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Regulatory Analysis and Development
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To whom it may concern:

The Center for Food Safety (CFS) submits the following comments on the draft environmental assessment (EA) conducted by USDA's Animal and Plant Health Inspection Service (APHIS) on its determination of nonregulated status for the Pioneer Hi-Bred International, Inc. ("DuPont-Pioneer")[1] soybean designated as transformation event 356043, (hereinafter "GAT soybeans" or "356043 soybeans"). GAT soybeans are tolerant to two different classes of herbicides: glycines (i.e. glyphosate) and ALS inhibitors. GAT soybeans present several new food safety and environmental/agronomic issues that APHIS has failed to address, or address adequately, in its EA.

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.

[1] Pioneer is a wholly owned subsidiary of DuPont. Thus, CFS refers to Pioneer Hi-Bred as DuPont-Pioneer.

CFS strongly opposes the cultivation and commercial use of genetically engineered food crops due to unexplored risks to the environment, biodiversity, specific protected species, and potential risks to human health that could result. Genetic engineering is a novel technology that fundamentally alters agriculture, our food supply, and the environment. Neither standard corporate testing practices for, nor U.S. government oversight of, genetically engineered (GE) crops is sufficiently stringent to rule out, with reasonable scientific certainty, unintended adverse impacts to human health or the environment.[2] CFS therefore supports a moratorium on GE crops until the U.S. government establishes a rigorous, science-based regulatory system.

Short of such a blanket moratorium on GE crop commercialization, the deregulation and commercialization of GAT Soybeans and progeny derived from it requires the preparation of an EIS under the National Environmental Policy Act ("NEPA"), because the EA contains unanswered or inadequately answered health and safety questions. Specifically, CFS requests that APHIS institute a moratorium on the commercial introduction, dissemination, interstate movement or conveyance of GAT soybeans, including but not limited to all food products containing any ingredients or material derived from this genetically engineered soy, until the USDA, as mandated under §102 of NEPA, fully evaluates the environmental, human health and socio-economic impacts caused by the commercialization of GAT soybeans. Such action and analysis should include completion of an environmental impact statement analyzing the effects on the human environment resulting from any USDA actions deregulating (or other action allowing commercial distribution, sale and planting) GAT Soybeans.

We have numerous serious concerns about this deregulation, as discussed in detail below. The deregulation should be denied because APHIS has not met its burden for supporting the deregulation and because APHIS has failed to do the proper environmental analysis supporting the deregulation.

BACKGROUND

On September 28,, 2006 APHIS received a petition seeking a determination of nonregulated status from Pioneer Hi-Bred for GAT Soybeans. (APHIS Petition # 06-271-01p). In the October 5, 2007 *Federal Register*, USDA APHIS announced a public comment period on a draft environmental assessment (EA). 72 Fed. Reg. 56981 (Oct. 5, 2007). Comments are due December 4, 2007. *Id.*

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[2] Freese, W. and D. Schubert (2004). "Safety Testing and Regulation of Genetically Engineered Foods," *Biotechnology and Genetic Engineering Reviews*, Volume 21, November 2004. http://www.foe.org/camps/comm/safefood/gefood/testingregbackgrounder.pdf

CFS COMMENTS

Summary

The draft EA is wholly inadequate and defective because APHIS failed to take the "hard look" required by NEPA. An EIS must be prepared for the deregulation of GAT soybeans to properly address the significant environmental impacts that may result from APHIS' approval. The draft EA fails to adequately discuss numerous significant environmental, public health and agronomic impacts of GAT soybeans, including increased glyphosate use and increased prevalence of glyphosate-resistant weeds. The draft EA also fails to adequately address a number of novel food safety concerns raised by GAT soybeans. APHIS has failed to analyze any alternatives beyond outright rejection or granting of DuPont-Pioneer's petition. Finally, APHIS has not provided a meaningful analysis of the cumulative impacts from introduction of GAT soybeans in association with the current widespread use of other glyphosate-tolerant soybeans and other glyphosate-resistant crops. The draft EA is arbitrary and capricious. Center for Food Safety therefore requests that APHIS prepare an Environmental Impact Statement (EIS) to fully analyze the environmental and public health affects of the deregulation prior to making any decision.

Herbicide-tolerant transgenic crops promote the use of chemical herbicides. The rise in herbicide use associated with these crops has been particularly steep in recent years, in part to address an epidemic of herbicide-resistant weeds. The GAT soybean is the first transgenic crop being considered for deregulation by APHIS that is tolerant to two herbicides rather than just one. APHIS has failed to adequately analyze the potential for increased use of two herbicides on GAT soybeans to increase the prevalence of resistant weeds, despite the fact that the most prevalent herbicide-resistant weeds in the United States are resistant to ALS inhibitors and/or glycines (i.e. glyphosate), the two classes of herbicides GAT soybeans have been engineered to tolerate.

GAT soybeans are also engineered for a higher level of tolerance to glyphosate than currently commercialized glyphosate-tolerant (Roundup Ready) soybeans. DuPont has stated its intention of further increasing the level of glyphosate-tolerance of GAT soybean by stacking it with one or two other mechanisms of glyphosate tolerance. While presented as a means to better control resistant weeds, increased glyphosate tolerance would in fact further increase glyphosate use, worsen weed resistance, and exacerbate other problems fostered by the already excessive use of this herbicide, such as environmental harms associated with glyphosate and Roundup, for example toxicity to certain amphibian species. Worsening glyphosate-resistance in weeds will also accelerate a trend towards increased use of other toxic herbicides, with adverse environmental and human health impacts.

APHIS has failed to adequately address these matters, despite: 1) A long-standing commitment of USDA and EPA to promote increased use of integrated pest management as a means to ***decrease*** chemical pesticide use; and 2) A joint initiative of USDA-EPA to manage herbicide-resistance in weeds specifically with respect to introduction of transgenic herbicide-tolerant crops.

GAT soybeans also present novel food safety concerns. GAT soybeans incorporate the "glyphosate acetyltransferase" (GAT) enzyme, which renders glyphosate non-herbicidal via a process known as acetylation. The GAT enzyme engineered into 356043 soybeans is modeled on an enzyme isolated from certain rare strains of *Bacillus licheniformis*. APHIS regulations for field tests require that "the function of the introduced genetic material is known." 7 C.F.R. 340.3(b)(3) The natural function of GAT in *B. licheniformis* is unknown. However, its source organism (*B. licheniformis*) has been identified as the toxigenic agent responsible for several cases of food poisoning. APHIS is thus poised to deregulate, for widespread commercial use, a genetically engineered crop that contains genetic material of unknown native function from a species of bacteria, some strains of which are known to be toxigenic.

GAT acetylates glyphosate, but is also known to acetylate several naturally occurring compounds (amino acids) in food. Via this process of acetylation, the GAT enzyme generates significant levels of several, perhaps 13, compounds in GAT soybeans that have no history of safe food use. Some would be completely novel additions to the food supply, others are found only at extremely low levels in foods; none have been subjected to toxicological assessment for potential adverse health impacts on humans or animals.

As part of its environmental analysis APHIS must consider **"**the degree to which the proposed action affects public health or safety."[3] APHIS has ignored this obligation.

The National Environmental Policy Act ("NEPA")

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."[4] 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."[5]

Recognizing the affects of new technologies on the environment, Congress explicitly stated in NEPA that "new and expanding technological advances" are activities that could threaten the environment.[6] In the legislative history, Congress expressed its concern with "[a] growing technological power … far outstripping man's capacity to understand and ability to control its impact on the environment."[7] Thus, in order to understand and control the effects of new technologies, Congress required federal agencies to consider their environmental effects by prescribing the requirements of NEPA. In addition to

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[3] 40 Fed. Reg. § 1508.27(b)(2).
[4] 42 U.S.C. § 4332(2)(C).
[5] *Robertson v. Methow Valley Citizens Council,* 490 U.S. 332, 349(1989).
[6] 42 U.S.C. § 4331(a).

[7] Found. on Economic Trends v. Heckler, 756 F.2d 143, 147 (D.C. Cir. 1985) (quoting S. Rep. No. 91-296 (1969)).

environmental concerns, the proposed action's possible direct, indirect, and cumulative impacts on public health must be reviewed.[8]

A threshold question is whether a proposed project will "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]

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]

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.[14] 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."[15] CEQ's regulations are applicable to and binding on all federal agencies.[16] Among other requirements, CEQ's regulations mandate that federal agencies address all "reasonably foreseeable" environmental impacts of their proposed programs, projects, and regulations.[17]

The CEQ regulations list factors that determine whether a federal action, such as deregulating GAT soybeans is "significant." The CEQ regulations define the term 'significantly' for purposes of NEPA as requiring analysis of both the 'context' and the 'intensity' of the action."[18] Context is the scope of the agency action.[19] Intensity "refers to the severity of the impact" and is defined by the factors in 40 C.F.R. section 1508.27(b).

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[8] 40 C.F.R. § 1508.8(b); Baltimore Gas & Elec. Co. v. NRDC, 462 U.S. 87, 106 (1983)(explaining that "NEPA requires an EIS to disclose the significant health, socioeconomic, and cumulative consequences of the environmental impact of a proposed action.").

[9] 42 U.S.C. § 4332(2)(C).
[10] 40 C.F.R. § 1508.9.
[11] *Id.*
[12] *Save the Yaak v. Block*, 840 F.2d 714, 717 (9th Cir. 1988).
[13] Id.
[14] See 42 U.S.C. §§ 4321, 4344.
[15] 40 C.F.R. § 1500.3.
[16] 40 C.F.R. §§ 1500.3, 1507.1; see, e.g., Hodges v. Abraham, 300 F.3d 432, 438 (4th Cir. 2002).
[17] See 40 C.F.R. §§ 1502.4, 1508.8, 1508.18, & 1508.25.
[18] *Anderson v. Evans*, 371 F.3d 475, 487 (9th Cir. 2004).
[19] *National Parks & Conservation Ass'n v. Babbitt*, 241 F.3d 722, 731 (9th Cir. 2001).

Courts rely on these factors to determine "significance." Even meeting just one of the factors in 1508.27(b) may require the preparation of an EIS.[20]

USDA specifically adopted these CEQ regulations in relation to APHIS' review of genetically engineered crop.[21] The factors include:

- the degree to which the proposed action affects public health or safety;[22]

- the unique characteristics of the geographic area such as proximity to historic and cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.[23]

- the degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks;[24]

- Whether the action is related to other actions with individually insignificant but cumulatively significant impacts.[25]

As discussed herein, the commercial introduction of genetically engineered GAT Soybeans poses novel human health threats that constitutes unique environmental impacts and impacts to human health and safety. Following is a description of the impacts that the USDA must evaluate.

