unintended changes that often accompany genetic engineering of plants, discussed above (e.g., off-target RNAi effects, insertion site mutations, and tissue culture induced genetic and epigenetic changes). One of the only studies to examine changes in nectar after introducing a defense compound via genetic engineering is relevant here.[234]

Sweet orange trees were genetically engineered to express an antibacterial peptide, sarcotoxin IA, to see if this peptide protected the orange trees against bacterial citrus canker. Because orange blossoms attract a variety of pollinators, and nectar composition is important to the health of pollinators, these researchers wanted to know if there were differences in nectar constituents:

> Nectar varies in chemical composition and these characteristics reflect the type of pollinator (Baker and Baker 1983). Also, the nectar is not sterile. Insects or avian pollinators certainly transfer microorganisms from flower to flower and, for this reason, a chemical variation in the nectar composition could alter the microflora present in the nectar . . . Results so far suggest that transgenic plant impacts on pollinators will depend on a case-by-case analysis of the gene concerned and its expression in the parts of the plant ingested by insects (Malone and Pham-Delegue 2001). Considering this aspect, studies of the nectar chemical composition are important to access environmental impacts of transgenic plants.[235]

And they did, in fact, find differences that could impact pollinators:

> In summary, the floral nectar components of the conventional and transformed STX IA sweet orange trees were analyzed to study possible quantitative and qualitative modifications. The results showed that there are significant differences in the primary and secondary metabolites contents. These data suggest that the introduction of the gene responsible for the production of the antibacterial peptide sarcotoxin IA could modify the amino acids, triacylglycerides and purine alkaloids contents present in the sweet orange nectar. Such nectar with altered composition may affect floral visitors, such as nectar robbers, generalist pollinators and specialized pollinators. <u>This work shows that deeper investigations are required to enlarge our understanding of multispecies interactions, as plant–pollinator, plant–herbivore and plant–microorganisms and to evaluate the impact of gene insertions on the nectar composition of genetically modified plants</u>.[236]

Another recent study found differences in flowering behavior and pollinator attraction to a genetically engineered squash.[237] These virus-resistant squash were engineered, using RNAi

[234] (Sala Junior et al. 2008)
[235] (Sala Junior et al. 2008) p. 2.
[236] (Sala Junior et al. 2008 p. 6)
[237] (Prendeville & Pilson 2009)

technology, to resist *Zucchini yellow mosaic virus* and *Watermelon mosaic virus*. They did see pleiotropic effects of transgenic virus resistance that affected how often pollinators visited squash flowers, but were not able to determine which floral changes were responsible. They recommended that "future risk assessments examine pleiotropic effects of transgenes on native and introduced pollinators in different environments." However OSF did not provide data about pollinator behavior in GD743 and GS784 flowers, and APHIS did not assess it.

4. Generalizations About RNAi and PPO Impacts: Pollinators to Other Non-Target Species

More is known about the biology of honey bees and how they interact with flowers than is known about any other organism likely to be found in, on or around apple trees. But the study of how the RNAi system functions in honey bees is just beginning (Adee 2013). Every new publication changes our understanding, showing how RNAi is involved in the bee's immune system, how target and off-target effects depend or don't depend on particular sequences, specific responses at different life stages, chronic effects, and so forth. And when RNAi effects are compared across species, honey bees are similar to some insects and different from others, with no clear pattern for making predictions.

Other organisms that are likely to be exposed to the PGAS transgene products include mycorrhizae that are exposed via root tissues—to dsRNA made in the root itself on seedling trees or translocated to grafted roots,[238] if apples have systemic movement of small RNAs. Birds may be exposed via sap, buds, or even by eating insects that have eaten GD743 and GS784 tissues—there is some evidence that dsRNAs may be able to move up a food chain. Some organisms may be exposed through inhalation of pollen or plant dust, others via absorbing small RNAs as they invade spaces between cells. These are just a few of many examples.

APHIS focuses on the compositional quality of fruit <u>as determined for human consumption</u> *in assessing risk to wildlife*. This is wholly inadequate, not only because many organisms consume other parts of the tree whose compositions have not been studied, but also because the compositional profile does not include RNAs from the transgene that could have wide-ranging impacts on gene expression in the exposed organisms, and that have not been tested in any way.

Without being able to predict which dsRNA sequences will change the expression of which genes via what exposure routes in particular organisms, GD743 and GS748 will have to be tested experimentally for impacts on each important nontarget organism, a conclusion reached by other scientists who are grappling with how to assess plants engineered to silence genes via the RNAi pathway, including EPA.[239] APHIS's EA is thus fundamentally lacking, and consequently contrary to sound science.

[238] (Molnar et al. 2010)
[239] (Heinemann et al. 2013; Lundgren & Duan 2013; US EPA 2013)

5. Gene Flow: Behavior of Wild Pollinators and Wild Trees Not Adequately Assessed

The history of movement of transgenes from GE crops to non-GE varieties and wild relatives shows that if gene flow is possible, it will occur. Seeds from GE crops escape into the environment, from field tests and from commercial production, where they sometimes establish feral populations (populations that survive without human intervention) and become weeds. Also, several GE crops cross-pollinate related plants, causing widespread transgenic contamination—gene flow from GE crops to related conventional or organic cultivars or wild species.[240]

There are many examples of GE crops escaping into the wild where they become feral and of transgenic contamination by pollination of feral or wild relatives of GE crops, as summarized in our recent report on GE trees[241]:

- Roundup ready alfalfa has contaminated non-GE alfalfa seed stocks and hay in western North America.[242]

- Feral canola populations with GE glyphosate resistance have established around the world, wherever GE canola is grown and are crossing with each other to produce transgene combinations not found in commercial varieties. Additionally, GE canola has the potential to cross with several weedy relatives, creating new weed problems.[243]

- GE glyphosate-resistant bentgrass has spread by seed escape miles beyond field test sites in eastern Oregon, and has even formed hybrids by cross-pollination with wild species in different genera, spreading the transgene to other wild grasses.[244] These glyphosate-resistant wild grasses are becoming a serious weed problem along irrigation ditches and are difficult to control with standard herbicide regimes.

