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July 23, 2007

Regulatory Analysis and Development
USDA APHIS PPD
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Submitted via Federal eRulemaking Portal: Docket No. APHIS-2007-023

The Center for Food Safety (CFS) submits the following comments on the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)'s environmental assessment and preliminary decision to allow SemBioSys Genetics, Inc., to plant genetically engineered proinsulin-producing safflower in Washington.

CFS is a non-profit public interest and environmental advocacy membership organization established in 1997 by its sister organization, International Center for Technology Assessment, for the purpose of challenging harmful food production technologies and promoting sustainable alternatives. CFS combines multiple tools and strategies in pursuing its goals, including litigation and legal petitions for rulemaking, legal support for various sustainable agriculture and food safety constituencies, as well as public education, grassroots organizing and media outreach.

CFS strongly opposes the use of genetically engineered food crops to produce experimental pharmaceuticals, due to unexplored risks to the environment and potential risks to human health that could result from contamination of food crops with experimental pharmaceutical substances. Like many others, we regard the outdoor cultivation of genetically engineered food crops that produce novel, bioactive substances that have not undergone review or received approval by U.S. food safety authorities as highly irresponsible. Besides posing unexplored risks to human health and the environment, this practice also undermines confidence in the integrity of the U.S. food supply, and in the "coordinated framework" for regulation of agricultural biotechnology products.

BACKGROUND

In the June 22, 2007 *Federal Register,* USDA APHIS announced a public comment period on a draft environmental assessment (EA) and on a preliminary decision to allow SemBioSys Genetics, Inc. (SemBioSys), to grow genetically engineered (GE) proinsulin-producing safflower (proinsulin safflower) in Washington State this year.

Safflower is a food crop valued primarily for its oily seeds, which are used to produce edible oil for human consumption, birdseed, and as supplements for fish and animal feed.

SemBioSys' application

SemBioSys Genetics, Inc. has submitted a request for a permit (APHIS Number 06-363-103r) for the planting and release of genetically engineered (transformed) safflower *(Carthamus tinctorius)*. The genetically engineered (GE) safflower *(Carthamus tinctorius)* has been developed to "express an oleosin-human proinsulin protein exclusively within its seed."[1] The purpose of this release is "to obtain a source of seed containing proinsulin to be used to develop an insulin purification process."[2]

SemBioSys engineered the safflower in response to the anticipated market growth of human insulin pharmaceuticals.[3] SemBioSys has initiated the application process with the FDA to obtain approval for human use.[4]

CFS COMMENTS

Summary

The draft EA is wholly inadequate. APHIS's cursory assessment assumes that any human or environmental exposure to the proinsulin-containing fusion protein will be negligible and of absolutely no concern. This conclusion is based on a chain of assumptions concerning the unlikelihood of human or animal exposure to insulin safflower, together with a casual and deeply flawed attempt to estimate the impacts of any exposure that does occur.

Errors in the EA include the mistaken assumption that proinsulin has no biological activity when ingested, when in fact proinsulin does have biological activity upon ingestion, and SemBioSys has stated its intention of developing plant-produced insulin for oral delivery. APHIS's inhalational exposure assessment is also flawed, in that it greatly underestimates the insulin-type activity of proinsulin by this route. APHIS also glosses over potential adverse impacts from exposure to proinsulin that do not involve insulin-type activity, such as hazardous autoimmune reactions. APHIS's cursory assessment of the potential allergenicity of the modified proinsulin-oleosin fusion protein does not even meet relevant international standards for allergenicity assessments of novel proteins in transgenic plants meant for food use. In short, there is abundant

[1] EA at 3.
[2] EA at 4.
[3] EA at 3.
[4] EA at 4.

evidence to suggest that exposure to proinsulin safflower could have serious adverse consequences to humans and/or animals, and APHIS's faulty assessment has completely failed to rule out such harmful effects. An EIS is required to address these failings.

Thus, the presumption in the EA that proinsulin safflower is safe is highly uncertain and highly controversial. The alternatives to the preferred action are inadequate. Information crucial to the evaluation of the cumulative and immediate health and safety impacts has been withheld, making informed public comment impossible. For each of these reasons, the draft EA does not meet the legal standards set by NEPA, the Council on Environmental Quality (CEQ)'s NEPA-implementing regulations, and applicable precedent.

USDA APHIS regulations

Under the Plant Protection Act of 2002 and regulations governing GE organisms,[5] companies must secure permits to plant pharmaceutical-producing plants. Typically, the department's EAs on engineered crops include an analysis of the crop's plant pest and other environmental risks that underlie the determination of whether to grant or deny a permit.

The analysis in USDA's EA of SemBioSys' proposed proinsulin safflower cultivation must inform the department's determination of whether a full environmental impact statement (EIS) is required under the National Environmental Policy Act (NEPA) and whether a permit should be issued under USDA's Plant Protection Act regulations.[6]

The National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires a federal agency such as USDA 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]

A threshold question is whether a proposed project *may* "significantly affect" the environment, thereby triggering the requirement for 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] An EA must "provide sufficient evidence and analysis for determining whether to prepare an EIS or a finding of no significant impact."[11]

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[5] 7 C.F.R. § 340
[6] 40 C.F.R. § 1508.9; 7 C.F.R. § 340; and 42 USC § 4332.
[7] 42 U.S.C. § 4332(2)(C).
[8] *Robertson v. Methow Valley Citizens Council,* 490 U.S. 332, 349(1989).
[9] 42 U.S.C. § 4332(2)(C). See Nat'l Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 730 (9th Cir. 2001) ("If the EA establishes that the agency's action *may* have a significant effect upon the environment, an EIS must be prepared.") (internal quotation marks omitted).
[10] 40 C.F.R. § 1508.9.
[11] *Id.*

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.[12] "The statement of reasons is crucial to determining whether the agency took a "hard look" at the potential environmental impact of a project."[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]

The Council on Environmental Quality (CEQ)

NEPA also established the Council on Environmental Quality and charged CEQ with the duty of overseeing the implementation of NEPA.[16] The regulations subsequently promulgated by CEQ, 40 C.F.R. §§ 1500-08, 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."[17] CEQ's regulations are applicable to and binding on all federal agencies.[18] Among other requirements, CEQ's regulations mandate that federal agencies address all "reasonably foreseeable" environmental impacts of their proposed programs, projects, and regulations.[19]

I. APHIS has Withheld Information Crucial to Adequate Public Comment, in Violation of NEPA.

APHIS has failed to provide adequate information to enable informed and meaningful public comment on this proposed field test. A decision should be delayed until the public is provided with such information and given adequate opportunity to consider and offer comment on it. The public's opportunity for comment "must be a meaningful opportunity."[20] APHIS' withholding here has denied the public that right.

A. The EA lacks crucial information concerning the nature and activity of the proinsulin fusion protein and related genetic elements, and safety tests conducted by SemBioSys, that are necessary for assessment of potential adverse impacts from exposure

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[12] *Save the Yaak v. Block*, 840 F.2d 714, 717 (9th Cir. 1988).
[13] Id.
[14] See 40 C.F.R. §§ 1508.8, .9, .13, .18.
[15] *Blue Mountains Biodiversity v. Blackwood*, 161 F.3d 1208, 1211 (9th Cir. 1998).
[16] See 42 U.S.C. §§ 4321, 4344.
[17] 40 C.F.R. § 1500.3.
[18] 40 C.F.R. §§ 1500.3, 1507.1; see, e.g., Hodges v. Abraham, 300 F.3d 432, 438 (4th Cir. 2002).
[19] See 40 C.F.R. §§ 1502.4, 1508.8, 1508.18, & 1508.25.
[20] Gerber v. Norton, 294 F.3d 173, 179 (D.C. Cir. 2002) (finding the public could not meaningfully comment on an ESA incidental take permit application which lacked a necessary map); see Fund for Animals v. Norton, 281 F.Supp.2d 209, 228-29 (D.D.C. 2003) (draft EA held to be insufficient after agency failed to provide certain pertinent information because that lack of information had a significant impact on the public's ability to provide meaningful comment).