I. The EA's "Analysis" of the Potential Environmental Impacts Is Wholly Inadequate Because APHIS Failed to Take the "Hard Look" Required By NEPA. These Impacts Require An EIS.

As mandated by Congress, APHIS must comply with NEPA before it attempts to deregulate and allow the commercialization of genetically engineered GAT Soybeans and any progeny derived from it. USDA is the lead federal agency designated to undertake NEPA analysis for the commercialization of genetically engineered plant varieties. USDA's decision whether to deregulate a genetically engineered soy variety is a major federal action that may significantly affect the environment. The commercial planting of genetically engineered GAT Soybeans could impact a vast number of acres of prime farm land and will have significant impacts on the environment, including impacts to human health, as well as cumulative impacts.[26]

The draft EA glosses over important issues, making nothing more than a perfunctory attempt to appear to cover the impacts stemming from this deregulation. The EA only

[20] *Ocean Advocates v. U.S. Army Corps of Engineers*, 402 F.3d at 865 (9th Cir. 2005) (*citing Nat'l Parks*, 241 F.3d at 731).
[21] 7 C.F.R. § 372.4
[22] 40 Fed. Reg. § 1508.27(b)(2).
[23] 40 Fed. Reg. § 1508.27(b)(3).
[24] 40 Fed. Reg. § 1508.27(b)(5).
[25] 40 Fed. Reg. § 1508.27(b)(7).
[26] 40 Fed. Reg. §§ 1508.27(b)(2), (3), (5), (7)

superficially covers a number of possible significant environmental impacts, as detailed below. The draft EA also relies uncritically on DuPont-Pioneer 's petition, which lacks a variety of important data that APHIS should have required and assessed in its draft EA.

A. The EA Failed to Examine Significant Environmental Impacts

DuPont-Pioneer's 356043 soybean represents still another pesticide-promoting, herbicide-tolerant transgenic crop. As such, it presents many of the same issues relating to increased use of herbicides that are presented by other crops in this class – issues that APHIS has failed to adequately analyze in this environmental assessment. However, DuPont-Pioneer's soybeans are also novel in that they are the first biotech crop being considered for deregulation that have been genetically engineered for tolerance to two herbicides rather than just one. In this section, we address concerns relating to this novel aspect of DuPont-Pioneer's soybeans and APHIS's failure to adequately address them.

Both DuPont-Pioneer and APHIS present 356043 soybeans as an answer to herbicide-resistant weeds. According to DuPont-Pioneer: "The commercialization of herbicide tolerant 356043 soybean is expected to have a beneficial impact on weed control practices, as growers will have another tool available to address their regional weed problems. 356043 soybean will enable growers to choose an optimal combination of glyphosate, ALS herbicide, and other complementary herbicides to best manage their individual weed populations" (Petition, p. 104). DuPont-Pioneer also states: "***We anticipate no increase in the usage of glyphosate***, but we do expect an increase in the use of ALS-inhibiting herbicides, as this family of herbicides currently is not widely used for control of weeds in soybean (emphasis added, Petition, p. 107). APHIS states: "A primary use of 356043 soybean is that it can enable effective weed control using an ALS-inhibitor herbicide in areas where glyphosate tolerant weeds are present" (EA, p. 10).

Our analysis suggests precisely the contrary: 356043 will exacerbate the serious agronomic problem of herbicide-resistant weeds (particularly glyphosate-resistant weeds) and contribute to increased use of herbicides to control them. As discussed further in the Cumulative Impacts section, APHIS officially recognized the need to manage herbicide-resistant weeds fostered by transgenic herbicide-tolerant crops in 2002, but has failed to act or even analyze the issue. USDA also has a long-standing commitment to promote integrated pest management to reduce use of chemical pesticides, but has failed to act on it. Deregulation of 356043 soybeans would run directly contrary to both commitments by exacerbating resistant weeds and increasing chemical pesticide use.

DuPont-Pioneer's observation that ALS inhibitors are "not widely used for control of weeds in soybeans" (Petition, p. 107) fails to explain why this is the case. In fact, ALS inhibitors were once much more widely used on soybeans. In 1994, for instance, 2.013 million lbs. of ALS inhibitor herbicides were applied to soybeans, falling to 265,000 lbs. in 2006.[27]. This substantial (more than 7-fold) decrease in ALS inhibitor use is explained in part by its decreasing effectiveness. Weed scientists have documented weeds resistant to ALS

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[27] CFS internal analysis of USDA NASS agricultural chemical use data for the pertinent years and crops, available at: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1560. Subsequent references to pesticide usage also based on these data.

inhibitor-class herbicides on up to 150,000 sites covering 9.9 million acres of land in 35 states. 37 species of weeds have developed resistance to ALS inhibitor herbicides, including many of those most troublesome in soybeans (as listed in Petition, Table 28, p. 102).[28] In fact, far more weeds have developed documented resistance to this class of herbicides than to any other, as measured by acreage of land infested.

The other reason for declining use of ALS inhibitors in soybeans is farmers' increasing reliance on glyphosate as the sole means of weed control fostered by adoption of the Roundup Ready soybean system. USDA data show an astounding 26.7-fold increase in glyphosate use on soybeans from 1994, the year before Roundup Ready soybeans were introduced, to 2006 (from 3.476 to 92.856 million lbs.). Over the same period of time, overall herbicide use on soybeans more than doubled, from 49.258 to 103.156 million lbs.

This massive increase in glyphosate use has fostered the latest epidemic of resistant weeds. Confirmed glyphosate-resistant weeds have now been reported on an estimated 2.4 million acres in the U.S., from just 10,000 acres as recently as 1999. Ironically, the two classes of herbicides that 306543 soybeans have been engineered to tolerate are the very herbicide classes to which weeds have developed the most resistance, as measured by acres infested. APHIS failed to adequately consider these impacts will have on prime farmlands.[29]

It is probably true that deregulation and adoption of 306543 soybeans would foster increased use of ALS inhibitor class herbicides, as DuPont-Pioneer projects. But contrary to DuPont-Pioneer's anticipation of "no increase in the usage of glyphosate" (Petition, p. 107), it is likely that use of glyphosate will increase substantially more. Consider the following factors. First, the prevalence of weeds resistant to ALS inhibitors will greatly limit their usefulness in many situations. Second, there are strong historical trends towards decreased use of ALS inhibitors and increased reliance on glyphosate as the sole means of weed control in soybeans. This overreliance on glyphosate has been demonstrated in many ways. As noted above, glyphosate use on soybeans increased 26.7-fold from 1994-2006, while use of ALS inhibitors on soybeans decreased 7-fold from 1994-2006. APHIS's tabulation of percentage of U.S. soybean acres treated with various herbicides in 1995, 2001 and 2006 corroborates these trends (EA, p. 10). Significantly, 12 herbicides were used on 10% or more of U.S. soybean acres in 1995, including glyphosate (20%). In 2006, glyphosate was applied to 96% of U.S. soybeans; the next-most frequently used herbicide, the ALS inhibitor chlorimuron, was used on just 4% of soybean acres, with many of the others applied to <1% of soybeans acres.[30] These statistics are further supported by a recent survey of 400 farmers in the U.S. Midwest conducted by Syngenta, which revealed that ***56% of soybean growers in northern states and 42% in southern states use glyphosate as their sole herbicide***.[31]

The prevalence of weeds resistant to ALS inhibitor herbicides and their dramatically decreasing use by farmers relative to glyphosate suggest strongly that farmers will apply more

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[28] CFS internal analysis of herbicide-resistant weed data accessed from www.weedscience.org, 11/21/07. Subsequent figures on herbicide-resistant weeds also based on this analysis.
[29] 40 Fed. Reg. § 1508.27(b)(3).
[30] Four ALS inhibitor herbicides were used on 10% or more of soybean acres in 1995: imazethapyr (44%), chlorimuron (16%), imazaquin (15%) and thifensulfuron (12%); the corresponding percentages of soybean acres treated with these four herbicides in 2006 were 3%, 4%, 1% and 1%, respectively (EA, table on p. 10).
[31] Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.

glyphosate than ALS inhibitors to 306543 soybeans. But if farmers will likely not make significant use of the ALS inhibitor tolerance trait, why would they purchase DuPont-Pioneer's soybeans rather than Roundup Ready soy? One answer is that DuPont-Pioneer's soybeans may well provide a much higher level of tolerance to glyphosate than Roundup Ready soybeans. This higher tolerance would allow for application of higher rates of glyphosate, for instance to kill weeds that show partial rather than complete resistance.[32]

First, we note that DuPont-Pioneer scientists reported that maize plants transformed with one version of its GAT enzyme were tolerant to six times the dose of glyphosate normally applied to Roundup Ready corn (Castle et al 2004, p. 1154, see also Figure 4, p. 1153). 306543 soybeans may exhibit a similar level of enhanced glyphosate tolerance.

Secondly, DuPont-Pioneer has announced its intention to develop super-glyphosate-tolerant crops that combine its GAT mechanism of glyphosate tolerance with one or both of the two glyphosate-tolerance traits developed by Monsanto. In its 2005 patent, "Novel Glyphosate-N-Acetyltransferase (GAT) Genes," DuPont-Pioneer claims:

> "A transgenic plant or transgenic plant explant having ***an enhanced tolerance to glyphosate***, wherein the plant or plant explant expresses a polypeptide with glyphosate-N-acetyltransferase activity… and ***at least one polypeptide imparting glyphosate tolerance by an additional mechanism***." (emphasis added, GAT Patent 2005, claim 111, p. 89).

DuPont-Pioneer is clearly interested in super-glyphosate-tolerant crops even without tolerance to ALS inhibitors or other herbicides, and regards them as a legitimate means of controlling glyphosate-resistant weeds:

> "The invention provides methods for controlling weeds in a field and preventing the emergence of glyphosate-resistant weeds in a field containing a crop which involve planting the field with crop seeds or plants that are glyphosate-tolerant as a result of being transformed with a gene encoding a glyphosate-N-acetyltransferase [GAT] and a gene encoding a polypeptide imparting glyphosate tolerance by another mechanism, such as a glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase and/or a glyphosate-tolerant glyphosate oxido-reductase[33] and applying to the crop and the weeds in the field ***a sufficient amount of glyphosate to control the weeds without significantly affecting the crop.***" (emphasis added, GAT Patent 2005, par. 0032).