- Transgenes have also contaminated wild cotton populations in Mexico,[245] the place of origin of the major cotton species grown throughout the world today. Within fifteen years of growing GE cotton in Mexico, transgenic contamination has spread hundreds of miles through wild cotton populations; the wild cotton includes almost all the traits from commercial GE cotton. These wild cotton plants have crossed with each other, resulting in new combinations of herbicide-, insect-, and antibiotic-resistance traits. In other words, these cotton plants have "stacked" transgenes into novel combinations without human intervention.

- In Hawaii, there is widespread transgenic contamination of feral papaya found in abandoned fields, roadsides, and other areas due to seed escape and cross-pollination.

[240] (reviewed in: CFS 2013b)
[241] (CFS 2013a)
[242] (Jenkins 2007).
[243] (CFS 2013b)
[244] (Snow 2012; Zapiola & Mallory-Smith 2012)
[245] (Wegier et al. 2011)

There is also transgenic contamination of non-GE cultivated papaya (Bondera and Query 2006).

- In Hong Kong, gardeners have planted seeds from Chinese and Hawaiian GE papayas in their back yards, from fruits purchased in markets, showing how gene flow can occur across wide geographical regions via trade (USDA Foreign Agriculture Service 2013).

In addition to potential ecological impacts when GE crops escape from the farm, transgenic contamination of cultivated non-GE crops can result in significant economic harm for farmers and rural communities, as recently demonstrated with rice, and more recently with wheat.[246] In addition, if organic crops are tainted with GE traits, farmers can lose their certification, their customers, their markets, and their reputations.

From experience to date with GE crops, it is clearly very difficult for GE crops to co-exist with other types of agriculture and with other ecosystems. Transgenes from some GE crops pose contamination risks for non-GE farmers, reducing their market opportunities. Although growers can take steps towards reducing the risk of transgenic contamination, the vagaries of weather, uncertainties of pollinator behavior, unknown locations of feral plants, and ever-present human error conspire to ensure gene flow regardless of precautionary measures.[247] In addition, wild relatives of GE crops can be at risk of transgenic contamination with negative impacts for the environment.

Because of their special biological characteristics—long lives, numerous flowers, close kinship with wild relatives[248]—GE trees pose an even greater risk of escape and transgenic contamination than do most crops, with potential to cause more serious environmental consequences in forests as well as significant economic harm to fruit growers.

Gene flow from GD743 and GS748 apple trees—the movement of the PPO-silencing transgene out of GD743 and GS748 apple trees and into wild or cultivated relatives of apple (crabapples), or into different apple varieties—will occur if GD743 and GS748 are deregulated. Apple trees have many flowers, each with copious pollen that is moved by pollinating insects, some of which go long distances between trees.[249] Seeds with transgenes will occasionally occur in fruits of non-GE trees as a result of pollination. Also, seeds from GD743 and GS748 fruits themselves will end up far from the parent trees (Petition, p. 103), wherever fresh fruits are sold

[246] (CFS 2013)
[247] (Marvier & Van Acker 2005)
[248] (Miller & Gross 2011)
[249] See e.g., Harris A & Beasley D. 2011. "Bayer Agrees to Pay $750 Million to End Lawsuits over Gene-Modified Rice." *Bloomberg*, July 1. http:// www.bloomberg.com/news/2011-07-01/bayer-to-pay-750-million-to-end-lawsuits-over-genetically-modified-rice.html. (Reporting that the multinational chemical company Bayer AG will pay $750 million to approximately 11,000 U.S. rice farmers whose rice harvests were contaminated by a Bayer-developed experimental GE rice in 2006); US Government Accountability Office. 2008. "Genetically Engineering Crops: Agencies Are Proposing Changes to Improve Oversight, but Could Take Additional Steps to Enhance Coordination and Monitoring." www.gao.gov/new.items/d0960.pdf. (Analyzing several major transgenic contamination incidents from the past decade and concluding that "the ease with which genetic material from crops can be spread makes future releases likely").

and consumed by people, or moved by other animals. Some seeds will germinate and grow into mature trees that flower and cross with compatible trees, transferring the PGAS transgene to new populations.

APHIS has concluded that successful crossing between GD743 and GS784 and crabapples or apples is possible, but not likely to increase weediness or fitness in crabapples or wild apples, or to be propagated in cultivated apples because they are not grown from seeds, (dPPRA at 13), and so they conclude gene flow will have no significant impacts on weediness, their only concern. However as explained *supra*, APHIS has no data to allow it to make sound conclusions about weediness or fitness of GD743 and GS748, so the agency cannot legitimately extrapolate impacts of the transgene to hybrids.

OSF focuses most of its discussion of gene flow on movement of pollen by honey bees, ignoring evidence that even when honey bees are present, wild pollinators often are more effective at accomplishing pollination in fruit orchards,[250] depending on the surrounding landscape.[251] In order to assess likelihood of gene flow, specific characteristics of wild pollinators must be taken into account. OSF and APHIS failed to so account for them.

OSF did not intentionally engineer GD743 and GS748 to mitigate gene flow. The trees do not have reduced fertility, such as lack of pollen. In other words, GD743 and GS748 flowers are likely to be fully functional, with viable pollen that can participate in mating with inter-fertile species, just as non-engineered pollen can.

OSF does recognize the need to mitigate gene flow, and plans to provide "stewardship guidelines as part of their licensing requirements" that include measures designed to minimize transfer of pollen from orchards of GD743 and GS784 to orchards or parts of orchards with other apple cultivars. They will ask growers not to transfer honey bee hives to another apple block after pollination is complete. Also, obligations will purportedly include providing "suitable isolation distances" between GD743 and GS784 trees and non-GE blocks of trees, with distances greater for organic apples. OSF plans to recommend border rows or other gene flow mitigation measures "as required."[252] However, these mitigation measures do not take into account the important role of wild pollinators, and thus are unlikely to stop transgenic contamination.

In addition to gene flow via pollen, the movement of seeds must be assessed, which APHIS also fails to do. Most seeds in fruits of GD743 and GS784 will contain a transgene, so most trees that grow from seeds will be transgenic:

> It is important to note that because apple trees are an outcrossing species, any apple seeds that are produced will be hybrids and would have characteristics of both parents. In the case of GD743 and GS784 apples, a portion of the seeds would carry the transgene responsible for the non-browning trait. GD743 carries

[250] Center for Food Safety. 2013. "Class Action Lawsuit Filed Against Monsanto." http://www.centerforfoodsafety.org/press-releases/2284/class-action-lawsuit-filed-against-monsanto
[251] (Watson et al. 2011)
[252] (Petition, p. 105).