Crucial information that is lacking includes the structures of the modified proinsulin protein and the proinsulin-oleosin fusion protein (essential to assess activity and risk factors); the conditions under which certain tests were carried out by SemBioSys (e.g. simulated digestive studies), which can have a substantial influence on the results obtained; complete information on sequence homology between the proinsulin fusion protein and known allergens (only inadequate partial information is provided); and the identity of the selectable marker gene, which is claimed as confidential business information (CBI) despite the assertion that it is one of the most commonly used selective markers in plants (EA, p. 25).

B. The EA lacks crucial information relevant to the protocols for planting, harvesting and storing genetically engineered material to ensure a confined field release.

APHIS has failed to make available to the public many of SemBioSys's protocols relevant to the proposed field trial, in contrast to its practice for many environmental assessments of past applications by other pharma crop companies (e.g. Prodigene and Ventria) to conduct field trials. In particular, APHIS has failed to make available to the public the "Standard Operating Procedures" (SOPs) that SemBioSys says it will follow in conducting this field trial. The SOPs have been withheld from the proposed EA as confidential business information (CBI).[21] These SOPs contain crucial information about SemBioSys' planting, harvesting, storage, and containment measures.[22] This is information that public interest groups like CFS need to offer informed public comment, particularly as regards to the adequacy or inadequacy of safety measures, and the potential for inadvertent exposure of humans and animals to the proinsulin fusion protein. APHIS relies upon the SOPs and other confidential business information throughout the EA to assert its conclusions about health and environmental risks. APHIS's judgment that the SOPs are adequate to prevent or mitigate health and environmental impacts requires critical, independent review.

An EA such as this one that withholds key scientific information is legally inadequate and circumvents the main purposes of NEPA: informed public participation, government accountability and transparency. 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."[23] Moreover, NEPA "insure[s] that environmental information is available to public officials and citizens before decisions are made and before action is taken."[24] This withholding greatly compromises the public review process, because it makes the public reliant on the interpretation of the data by the submitter, which is not a disinterested or unbiased party. APHIS has purportedly evaluated the data in its EA, but this is not a substitute for the public review process, which is mandated by NEPA. Public review is especially crucial in this case, in light of the fact that APHIS has made fundamental errors in its assessment, as demonstrated below.

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[21] EA at 5.
[22] EA at 5.
[23] Idaho Sporting Cong. v. Thomas, 137 F.3d 1146, 1149 (9th Cir. 1998) (internal quotation marks omitted).
[24] 40 C.F.R. § 1500.1(b), (c).

The proposed EA inadequately fails to give a meaningful opportunity for informed public comment. The final EA will be inadequate if it does not provide an open analysis and summary of the CBI and SOPs "detailing protocols for planting, harvesting and storing genetically engineered material to ensure a confined field release."[25]

This failure to disclose is particularly egregious in that APHIS admits in the draft EA that one major route of potential contamination and harm from the field test is "human error."[26] It is difficult if not impossible to adequately assess the potential hazards of the field test and any mitigation measures without full disclosure of the measures that SemBioSys is undertaking and APHIS is mandating.

II. The EA Insufficiently Addresses Human Health and Safety Risks, in Violation of NEPA.

A. *If there is a finding of no significant impact (FONSI), the EA must convincingly state the reasons that the potential public health and safety impacts are not significant. The EA is in deficient in this regard.*

NEPA mandates that public health and safety issues be addressed in an EA because health and safety issues may be significant environmental impacts requiring the preparation of an EIS.

Public health and safety effects may be significant effects of major agency actions and therefore require environmental impact statements. As noted above, a threshold question is whether a proposed project may "significantly affect" the environment, thereby triggering the requirement for an EIS.[27] An EA must "provide sufficient evidence and analysis for determining whether to prepare an EIS or a finding of no significant impact."[28] Thus, an EA must consider factors that may significantly affect the environment.

The CEQ regulations articulate what factors may be significant effects on the human environment and therefore require EISs. One such factor is "[t]he degree to which the proposed action affects public health or safety." 40 C.F.R. § 1508.27(b)(2). The presence of one or more of the factors in 40 C.F.R. § 1508.27 may be sufficient to require the preparation of an EIS.[29] Therefore, the EA must address any potential human health or safety risks and determine whether those human health and safety impacts may be significant. If those impacts are found not to be significant, there must be a convincing statement of reasons.[30]

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[25] EA at 5.
[26] EA at 12.
[27] 42 U.S.C. § 4332(2)(C). See Nat'l Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 730 (9th Cir. 2001) ("If the EA establishes that the agency's action *may* have a significant effect upon the environment, an EIS must be prepared.") (internal quotation marks omitted).
[28] 40 C.F.R. § 1508.9. See also Nat'l Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 730 (9th Cir. 2001) (holding that an EA finding of no significant impact must be accompanied by a convincing statement of reasons why an agency action's impacts are insignificant).
[29] National Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 731 (9th Cir. 2001); Public Service Co. of Colorado v. Andrus, 825 F.Supp. 1483, 1495 (D. Idaho 1993).
[30] National Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 731 (9th Cir. 2001).

The requirement that an EA must include human health and safety has been stated by at least one court ruling in the food and drug context (the bovine growth hormone Posilac). The court in Stauber v. Shalala stated that NEPA "require[s] a thorough evaluation of Posilac's effects on human and bovine health and safety."[31] The court noted:

> Such incorporation of the health and safety data by reference in the environmental assessment and finding of no significant impact would provide an interested party (or reviewing court) with a complete picture of all analysis bearing on the agency's obligations under the National Environmental Policy Act.[32]

In this case, there are significant potential human health impacts of the planting of this experimental pharma-crop. As explained below, the EA's treatment of these issues is wholly inadequate and demand, at the very least, the preparation of an EIS.

APHIS's attempt to assess the impacts of exposure to insulin safflower are shot through with fundamental misunderstandings concerning the nature and activity of (pro-)insulin, the behavior of proteins in the gastric system, the science of autoimmune disease, and the functioning of the human immune system. In Appendix 1, we present several examples in the EA demonstrating APHIS's incompetence to assess potential human or animal health impacts from exposure to the potent hormone at issue here, and which are sufficiently grave to completely invalidate this crucial part of its draft EA. These deficiencies in the draft EA lead CFS to call on APHIS to perform an environmental impact statement (EIS). However, such an EIS would have little value if not prepared by personnel with a much better grounding in medical science than the reviewer(s) who prepared the draft EA. Therefore, we urge APHIS to enlist the support of the U.S. Food and Drug Administration and/or independent medical scientists who have expertise with insulin in the preparation of the EIS. Precedent for the latter approach is provided by the U.S. Environmental Protection Agency, which frequently convenes Scientific Advisory Panels comprising independent experts to advise it on issues on which Agency officials do not have adequate expertise. A National Academy of Sciences panel explicitly recommended that APHIS seek more independent scientific support in its assessment and decision-making with respect to transgenic plants (NAS 2002).

We note at the outset that our assessment of SemBioSys's proinsulin-oleosin fusion protein is based primarily on the properties of native human proinsulin as well as porcine and bovine forms of the molecule. This is made necessary by the paucity of the data supplied by APHIS on the SemBioSys fusion protein. We simply do not know such basic facts as the structure of SemBioSys's proinsulin-oleosin fusion protein, its activity, its digestive stability, and its potential unintended effects. Either SemBioSys has not developed these data or reported them to APHIS, or APHIS has not reported them in the draft EA.