The stacking of up to three mechanisms of glyphosate-tolerance in a single plant will allow more frequent applications of higher doses of glyphosate, perhaps over the entire growing season of the crop. Such super-tolerance will enable vastly increased use of glyphosate (over already exorbitant and growing levels) in an attempt to keep up with the rapidly growing

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[32] Complete immunity to an herbicide is a rare phenomenon. The term "resistant weed" is normally used to refer to a weed that tolerates application of more than the standard dose of the pertinent herbicide. Glyphosate-resistant weeds that survive 2-fold to 10-fold and more the normal dose are becoming increasingly common.

[33] These are two additional mechanisms of glyphosate-tolerance that are utilized by commercially by Monsanto in Roundup Ready crops. It is unclear if DuPont-Pioneer plans to license the use of these patented mechanisms from Monsanto, or alternately utilize them when the pertinent patents lapse.

level of glyphosate-resistance found in various weed species. The end result is a vicious circle of rising glyphosate use to control resistant weeds, followed by increased weed resistance, which in turns drives still more chemical use.

DuPont-Pioneer also claims a plant with GAT glyphosate-tolerance and tolerance to one additional herbicide, corresponding to 356043 soybeans (claim 112), as well as plants that incorporate ***super***-glyphosate tolerance plus tolerance to one of a whole battery of additional herbicides (claim 113), described as follows:

> "In a further embodiment the invention provides for …[super-glyphosate tolerance as described above] … and a gene encoding a polypeptide imparting tolerance to an additional herbicide, such as a mutated hydroxyphenylpyruvatedioxygenase, a sulfonamide-tolerant acetolactate synthase, a sulfonamide-tolerant acetohydroxy acid synthase, a phosphinothricin acetyltransferase and a mutated protoporphyrinogen oxidase and applying to the crop and the weeds in the field a sufficient amount of glyphosate and an additional herbicide, such as, a hydroxyphenylpyruvatedioxygenase inhibitor, sulfonamide, imidazolinone, bialaphos, phosphinothricin, azafenidin, butafenacil, sulfosate, glufosinate, and a protox inhibitor to control the weeds without significantly affecting the crop." (DuPont-Pioneer Patent, par. 0033)

APHIS concedes that weeds (waterhemp and horseweed) with confirmed resistance to both ALS inhibitors and glyphosate have already developed (EA, p. 10). Our detailed internal analysis of the www.weedscience.org website cited by APHIS reveals that weeds with confirmed cross-resistance to two or three major classes of herbicides now infest up to 1459 sites covering 245,755 acres in the U.S. alone. Since just 2003, weeds with cross-resistance to ALS inhibitors and glyphosate have been confirmed in Ohio, Missouri and Illinois. Deregulation of 356043 soybeans will foster increased use of glyphosate, and probably increased use of ALS inhibitors as well in those areas where weeds resistant to these herbicides are not yet legion. Thus, deregulation of 356043 soybeans will likely foster more rapid development of cross-resistant weeds.

APHIS completely failed to analyze the significant potential for increased glyphosate use and exacerbation of glyphosate-resistant weeds from deregulation of 356043 soybeans or super-glyphosate-resistant progeny derived from it. Instead, APHIS incorrectly assumes that displacement of Roundup Ready soybeans by GAT soybeans would have no impact on agronomic practices. Deregulation decisions normally cover not only the deregulated event, but all progeny of that event, including crosses with other deregulated GE crops. But this need not be the case. APHIS has the option of imposing conditions on deregulation, and in fact recently proposed altering its regulatory practices to provide for "conditional deregulation," including restricting the scope of deregulation to exclude "stacking" of the deregulated crop with certain other deregulated crops.[34] Clearly, APHIS should consider such an alternative that would exclude development of super-glyphosate versions of GAT soybeans, as discussed above, in the context of an Environmental Impact Statement. We address cumulative impacts related to deregulation of GAT soybeans later in these comments.

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[34] USDA APHIS: Draft Programmatic Environmental Impact Statement for the Introduction of Genetically Engineered Organisms, 72 Fed. Reg. 39021 (July 17, 2007).

B. The EA Is Defective Because APHIS Improperly Relied on EPA's and FDA's Regulations Instead of Conducting an Independent NEPA Evaluation of the Environmental and Health and Safety Impacts of GAT Soybeans.

In its EA, APHIS impermissibly relied on EPA's Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq*.) and Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq*.) regulations and failed to conduct an independent NEPA evaluation of the environmental effects associated with the deregulation.[35] APHIS cannot solely rely on another agency's evaluation of environmental effects under a separate statute to adequately fulfill its own NEPA obligations.[36] In *Save Our Ecosystems*, the Ninth Circuit held that the Forest Service could not rely on EPA's registration process for herbicides under FIFRA to address environmental impacts pursuant to NEPA.[37]

APHIS' reliance on FDA is more problematic given the food safety issues posed by this deregulation and the limited nature of FDA's process. FDA created a voluntary consultation process in which FDA receives from biotechnology companies a bare summary of food safety and nutritional data regarding its proposed crop. The agency does not even make its own determinations of safety; rather, it merely states its understanding that a biotechnology company has concluded the crop is safe, and that further FDA approval or premarket review is not required.[38] This cursory process in no way resembles or can be considered sufficiently equivalent in scope nor depth to the searching, "hard look" required of APHIS by NEPA. APHIS must analyze the degree to which the proposed action affects public health or safety.[39] Thus, APHIS must prepare an EIS to address the food and pesticides issues that it impermissibly deferred to the other agencies.

DuPont-Pioneer's GAT soybeans raise a number of food safety concerns. One class of concerns relates to known and potential unintended effects of the "glyphosate acetyltransferase" (GAT) enzyme introduced into GAT soybeans. A second class relates to numerous unintended effects of the genetic engineering process on the composition of GAT soybeans. Finally, increased residues of both glyphosate and ALS inhibitor herbicides on GAT soybeans present a third class of concerns.

GAT soybeans incorporate a novel method for resistance to the herbicide glyphosate. While Roundup Ready crops tolerate applications of glyphosate via introduction of a gene encoding a glyphosate-insensitive version of the EPSPS enzyme and/or a gene encoding a glyphosate-oxidoreductase (GOX) enzyme, the GAT enzyme in 356043 soybeans renders

[35] EA at 5.
[36] *Save Our Ecosystems v. Clark*, 747 F.2d 1240, 1248 (9th Cir. 1984); *Or. Envtl. Council v. Kunzman*, 714 F.2d 901, 905 (9th Cir. 1983).
[37] *747 F.2d at 1248 (explaining that FIFRA only requires a cost-benefit analysis and holding that FIFRA "does not require or even contemplate the same examination that the [agency] is required to undertake under NEPA"); see also Wash. Toxics Coal. v. U.S. EPA, 413 F. 3d 1024, 1032 (9th Cir. 2005).*
[38] See 57 Fed. Reg. 22984 (FDA's Policy on Foods Derived from New Plant Varieties).
[39] 40 Fed. Reg. § 1508.27(b)(2).

glyphosate non-herbicidal via the process of acetylation. GAT transfers an acetyl group (C_2H_3O) to glyphosate's nitrogen atom, generating N-acetyl glyphosate, which is not toxic to plants.

Development of the GAT enzyme

Scientists with DuPont-Pioneer and its collaborator Verdia developed GAT soybeans by first identifying naturally occurring N-acetyltransferase enzymes with limited ability to acetylate glyphosate in three strains of the saprophytic bacterium, *Bacillus licheniformis* (Castle 2004). They dubbed these enzymes "glyphosate acetyltransferase" or "GAT." They then used a process known as gene shuffling to recombine portions of the *gat* genes[40] from these three strains to create numerous synthetic variants. Some of these variants generated GAT enzymes with improved glyphosate acetylation (i.e. deactivation) activity, but none sufficient for development of a glyphosate-tolerant plant. Numerous additional *gat* gene variants were created by introducing genetic diversity from the hypothetical gene sequences corresponding to GAT-like enzymes derived from the related bacterial species, *B. subtilis* and *B. cereus* (Petition, pp. 53-54; Castle et al 2004, p. 1153; Castle et al 2004 Supplementary, Figure S2A).

GAT gene derived from source organisms that can be toxic; role of GAT unknown

DuPont-Pioneer's choice of source organisms (*B. licheniformis, B. cereus, B. subtilis*) for derivation of its GAT enzyme poses potential risks to food safety. Toxin-producing strains of *B. licheniformis* in raw milk and industrially-produced baby food have been identified as the cause of several food poisoning episodes (Salkinoja-Solonen et al 1999). *B. licheniformis* has also been associated with septicemia, peritonitis, ophthalmitis and other cases of food poisoning in humans, as well as bovine toxemia and abortions (Johnson et al 1994; Turnbull & Kramer 1995). Salkinoja-Solonen et al (1999) note that recombinant versions of *B. licheniformis* are used to produce industrial enzymes used in food processing. Based on their findings, they urge FDA to reassess the safety of products derived from *B. licheniformis*. While the synthetic GAT enzyme in 356043 soybeans is most similar to the GAT of *B. licheniformis*, it is also modeled on the GAT-like enzymes of *B. cereus* and *B. subtilis*. *B. cereus* is the most common Bacillus species associated with food poisoning, while *B. subtilis* is increasingly recognized as a food poisoning organism (Salkinoja-Solonen et al 1999).

Pathogenic organisms should not be used as the source of genes for insertion into genetically engineered crops. At the very least, genes derived from pathogenic organisms should be thoroughly understood, and used only if it is certain that the gene does not have a pathological role. That is not the case here. First, DuPont-Pioneer denies the very existence of the easily accessible, peer-reviewed scientific literature discussed above that conclusively demonstrates that *B. licheniformis* can cause food poisoning: "B. licheniformis is widely known as a contaminant of food but is not associated with any adverse effects" (Petition, p. 63). As documented above, this is not true. Far from correcting DuPont-Pioneer's false assertion, APHIS further confuses the issue with a startling error of its own: "The GAT protein sequence, which is derived from the bacterium *Bacillus licheniformis*, has been considered safe for food and feed in the U.S., Canada and Europe (EU Commission, 2000; FDA, 2001)" (EA, p. 17). Yet the references cited by APHIS say absolutely nothing about a "GAT

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[40] The information needed to generate the GAT enzyme is encoded by the *gat* gene.

protein sequence," from *Bacillus licheniformis* or from any other organism. Indeed, it would be quite surprising if they did, since there is not a single scientific paper that even mentions "glyphosate acetyltransferase" until 2004, three and four years later than the references cited by APHIS.[41] We explain in detail why the sources APHIS cites have absolutely no bearing on the safety or potential harms from GAT in Appendix I.