> two copies of the transgene while GS784 carries four copies (OSF, 2012). Therefore three quarters of the GD743 seeds would carry at least one copy of the transgene and 15/16 of the seeds of GS784 would carry at least one copy of the transgene.[253]

In assessing impacts of gene flow via movement of seeds, APHIS must, but failed to, consider impacts in any region of the country or world where fresh GD743 and GS748 apples could be sold, or taken by consumers. That would be at least the entire U.S. if APHIS grants the Petition. Wherever apples are consumed, it is likely that apple cores with seeds will be discarded, and that some seeds will germinate and grow into trees that flower and can cross with local crabapples and apples. Instead, in the EA, APHIS states that it cabined its scope to the impacts of GD743 and GS748 in apple growing areas of the US:

> Although the preferred alternative would allow for new plantings of GD743 and GS784 anywhere in the U.S., APHIS will limit the environmental analysis to those areas that currently support apple production. To determine areas of apple production, APHIS used data from the USDA-NASS 2011 Noncitrus Fruits and Nuts Report (USDA-NASS, 2012b).[254]

APHIS's narrow geographic scope fails to account for important risks. Further, apple seedling trees are more likely to thrive in some parts of the country,[255] thus impacts from gene flow via seeds are likely to differ by region. APHIS did not take these regional differences in seedling apple success into account in its environmental assessment, which is a fundamental omission that renders its analysis arbitrary and capricious.

D. APHIS Fails to Adequately Consider Impacts to Public Health: The Agency Lacks Evidence to Support Its Conclusions About Nutritional Composition and Entirely Failed to Consider Possible Changes to Pathogen Resistance.

GD743 and GS784, which were designed for human consumption, have the potential to significantly impact human health. The vast majority of GE crops are not eaten directly by the public, but instead fed to animals, or used in highly processed foods. As noted above, public health issues may be significant environmental impacts requiring the preparation of an EIS. CEQ regulations explain what factors may be significant effects on the human environment and one such factor is "[t]he degree to which the proposed action affects public health or safety."[256] The presence of one or more of the factors in 40 C.F.R. section 1508.27 may be sufficient to require the preparation of an EIS.[257] Accordingly, APHIS's analysis must address any potential human health or safety risks and determine whether those human health and safety impacts may

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[253] (EA, p. 31)
[254] (EA, p. 30)
[255] (Petition, Biology of Apple, p. 9), and
[256] 40 C.F.R. § 1508.27(b)(2).
[257] *Nat'l Parks & Conservation Ass'n v. Babbitt*, 241 F.3d 722, 731 (9th Cir. 2001); *Pub. Serv. Co. of Colo. v. Andrus*, 825 F.Supp. 1483, 1495 (D. Idaho 1993).

be significant. If those impacts are found not to be significant, there must be a convincing statement of reasons.[258]

Here, APHIS lacks evidence to support its conclusions about nutritional composition and entirely failed to consider possible changes to pathogen resistance. Specifically, APHIS relied solely on incomplete and inadequate data from OSF to conclude that GD743 and GS784 are "nutritionally equivalent" to their respective controls. Further, APHIS failed to undertake or consider tests to determine whether these GE apples are more susceptible to infection by pathogens that cause foodborne illnesses; for example, by challenging GD743 and GS784 with common fruit pathogens and then monitoring infection during realistic storage scenarios Accordingly, APHIS's conclusions about nutritional composition and safety are arbitrary and capricious.

1. Nutritional Composition

APHIS evaluated the data from OSF on the nutrient composition of GD743 and GS784 apples compared to the recipient apple cultivars, and concluded that they are "nutritionally equivalent to their respective controls GD [Golden Delicious] and GS [Granny Smith] and fall within or close to the range for NDB09003 USDA standard" derived from a composite sample of fruits of common apple varieties. APHIS relies on this conclusion throughout its assessments of health impacts.[259]

However, APHIS's conclusion of nutritional and compositional equivalence lacks evidential support, for several reasons:

a. OSF failed to assess the nutritional composition of whole apples, but rather only apple slices, and made illegitimate comparisons of nutrient levels in whole and sliced apples.
b. OSF's claim that vitamin C levels are higher in GE apples is unfounded and likely false.
c. OSF used inappropriate reference data to characterize the range of variation in apple nutrient levels.
d. OSF assesses far too limited an array of nutrients to give an adequate nutritional profile of its GE apples.
e. OSF makes little or no attempt to control for numerous environmental and other factors in growing apple trees and selecting fruit for testing that are known to influence nutrient levels, rending its results suspect.
f. OSF's failure to report on the methods used to assess nutrients also renders its results suspect, especially in the case of Vitamin C.
g. OSF did not test for unintended production of harmful compounds in GE apples due to the genetic manipulation and tissue culture techniques used in their development.

APHIS's reliance on the lack of data and its expectations for no impacts is improper because NEPA requires it to take a hard look at environmental impacts itself, not assume that if any impacts were to exist they would be disclosed by the applicant.

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[258] *Nat'l Parks & Conservation Ass'n*, 241 F.3d at 731.
[259] (e.g., dPPRA at 7–8; EA at 49–50, 61–62).

a. Apple Slices Rather than Whole Apples

Instead of sending whole, fresh fruits for nutrient analysis, OSF sliced the apples first, put the slices on ice, and sent those to an outside lab:

> Mature fruit was harvested in the fall of 2009 from the Washington and New York field trials. For each event (GD743 and GS784) and control (GD and GS), fruit was harvested from 3 trees in Washington and 3 trees in New York (n = 6). Golden Delicious apples were harvested approximately one month prior to Granny Smith in both Washington and New York, and were stored at 2°C. Immediately after the Granny Smith harvest, all apples were sampled and sent for proximate and phenolic analysis. Composite samples were created by combining one-quarter slices from four apples from one tree, providing, in total, one whole apple equivalent. Samples were cut, cored and placed in a Ziploc™ bag. The samples were packed in a cooler on wet ice and sent overnight to Exova for the proximate analysis and Brunswick Laboratories for the ORAC and total phenolics analysis.[260]

The length of time between slicing of the apples and their processing for analysis was reported to be "as long as 24 hours" (Petition, p. 90), and during that time the control apple slices exhibited enzymatic browning, presumably consuming vitamin C and phenolics in the process (or perhaps not, see next section), so that by the time nutrients were measured, GD743 and GS748 appeared to differ from GD and GS in these compounds.