This information is vital for assessment purposes. In its absence, and in light of the novelty and potency of SemBioSys's experimental proinsulin fusion protein, we urge that assessment of proinsulin safflower in the context of an EIS be conducted with a ten-fold safety margin to account for its largely unknown properties. In other words, all appropriate studies should be

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[31] Stauber v. Shalala, 895 F.Supp. 1178, 1195 (W.D. Wis. 1995).
[32] Stauber v. Shalala, 895 F.Supp. 1178, 1195 (W.D. Wis. 1995).

conducted on the assumption that human and environmental exposure to the fusion protein is ten times the maximal estimated exposure. This is common practice, for instance, in the assessment of pesticides. The Food Quality Protection Act stipulates that pesticides be proven reasonably safe at concentrations at least ten-fold greater than estimated human exposure levels to account for factors such as the greater sensitivity of children to many toxic chemicals, and the inherent uncertainties in extrapolating laboratory animal test results to humans (i.e. humans are sometimes more sensitive than lab animals). It is entirely reasonable, and indeed conservative, to demand a similar margin of safety with respect to exposure to a potent hormone that is specifically designed to exert powerful and potentially hazardous effects on human physiology at microgram (millionths of a gram) levels.

Basic Facts on Proinsulin, Insulin and Diabetes

Insulin is an extremely important and potent hormone that regulates blood sugar levels, active at the microgram level. It is generated primarily in the pancreas and to a lesser extent in the thymus, but insulin-producing cells are also sometimes found in bone marrow, fat cells, the spleen, and other tissues (SoRelle 2004). Insulin functions in a homeostatic system with glucagon and other regulatory elements to keep blood sugar levels within an optimal range. Insulin is secreted in response to high blood sugar levels, and triggers removal of blood glucose from the circulatory system by initiating its conversion to storage forms of glucose such as glycogen, which are stored in muscle and fat tissue. Glucagon secretion is stimulated in response to low blood sugar levels, and acts to convert glycogen and other energy-rich stores to glucose and release it into the bloodstream (see Figure 1). Insulin also inhibits glucose production in the liver, inhibits breakdown of fat for energy production, inhibits breakdown of proteins, and enhances protein synthesis (Exubera Label 2006).

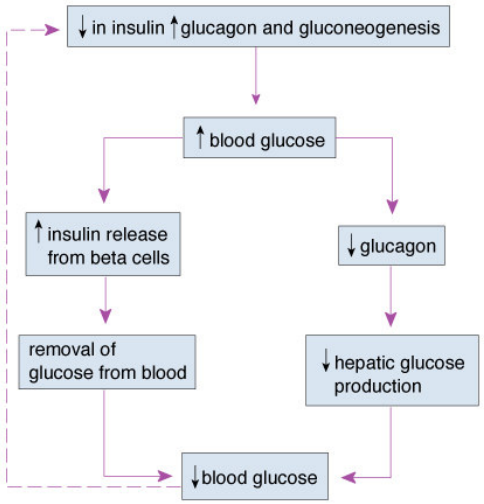

Figure 43-1 Hormonal and hepatic regulation of blood glucose.

Figure 1. Hormonal and hepatic regulation of blood glucose[33]

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http://images.google.com/imgres?imgurl=http://connection.lww.com/Products/porth7e/documents/Ch43/jpg/43_003.jpg&imgrefurl=http://connection.lww.com/Products/porth7e/Ch43.asp&h=502&w=700&sz=103&hl=en&start=2&um=1&tbnid=MrLVN-i4dIp4tM:&tbnh=100&tbnw=140&prev=/images%3Fq%3D%2522proinsulin%26svnum%3D10%26um%3D1%26hl%3Den

Diabetes is a disease that disrupts blood sugar homeostasis. Type 1 diabetes is an auto-immune disease in which the body's immune system generates autoantibodies that attack and destroy the pancreatic beta cells that generate insulin. This renders the afflicted person unable to produce insulin, or sufficient amounts of it. Type 2, or acquired, diabetes is caused by "insulin resistance," or the body's failure to respond (adequately) to insulin for a variety of reasons. As a consequence, diabetics can experience large swings in blood glucose levels outside of the optimal (and safe) range. Both hyperglycemia (excessive glucose levels) and hypoglycemia (inadequate glucose levels) pose serious health risks. Diabetics often self-administer insulin to help stabilize their blood sugar levels. The challenge of treatment regimens for diabetes is to mimic, to the greatest extent possible, the healthy person's fine-grained homeostatic control system, which involves frequently repeated, very small, doses of insulin secreted by the pancreatic beta cells, by means of exogenously introduced insulin delivered, of necessity, much less frequently and in higher doses.

The potent nature of the insulin hormone and the potentially serious consequences of improper exposure to insulin demand a serious assessment of SemBioSys's proinsulin safflower.

APHIS Mistakenly Asserts That Proinsulin Has No Activity When Ingested

In Appendix IV, titled "Human Proinsulin," APHIS baldly states that: "Proinsulin has no biological activity when ingested" (EA, p. 28). This statement is incorrect. Ni et al (2007) have demonstrated that feeding a fungus genetically engineered to express a form of human proinsulin to rats resulted in significant reductions in blood glucose levels relative to rats fed non-transgenic fungus that lacked proinsulin, a sure sign of proinsulin's activity. Ruhlman et al (2007) have demonstrated that feeding non-obese diabetic (NOD) mice an oral powder derived from tobacco leaves engineered to express a fusion protein comprising human proinsulin and cholera toxin B subunit prevents development of pancreatic insulitis and preserves the animals' insulin-producing beta cells, evidence of proinsulin's activity. These two studies, both involving forms of human proinsulin, demonstrate conclusively that APHIS is wrong in assuming that "[p]roinsulin has no biological activity when ingested." In fact, both studies cited above found not only activity, but activity significant enough to give the authors confidence in the development of a successful oral delivery method for proinsulin.

We also note that a patent recently obtained by SemBioSys, entitled "Methods for the Production of Insulin in Plants," shows quite clearly that the company regards the oral route as one of "the most likely" methods to administer plant-produced insulin, which further "may be delivered in any desired manner:"

> "The final formulation of the insulin preparation will generally depend on the mode of insulin delivery. The insulin prepared in accordance with the present invention ***may be delivered in any desired manner***; however parenteral, ***oral*****,** pulmonary, buccal and nasal

> forms of delivery are considered the most likely used modes of delivery." (SBS Patent 2005, par. 0178, emphasis added)[34]

This same patent lists safflower as a "particularly preferred" host organism for production of insulin or proinsulin (SBS Patent 2005, par. 0142), and also includes an embodiment involving fusion of proinsulin to *Arabidopsis*-derived oleosin protein (Ibid, par. 0057), as does the safflower line at issue in this EA.

Thus, the proinsulin in the transgenic safflower proposed for cultivation may in fact be specifically designed to retain activity upon ingestion.

APHIS Fails to Consider the Enhanced Activity of Proinsulin Derivatives

The pancreas's beta cells first produce a 110 amino-acid (AA) polypeptide known as preproinsulin. The 86-AA proinsulin molecule is generated from preproinsulin by removal of a 24 AA signal peptide during its insertion into the endoplasmic reticulum.[35] Proinsulin is the body's main storage form of insulin. Insulin is generated from proinsulin by removal of a connecting peptide (C-peptide). Insulin is a 51 AA protein consisting of an alpha-chain (21 AA) and a beta-chain (30 AA) connected by two disulphide bonds. The structures of human proinsulin and insulin are portrayed in Figure 2.