In contrast to APHIS's mistaken assumption of GAT's safety, DuPont-Pioneer scientists admit that the physiological role of GAT in *B. licheniformis* remains unknown (Petition, p. 143; Siehl et al 2005, p. 240; Siehl et al 2007, p. 11453). APHIS regulations for environmental release of regulated genetically engineered organisms grown in field trials require that "the function of the introduced genetic material is known." 7 C.F.R. 340.3(b)(3). Thus, a strong argument could be made that GAT soybeans should never have been field-tested until the physiological role of GAT enzymes was elucidated. This argument applies with still more force to deregulation, which allows for unrestricted commercial use of the deregulated GE crop. The fact that DuPont-Pioneer has identified one non-physiological role of rare GAT variants is hardly enough to satisfy 7 C.F.R. 340.3(b)(3), still less when one considers that the GAT variant incorporated into 356043 soybeans has been extensively modified, and as discussed further below has many additional functions beyond deactivation of glyphosate.

Enzymes like GAT perform an extremely diverse range of functions. One role of acetylation has been discovered in the plague bacterium, *Yersinia pestis* (Worby & Dixon 2006). One of the proteins that *Yersinia pestis* injects into human cells is an acetyltransferase enzyme (YopJ) of the same general family as GAT. This YopJ enzyme acetylates two key proteins of the human immune system, thereby shutting down the immune system response that would otherwise defend against plague infection. Worby & Dixon (2006) conclude their article as follows:

> "Knowing that YopJ functions as an acetyltransferase is not the end of the story. It is really the beginning of what is likely to be the exciting search for other acetyltransferases in bacterial pathogens and viruses as well as in eukaryotic cells."

GAT soybeans hence contain genetic material encoding proteins of unknown physiological function in their host organisms, some strains of which have been positively identified as the cause of food poisoning incidents. Furthermore, the acetylation activity of a GAT-like enzyme in at least one pathogen, the plague bacterium *Yersinia pestis*, has been demonstrated to be a virulence factor that suppresses the immune system response to infection.

GAT enzymes have high potential for potentially hazardous unintended effects

The compounds modified by an enzyme are known in scientific terms as "substrates." Some enzymes have a high degree of "substrate specificity," which means that they act on (i.e. modify) just one substrate, or on several that have very similar biochemical structures.

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[41] Based on a search of the keyword "glyphosate acetyltransferase" on the comprehensive PubMed database (http://www.ncbi.nlm.nih.gov/sites/entrez). We note that to the best of our knowledge, DuPont-Pioneer and its collaborator Verdia (since acquired by DuPont-Pioneer) have been solely responsible for the discovery of glyphosate acetyltransferase in B. licheniformis and its subsequent development for use in GAT soybeans.

Other enzymes are less specific, and can modify a broad range of dissimilar substrates. The activity and substrate specificity of enzymes are also influenced by the cellular environment, including factors such as potassium ion concentration and pH. When an enzyme is engineered into a genetically engineered crop, it is desirable that it perform ***only*** its intended function under all conditions it will experience in the field. This requires that it have a high degree of substrate specificity. This is not the case for the GAT enzyme. DuPont-Pioneer has reported that it acetylates not only glyphosate, but also from seven to 13 amino acids (discussed further below), indicating a low degree of substrate specificity and high potential for unintended effects. Amino acids can exist in free form, but most (~99%) become incorporated into proteins. Below, we discuss the potential for GAT to acetylate amino acids in free form as well as incorporated into proteins.

GAT acetylates amino acids

There are striking disparities in the activity and substrate specificity of GAT enzymes reported by DuPont-Pioneer scientists in various publications. Activity refers to the efficiency of an enzyme's modification of its substrate; substrate specificity refers to the relative selectivity of an enzyme in "choosing" its substrates. The less specific an enzyme is in choosing its substrates, the more unintended compounds will be generated, and the higher the potential for hazardous unintended effects. Therefore, it is important to understand the reasons for the discrepancies in DuPont-Pioneer's figures.

In its 2004 patent, DuPont-Pioneer reported that the native GAT enzyme acetylates not only glyphosate, but 13 amino acids as well (GAT Patent 2005, par. 548-549). Elsewhere, company scientists reported that the same enzyme acetylates just seven amino acids (Siehl et al 2005). The reason for this discrepancy is unclear, especially since the results seem to be based on the same set of assays (see Table 1 for details). Whether seven or 13, the diverse nature of these amino acid substrates, in both structure and biochemical properties, corroborates the low substrate specificity of GAT, and the broad range of dissimilar compounds it can modify (Table 2).

DuPont-Pioneer altered the native GAT enzyme via gene shuffling as described above to make it more efficient at deactivating glyphosate. The R7 round of synthetic GAT enzymes exhibited roughly 1100-fold greater activity towards glyphosate than native GAT, with the best variant having over 2400-fold greater activity (Siehl et al 2005, Tables 2 & 3; Petition, p. 53). Synthetic GAT variants also had altered activity towards amino acids. For instance, the R7 round GAT had 40 times greater activity in acetylating the amino acid aspartate than native GAT (Siehl et al 2005, pp. 238-39).

The GAT4601 enzyme deployed in GAT soybeans is "similar to" the R7 variants (Petition, p. 143). DuPont-Pioneer reports that GAT4601 has a k_{cat}/K_M value towards glyphosate of 267 $min^{-1}mM^{-1}$ (Petition, Appendix 7, Table 1). This is many-fold lower than the values for R7 variants. For instance, DuPont-Pioneer reports a k_{cat}/K_M value of 10,340 for the most active of the R7 variants, and 4573 for a more typical variant of the R7 class (see Siehl et al, 2005, Tables 2 & 3). This represents a striking 17- to 39-fold reduction in activity towards glyphosate (4573/267 and 10340/267, respectively).

There are several possible explanations for this huge discrepancy. GAT4601 may be slightly altered from the R7 variants tested previously, with different activity; however, this is unlikely to explain a reduction in activity so large as 17- to 39-fold, given DuPont-Pioneer's statement that GAT4601 is "similar to" the R7 variant reported in Siehl et al (2005). A more likely explanation is the substantially different assay conditions employed in the testing of GAT4601 and the previously reported R7 GAT enzymes. In particular, DuPont-Pioneer used an assay buffer containing 100 mM KCl to test the activity and substrate specificity of GAT4601 (Petition, p. 143), while no KCl whatsoever was used in the assay buffers for the R7 activity tests (Siehl et al 2005, Section 2.3.1, p. 236). Potassium chloride (KCl) is known to strongly inhibit the activity of GAT. In fact, DuPont-Pioneer tested the effect of KCl on GAT activity in its 2004 patent "[t]o better approximate the physiological conditions under which the GAT enzymes of the invention are intended to be used (e.g., plant cells)…" (GAT Patent 2005, paragraph 0520).

Table 1
Discrepancies in Data on GAT Activity Reported by DuPont-Pioneer

	GAT Patent (2005)		**Siehl et al (2005)**	
Substrates	Activity as % of activity towards glyphosate (=100%)	Activity (k_{cat}/K_M) of native GAT ($min^{-1}mM^{-1}$)	Activity (k_{cat}/K_M) of native GAT as % of activity towards glyphosate (= 100%)	
Glyphosate	100%	4.21	100%	
Serine	470%	19.8	470%	
Threonine	250%	10.5	250%	
Phospho-L-serine	200%	8.4	200%	
Aspartate	86%	3.6	Roughly = glyphosate	
Glutamate	51%	2.1	51%	
Cysteine	**50%**	**2.1**	**4%**	
Alanine	**33%**	**1.4**	**nil or < 3%**	
Glutamine	**32%**	**1.3**	**nil or < 3%**	
Asparagine	27%	1.1	27%	
Glycine	**21%**	**0.9**	**nil or < 3%-**	
Tyrosine	**18%**	**0.8**	**nil or < 3%**	
Valine	**12%**	**0.5**	**nil or < 3%-**	
Histidine	**10%**	**0.4**	**nil or < 3%**	

Notes: GAT Patent (2005) reports k_{cat}/K_M values of 4.21 $min^{-1}mM^{-1}$ for glyphosate, 19.8 for serine, and 3.6 for aspartate (Table 21, p. 72). The remaining values are reported as % of activity towards glyphosate (paragraph 0549), from which the corresponding k_{cat}/K_M values have been calculated. For some reason, the activity towards cysteine has dropped ten-fold from GAT Patent (2005) to Siehl et al (2005), and the activity towards six other amino acids (boldface) has dropped from 10-50% to "nil or < 3%." The figures reported on p. 239 of Siehl et al (2005) appear to derive from the same set of assays as those reported in GAT Patent (2005). First, the activities for glyphosate and six amino acids (unbolded) are **identical** to those listed in GAT Patent (2005). Second, a comparison of GAT Patent (2005), Table 21 to Siehl et al (2005), Table 3 reveals that they are essentially identical. Finally, assay conditions in GAT Patent (2005) and Siehl et al (2005) were identical: compare GAT Patent (2005), paragraph 0549; and Siehl et al (2005), Section 2.3.1, p. 236.

DuPont-Pioneer assayed 11 GAT variants (3 native and 8 synthetic) for activity towards glyphosate in assay buffers with KCl (20 mM) and without KCl. These tests showed that 20 mM KCl reduced glyphosate acetylation activity by 2-fold to 7-fold (Patent, Figures 15A & 15B). DuPont-Pioneer concluded:

> "Figure 15A shows that addition of salt (20 mM KCl) to the assay buffer significantly increases the KM value for glyphosate. The kcat value remains relatively unchanged or increases slightly, the net result being a lower observed kcat/KM value for GAT enzymes assayed in the presence of 20 mM KCl than in the absence of added KCl (Fig. 15B)" (GAT Patent 2005, par. 0520).

To summarize, KCl in assay buffer at 20 mM reduces the activity of GAT enzymes by 2- to 7-fold, while 100 mM appears to reduce GAT activity by 17- to 39-fold. It is puzzling that DuPont-Pioneer would choose to test GAT4601 at 100 mM KCl when all its previous assays used either no KCl or just 20 mM. DuPont-Pioneer explains that it used assay buffer containing 100 mM KCl "to represent physiological conditions in the plant cytosol and chloroplast" (Petition, p. 143). However, this begs several questions. First, GAT is not expressed in soybean chloroplast, so this observation is irrelevant. Second, why was the majority of the company's GAT activity testing in published literature conducted without the use of any KCl in assay buffer? More importantly, why did DuPont-Pioneer use assay buffer with just 20 mM KCl as representative of plant physiology in its 2005 patent, then switch to 100 mM KCl for the purposes of the GAT soybean petition? DuPont-Pioneer cites two papers to back its assertion that 100 mM KCl represents physiological conditions in plant cytosol and chloroplast, but ignores its own prior assumption of much lower potassium ion concentrations, as well as other published literature reporting variable and generally lower plant intracellular potassium concentrations (e.g. 40 mM in barley, see Kronzucker et al 2003).