Whether the whole apples also differed in vitamin C, phenols, or any other nutrients can only be guessed because whole apples were not compared. OSF says that the "[e]vidence provided here is consistent with the concept that Arctic™ Apple cultivars GS743 and GS784 are nutritionally equivalent with their parent cultivars, prior to slicing."[261] Nutritional comparisons should be a straightforward procedure providing clear results, not something that requires guesswork. OSF and APHIS used an improper baseline for comparison here. APHIS must require data from OSF on fresh, whole fruits that provides meaningful comparisons between GS743 and GS784 and controls.

The nutritional profile of whole Arctic Apples is also required because if deregulated, they could enter commerce and be consumed as whole apples as well as in sliced form.

b. No Basis for Claim that GE Apples or Apple Slices Are Higher in Vitamin C Content

OSF claims that its GE apple slices have higher vitamin C content than non-GE control apple slices.

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[260] (Petition, p. 81)
[261] (Petition, p. 90, emphasis added).

> "Apple events GD743 and GS784 had significantly higher ORAC (Table 37), total phenolics (Table 38), and vitamin C (Table 39) than the control cultivars GD and GS." (Petition, p. 88)

As part of its unfounded claims that this GE apple is "beneficial," APHIS repeats this claim in several passages: "GD743 and GS784 had higher Vitamin C, ORAC and total phenolics as compared to the GD and GS controls." "Moreover, the resulting elevated Vitamin C content and increased total phenolics after slicing contributes to an increase in chemical compounds with antioxidant capacity for the GD743 and GS783 events…" These claims are unfounded. Vitamin C is found in two different forms in apple: reduced ascorbic acid (RAA) and dehydroascorbic acid (DHA). Vitamin C content (also called "total ascorbic acid") is the sum of AA and DHA.[262]

The putative "vitamin C" levels reported in the petition are actually measurements of just one component (RAA), and exclude the second component (DHA). Despite this fact, OSF falsely and repeatedly presents the RAA results as if they represented the total vitamin C content of the GE and control apple slices. RAA levels are also routinely mischaracterized as vitamin C elsewhere in the petition. APHIS conclusion is contrary to the evidence.

PPO enzymes convert RAA to DHA in the presence of oxygen (e.g., upon slicing). Because PPO is absent or at very low levels in GE apples, one would expect little or no RAA to be converted to DHA upon slicing. However, this is not the case for control apple slices, in which some unknown portion of the RAA is converted to DHA by virtue of the presence of PPO. By mischaracterizing RAA as total vitamin C, OSF has created the false and misleading impression that natural apples slices have lower vitamin C content.

Testing methods are available that accurately measure the total vitamin C content of apples (RRA+DHA),[263] with high-pressure liquid chromatography (HPLC) with UV or electrochemical detection regarded as one of the most reliable,[264] but OSF chose not to utilize them. APHIS must ensure that proper tests are conducted to determine the total vitamin C content of: 1) GD743 and GS784 applies, in whole and in sliced form; and 2) GD and GS control apples, in whole and in sliced form.

c. Inappropriate Reference Data

OSF compares the compositional results for both the GE and control apple slices to USDA data on apple nutrients:

> The USDA nutrient values for apples, raw with skin (NDB09003) are based on data for Red Delicious, Golden Delicious, Gala, Granny Smith, and Fuji cultivars of apple. These are the five most popular apple cultivars in the US, representing almost 70% of US production (Table 46). Data is compiled from a variety of sources (USDA, 2009). It is not possible from the data provided, to determine the

[262] (Yu, undated)
[263] (e.g., Gillespie & Ainsworth 2007, Yu undated)
[264] (Planchon et al. 2004)

> specific growing region the apples are from, or any specifics regarding the individual apple samples or the contribution of the different apple cultivars to the final values provided by the USDA. It is obvious however, that only a limited number of apple samples are included in the final numbers provided. As such, this data provides an approximation of nutrient composition that might be expected in the most commonly consumed apple cultivars grown under a variety of conditions.[265]

These USDA reference values are inappropriate for comparison purposes in two respects. First, the USDA data apparently apply to whole apples ("apples, raw with skin") rather than apple slices (slices exposed for up to 24 hours prior to testing). Because exposure of the apple flesh upon slicing can affect the levels of Vitamin C, phenolics and perhaps other nutrients, comparisons between whole apples and apple slices are illegitimate and may give rise to false inferences. For instance, OSF uses this illegitimate comparison as the basis for the following statement:

> ORAC, total phenolic and vitamin C levels in events GD743 and GS784 fell within, or very close to, the published range for apple (NDB09003) [USDA values for whole apples]. This indicates that Arctic Apple cultivars GD743 and GS784 are, in all aspects (proximates, phenolic antioxidants and vitamin C), nutritionally equivalent to the published norms for apple. By contrast, it is the GD and GS control values that fell well below the minimum values established for apple.[266]

One cannot conclude that either Arctic apple cultivars are nutritionally equivalent to, or that control apples are less nutritious than, the "published norms for apple" when the nutritional comparison that is made is between apple slices and whole apples. OSF here commits the basic fallacy of comparing "apples and oranges" (substitute "apple slices" for "oranges").

Secondly, OSF inappropriately constructs its nutrient reference ranges and averages from pooled data for five different apple cultivars (Red Delicious, Golden Delicious, Gala, Granny Smith, and Fuji). Because nutrient levels vary by cultivar,[267] OSF should have utilized USDA datasets specific to Golden Delicious and Granny Smith apples for reference purposes.[268] In addition, as noted above OSF needs to generate nutritional data for whole apples to enable meaningful comparison to the reference values for Golden Delicious and Granny Smith. APHIS's health assessment based on the OSF submission is contrary to sound science.

d. Too Few Nutrients/Components Assessed

OSF's nutritional profile is comprised of data on just 12 apple components: fat, protein,

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[265] (Petition, p. 81)
[266] (Petition, p. 88)
[267] (Planchon et al. 2004)
[268] For Golden Delicious, see http://ndb.nal.usda.gov/ndb/foods/show/2528; for Granny Smith, http://ndb.nal.usda.gov/ndb/foods/show/2529?qlookup=Apples%2C+raw%2C+granny+smith%2C+with+skin&fg=&format=&man=&lfacet=&max=25&new=1/

moisture, ash, carbohydrates, calories, sugar profile, dietary fiber, potassium, Vitamin C, oxygen radical absorbance capacity (ORAC), and phenolics. OSF arbitrarily provides no explanation as to why it chose to measure these components, or exclude others. USDA's Nutrient Database contains a much fuller compositional characterization of Golden Delicious and Granny Smith apples that includes many additional nutrients: calcium, iron, magnesium, phosphorus, sodium, zinc, thiamine, riboflavin, niacin, Vitamin B-6, Vitamin A, Vitamin E, and Vitamin K.