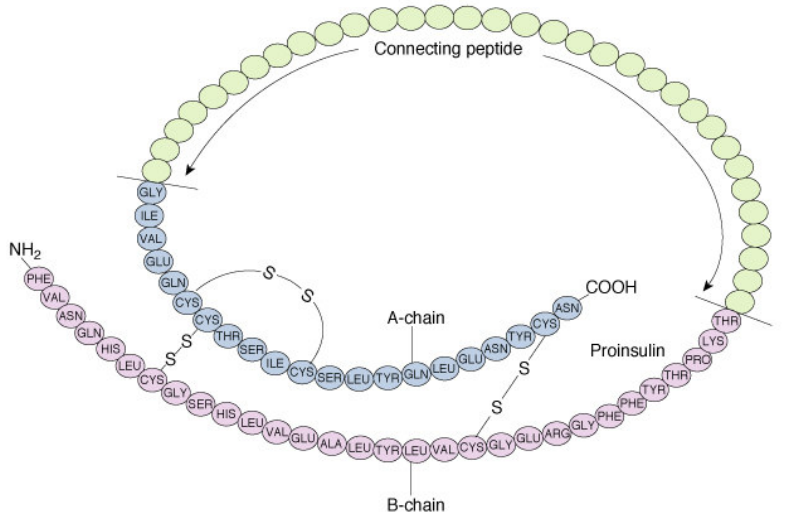

Figure 43-3 Structure of proinsulin. With removal of the connecting peptide (C-peptide), proinsulin is converted to insulin.

Figure 2: Structure of human proinsulin[36]

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[34] We note that as used by SemBioSys in its patent, the term "insulin" covers proinsulin and a wide range of modified versions of (pro-)insulin.

[35] http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2005/Dresser/My%20favorite%20Protein.html.

[36] http://connection.lww.com/Products/porth7e/documents/Ch43/jpg/43_003.jpg

APHIS cites several studies in support of its assertion that human proinsulin has just 10% the activity of human insulin, and uses this 10% figure as the basis of a casual attempt to assess inhalational exposure assessment. Two of these studies were conducted on porcine and beef forms of (pro-)insulin (Kitabchi 1970; Yu & Kitabchi 1973), the third on human versions (Rosak et al 1988). What APHIS fails to note is that Yu & Kitabchi (1973) also test the activity of a number of proinsulin derivatives that are thought to be intermediate forms in the conversion of proinsulin to insulin. Yu & Kitabchi established that porcine proinsulin intermediates lacking 2, 9 and 13 amino acid-long segments had 36%, 39% and 42% the biological activity, respectively, of porcine insulin. Similarly, a bovine proinsulin intermediate lacking a 2 amino acid-segment (i.e. a dipeptide) had 33% the activity of bovine insulin. If similar intermediates are generated in the conversion of human proinsulin to insulin, and are formed in the gut upon ingestion of the proinsulin-oleosin fusion protein, the activity of a given exposure could be four times the level estimated by APHIS.

APHIS also fails to account for the possibility that SemBioSys has modified human proinsulin to be more active. APHIS reports that the modified proinsulin portion of the SemBioSys fusion protein "has two basic amino acids removed for added stability in plants..." (EA, p. 25). Interestingly, the porcine and bovine proinsulin derivatives lacking dipeptides that were tested by Yu & Kibatchi lacked 2 ***basic*** amino acids (lysine and arginine). APHIS does not report which basic amino acids were removed in the SemBioSys fusion protein, but if they are the same as those missing in the proinsulin derivatives tested by Yu & Kibatchi, the SemBioSys proinsulin may already (before any enzymatic breakdown) be roughly four times more potent than assumed by APHIS. In addition, Yu & Kibatchi found that porcine proinsulin derivatives lacking 9 and 13 amino acid-long peptides were somewhat more active than those lacking dipeptides. In all cases, the amino acids removed comprised various segments of the C-peptide portion of proinsulin. In the SemBioSys patent, shortening of the C-peptide of native proinsulin to form "mini-insulin" is a preferred embodiment of the invention (SBS Patent 2005, par. 0099). If the proinsulin fusion protein at issue here contains a mini-insulin molecule with shortened C-peptide, it would likely be still more active than a proinsulin molecule lacking just the lysine-arginine dipeptide.

APHIS's Assessment of Exposure to Proinsulin is Deeply Flawed

Clearly, exposure of humans and animals to plant-produced pharmaceuticals (PMPs) grown in the open air is a novel issue that has only arisen with the rise of transgenic plants engineered to produce pharmaceutical proteins. The only possible precedent is exposure from contamination of the environment with drugs (e.g. drugs in drinking water supplies). Drugs are developed for controlled use via defined routes of administration, whereas exposure to PMPs will often occur via routes that have not been subject to any study. PMPs in the experimental phase (such as SemBioSys's proinsulin) have not been adequately studied to ensure safety or establish proper dosage ***even via the intended route of administration***. To our knowledge, no one has devised a protocol for determining safe exposure levels to any experimental PMP via any route of exposure, much less potent, experimental hormones. In the absence of such a protocol, an approach that errors on the side of caution is obviously desirable.

We discuss APHIS's casual and flawed attempt to assess potential impacts from inadvertent exposure to proinsulin below.

1) Inhalational Exposure:

APHIS's cursory assessment of inhalational exposure to proinsulin (EA, pp. 14-15) is flawed for several reasons. First, the assessment comes in Section VI.3 (Potential Environmental Impacts: Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species), and yet incidentally addresses ***human*** exposure to proinsulin. It is not clear if APHIS regards human beings as a non-target or beneficial organism, or a threatened or endangered species. Human health impacts alone can be significant enough impacts to the "human environment" to require an EIS and demand adequate assessment in order to comply with NEPA. 40 C.F.R. § 1508.27(b)(2). If those impacts are found not to be significant, there must be a convincing statement of reasons. Here there is no such analysis by the agency of potential human health impacts. APHIS's incidental treatment of human exposure in a section meant to address potential impacts on non-target organisms is insufficient, and is symptomatic of the deep confusion reigning in U.S. regulatory agencies over how to deal with pharmaceutical-producing plants.

APHIS cites SemBioSys for the figure of 66 µg of proinsulin-oleosin fusion protein per safflower seed (EA, p. 14). For some unexplained reason, APHIS chooses to assess 1,000 safflower seeds, which would contain 66 mg of fusion protein. Assuming that proinsulin has 10% the activity of insulin (critiqued in the last section), APHIS then assumes that 66 mg of fusion protein would have 6.6 mg of insulin-type activity. APHIS then translates this 6.6 mg into 171 units of insulin activity (based on a 38.5 µg/unit conversion factor). Finally, the APHIS reviewer, assuming that only 10% of insulin delivered nasally or via lungs is actually absorbed, arrives at the conclusion that 1,000 proinsulin-containing seeds would deliver 17.1 units of insulin-like activity.

This analysis is flawed in several respects. First, APHIS mistakenly equates 66 mg of fusion protein with 66 mg of proinsulin. In fact, since the oleosin portion of the fusion protein has no insulin-like activity, the reviewer should have corrected for this; alternately, the reviewer may have mistakenly stated that one safflower seed contains 66 µg of fusion protein, when in fact it contains 66 µg of proinsulin. We do not have sufficient information to decide which error the APHIS reviewer made, and so proceed on the latter assumption. Second, we need to correct the 10% proinsulin activity factor to 40% to account for the increased activity of proinsulin derivatives and/or SemBioSys's modified version of proinsulin (see discussion in last section). Finally, careful examination of the source APHIS cited for the assertion that only 10% of insulin delivered nasally or by lung "is transported across the membranes..." (Hite et al 2006) reveals that it says no such thing. Hite et al (2006), in an article on Exubera (the first FDA-approved inhalant insulin), instead state that: "After the powder is delivered to the alveoli [lung cells], approximately one-third of the administered dose is absorbed into the bloodstream" (p. 111). Thus, it appears that 33% is a more correct factor than APHIS's 10%.