Table 2
Broad Substrate Activity of Native GAT Enzyme

Amino Acid (L forms)	**Activity (Glyphosate = 100%)**[1]	**R Group**[2]	**Molecular weight**[2]	**Biochemical Properties (R Group)**[2]
Serine	470%	CH_2OH	105	Uncharged, polar
Threonine	250%	C_2H_4OH	119	Uncharged, polar
Phospho-L-serine	200%			
Aspartate	100%	$CH_2CO_2^-$	133	Negatively charged (pH = 6.0)
Glutamate	51%	$C_2H_4CO_2^-$	147	Negatively charged (pH = 6.0)
Cysteine	50%	CH_2SH	121	Uncharged, polar
Alanine	33%	CH_3	89	Uncharged, nonpolar
Glutamine	32%	$C_2H_4CONH_2$	146	Uncharged, polar
Asparagine	27%	CH_2CONH_2	132	Uncharged, polar
Glycine	21%	H	75	Uncharged, polar
Tyrosine	18%	$CH_2C_6H_4OH$	181	Uncharged, polar, aromatic ring
Valine	12%	$CH(CH_3)_2$	117	Uncharged, nonpolar
Histidine	10%	$CH_2C_3N_2H_4$	155	Positively charged (pH = 6.0)
Sources: [1] GAT Patent (2005), par. 0548 – 0549 of the patent; [2] Lehninger, A.L. (1975) Biochemistry, 2nd edition, Figures 4.2-4.4.				

If all that were at stake here were GAT4601 activity towards glyphosate, concerns might be limited to determining the true level of glyphosate-tolerance conferred to soybeans by GAT4601 under whatever potassium ion concentrations actually obtain in soybean cells. But much more is at stake. GAT enzymes acetylate amino acids as well as glyphosate. Did DuPont-Pioneer's use of an unnaturally high potassium ion concentration in its GAT4601 substrate tests kill or greatly suppress the enzyme's activity towards amino acids, as it did towards glyphosate? If so, GAT soybeans may in fact generate much higher levels of many more N-acetylated amino acids than is indicated by DuPont-Pioneer's treatment in the Petition (pp. 143-144), to which we now turn.

First, DuPont-Pioneer's substrate testing of the GAT4601 enzyme was conducted with microbially-generated enzyme rather than plant-produced enzyme. We have no way of knowing whether the soybean-expressed GAT4601 has the same activity and substrate specificity as the microbial surrogate used in its tests. While DuPont-Pioneer claims to have demonstrated equivalence between the two, such equivalence testing is fraught with difficulties and has frequently been criticized by experts in GM foods testing, who recommend use of the plant-produced transgenic protein for testing purposes. For a general treatment of this subject with respect to pesticidal (Cry) proteins found in Bt crops, see Freese & Schubert (2004).

Second, DuPont-Pioneer's tests revealed that GAT4601 acetylates five amino acids in addition to glyphosate, ***even with use of the enzyme-suppressing 100 mM KCl in assay buffers***. N-acetylated aspartate (NAA) levels increased 230-fold over the trace levels detected in control soybeans, while N-acetylated glutamate (NAG) increased 8-fold. These dramatically increased levels of NAA and NAG occurred despite DuPont-Pioneer's finding that GAT4601's activity towards aspartate and glutamate was just 2.9% of that towards glyphosate. Activity towards serine, threonine and glycine was estimated at 0.2-3% that of glyphosate activity (Petition, Appendix 7, Table 1, p. 144). ***Despite this finding, DuPont-Pioneer did NOT test for the presence of N-acetylated forms of these amino acids in GAT soybeans.*** Clearly, if the low level of activity towards aspartate and glutamate (7.7 to 7.8 k_{cat}/K_M) can result in one to two orders of magnitude increases in the N-acetylated forms of these amino acids, the lesser activities reported towards the other three amino acids could generate significant levels of their N-acetylated forms as well. DuPont-Pioneer appears to have followed a "don't look, don't find" methodology.

More important, however, is DuPont-Pioneer's use of an unnaturally high concentration of K^+ in the assay buffers to "kill" or greatly suppress GAT4601's modification of amino acids. At the lower K^+ concentrations that are likely found in GAT soybean cells, ***GAT4601 may well be generating substantial levels of N-acetylated forms of many more amino acids, and much higher levels of NAA and NAG, than suggested by the results reported in the petition.*** This possibility is also suggested by DuPont-Pioneer's earlier finding that native GAT has the ability to acetylate up to 13 amino acids (see Tables 1 and 2 above).

Acetylated Amino Acids Have No History of Safe Use in Food or Feed

DuPont-Pioneer purports to assess the safety of NAA and NAG in Addendum 2 to the petition. This "safety assessment" is seriously lacking in a number of respects. For instance, DuPont-Pioneer states that "***acetylation of proteins is commonly employed in the food industry*** to alter the solubility, water absorption capacity and emulsifying properties of protein concentrates (El-Adway 2000, Ramos and Bora 2004)" (emphasis added, Petition, Addendum 2, p. 3). However, the two studies cited by DuPont-Pioneer are purely experimental in nature and in no way support this statement. In "Functional Characterization of Acetylated Brazil Nut (*Bertholletia* excelsa HBK) Kernel Globulin," Ramos and Bora (2004) state that: "Defatted Brazil nut kernel flour… is presently being utilized in the formulation of animal feeds. ***One of the possible ways to improve its utilization for human consumption*** is through improvement of its functional properties" [i.e. by acetylation] (emphasis added, p. 134). Furthermore: "Modification [i.e. acetylation] of [Brazil nut] proteins ***may possibly lead to an improvement in functional properties***" (emphasis added, p. 134). An experimental study such as this in no way suggests that acetylated proteins are used at any level, much less "commonly employed," in the food industry…" as DuPont-Pioneer states. If anything, experimentation such as this suggests the contrary. The second study cited by DuPont-Pioneer is entitled "Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate" (El-Adway 2000); it is also experimental and in no way supports DuPont-Pioneer's claim.

DuPont-Pioneer makes similar mischaracterizations with respect to acetylated amino acids in animal feeds, stating: "One well-characterized industrial application of acetylated amino acids is the use in livestock feed [sic] in cases when it is unsuitable to use free amino acids" (Petition, Addendum 2, p. 3). Once again, DuPont-Pioneer cites two ***purely experimental studies*** (in rats, not livestock!) on the bioavailability of acetylated methionine (not NAA or NAG) when fed to rats at low levels (Boggs et al 1975; Amos et al 1975). ***Rats are not livestock.*** Acetylated methionine is not acetylated aspartate or glutamate. DuPont-Pioneer presents no evidence that any acetylated amino acid or protein is used at any level in livestock feed, in direct contradiction to its claim.

USDA improperly relied on DuPont-Pioneer's misleading statements. USDA reiterates (twice) DuPont-Pioneer's unsubstantiated claims that acetylated amino acids are used in the food and feed industries (EA, pp. 14, 16). We are not aware of any acetylated amino acids that have been reviewed or approved by the U.S. Food and Drug Administration (FDA) as food additives, nor of any such compound having been designated as a "generally recognized as safe" (GRAS) food ingredient.[42] One must presume that if real evidence were available to document the company's claims, DuPont-Pioneer would have found and cited it. Thus, ***we conclude that there is no history of safe use of these unusual amino acid variants for either human or animal consumption.***

DuPont-Pioneer mischaracterize three other studies. DuPont-Pioneer claims that Magnusson et al (1989), Neuhauser & Bassler (1986) and Arnaud et al (2004) support its

[42] Based on a search for keywords "acetyl," "acetylated" and "amino" on FDA's GRAS Notice website http://www.cfsan.fda.gov/~rdb/opa-gras.html. This website lists 234 GRAS notices submitted to FDA for novel food ingredients from 1998 to the present.

assertion that nutritional and metabolic studies on the N-acetyl form of ***glutamate*** substitutes for glutamate via metabolic deacetylation (Petition, Addendum 2, p. 3). In other words, that acetylated glutamate is converted to glutamate by enzymes in the body. We are certain that DuPont-Pioneer would like this to be true, since NAG levels in its GAT soybeans are at least 8-fold higher than in control soybeans. However, none of these three studies have anything to do with glutamate or acetylated glutamate (NAG). They were all conducted on ***glutamine*** and N-acetyl ***glutamine***, as revealed in their titles and examination of their abstracts. Glutamine is a different amino acid than glutamate (see Table 2 for structure of R group), and studies on the former have no bearing on assessment of the latter. Two of these studies are not even relevant to human consumption of acetylated glutamine, involving rather ***intravenous*** administration of the compound to humans and rats. The third study (Arnaud et al 2004) involved feeding it to pigs, and found slightly lower digestibility of the acetylated vs. natural form of glutamine.

DuPont-Pioneer's "safety assessment" rests heavily on the speculation that deacetylating enzymes will convert acetylated amino acids like NAA and NAG consumed in foods containing GAT soybeans to their normal forms, but offers not a single citation specific to deacetylation of either NAA or NAG. One study cited by DuPont-Pioneer (Endo 1980) examined only N-acetyl forms of histidine and tryptophan, while the second examined only N-acetylated lysine (Neuberger and Sanger 1943). None of these three amino acids are among the list of 13 previously reported to be acetylated by native GAT (Table 1), making these studies still less relevant. DuPont-Pioneer refers to broader-ranging speculations and hypotheses advanced in those articles, but such speculations cannot substitute for hard data. If such data were available, DuPont-Pioneer would surely have found and cited them.

Lacking any evidence for use of acetylated amino acids in the food or feed industries, DuPont-Pioneer searched the scientific literature to discover whether they are naturally occurring in the food supply. Its conclusion: "Literature values for NAA and NAG are not available" (Petition, p. 87). There is no evidence presented on the levels, if any, of other acetylated amino acids in foods. The absence of data on the presence in food of these unusual compounds in the voluminous medical and food science literature is striking, and testifies to the scientific community's ignorance of their properties.