For an accurate scientific assessment, OSF must complete a much fuller nutritional/compositional assessment that includes at least these additional nutrients. While apples generally contribute small amounts of these nutrients in a typical diet, they are not inconsequential. For instance, a single apple typically supplies five percent of the daily value of Vitamin K; two to three and a half percent of Vitamins A, E, B1, B2, and B6; and two to three percent of the minerals copper, magnesium, manganese and phosphorus.[269] For comparison's sake, OSF did measure potassium levels, and a single apple contributes a similar amount of potassium to the typical diet (five to six percent of daily value, see last footnote) as the above-listed nutrients that were not assessed. Apples' contribution of these nutrients would be correspondingly greater in heavy apple consumers.

The genetic manipulations carried out to develop Arctic apples are not intended to alter nutrient composition, yet nutritional profiling is a standard procedure for GE food crops, and OSF had 12 components/nutrients assayed here. The implicit rationale for OSF's nutritional profiling is that unintended effects of the genetic engineering process or RNAi might have adversely altered the nutrition of GE apples. The same rationale applies to other nutrients, including those listed above, which must therefore be tested to provide a fuller test of the hypothesis that GE apples are nutritionally equivalent to control apples. APHIS's reliance on such OSF omissions is arbitrary and capricious and contrary to sound science.

e. Controls for Environmental Influences and Fruit Selection

Nutrient content in fruit can vary dramatically depending on numerous factors, and OSF's one-paragraph description of its protocol (Petition, p. 81, reproduced above under "Apple Slices Rather Than Whole Apples") provides no assurance that it made any attempt to control for such factors. Speaking of Vitamin C content, Pissard et al. (2013) state:

> Its content in fruits can be influenced by various factors, such as genotypic differences, pre-harvest climatic conditions, cultural practices, maturity, harvesting methods and post-harvest procedures.[18] The variability of vitamin C levels among fruits of the same cultivar and between years can be very high.[19] The vitamin C content in apples also varied according to fruit position in trees, fruit size and fruit skin colour, and varied greatly in content, from 3 to 26 mg 100 g−1 , depending on the cultivar.[20] The same authors also demonstrated that some old cultivars contain several times more ascorbic acid than new commercial cultivars (emphasis added)

[269] See nutrient analysis at http://www.whfoods.com/genpage.php?tname=nutrientprofile&dbid=94, last visited 12/12/13.

Did OSF control for fruit position in trees, fruit size and fruit skin color? All have been shown to influence vitamin C levels.[270] If not, then disparities in the fruit selection process for GE versus control apples could skew results for Vitamin C and perhaps other nutrients. OSF's sample sizes also appear much too small to give statistically meaningful results, especially in view of this variability. OSF should provide much fuller information on its methodology here, and if it was inadequate to control for environmental and fruit selection factors that could influence nutrient composition, the trials should be repeated to achieve more meaningful results.

f. Lack of Methodology for Nutrient Measurement

OSF provides almost no description of the testing methods or methodology used to assess nutrient levels. We are told only that "Exova" conducted the proximate analysis and Brunswick Laboratories the ORAC and total phenolics measurements (Petition, p. 81). A variety of testing methods are available for proximate and specific nutrient tests, yet OSF fails to provide even the names of the specific procedures that its contract labs utilized. As discussed above, the use of a substandard assay for vitamin C content (one that measured only the RAA but not the DHA component of vitamin C) was exploited by OSF to make unfounded and likely false claims as to the putative nutritional superiority of GE versus non-GE apple slices. There may be similar issues with other nutrients. Without specification of test methods and methodology, there is no way for other scientists to repeat, and thus check the accuracy, of scientific findings. APHIS should require that OSF submit complete methodology for the nutrient and compositional testing reported in the petition. Where the methods used were deficient (e.g., vitamin C), the relevant tests must be repeated using accepted procedures.

g. Adverse Alterations Affecting Food Safety

The rationale for nutritional profiling of GE crops such as the Arctic Apple is that unintended effects occur more frequently with genetic engineering than with traditional cross-breeding, potentially resulting in lower nutritional value. By the same token, GE and RNAi could trigger production of harmful substances. For example, apple genes not normally expressed in fruits could be activated by proximity to inserted transgenes, from tissue culture induced changes, or from off-target RNAi effects, as we discuss in these comments. Such changes could result in production of anti-nutrients, toxins, or allergens that are found in other parts of the tree but not normally produced in the fruit, and are not measured in compositional assays.

The unpredictability of such unintended effects limits the usefulness of the standard compositional assessment procedures used with GE crops, which somewhat arbitrarily "target" a very limited range of components for assessment:

[270] (Planchon et al 2004)

> unexpected changes are merely identified by chance. The targeted approach has severe limitations with respect to unknown anti-nutrients and natural toxins . . .[271]

The inadequacies of this approach make it necessary to apply more sophisticated, "non-targeted" profiling methods. Profiling methods currently available or under development include DNA expression analysis, proteomics, two-dimensional gel electrophoresis, and chemical fingerprinting. These techniques—used singly or in combination—permit simultaneous, small-scale, quantitative analysis of a large array of plant components, including messenger RNA, proteins and metabolites.[272]

APHIS should demand compositional profiling of GD743 and GS784 to provide fuller information on their composition, both nutrient levels as well as potential antinutrients, toxins or allergens. Such profiling techniques should be accompanied by long-term animal feeding trials with GD743 and GS784 apples.[273] The need for more comprehensive assessments of this sort is magnified by the fact that these GE apples would be consumed in whole or minimally processed form rather than (as most GE crops are) fed to animals or utilized as ingredients in processed foods. Failure to undertake, consider and analyze this compositional assessment would be arbitrary and capricious and contrary to sound science. Failing to provide the necessary comprehensive nutritional profiling is contrary to the mandates of NEPA.