When these corrections are made, the 17.1 units of insulin-activity calculated by APHIS as the exposure from 1,000 proinsulin safflower seeds turn out to be 229 units, or over 13 times more. What effect would this much insulin have? One unit of insulin is administered to diabetics for

each 10 to 20 grams of ingested carbohydrate.[37] 229 units of insulin-like activity would be sufficient for 2,290 to 4,580 grams, or 5-10 lbs, of ingested carbohydrates. The hypoglycemic effects of such a massive dose would surely be severe, and probably life-threatening. As we stated at the outset, however, it is not clear why the reviewer chose to assess 1,000 seeds. But even if one assumes inhalant exposure to the proinsulin contained in just 10 seeds rather than 1000 (2.29 units), the dose of insulin would be suited to 23 to 46 grams of ingested carbohydrate, or roughly one to two ounces. This would still exert a significant hypoglycemic effect, and is in fact close to the 3 unit dose of insulin that diabetics often self-administer subcutaneously prior to meals (Exubera Label 2006).

We stress that without more detailed knowledge of the structure and activity of SemBioSys's version of modified proinsulin, we cannot draw any firm conclusions as to the impacts of inhalant exposure to safflower seed dust, for instance by workers who process the seeds. The purpose of APHIS's flawed assessment is also unclear. APHIS does not impose any requirement that seed processors wear protective equipment to guard against inhalant exposure, but merely notes that SemBioSys says it will take this measure.

2) Oral Exposure

APHIS does not carry out a similar assessment for oral exposure on the mistaken assumption that "[p]roinsulin has no biological activity when ingested" (EA, p. 28). As discussed above, not only do two recent studies (Ni et al 2007, Ruhlman et al 2007) establish that ingested proinsulin is in fact active, but ***SemBioSys is actively pursuing oral delivery of plant-produced insulin as a preferred delivery method*** (SBS Patent 2005, par. 0178)***,*** as are others (e.g. Ruhlman et al 2007). In an attempt to buttress its mistaken notion that ingested proinsulin lacks biological activity, APHIS states: "Even the task of developing an oral insulin delivery method has been unsuccessful in the last several decades because both insulin and proinsulin are peptides that are easily and quickly digested" (EA, p. 28). While problems with preventing (pro-)insulin from being degraded in the gut may have been one obstacle to development of an oral (pro-)insulin delivery method in the past, Ni et al (2007) and Ruhlman et al (2007) have demonstrated that such problems can be overcome. SemBioSys, which intends oral delivery as one preferred route of administration, may have also developed its modified proinsulin-oleosin fusion protein in such a way as to overcome this obstacle. However, a more significant reason that proinsulin was never successfully developed for delivery ***by any route of administration*** had to do with concerns about its adverse impacts, such as the potential for increased risk of myocardial infarctions (see next section), which APHIS missed entirely.

The only empirical evidence offered by APHIS on this question involves simulated digestive studies conducted by SemBioSys (EA, p. 28), in which "active insulin" was digested in 60 minutes and the proinsulin seed fusion protein digested in 15 minutes. Simulated digestive stability tests normally involve placing a protein in a test tube containing an acidic solution with pepsin (a digestive enzyme). Despite their name, such simple tests do not come anywhere

[37] "The mealtime bolus (MB) is delivered on demand according to the amount of carbohydrates ingested in that meal and, on average, one insulin unit is used for each 10 to 20 grams of ingested carbohydrate." From: http://www.ijathero.com/2007/june/pdf/1-BrazilianGuidelines-partIII.pdf

simulating the human gastric system. Choice of test conditions is crucial to outcome. In most cases, biotech companies choose to test their novel transgenic proteins in a very acidic solution (e.g. pH = 1.2) that represents the lower end of the range of gastric pH in the human stomach (fasting), which can increase to the (much milder) pH = 4 or even 5 after a meal. Another crucial variable is the concentration of digestive enzyme or enzymes (usually pepsin). Higher concentrations facilitate more rapid degradation. Without reporting of test conditions, we cannot assess the meaning, if any, of SemBioSys's findings.

FAO-WHO (2001) presents a protocol for assessment of novel proteins in transgenic food crops for "digestive stability," a characteristic property of food allergens. This protocol, developed by leading international allergists, recommends that such studies be conducted at pH = 2.0 and a certain ratio of pepsin to test protein, conditions that biotech companies have rarely if ever followed (they normally choose harsher conditions that foster more rapid breakdown). A detailed treatment of the pitfalls of simulated digestive studies in the assessment of novel proteins in transgenic crops is provided by Freese (2001).

However, even the FAO-WHO (2001) protocol was not formulated to assess potent, novel hormones generated in transgenic plants. Clearly, novel hormones (such as the modified human proinsulin at issue here) that trigger profound effects on human physiology at the microgram level should be subject to much stricter testing standards that ensure truly complete digestion in a range of gastric conditions. Simple *in vitro* testing is inappropriate for a potent hormone such as proinsulin. We note also that SemBioSys has modified the proinsulin protein for "added stability in plants." It is unclear whether this would also mean increased stability to digestion, though this is certainly possible. In addition, both proinsulin and insulin possess two disulphide bonds (see Figure 1). Allergy experts state that disulphide bonds are one feature increasing the digestive stability of a protein (Breiteneder & Mills 2005). We also find it suspicious that the much larger fusion protein should have been found to degrade more rapidly than the smaller "active insulin." The standard used to assess what constitutes breakdown is also important, in view of the evidence we have presented that small, 10-17 amino acid segments of the proinsulin molecule may in fact be sufficient to elicit harmful autoimmune responses.

Finally, and most importantly, these simple *in vitro* tests have little meaning in comparison to *in vivo* testing on experimental animals. Two such studies with other versions of transgenic human proinsulin have found that it exerts activity upon ingestion, which testifies to proinsulin's stability to digestion.

Proinsulin Associated With a Number of Diseases

There is considerable suggestive evidence that proinsulin has other effects, some of them adverse, beyond insulin-like activity. As described above, proinsulin is the storage form of insulin. Only small amounts enter the circulation (approximately 3% as much as insulin), yet fasting proinsulin concentrations in the blood are 10-15% those of insulin. This large discrepancy between the amount of proinsulin that enters the bloodstream and the concentration found in the bloodstream (each relative to insulin) is explained by the two- to three-fold longer half-life of proinsulin vs. insulin, which in turn is due to the four-fold slower clearance of

proinsulin vs. insulin from the bloodstream by the liver (Labcorp, undated). In short, any proinsulin that enters the bloodstream persists for two- to three-fold longer than insulin.

High proinsulin levels in the bloodstream have been:
1) Associated with several forms of pancreatic cancer (Labcorp, undated);
2) Identified as a risk factor for the development of type 2 diabetes (Zethelius et al 2003; Hanley et al 2002);
3) Identified as an independent predictor of cardiovascular mortality and mortality in general (Alssema et al 2005; Zethelius 2002); and
4) Found in patients with chronic renal failure, cirrhosis and hyperthyroidism (Labcorp, undated).

Whether high proinsulin levels are merely a marker of, or play a causal role in, any of these conditions is not known with certainty. However, it is known that ***Eli Lilly stopped human clinical trials on transgenic proinsulin in February 1988 after an independent review corroborated the company's own concerns that proinsulin was not safe*** (Galloway et al 1992). The authors noted that in one multicenter study, six patients receiving human proinsulin had myocardial infarctions, two of whom died, while there were no myocardial infarctions in the control group. The expert group concluded that proinsulin may not have "unique efficacy," meaning that proinsulin may have effects on human physiology (some adverse) other than controlling blood sugar levels.