DuPont-Pioneer then purchased a handful of food items and conducted its own tests for levels of NAA and NAG. It chose items with high concentrations of aspartic and glutamic acid on the assumption that "this might be correlated with high levels of NAA and NAG, respectively." (Petition, Addendum 2, p. 3). Significantly, DuPont-Pioneer does not report its methodology for these tests. The absence of literature values for NAA and NAG suggests that standardized, validated tests are not available for these compounds. Errors in DuPont-Pioneer's testing methods – or intentional manipulation of test protocols – could give results that greatly overestimate the naturally levels of NAA and NAG in the foods it tested.

Exposure to novel compounds in the food supply, or at least vastly increased dietary exposure to poorly characterized compounds present only at low levels, requires a much more rigorous assessment than that presented by DuPont-Pioneer. Due to the inadequacies in DuPont-Pioneer's assessment, including failure to report test methodology for NAA and NAG test results and the failure to test for acetylated forms of any other amino acids likely to be found in 356043 soybeans, the safety assessment in the petition should be disregarded. Since the animal and human exposure assessments (Petition, Addendum 2, pp. 5-8) are based on DuPont's undocumented and incomplete testing, they should be disregarded as well.

Acetylation of proteins

Amino acids are the building blocks of proteins. Acetylation is the most common modification to proteins that occurs in eukaryotic (higher) organisms, including plants. It is estimated that amino terminal acetylation occurs on 85% of all eukaryotic proteins, and the modification is irreversible. Reversible acetylation of chromosomal proteins (histones, HMG proteins and transcription factors) plays a key role in controlling transcription of DNA.[43]

> "Acetylation affects many protein functions, including enzymatic activity, stability, DNA binding, protein-protein interaction, and peptide-receptor recognition" (Polevoda & Sherman 2002 p. 1).

One interesting role of acetylation is modification of regulatory hormones. N-acetylation of alpha melanocyte-stimulating hormone enhances its pigment-producing activity. Acetylation of growth hormone releasing factor increases its potency 50-fold. In contrast, acetylation of beta-endorphin decreases its opioid activity (Polevoda & Sherman 2002). Abnormal acetylation can disrupt normal functioning of the cell. For instance, acetylation of the N-terminal catalytic threonine residue of various 20 S proteasome subunits causes the loss of specific peptidase activities (Polevoda & Sherman 2000).

In short, N-acetylation is a very common modification of many diverse proteins. However, it has an extremely broad range of functions, most of which remain undiscovered or poorly understood. It is possible that N-acetylation of amino acids incorporated in soybean proteins could alter the function of plant enzymes or plant hormones, interfere with DNA transcription (if the protein binds DNA), or disrupt cellular metabolism in some other hard to predict way. Such unintended changes could lead to generation of novel, potentially harmful, compounds; or increased production of toxins or allergens that naturally occur at low and unobjectionable levels.

Interestingly, DuPont-Pioneer says nothing in the petition about the potential of GAT to acetylate proteins and have such unintended and potentially hazardous unintended effects on protein function. The company provides only a brief discussion of protein acetylation in general, mentioning only one role of N-acetylation, protection of proteins from breakdown, citing references more than 25 years old. The many additional roles for N-acetylation cited above that raise concerns with respect to GAT are not discussed at all (Petition, Addendum 2, p. 2). In fact, DuPont-Pioneer's treatment of N-acetylation appears to assume, without argument, that GAT will only acetylate free amino acids rather than also acetylating amino acids that have been (or become) incorporated in proteins (Petition, Table 25, p. 91). We see no substantiation for this implicit assumption.

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[43] Transcription of DNA is the first step in generation of a protein from a gene.

Acetylated AMPA

Soybeans convert roughly 50% of absorbed glyphosate to the metabolite aminomethylphosphonic acid (AMPA) (Sandermann 2006). Siehl et al (2007) report that AMPA is acetylated at the same rate (k_{cat} value) as glyphosate, with a K_{cat}/K_M value that is 6% that of glyphosate (Table 3, R7 variant). DuPont-Pioneer presents no data in the petition regarding the levels of acetylated AMPA in soybeans, and no toxicology studies to determine whether or not it is safe for animal or human consumption. Acetylated AMPA is a novel compound that will likely accumulate at significant levels in 356043 soybeans. Yet DuPont-Pioneer has failed to provide any analysis of it or its toxicology. Acetylated AMPA is also not mentioned by APHIS.

Compositional Analysis

There are several serious general flaws in DuPont-Pioneer's analyses for potentially hazardous compositional changes in 356043 soybeans. First, it is unclear whether or not the GAT soybeans used for the compositional assessment were sprayed with glyphosate and/or an ALS-inhibitor type herbicide. In the petition, DuPont-Pioneer states only that: "Normal agronomic practices were employed throughout the growing season" (Petition, p. 77, citing Experiment C, described on p. 72). Application of glyphosate and/or ALS inhibitor alters the cellular metabolism of 356043 soybeans. Glyphosate is absorbed by the plant; as noted above, roughly 50% is converted to AMPA, which may then be acetylated, forming acetylated AMPA. Glyphosate application may also alter the GAT enzyme's acetylation activity with respect to amino acids, or cause other unintended changes in soybean composition. Similar unpredicted changes in soybean composition may be caused by application of an ALS inhibitor. Even if neither chemical alone significantly changes soybean composition, the simultaneous or sequential application of the two herbicides may effect such unintended compositional changes. DuPont-Pioneer's failure to report whether "normal agronomic practices" did or did not involve use of glyphosate/ALS inhibitors thus leaves an important variable undefined. If the herbicides were not applied, or not applied in a manner simulating field conditions, the compositional assessment reported in Section VIII of the Petition (pp. 77-97) may misrepresent the actual composition of 356043 soybeans grown under field conditions.

There is suggestive evidence that herbicide application does in fact change the composition of 356043 soybeans. In the broiler study mentioned above, McNaughton et al (2007) provide a crude compositional assessment of 356043 soybeans grown with and without application of herbicides. The treated soybeans were sprayed with "2 sequential broadcast applications of a herbicide tank mix containing the active ingredients glyphosate (Touchdown IQ, Syngenta, Greensboro, NC), chlorimuron (Spin, DuPont-Pioneer, Wilmington, DE), and thifensulfuron (Refine, DuPont-Pioneer)" (p. 2570). Soybean meal prepared from unsprayed and sprayed 356043 soybeans exhibited potentially significant compositional changes (Table 1). The protein content of soy meal from sprayed soybeans was 6% higher (49.0%) than that of unsprayed soybeans (46.3); the fiber content of meal from sprayed soybeans was 16% lower relative to unsprayed; fat content was 29% higher and ash content was 6% lower. The lower ash content may be indicative of lower mineral content in sprayed soybeans. As discussed further below, there is great scientific concern that glyphosate application to glyphosate-tolerant crops blocks the plant's uptake of essential minerals in several ways. Other notable differences include the following: meal from sprayed vs. unsprayed soybeans contained 12% more arginine; 7% more aspartate; and 8% more glutamate.[44] The latter two differences are interesting given the fact that 356043 soybeans are known to generate substantially increased levels of acetylated forms of aspartate (at least a 230-fold increase) and glutamate (at least an 8-fold increase) vs. control soybeans.

The crude compositional assessment of hulls derived from sprayed and unsprayed 356043 soybeans also shows potentially significant differences (McNaughton et al 2007, Table 2). Protein content of hulls from sprayed soybeans was 10% higher than hulls from unsprayed beans; fiber content was 5% lower, ash content 13% lower; arginine content 11% higher; valine and aspartate content 10% higher; aspartate content 12% higher; proline content 15% higher; and tyrosine content 47% lower. We note that these results mirror the results found for soybean meal with respect to protein, fiber, ash, arginine, aspartate and glutamate, suggesting that application of glyphosate/ALS inhibitors has generally consistent effects on soybean metabolism expressed in similar changes in disparate tissues (grain and hull). While these changes may not be of concern in themselves, they demonstrate that application of glyphosate/ALS inhibitors to 356043 soybeans alters the soybean's metabolism, and raise the issue of whether other unintended but less benign changes may also be induced. If so, such changes would not be captured in DuPont-Pioneer's compositional assessment as reported in the petition, which did not involve a comparison of the composition of treated and untreated 356043 soybeans, and which leaves unclear whether or not they were treated. The crude compositional assessment in the broiler study is suggestive, but not detailed enough to capture potentially harmful unintended changes resulting from herbicide application to 356043 soybeans.

Another general flaw in DuPont-Pioneer's compositional assessment is its unjustified use of "reference varieties" of soybeans for calculation of a "tolerance interval" (Petition, p. 77). First, DuPont-Pioneer does not report which soybean varieties it used for this purpose, beyond noting that they were four commercial conventional varieties. We have no way of knowing whether or not they are mainstream soybean varieties or specialty lines bred for some specific property that would make them unsuitable as comparators. Second, for some unexplained reason, DuPont-Pioneer grew these reference varieties in mostly different locations than the 356043 and control Jack soybeans used in the compositional assessment (Petition, compare locations listed on p. 77 to locations for Experiment C in Figure 26, p. 68). Differing environmental conditions in different growing regions can have large effects on the composition of soybeans. The purpose of a compositional assessment of a transgenic crop should be to identify changes in composition due to the genetic engineering process, for which purpose strict control of environmental variables is key. The "tolerance interval" DuPont-Pioneer constructed from assessment of unidentified soybean lines grown under different conditions of soil, weather, etc. than its 356043 and control lines is simply a device

[44] Calculated from figures reported in McNaughton et al (2007), Table 1. No values are listed for asparagine (Asn) or glutamine (Gln). The levels for these amino acids are likely grouped with those for aspartate (Asp) and glutamate (Glu), respectively, even though proper nomenclature calls for different designations for such groupings (Asx = asparagine + aspartate; Glx = glutamine + glutamate).

to expand the "allowable" ranges of component levels and hence obscure significant differences resulting from the genetic engineering process, and should be disregarded.

DuPont-Pioneer also used a novel and highly dubious statistical method (adjusted p value) to discount significant differences in composition resulting from the genetic engineering process that are considered significant using standard and long-accepted statistical measures (p value). DuPont-Pioneer's "adjusted p value," like the "tolerance range," provides the company with expanded wiggle room to claim no significant difference when in fact such a difference exists. Hence, adjusted p values should be disregarded. For instance, DuPont-Pioneer ignores a statistically significant 13% increase in mean levels of neutral detergent fiber in 356043 soybeans vs. the near-isoline control Jack soybeans ($p < 0.05$) (Petition, Table 20, p. 80). Instead of ignoring this significant difference, DuPont-Pioneer should explain the significantly higher level of fiber in 356043 soybeans relative to control Jack soybean; fiber is indigestible to non-ruminants and may decrease the feed value of soybean meal from 356043 soybeans. We should note here that in the broiler study, McNaughton et al (2007) intentionally mask differences in the nutritional content of the different soybean varieties used to make feed for the different test groups by supplementing each, as needed, with protein, lysine, methionine, cysteine, Ca and P to standardized levels for each of these important feed components (p. 2572).