2. Changes in Susceptibilities of Apple Slices to Diseases: No Tests Reported

The ability of GS743 and GS784 apple fruits to sustain damage without showing the normal browning associated with cellular injury is being marketed by OSF as a boon to the minimally processed apple industry. Apple slices from GS743 and GS784 can presumably be sliced and then stored in plastic bags for weeks without browning, whereas normal apples begin to turn brown rapidly after slicing and need to be treated specially to inhibit browning and retain appeal during storage.[274]

Lack of browning in GS743 and GS784 is a consequence of lower levels of at least some apple PPOs that could be important for resistance to pathogens (see our discussion of PPO functions in the Background section). In addition, differences in nutrient content of GS743 and GS784 apple slices could result in changes in growth of specific pathogens during storage.[275] And even if there are no differences in growth of pathogens, it is possible that lack of browning could mask the presence of pathogens, making them more difficult to detect visually.[276]

Apple slices are prone to infection by bacteria and fungi because the cut surfaces exude nutrients, and, without the protective covering of the apple skin, fruit flesh is more exposed to

[271] (Kuiper et al. 2001)
[272] (Kuiper et al. 2001)
[273] (Freese and Shubert 2004)
[274] (Corbo et al. 2010)
[275] (Cocci et al. 2006; Francis et al. 2012)
[276] (Ragaert et al. 2007)

them.[277] Some bacteria and fungi that grow on apple slices are harmless, but others can cause serious foodborne illnesses in humans.[278] Sliced apples have been subject to several high profile recalls in the last few years.[279]

Some microorganisms that infect apple slices might cause enzymatic browning during the infection process, and if so, GS743 and GS784 would have different phenotypes in response to such infections: the infections would not produce a color change in GS743 and GS784 that would be present under the same circumstances in the recipient apple cultivars. For example:

> One visual defect occurring during storage of some minimally processed vegetables is enzymatic browning . . . [caused in part by PPO], which converts these phenols into quinones, which rapidly condense to produce relatively insoluble brown polymers (melanins). Bruised or ruptured cells in damaged areas of tissue result in cellular enzymes such as . . . PPO coming into contact with substrates, with subsequent phenol oxidation and eventually melanin formation. Moreover, wounds induce changes in phenolic compound composition such as increases in chlorogenic acid, dicaffeoyl tartaric acid, and isochlorogenic acid. Enzymatic browning can be delayed by modified atmospheres whether or not in combination with anti-browning agents. Although, these studies did not involve microbiological counts, it should be noted that pectinolytic micro-organisms could break down cell walls resulting in stress-related exposure of enzymes and substrates, which also could lead to enzymatic browning.[280]

Thus in order for APHIS to assess these possible changes in resistance of GS743 and GS784 to pathogens, and the significant risks from possible masking of infections, OSF must supply, and APHIS must consider, data from appropriate studies where apple slices are challenged with common pathogens of fruit and then monitored during realistic storage scenarios. Results of such tests are not reported by OSF in its Petition or by APHIS in the assessment documents. Approval absent analysis of these risks would be arbitrary and capricious and contrary to sound science.

3. dsRNA and Humans: No Tests Reported

Impacts of the gene products of the PGAS transgene in GD743 and GS784 apple fruits on humans and other mammals must be assessed by APHIS. There is some evidence that small RNAs from plants can transfer from food to humans and regulate human gene expression.[281]

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[277] (Francis et al. 2012; Bhagwat 2004)
[278] (Corbo et al. 2010)
[279] For example, minimally processed apples have been recalled for possible *Salmonella* or *Listeria* contamination in all of the last 3 years, including some that involved thousands of pounds of red and green apple slices in small bags (http://www.recallowl.com/Food+Recalls/Food/Freshway+Foods+Voluntarily+Recalls+Out-of-Date+Sliced+Apples+Because+of+Possible+Health+Risk), and hundreds of thousands of cases and "individually distributed units" of apple slices (http://www.fda.gov/safety/recalls/ucm315249.htm).

[280] (Ragaert et al. 2007)
[281] (Zhang et al. 2011; Vaucheret & Chupeau 2011)

Although these studies need to be repeated and extended, they should not be ignored in risk assessments,[282] as summarized by Heinemann and colleagues:

> While some GMOs have been designed to make new dsRNA molecules, in other GMOs such molecules may occur as a side-effect of the genetic engineering process. Still others may make naturally occurring dsRNA molecules in higher or lower quantities than before. Some dsRNA molecules can have profound physiological effects on the organism that makes them. Physiological effects are the intended outcomes of exposure to dsRNA incorporated into food sources for invertebrates; biopesticides and other topically applied products, and could be the cause of off-target effects and adverse effects in nontarget organisms. **"**A daunting outcome is raised, that each [dsRNA] formulation might have its own risks**"** (p. 514 Aronin, 2006).
>
> Two separate studies have now provided evidence for miRNAs of plant origin in the circulatory system or organs of humans or mammals (Zhang et al., 2012a, 2012b). In addition, there is experimental evidence demonstrating that some dsRNA molecules can be transmitted through food or other means and can affect those organisms through alterations in gene expression (Zhang et al., 2012a).
>
> Production of intended dsRNA molecules may also have off-target effects due to silencing genes other than those intended. Unanticipated off-target adverse effects can be difficult to detect and they are not possible to reliably predict using bioinformatics techniques.
>
> Regulatory bodies are not adequately assessing the risks of dsRNA producing GM products.
>
> As a result, we recommend a process to properly assess the safety of dsRNA-producing GM organisms before they are released or commercialized (Fig. 3). This process includes the following: (1) bioinformatics to identify any likely, unintended targets of the dsRNA in humans and other key organisms; (2) experimental procedures that would identify all new intended and unintended dsRNA molecules in the GM product; (3) testing animal and human cells in tissue culture for a response to intended and unintended dsRNAs from the product; (4) long-term testing on animals; and possibly (5) clinical trials on human volunteers.[283]

Neither APHIS nor OSF even mentioned the possibility of any risks to humans or mammals of dsRNA from the PGAS transgene in apples, much less performed any of the steps required to assess such risks.