One such potential effect of proinsulin is hazardous autoimmune responses. Proinsulin has been identified as an autoantigen (partially) responsible for stimulating the aberrant, destructive immune system response implicated in type 1 diabetes (You & Chatenoud 2006; Krishnamurthy et al 2006). Further studies have identified specific parts of the proinsulin molecule (epitopes) that are the target of the destructive immune system response in type 1 diabetes. In a study on rats, Griffin et al (1995) identified a 17 amino acid epitope on the rat proinsulin molecule that appears to be the major target of immune system attack. In a study on mice, Chen et al (2001) identified a 10-amino acid epitope on the mouse proinsulin molecule that is targeted by the immune system. Interestingly, in both cases, these epitopes span a portion of the proinsulin molecule that is not present in insulin, being removed in the processing of proinsulin to insulin. In other words, it appears that proinsulin, but not insulin, is the target of the aberrant and destructive autoimmune response that characterizes type 1 diabetes.

In addition, these studies suggest that the entire proinsulin molecule is not required to trigger such responses. Relatively small peptides of the proinsulin molecule – just 10-17 amino acids in length – could be sufficient to trigger hazardous autoimmune reactions. Thus, digestive breakdown of proinsulin that leaves such peptides intact for a sufficient amount of time to allow absorption into the bloodstream could be sufficient to trigger an autoimmune response. While most people would probably not mount such an autoimmune response, some could. In addition, the fact that SemBioSys's proinsulin has been modified relative to native human proinsulin increases the potential for aberrant autoimmune reactions in the general public. We discuss the modification of the SemBioSys proinsulin in the following section.

Structural Differences Between the SemBioSys Fusion Protein and Native Human Proinsulin

We have not been able to find the precise structure of the proinsulin-oleosin fusion protein generated in SemBioSys's transgenic safflower, either in the EA or elsewhere. However, a patent obtained by SemBioSys suggests that the structure of the proinsulin portion of the transgenic fusion protein could differ substantially from that of native human proinsulin, in that the patent covers sequences that have as little as 75% sequence identity to native human proinsulin (SBS Patent 2005, par. 0088). APHIS provides another clue, stating that: "The modified human proinsulin gene [sic][38] has two basic amino acids removed for added stability in plants plus eleven C-terminal amino acids. The added C-terminal amino acids act as a protein signal that ensures the retention of the fusion protein in the endoplasmic reticulum of the plant seed cell and the removal in downstream processing" (EA, p. 25).

Thus, assuming that APHIS has reported all of the modifications, SemBioSys's proinsulin is 9 amino acids longer (11 added minus 2 removed) than native human proinsulin; that is, 95 amino acids vs. 86, or 10% larger.

APHIS provides no information on the structure or even the length of the *Arabidopsis*-derived oleosin protein to which the modified human proinsulin is fused. However, SemBioSys's patent may provide a rough indication. In Figure 2 of this patent, the Arabidopsis-derived oleosin protein fused to insulin is 171 AA (18 kDa). The sequence reported in Figure 2 of the invention includes several other expressed amino acid sequences which may or may not also be present in the fusion protein of interest here.

In short, the SemBioSys fusion protein differs substantially from the native human, porcine, and bovine (pro-)insulin molecules that form the basis of APHIS's cursory treatment. The proinsulin portion has had two amino acids removed, and 11 added, to form a roughly 10% larger molecule that, moreover, has "added stability in plants." And fusion of the modified proinsulin to the oleosin protein likely yields a protein on the order of three times longer than proinsulin.[39] Both the modification to proinsulin and the fusion to the oleosin protein generate concerns that APHIS has largely failed to address. We address potential autoimmune hazards with respect to recombinant pharmaceuticals in general in Appendix 2. These concerns are especially acute in the case of recombinant human proinsulin, since the native version of the molecule, as discussed above, triggers hazardous autoimmune reactions implicated in type 1 diabetes. If the native molecule can trigger such reactions in a subset of the population (type 1 diabetes), it is reasonable to assume that novel versions like SemBioSys's modified proinsulin have a heightened potential to elicit such reactions in broader, non-diabetic, segments of the general population. The next section discusses potential immune system hazards with respect to the fusion protein.

[38] A gene is not composed of amino acids, as APHIS implies, but rather of nucleotides. APHIS meant to say "protein" rather than "gene."

[39] 95 AA for modified proinsulin + an assumed 171 AA for Arabidopsis oleosin protein = 266 AA.

Potential for Allergic and Other Hazardous Immune Responses to the Fusion Protein

APHIS addresses the potential for the SemBioSys fusion protein to elicit allergic reactions in two sections of Appendix V: Threatened and Endangered Species Analysis (EA, pp. 29 and 32-33). On page 29, APHIS states that: "the amino acid sequence of the human proinsulin-oleosin domains did not reveal any significant homology (>50%) to the amino acid sequence of proteins other than oleosin and human proinsulin proteins." APHIS then concedes that the oleosin portion of the fusion protein "shares a 70% sequence homology within its hydrophobic domain (central part) to a filbert (hazelnut) oleosin that has been implicated as a candidate allergen…" APHIS discounts the significance of this substantial homology with the argument that the hydrophobic domain is highly conserved (very similar) among oleosins of many food species that are consumed by humans and animals, implying that the hydrophobic portion of the Arabidopsis oleosin protein cannot be allergenic since similar oleosins of other food species do not appear to be allergenic.

There are several problems with this argumentation. First, APHIS's standard for what constitutes significant overall homology to known allergens (>50%) is faulty. In the internationally-accepted Codex Alimentarius standard for assessment of novel proteins in transgenic plants for potential allergenicity, any homology of the novel protein to known allergens exceeding 35% (not 50%) indicates potential allergenicity and calls for additional assessment.

Secondly, this Codex standard was developed to assess food allergenicity risks presented by transgenic food plants, not allergenicity or other immune system hazards from transgenic plants expressing a potent hormone. Any allergenicity of the Arabidopsis oleosin protein must be assessed in the context of the entire fusion protein, which also contains a potent hormone, proinsulin. As discussed above, proinsulin presents its own set of serious concerns with respect to a different class of aberrant immune system response, autoimmune reactions. In short, the modified proinsulin-oleosin fusion protein poses two potential immune system hazards – autoimmune reaction to the modified proinsulin, and allergic response to the oleosin protein. The fusion of these two proteins may give rise to synergistic effects that amplify either or both hazards.

Finally, several studies indicate that oleosin proteins in sesame seeds are allergenic (Leduc et al 2006; Cohen et al 2007), not merely the oleosin proteins in hazelnuts (Akkerdaas et al 2006). This casts doubt on APHIS's assumption that oleosin proteins present in food crops are generally non-allergenic.

B. *The EA is inadequate because it fails to convincingly state the reasons that the reasonably foreseeable human health and safety consequences are not significant.*

The EA must consider reasonably foreseeable effects when determining whether any effects are significant and therefore require an EIS. "Effects and impacts" are synonymous and include "reasonably foreseeable" indirect effects.[40] The above delineated human health risks are "reasonably foreseeable."

[40] 40 C.F.R. § 1508.8.

In addition, while the EA discusses containment efforts on the release site itself, it withholds all information regarding the facilities and procedures with which the proinsulin expressing seeds will be transported, ground into dust, and further processed. This SOP information is withheld as CBI. Also withheld are the procedures for protecting workers from exposure to the dust containing proinsulin. The EA acknowledges that foreseeable risks include both worker exposure to dust[41] and the spread of seeds through human error.[42] Given such risks, it is inadequate to withhold all information regarding the mitigation of those risks. The draft EA is inadequate because it does not adequately analyze reasonably foreseeable human health impacts and it does not provide convincing reasons those impacts will be prevented or mitigated.