DuPont-Pioneer reports roughly three-fold higher mean levels of two unusual fatty acids in 356043 soybeans vs. control Jack soybeans: heptadecanoic acid (17:0) and heptadecenoic acid (17:1) (Petition, Table 21, p. 83). These levels are also nearly three-fold higher than the highest values reported for reference varieties and in the literature. DuPont-Pioneer states generally that these compounds are also found in other foods, but fails to specify relative levels. Consultation of the USDA nutrition database (www.nutritiondata.com) reveals that by far the highest levels of both compounds are found in "tofu, extra firm, prepared with nigari." On a caloric basis, this product contains 1191.3 mg of heptadecanoic acid (17:0) and 2385.0 mg of heptadecenoic acid (17:1) per 200 calories of tofu. The products listed as #2 for content of these compounds – cooked Australian lamb (17:0 fatty acid) and broccoli raab, raw (17:1 fatty acid) – contain far less. Cooked Australian lamb contains just 363.0 mg 17:0 fatty acid per 200 calories, or just 30% the level found in tofu; broccoli raab, raw, contains just 154.6 mg 17:1 fatty acid per 200 calories, or just over 6% the level of tofu.

Tofu is made from soybeans, and these unusual fatty acids are presumably present in raw soybeans or generated in the processing of soybeans into tofu. DuPont-Pioneer's finding that 17:0 and 17:1 fatty acids are three-fold higher in 356043 soybeans than control soybeans would therefore likely mean that tofu made from 356043 soybeans would have three-fold higher levels of these compounds than tofu made from conventional soybeans. One would thus expect on the order of 3600 mg of 17:0 fatty acid and 7000 mg of 17:1 fatty acid in tofu made from DuPont-Pioneer's soybeans. These higher levels would represent roughly 10-fold more 17:0 fatty acid, and 45-fold more 17:1 fatty acid, on a caloric basis, than is found in the second-leading food item for each fatty acid in USDA's extensive nutrition database. Thus, consumption of tofu made from 356043 soybeans could greatly increase exposure to these unusual fatty acids. While this may not be problematic, it may have long-term health implications, and it is troubling that neither DuPont-Pioneer nor APHIS assessed this matter. We note that other types of fats long assumed to pose no human health concerns have been found to adversely affect human health. For instance, concerns over the adverse impacts from long-term consumption of trans-fats has led to a food industry-wide phase-out or reduction of these fats in the food supply. It is irresponsible to introduce vastly increased levels of unusual, poorly understood and little consumed fats into the food supply.

356043 soybeans also have significantly increased mean levels of four isoflavones relative to control soybeans: 33% more glycitin, 23% more malonylglycitin, 15% more daidzin, and 12% more malonyldaidzin (Petition, Table 26, p. 93).[45] As noted by DuPont-Pioneer, isoflavones have been attributed with both negative (antinutrient, estrogenic) and positive (anticancer) effects. DuPont-Pioneer provides no explanations for these alterations.

Need for Toxicological Assessments

The many food safety concerns noted above demand that GAT soybean be subject to rigorous toxicological assessment prior to any decision on its deregulation. Such toxicological assessments should include multi-generational feeding trials with well-characterized lab animals with toxicological endpoints, such as tests for digestive system damage, carcinogenic and adverse reproductive effects. The broiler performance feeding study cited by DuPont-Pioneer in Addendum 2 to the petition (McNaughton et al 2007) is not a toxicological assessment. It is a short performance study whose primary criterion is weight gain. It is not designed to detect potentially subtle health harms to humans or livestock from long-term exposure to food or feed derived from GAT soybeans and the many novel compounds it contains. As such, it is wholly inadequate to support any claims as to the safety of GAT soybeans.

C. The EA's "Analysis" of Alternatives is Inadequate.

APHIS' analysis of alternatives in the EA was insufficient because APHIS failed to adequately analyze the alternatives it identified in the EA.[46] EAs must include analysis of the alternatives to the proposed action.[47] Here, APHIS does not have a sufficient alternatives analysis. APHIS only identifies a no action alternative and the approval of the project. No other alternatives are considered, even though APHIS' regulations authorize the agency to approve a petition in part.[48] The EA contains no analysis of other alternatives besides the

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[45] Based on the standard statistical measure of significance: $p\text{-value} < 0.05$. We disregard DuPont-Pioneer's "adjusted p value" as discussed above. Also, it is worth noting that the lower limit of DuPont-Pioneer's "tolerance interval" for each of the isoflavones reported in this table is either a negative number or 0, and hence presumably could be used to justify as "normal" the complete absence of any of these isoflavones in 356043 soybeans. This is clearly not justifiable – that is, the near complete absence in 356043 soybeans of one or more compounds that naturally occur in soybeans ***would*** represent a very significant effect, despite falling within DuPont-Pioneer's "tolerance interval." We note that the unrealistic nature of the lower-bound values of these intervals also casts grave doubt on the validity of the upper-bound figures.

[46] *See Bob Marshall Alliance v. Hodel*, 852 F.2d 1223, 1228 (9th Cir. 1988).

[47] 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.")

[48] 7 C.F.R. 340.6(d)(3)(ii).

preferred alternative. This is not meaningful analysis and is inadequate to comply with NEPA.[49]

NEPA requires that federal agencies consider alternatives to recommended actions whenever those actions "involve[ ] unresolved conflicts concerning alternative uses of available resources."[50] The goal of the statute is to ensure "that federal agencies infuse in project planning a thorough consideration of environmental values."[51] 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."[52] 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.[53] Informed and meaningful consideration of alternatives is thus an integral part of the statutory scheme.[54]

For the reasons discussed above, APHIS should conduct an EIS that, *inter alia*, includes consideration of an alternative that excludes from the scope of deregulation the progeny of crosses between 356043 soybeans and soybeans with either or both of the two commercialized glyphosate-tolerance mechanisms developed by Monsanto.

II. APHIS Failed to Adequately Assess the Cumulative Impacts of Deregulation of GAT Soybeans

The deficient draft EA failed to adequately address several significant issues, such as the cumulative impacts of increasing glyphosate use from GAT soybeans together with the widespread use of the Roundup Ready soybean system and other Roundup Ready crop systems. As discussed above, this includes potential impacts from the stacking of GAT soybeans with other mechanisms of glyphosate tolerance to "enhance" glyphosate tolerance.

NEPA requires agencies to consider the cumulative impacts of their proposed actions.[55] 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

[49] See Klamath-Siskiyou Wildlands Center v. Bureau of Land Management, 387 F.3d 989, 997 (9th Cir. 2004) (agency cannot narrowly define "alternatives until only the desired one survives").
[50] 42 U.S.C. § 4332(2)(E).
[51] Conner v. Buford, 836 F.2d 1521, 1532 (9th Cir. 1988).
[52] Calvert Cliffs' Coordinating Committee, Inc. v. United States Atomic Energy Commission, 449 F.2d 1109, 1114 (D.C.Cir.1971) (emphasis added).
[53] Id.
[54] *See* Bob Marshall Alliance v. Hodel, 852 F.2d 1223, 1228 (9th Cir. 1988).
[55] 40 C.F.R. § 1508.25(c); 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).

other actions."[56] Individually minor, but collectively significant actions, taking place over time, can generate cumulative impacts.[57]

Analyzing cumulative impacts in EAs is crucial: The Council on Environmental Quality has noted that "in a typical year, 45,000 EAs are prepared compared to 450 EISs.... Given that so many more EAs are prepared than EISs, adequate consideration of cumulative effects requires that EAs address them fully."[58] 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.[59]

In this case, APHIS has failed to provide a meaningful analysis of the cumulative impacts of deregulation of GAT soybeans in association with other past, present and future glyphosate-tolerant crops, which is violative of NEPA.[60] APHIS' cumulative impact assessment assumes, with justice, that deregulation of GAT soybeans would not lead to an overall increase in acreage planted to glyphosate-tolerant soybeans. However, APHIS incorrectly assumes that such displacement would have no environmental or agronomic impacts related to altered patterns of herbicide use or herbicide-resistant weed development.

In Section I(A) above, we discussed how the enhanced glyphosate tolerance of GAT soybeans will likely lead to increased glyphosate use. We also discussed DuPont's intentions, as expressed clearly in GAT Patent (2005), to further increase the glyphosate tolerance of its GAT soybeans by "stacking" them with one or two additional mechanisms of glyphosate tolerance in addition to GAT. We provided an analysis of the relative ineffectiveness of ALS inhibitor herbicides due to the 9.9 million acres of fields infested with weeds resistant to this class of herbicides, and farmers' decreasing use of ALS inhibitors on soybeans. This development was analyzed in association with the dramatically increasing use of glyphosate on soybeans. Our conclusion was that farmer adoption of GAT soybeans would be based primarily on its enhanced tolerance to glyphosate vs. currently available glyphosate-tolerant (Roundup Ready) soybeans. This enhanced tolerance – to be amplified in the future still more through stacking with other mechanisms of glyphosate tolerance – will spur already dramatically increasing glyphosate use to new heights. Finally, we discussed the epidemic of glyphosate resistant weeds that has developed with incredible rapidity due to already increasing glyphosate use, from just 10,000 acres in 1999 to 2.4 million acres today. Significantly, APHIS does not address any of these issues in its EA.

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[56] 40 C.F.R. § 1508.7.
[57] Id.
[58] Council on Environmental Quality, *Considering Cumulative Effects Under the National Environmental Policy Act* at 4, Jan. 1997, available at http:// ceq.eh.doe.gov/nepa/ccenepa/ccenepa.htm (last visited Feb. 26, 2002) (emphasis added).
[59] Grand Canyon Trust v. F.A.A., 290 F.3d 339, 345 (D.C. Cir. 2002).
[60] Kern v. U.S. Bureau of Land Mgmt., 284 F.3d 1062, 1076-77 (9th Cir. 2002).