E. APHIS Fails to Consult with Tribes

[282] (Heinemann et al. 2013; Hirschi 2012; US EPA 2013, Martineau 2013)
[283] (Henemann et al. 2013, Summary)

Native American tribes occupy a unique legal status, with certain rights established in the U.S. Constitution, treaties, Executive Orders, and by the judiciary. The federal government's trust obligation to tribes requires it to act in the best interest of Native American tribes and individuals. In addition, tribes have the right to government-to-government consultation with the federal government. This requirement is set forth in Executive Order 13175, Consultation and Coordination with Indian Tribal Governments (EO 13175).[284] Section 5(a) of EO 13175 states that "[e]ach agency shall have an accountable process to ensure meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications."

APHIS has made no showing in this EA to indicate that it has considered the potential impacts of this action upon tribes or whether it has sought out any input from tribal officials.

F. APHIS Failed to Properly Consider and Disclose Its Obligations to Migratory Birds

APHIS also fails to properly consider and disclose its obligations to migratory birds. The EA notes that Executive Order 13186, "Responsibilities of Federal Agencies to Protect Migratory Birds," requires federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations to develop and implement, within two years, a Memorandum of Understanding with FWS to promote the conservation of migratory bird populations.[285] Rather than properly studying this matter to determine whether deregulation of GD743 and GS784 apple trees would have measureable negative effects on migratory bird populations, APHIS summarily dismisses potential impacts. It finds it "unlikely that a determination of nonregulated status of GD743 and GS784 apples would have a negative effect on migratory bird populations."[286] This finding is based on the APHIS's belief that GD743 and GS784 apples are "not *expected* to be allergenic, toxic, or pathogenic in *mammals*."[287] APHIS's expectation is based on data submitted by the applicant that has "shown no difference in compositional and nutritional quality of these apples compared to other conventional apples."[288]

This finding is fundamentally flawed for three reasons. First, it wrongly assumes that if impacts to migratory birds were to exist, they would be spelled out in the data submitted by the applicant. APHIS's reliance on the lack of data and its expectations for no impacts is improper because NEPA requires it to take a hard look at environmental impacts itself, not assume that if any impacts were to exist they would be disclosed by the applicant. Secondly, APHIS bases its determination that GD743 and GS784 will not negatively impact migratory bird populations on

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[284]Executive Order No. 13,175, 65 *Fed. Reg.* 67249 (November 9, 2000). EO 13175 expanded the breadth of tribal consultation to "ensure the meaningful and timely input by tribal officials in the development of regulatory policies [rules, policies, and guidance] that have tribal implications." Tribal implications are defined as having substantial direct effects on one or more tribes, on the relationship between the federal government and tribes, or on the distribution of power and responsibilities between the federal government and tribes. Among other things, EO 13175 requires federal agencies to respect tribal self-government and sovereignty, honor tribal treaty and other rights, and strive to meet responsibilities arising from the unique relationship between the federal government and tribes.

[285] EA at 62.

[286] *Id.* at 63.

[287] *Id.* (emphasis added).

[288] *Id.*

its expectations regarding impacts to mammals. Birds are not mammals. APHIS's failure to actually consider impacts to birds prior to reaching a no effects finding is arbitrary and capricious and contrary to sound science. Analysis of impacts to migratory birds requires separate study from analysis of impacts to mammals, and this analysis must consider the wide array of habitat requirements of migratory birds. Finally, USDA's finding is based on the applicant's data that purportedly shows "no difference in compositional and nutritional quality of these apples."[289] As APHIS notes, migratory birds can be found in apple orchards feeding not just on the apples but also on various parts of the trees, nesting in their limbs and grassy understories, and foraging for insects and seeds in and around them.[290] Thus, it is arbitrary and capricious for APHIS to only consider potential compositional differences in the apples and not consider migratory bird impact resulting from consumption of these apple trees, seeds, and insects that feed upon them. The scope of APHIS's review is again narrowly cabined and fails to consider import aspects of the problem. For these reasons, APHIS's no effects conclusion constitutes a failure to take the hard look mandated by NEPA.

Further, while APHIS at least gave a cursory glance at impacts to migratory birds in consideration of its obligations under Executive Order 13186, it utterly failed to consider its obligations under the MBTA. The MBTA allows entities to obtain take permits in a limited number of situations if they adhere to narrowly proscribed requirements. Available permits include those for import and export,[291] banding or marking,[292] scientific collection,[293] taxidermists,[294] waterfowl sale and disposal,[295] Canada geese,[296] falconry,[297] raptor propagation,[298] rehabilitation,[299] depredation, [300]and special purposes.[301] The activity discussed in this EA is not covered by any of these permitting area, thus under the MBTA, this activity may not "take" even a single migratory bird. APHIS fails to properly consider whether migratory birds may be taken as a consequence of it deregulating GD743 and GS784. All of the issues raised *regarding* Executive Order 13186 also apply here, especially APHIS's failure to adequately analyze issues specific to migratory birds and its improperly narrow focus on only apples—not apple trees—when it considers impacts to deregulating GD743 and GS784.

Migratory birds nest, forage for insects and weed seeds, eat apples, and eat flower buds and sap from apple trees. For example, migrating hummingbirds (e.g., Ruby-throated hummingbird (*Archilochus colubris*)) and woodpeckers (e.g., Yellow-bellied sapsucker

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[289] *Id.* at 63.
[290] *Id.; see also id.* at 43 ("Mammals and birds may use apple orchards and the surrounding vegetation for food and habitat throughout the year.").)
[291] 50 C.F.R. § 21.2.
[292] *Id.* § 21.22.
[293] *Id.* § 21.23.
[294] *Id.* § 21.24.
[295] *Id.* § 21.25.
[296] *Id.* § 21.26.
[297] *Id.* § 21.29.
[298] *Id.* § 21.30.
[299] *Id.* § 21.31.
[300] *Id.* § 21.41.
[301] *Id.* § 21.27.