C. *If there is a finding of no significant impact, the EA must convincingly state the reasons that the human health and safety effects are neither highly uncertain nor highly controversial. The proposed EA is inadequate.*

Along with public health and safety, factors that must be considered in determining whether environmental effects are significant include whether the environmental effects are highly uncertain or highly controversial. CEQ regulations state that when determining whether environmental effects are significant, the following factors must considered:

> (4) The degree to which the effects on the quality of the human environment are likely to be highly controversial.
>
> (5) The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.[43]

Either of these factors may be sufficient to require the preparation of an EIS.[44] Therefore, in determining whether an effect is significant, an EA must include an analysis of both highly controversial and highly uncertain environmental effects, including public health and safety impacts.

The proposed EA does not address the highly uncertain and unknown risks and consequences accompanying any pharmaceutical that has not yet been approved by the FDA. The proposed EA also does not acknowledge or address the controversy over the human health effects that may occur if safflower seeds and dust containing proinsulin are ingested or inhaled. If there is a finding of no significant impact, the proposed EA is inadequate because it fails to address the highly uncertain human health effects resulting from the ingestion and inhalation of proinsulin and it also fails to address the controversy regarding the significance of those effects.

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[41] EA at 15.
[42] EA at 12.
[43] 40 C.F.R. § 1508.27(b).
[44] National Parks & Conservation Ass'n v. Babbitt, 241 F.3d 722, 731 (9th Cir. 2001); Public Service Co. of Colorado v. Andrus, 825 F.Supp. 1483, 1495 (D. Idaho 1993).

III. APHIS Failed To Analyze the Cumulative Impacts of the Proposed Field Tests.

The proposed EA inadequately addresses cumulative impacts. Cumulative effects are briefly mentioned three times in the memo, first in the context of pollen, then in the context of effects of the transgene on the seeds, and finally in the context of volunteer growth. [45] Nowhere is there mention, for instance, of the cumulative exposures to proinsulin seed dust inhaled by workers or other cumulative effects on the public health.

Cumulative impacts must be fully considered in an EA. "Given that so many more EAs are prepared than EISs, *adequate consideration of cumulative effects requires that EAs address them fully.*"[46] NEPA requires agencies to consider the cumulative impacts of their proposed actions.[47] By definition, cumulative effects must be evaluated along with direct and indirect effects of a project and its alternatives. "'Cumulative impact' is 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 or person undertakes such other actions."[48] Individually minor, but collectively significant actions, taking place over time, can generate cumulative impacts.[49]

A meaningful cumulative impact analysis, according to the D.C. Circuit, must identify

> (1) the area in which the effects of the proposed project will be felt; (2) the impacts that are expected in that area from the proposed project; (3) other actions–past, present, and proposed, and reasonably foreseeable–that have had or are expected to have impacts in the same area; (4) the impacts or expected impacts from these other actions; and (5) the overall impact that can be expected if the individual impacts are allowed to accumulate.[50]

The EA only briefly mentions cumulative impacts and does not offer a cumulative impact analysis encompassing these five elements. Therefore, the EA is inadequate.

It is reasonably foreseeable that SemBioSys or another company will request a permit for a greatly expanded release of proinsulin expressing safflower with acreage to produce enough insulin to meet the high market demand for human insulin. The EA clearly implies that meeting this market demand is the long term goal of SemBioSys.[51] An adequate EA must include an analysis of the cumulative effects of such foreseeable future permit requests.

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[45] EA at 11 and 13.
[46] Kern v. United States Bureau of Land Mgmt., 284 F.3d 1062, 1076 (9th Cir. 2002) ("We have held that an EA may be deficient if it fails to include a cumulative impact analysis or to tier to an EIS that has conducted such an analysis.").
[47] 40 C.F.R. § 1508.27(b)(7); Utahns for Better Transp. v. United States Dep't of Transp., 305 F.3d 1152, 1172 (10th Cir.2002); Kern v. United States Bureau of Land Mgmt., 284 F.3d 1062, 1076 (9th Cir.2002); Vill. of Grand View v. Skinner, 947 F.2d 651, 659 (2d Cir.1991).
[48] 40 C.F.R. § 1508.7.
[49] Id.
[50] Grand Canyon Trust v. F.A.A., 290 F.3d 339, 345 (D.C. Cir. 2002).
[51] EA at 3 and 4.

IV. The EA's Analysis of Alternatives is Inadequate.

APHIS' analysis of alternatives in the EA was equally insufficient because USDA failed to adequately analyze the alternatives it identified in the EA.[52] EAs must include analysis of the alternatives to the proposed action.[53] The preferred option is B,[54] so the only alternative to the preferred option is alternative A (the "no action" alternative). There are only three sentences discussing alternative A.[55] This is wholly inadequate.

NEPA requires that federal agencies consider alternatives to recommended actions whenever those actions "involve[ ] unresolved conflicts concerning alternative uses of available resources."[56] The EA "[s]hall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives . . . ."[57] The goal of the statute is to ensure "that federal agencies infuse in project planning a thorough consideration of environmental values."[58] The consideration of alternatives requirement furthers that goal by guaranteeing that agency decision makers "[have] before [them] and take [ ] into proper account all possible approaches to a particular project (including total abandonment of the project) which would alter the environmental impact and the cost-benefit balance."[59] NEPA's requirement that alternatives be studied, developed, and described both guides the substance of environmental decision-making and provides evidence that the mandated decision-making process has actually taken place.[60] Informed and meaningful consideration of alternatives -- including the no action alternative -- is thus an integral part of the statutory scheme.[61]

Because the proposed EA only includes one alternative, that of no action at all, and because that alternative is not discussed save three sentences, the proposed EA is inadequate.

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[52] *See* Bob Marshall Alliance v. Hodel, 852 F.2d 1223, 1228 (9th Cir. 1988).
[53] Id. at 1229 ("consideration of alternatives requirement is both independent of, and broader than, the EIS requirement. In short, any proposed federal action involving unresolved conflicts as to the proper use of resources triggers NEPA's consideration of alternatives requirement, whether or not an EIS is also required.")
[54] EA at 7.
[55] EA at 5.
[56] 42 U.S.C. § 4332(2)(E).
[57] 40 C.F.R. § 1508.9.
[58] Conner v. Buford, 836 F.2d 1521, 1532 (9th Cir. 1988).
[59] Calvert Cliffs' Coordinating Committee, Inc. v. United States Atomic Energy Commission, 449 F.2d 1109, 1114 (D.C. Cir.1971).
[60] Id.
[61] *See* Bob Marshall Alliance v. Hodel, 852 F.2d 1223, 1228 (9th Cir. 1988).

CONCLUSION

The EA inadequately analyzes the public health, environmental and cumulative impacts of the release that will commence if the permit is granted. Additionally, the alternatives to the preferred action are inadequate. Further, information crucial for the evaluation of the cumulative and immediate health and safety impacts has been withheld making informed public comment impossible. If a finding of no significant impact is made, the EA will not provide convincing reasons as to why the impacts are insignificant. For each of these reasons, the proposed EA does not meet the legal standards set by NEPA, the CEQ, and case law. An EIS must be prepared.

Respectfully submitted,

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Appendix I

Fundamental Scientific Misunderstandings in APHIS's Environmental Assessment

APHIS's treatment of the potential adverse impacts on humans and animals from exposure to the SemBioSys proinsulin fusion protein is limited to a few brief paragraphs or sentences scattered throughout the EA on pages 11, 14-15, in Appendix IV (p. 28), and Appendix V (pp. 32-33). APHIS provides no assessment of potential human health impacts from ingestion of the proinsulin fusion protein, and makes only an incidental attempt to address inhalational exposure. A bit of additional information on the fusion protein is supplied on pp. 25-26. This scattered treatment is deeply flawed, and in no way constitutes an acceptable assessment.

APHIS mistakenly asserts that proinsulin has no biological activity upon ingestion. How can one account for APHIS's error here, given published studies which demonstrate the contrary, and even more SemBioSys's stated preference (in a 2005 patent) for delivering plant-made insulin via the oral route? APHIS presents three arguments.

1) Researchers have not developed a successful oral delivery method for insulin "in the last several decades."
2) Like "most food proteins," (pro-)insulin will be broken down in the digestive system before being absorbed in the intestine and entering the circulatory system.
3) Two "simulated digestive studies" by SemBioSys

The first argument (presented in a single sentence) is completely beside the point. Proinsulin may indeed have oral activity when ingested, and even substantial activity, as Ni et al (2007) and Ruhlman et al (2007) clearly show, without leading to perfection of an oral delivery method for insulin. The latter requires not just (sufficient) activity, but reproducible delivery of a consistent dose under a variety of gastrointestinal conditions (e.g. encompassing inter-individual gastric differences in, for instance, pH); accomplish this without adverse health impacts; and accomplish this in a manner that provides benefits beyond accepted insulin delivery methods. The scientific naiveté of concluding that a drug has no activity via a particular route of adminstration from the failure to introduce a commercial product utilizing that particular route is breathtaking.

The second argument is based on the assumed equivalence of proinsulin to "food proteins," most of which are in fact rapidly degraded before they can be absorbed in whole form by the intestine. However, some food proteins and other types of proteins resist digestion. Some are absorbed by the intestine in whole or partially degraded form, in some cases with harmful effects (e.g. food allergens, which can cause life-threatening anaphylactic reactions). To simply assume that insulin will be degraded "like most food proteins" is highly irresponsible. It is important to understand that the fallacy of this argument does not depend on the two studies cited above, which clearly show that proinsulin can survive digestion and exert a substantial effect. The real problem is APHIS's apparent ignorance of the huge scientific literature demonstrating that many proteins, food and otherwise, do in fact survive (complete) digestion and become absorbed into the blood stream, in some cases with harmful effects. APHIS's argument here is at the level of a high-school biology truism – proteins are broken down in the stomach, and therefore pose no concern. To use such a truism as a supposed demonstration that a potent hormone will not pose

health risks is scientifically indefensible, and highly irresponsible of a regulatory agency charged with protecting public health and the environment.

The third argument is addressed in the text of our comments.

APHIS cites one paper on proinsulin's role as an autoantigen in a passage that reveals deep confusion about the paper's import, the science of autoimmune disease, and indeed, about basic facets of the human immune system:

> "It has also been suggested that high levels of proinsulin found in the blood may become an autoantigen [sic]; a protein that the body unfavorably reacts to causing an allergic response (e.g. inflammation) (You and Chatenoud 2006)" (EA, p. 28).

There are several fundamental errors here: 1) An autoantigen does not cause an allergic response. The APHIS reviewer is confusing two quite distinct classes of aberrant immune system activity. Allergic responses involve production of immunoglobulins that target an exogenously introduced protein (e.g. food protein or pollen protein) that a normal immune system does not attack, while pathologic autoimmunity involves an aberrant immune system response targeting one of the body's own proteins – a "self protein" or autoantigen; 2) Neither the paper cited (nor the study by Krishnamurthy et al (2006) on which it is based) discusses whether or not "high levels of proinsulin" are a prerequisite to proinsulin becoming an autoantigen, as the APHIS reviewer seems to think; 3) The reviewer fails to mention that autoimmune reaction to proinsulin is discussed in the context of elucidating its role in development of type 1 diabetes (perhaps because he/she mistakenly thinks that the autoimmune reaction is some sort of "allergic response"); 4) Finally, inflammation is not one form of "allergic response," as the APHIS reviewer's "allergic response (e.g. inflammation)" implies, but is rather a general symptom accompanying a wide range of conditions, including both proper immune system responses (e.g. when leukocytes attack an infectious agent) and aberrant ones such as allergic reactions.

We have dwelt on this passage because it demonstrates so clearly why an agency whose expertise is in agriculture does not have the competence to deal with the important medical issues raised by exposure to a novel and potent human drug. In preparation of an EIS, we urge APHIS to consult with the FDA, and/or with independent medical scientists who have expertise with insulin.

Appendix 2

Adverse Immunological Responses to Recombinant Pharmaceutical Proteins

There is a growing body of evidence demonstrating puzzling, unexpected and in some cases dangerous immunologic responses to biopharmaceuticals produced in genetically engineered cell cultures.[i] In these cell culture production systems, a human gene encoding a medically useful protein such as insulin is spliced into bacteria or mammalian cells, which then produce a recombinant version of the protein, known as a biopharmaceutical. While the immune system does not normally attack a bodily protein because it is recognized as "self," it may respond to the corresponding biopharmaceutical due to subtle differences that cause the body to recognize it as foreign. The precise nature of these differences has not been established in most cases and is a subject of intense research; they could involve differences in post-translational processing, tertiary structure, and/or primary amino acid sequence.

In some cases, the administered biopharmaceutical merely elicits an immune system response that reduces or eliminates the drug's potency. This phenomenon has been observed in some patients receiving recombinant blood clotting Factor VIII and the multiple sclerosis drug beta-interferon. In other cases, the immune system detects that the engineered drug is different (i.e. treats it as foreign), yet the antibodies produced against the engineered drug also target the natural counterpart, thereby leading to potentially disastrous consequences. For instance, a recombinant version of megakaryocyte growth and development factor (MGDF) produced by Amgen was discontinued in clinical trials because some patients receiving the drug mounted an immune attack on both Amgen's recombinant MGDF and their own natural version of MGDF, resulting in bleeding. A similar phenomenon might be responsible for up to 160 cases of red cell aplasia (virtual shutdown of red blood cell production) observed in patients treated with recombinant erythropoietin, a hormone that stimulates red blood cell production. The important fact to keep in mind here is that these reactions to recombinant biopharmaceuticals have taken biotech companies and regulators alike ***by surprise***. Dr. Burt Adelman, head of research & development at Biogen, found the immune reactions to MGDF "stunning."

> *"The conventional wisdom had been that this was a theoretical risk ... nobody saw it coming. If you're in my business, it's really unnerving."*[ii]

In other words, although the natural human protein and the corresponding engineered biopharmaceutical appear to be identical, the immune system is able to detect a difference that scientists, at present, cannot. The FDA has implicitly recognized this fact. At a meeting in 2002 about human plasma-derived drugs, the FDA's Chris Joneckis noted that:

> *"Despite best efforts to detect product differences and predict the impact of manufacturing changes, these surprises do continue to occur."*[iii]

If tightly-controlled fermentation production of mammalian cell-produced "human" drugs is causing such stunning, unpredicted and in some cases hazardous immune reactions, it is even more probable that biopharmaceuticals such as proinsulin produced in plants subject to the "manufacturing changes" imposed by nature in the form of widely varying microclimates and microhabitats, insect infestation, etc, will elicit similar reactions.

[i] For a fuller treatment of the following discussion, with references, see: "Comments on Draft Guidance for Industry: Drugs, Biologics and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals," Docket No. 02D-0324, by Friends of the Earth, submitted to FDA on January 10, 2003. www.foe.org/biopharm/commentsguidance.pdf.

[ii] As quoted in: Aoki, N. "Protein therapies spark scrutiny: researchers weigh potential risk of immune responses," *The Boston Globe*, Nov. 27, 2002.

[iii] Transcript of "Comparability Studies for Human Plasma-Derived Therapeutics," FDA CBER workshop, May 30, 2002, p. 42.