(*Sphyrapicus varius*)) are known to eat sap from apple trees,[302] and migrating warblers (e.g., Magnolia warbler (*Setophaga magnolia*)) have been observed eating flower buds, perhaps to get to insects.[303] APHIS then inappropriately uses nutritional analysis of apple fruit as a stand-in for nutritional equivalence of all other parts of the apple tree.[304][305] APHIS fails to analyze the potential impacts of all the other aspects of the genetically engineered tree, including its flower buds, bark, dependent insects, and sap, on migratory birds. Small RNAs produced by the transgene during the RNAi process have the potential to be toxic due to unintended gene silencing, and other RNAi-associated effects. Even though the process of genetically engineering this tree affects the whole tree, APHIS improperly focuses just on the fruit. APHIS failed to provide data or consider all of the possibilities that would allow a determination of risks to 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.

G. APHIS Fails to Adequately Assess Impacts on Threatened and Endangered Species

GD743 and GS784 may significantly affect threatened and endangered species ("TES"), 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."[306] 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.[307] The initial request for information from FWS and/or NMFS is a predicate to further agency action and cannot be ignored.[308]

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 GD743 and GS784 on threatened or endangered species. However, APHIS failed to take even the first step of consultation.[309] 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.[310] The

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[302] (U of ME, undated)
[303] (Crouch, pers. comm.)
[304] EA at 60.
[305] "Apple events GD743 and GS784 are nutritionally equivalent to their parents and may even have improved phenolic compound content and stability. Apple events GD743 and GS784 are nutritionally equivalent to their parents and may even have improved phenolic compound content and stability (OSF, 2012). The results presented by OSF show that there was no effect of the Arctic™ Apple trait on the composition of the apples, and no biologically-meaningful differences between GD743 or GS784 apples and their non-GE counterparts. *Therefore, based on these analyses, APHIS concludes that consumption of GD743 and GS784 apples or plant parts (seeds, leaves, stems, pollen, or roots) would have no effect on any listed threatened or endangered animal species or animal species proposed for listing*." EA at 60 (emphasis added).
[306] 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).
[307] *Id.*
[308] *Thomas v. Peterson*, 753 F.2d 754, 764 (9th Cir. 1985).
[309] *Ctr. for Food Safety v. Johanns*, 451 F. Supp. 2d 1165, 1182 (D. Hawaii 2006).
[310] *Id.*

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."[311]

APHIS states that it considered several factors when assessing impacts of GD743 and GS784 on TES, including a review of weediness potential; a "determination of where the new transgene and its products (if any) are produced in the plant and their quantity"; agronomic characteristics such as susceptibility to pests and pathogens, and impacts on the environment; whether there are known toxicants; and whether the GE plant is a host to any TES, among other information.[312]

Contrary to the agency's statement, as explained above, APHIS does not have enough information to assess any of those factors for GD743 and GS784. Plant characteristics that are relevant to weediness were not measured in unmanaged trees; the amounts and locations throughout the trees of the product of the transgene—an RNA designed to silence expression of the apple PPO gene family—were not determined; susceptibility to pests and pathogens was not adequately tested; and environmental impacts did not take into account potential toxicity of the gene product, particularly to non-target organisms that make up the biodiversity in, on, and around apple trees. Without these data, APHIS cannot assess impacts on TES of deregulating GD743 and GS784. This failing violates NEPA, the ESA and the APA.

APHIS claims that apple trees do not host any TES, without comment on whether unmanaged trees were considered, or how "host" was defined. It is likely that trees growing outside of cultivation and in abandoned orchards will interact with a wider array of wild organisms that may include TES. Abandoned apple trees and cultivated trees growing in proximity to forests and other wild areas have been shown to have higher insect diversity, including insects that move in from surrounding wild areas.[313] Arthropods on abandoned apple trees in South Moravia, Czech Republic, include some that are endangered.[314] Non-intensively managed apple orchards in Great Britain are home to endangered species, including fungi and insects, and management plans are being promoted specifically to protect those endangered species.[315] In the U.S., apple trees from old homesteads and abandoned orchards are being intentionally maintained for wildlife,[316] and may be used by TES.

APHIS also continues to equate the nutritional assessment of apple fruits by OSF with food quality and safety for wild animals, even though there is no evidence that the compositional qualities of twigs, buds, leaves, or other plant parts eaten by many wild animals are similar to fruit, or that animals eating fruit have the same nutritional requirements as humans:

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[311] *Id.*
[312] EA at 56.
[313] (Altieri & Schmidt 1986)
[314] Šťastná & Psota 2013)
[315] (PTES 2011)
[316] (Park 2012; U of ME n.d.),

> Apple events GD743 and GS784 are nutritionally equivalent to their parents and may even have improved phenolic compound content and stability (OSF, 2012). The results presented by OSF show that there was no effect of the Arctic™ Apple trait on the composition of the apples, and no biologically-meaningful differences between GD743 or GS784 apples and their non-GE counterparts. Therefore, based on these analyses, APHIS concludes that consumption of GD743 and GS784 apples or plant parts (seeds, leaves, stems, pollen, or roots) would have no effect on any listed threatened or endangered animal species or animal species proposed for listing.[317]

Equating and limiting potential impacts to protected species with the potential impacts to humans is arbitrary and capricious and contrary to sound science.

In order to determine impacts on TES that might use GD743 and GS784 apple trees from food or shelter, APHIS must consider the impacts of unmanaged trees in orchards and in wild settings where a wider array of organisms are likely to be present; and the food quality of the parts of apple trees the organisms actually use; in addition to the other factors APHIS identified as important but is unable to assess due to lack of data. Thus, APHIS lacks evidence to support its conclusion that GD743 and GS784 will not adversely affect TES.

IV. CONCLUSION

For the above reasons, and additionally based on the body of evidence submitted in this administrative record, it is CFS's position that APHIS's proposed approval and draft assessment is substantively, procedurally, scientifically, and legally inadequate. The petition should be denied, because approval would violate the mandates of NEPA, the PPA, the ESA, the MBTA, and the APA. In addition or in the alternative, the agency must prepare an EIS before considering any approval; analyze and fully disclose the impacts of the GE apples on the environment and agricultural economy, based on sound science, and make findings regarding those impacts pursuant to its entire PPA statutory authority; comply with the ESA and MBTA; and avoid taking action that is arbitrary and capricious, an abuse of discretion, or otherwise not in accordance with the law.

Respectfully submitted,

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[317] EA at 60, (emphasis added); *see also id.* at 45, 59.

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