Below, we present a detailed assessment of the twin threats of increasing use of herbicides in American agriculture, and the epidemic of herbicide-resistant weeds, focusing on glyphosate and glyphosate-resistant weeds, but also addressing recent increases in the use of other herbicides. This discussion provides essential background for a cumulative impacts assessment that APHIS should undertake in the context of an Environmental Impact Statement.[61]

APHIS Must Assess HT Crop Systems for Weed Resistance Threat

APHIS has not adequately analyzed the environmental effects of weed resistance resulting from transgenic herbicide-tolerant (HT) crop systems. An herbicide-tolerant crop system comprises an herbicide-tolerant crop and associated use of the herbicide that the crop is specifically engineered to tolerate.[62]

While weed scientists universally acknowledge that the Roundup Ready crop system has fostered rapid development of glyphosate-resistant weeds on millions of acres of cropland, APHIS turns a blind eye to these facts and instead cites studies – again, from the mid 1990s – that ***predict*** slow development of glyphosate resistant weeds (DEIS, p. 120). These predictions – in studies published in 1993 and 1997 – were made before Roundup Ready ("RR") crops were (widely) planted, and thus were necessarily made in the absence of the current empirical data that demonstrates that RR crop systems foster rapid development of glyphosate-resistant weeds.

Moreover, the DEIS does not discuss a significant use it could make of its noxious weed authority. This would be to assess, and regulate, transgenic herbicide-tolerant (HT) crop systems as noxious weed risks. An herbicide-tolerant crop system comprises an herbicide-tolerant crop and associated use of the herbicide that the crop is specifically engineered to tolerate. As explained *infra*, APHIS cannot meaningfully address the noxious weed risks posed by HT crops without considering them as part of such a crop system.

While judicious use of HT crop systems may offer benefits to growers and American agriculture, their present excessive and unregulated use poses a serious and growing threat. HT crop systems have two serious and inter-related impacts: they foster more rapid development of herbicide-resistant (HR) weeds, and greater use of detrimental weed control methods to kill them. Increased use of toxic herbicides to control resistant weeds has adverse environmental and public health impacts.

APHIS has previously refused to provide any meaningful assessment of HT crop systems. A recent federal district court ruling vacated APHIS's decision to deregulate (approve for commercial cultivation) Monsanto's herbicide-tolerant, Roundup Ready alfalfa. *Geertson Seed Farm*, 2007 WL 518624 (N.D. Cal.). The court's decision means that APHIS will be required

[61] This section is adapted from Center for Food Safety's comments on APHIS' Programmatic Environmental Impact Statement, cited above, which is referred to as DEIS in these comments.
[62] The concept of "HT crop system" is borrowed from Monsanto, which describes its latest HT soybean in these terms: "The utilization of Roundup agricultural herbicides plus Roundup Ready soybean, collectively referred to as the Roundup Ready soybean system…" From: "Petition for the Determination of Nonregulated Status for Roundup Ready2Yield™ Soybean MON 89788," submitted to USDA by Monsanto on June 27, 2006 (revised November 3, 2006), APHIS Docket No. APHIS-2006-0195, p. 4).

to prepare an environmental impact statement ("EIS") if Monsanto wishes to re-introduce Roundup Ready alfalfa commercially.

The court's decision requires APHIS to consider, in the context of the EIS, the impacts of commercial introduction of the Roundup Ready alfalfa system on the development of herbicide-resistant weeds. When confronted with the issue of Roundup resistance in the GE alfalfa context, APHIS "found that such a possible impact nevertheless does not warrant the preparation of an EIS because weed species often develop resistance to herbicides and the agricultural community is addressing the issues." *Id.* The court found APHIS' "cavalier response" to be unconvincing, stating that such rationale "is tantamount to concluding that because this environmental impact has occurred in other contexts it cannot be significant." *Id.* The Court also rejected APHIS' argument that "good stewardship" may be the only defense to herbicide-resistant weeds. *Id.* The court required APHIS to address how to in fact ameliorate this problem in its environmental review so that farmers in the real world can engage in practices to address the issue. *Id.*

Finally, the court held that APHIS must consider cumulative impacts of the Roundup Ready alfalfa system in combination with other Roundup Ready crop systems that have already received commercial approval and are widely planted across the country.

The court noted "that it is unclear from the record whether any federal agency is considering the cumulative impact of the introduction of so many glyphosate resistant crops; one would expect that some federal agency is considering whether there is some risk to engineering all of America's crops to include the gene that confers resistance to glyphosate." *Id.* at 11.

This decision sets a precedent for future APHIS decision-making with respect to HT crop systems. APHIS must assess the impacts of HT crop systems with respect to HT trait transfer, development of HR weeds from increased selection pressure, as well as cumulative impacts in future decisions regarding HT crop systems.

<u>USDA Has Officially Recognized the Need for Management of Resistant Weeds Fostered by HT Crop Systems, But Failed to Act</u>

In 2001, USDA and EPA set up an interagency work group to develop management programs to forestall or manage the emergence of herbicide-resistant weeds fostered by HT crop systems. 67 Fed. Reg. 60934 (Sept. 27, 2002). The formation of this work group represents official USDA recognition of the fact that herbicide-resistant weeds are a serious issue that needs to be addressed in assessments of HT crop systems. Despite the formation of this work group, there is no indication that EPA was ever consulted on these issues. As the Court stated in the recent GE alfalfa case: "one would expect that some federal agency is considering whether there is some risk to engineering all of America's crops to include the gene that confers tolerance to glyphosate." *Geertson Seed Farms*, 2007 WL 518624 at 11. However, there is no evidence to suggest that USDA has made any such assessment, or taken any action, to manage development of HR weeds fostered by any pesticide-promoting HT crop system.

Prevalence of HT Crop Systems: Present and Future

APHIS needs to analyze both the current state of use of HT crops systems and the impacts of these regulations on its use. HT crops comprise by far the largest class of GE crops, by several measures. Crops with HT traits comprised 81% of commercial GE crop acreage worldwide in 2006,[63] or 4 of every 5 acres. Four HT crops – soybeans, corn, cotton and canola – are commercially planted in the U.S. In 2006, HT soybeans comprised 89% of all soybeans grown in the U.S.; HT corn 36% of all corn;[64] HT cotton 86% of all cotton;[65] and in 2003, HT canola comprised over 75% of all canola.[66] Some of the HT corn and most of the HT cotton is stacked with one or more insect-resistance traits. Herbicide tolerance and/or insect resistance are virtually the only traits found in commercially-grown GE crops.

The vast majority (roughly 99%) of commercially grown HT plants are Monsanto's Roundup Ready crops, tolerant to the herbicide glyphosate, which were planted on over 114 million acres in 2006,[67] an area larger than the state of California. The remaining 1% are Bayer's LibertyLink crops, tolerant to the herbicide glufosinate.[68] There are virtually no commercially-grown transgenic HT crops tolerant to any other herbicide.

HT crop acreage, particularly acres planted to glyphosate-tolerant crops, will expand greatly in the near future. Most importantly, Roundup Ready corn adoption has been increasing dramatically in recent years, more than quadrupling from 7.8 to 32.7 million acres from 2002 to 2006. Sugar beet growers have announced their intention to begin growing glyphosate-tolerant sugar beets next year.[69] In addition, five of 11 GE crops being considered for deregulation (i.e. commercial approval) as of August 2, 2007 were herbicide-tolerant.[70] All five of these crops are tolerant to glyphosate; two of the five each have dual tolerance to glyphosate and one other herbicide.

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63 ISAAA 2006. "Global Status of Commercialized Biotech/GM Crops," Highlights of ISAAA Brief No. 35, International Service for the Acquisition of Agri-Biotech Applications. http://www.isaaa.org/Resources/Publications/briefs/35/highlights/pdf/Brief%2035%20-%20Highlights.pdf. Crops with HT alone = 68%, HT stacked with insect-resistance = 13%

64 "Adoption of Genetically Engineered Crops in the U.S.," USDA Economic Research Service. See: http://www.ers.usda.gov/Data/BiotechCrops/alltables.xls. Last visited Sept. 10, 2007. USDA AMS has more reliable data on HT cotton (see next footnote).

65 USDA AMS (2006). "Cotton Varieties Planted: 2006 Crop," U.S. Dept. of Agriculture, Agricultural Marketing Service, Cotton Program, August 2006. http://www.ams.usda.gov/cottonrpts/MNXLS/mp_cn833.xls.

66 Cerdeira, A.L. and S.O. Duke (2006). "The current status and environmental impacts of glyphosate-resistant crops: a review," *J. Environ. Quality* 35:1633-1658.

67 Based on Monsanto's latest figures at: "Monsanto biotechnology trait acreage: fiscal years 1996 to 2006," updated October 11, 2006. http://www.monsanto.com/pdf/pubs/2006/Q42006Acreage.pdf.

68 See Freese, B. (2007). "Cotton Concentration Report: An Assessment of Monsanto's Proposed Acquisition of Delta and Pine Land," ICTA/CTA, February 2007, Section 3.6.2, footnote 31, available at http://www.centerforfoodsafety.org/pubs/CFS-CTA%20Monsanto-DPL%20Merger%20Report%20Public%20Release%20-%20Final%20_2_.pdf (last visited September 11, 2007).

69 "Biotech beets gaining approval," Associated Press, August 22, 2007.

70 See USDA's website "Petitions of Nonregulated Status Granted or Pending as of August 2, 2007" at http://www.aphis.usda.gov/brs/not_reg.html. Last visited on August 23, 2007.

Field trial permit figures are the best prognosticator of longer-term trends in GE crop development. 36.3% of active field trial permits for GE crops involve an HT trait.[71] We note that this 36.3% figure slightly exceeds the historical proportion of GE crop field tests that involve the HT trait, cited by APHIS as "nearly one-third" (DEIS, p. 119). This indicates continued strong interest in the development of new HT crops.

The 352 active permits for field trials of HT crops encompass 18 different plant species and tolerance to more than eight different herbicides. Glyphosate-tolerance is by far the most common HT trait in field tests, though others, especially crops tolerant to dicamba herbicide, are also being extensively tested.[72]

In summary, both the present and future of transgenic agriculture are overwhelmingly dominated by herbicide-tolerant crops, and in particular by crops that tolerate the herbicide glyphosate.

HT crop systems can generate or foster the more rapid development of HR weeds via two major mechanisms:

I. Selection of naturally herbicide-resistant weeds due to the increased selection pressure from greater and more frequent use of the herbicide to which the HT crop is tolerant;

II. Transfer of the HT trait to sexually-compatible relatives, either commercial cultivars or weedy species, via pollen flow or pollen flow in combination with seed dispersal.

We first discuss selection for herbicide-resistant weeds.

HR Weeds From Increased Selection Pressure on HT Crop Systems

Factors That Foster Development of Herbicide-Resistant Weeds

Weeds resistant to an herbicide such as glyphosate can emerge via two different mechanisms. First, frequent and extensive use of a particular herbicide tends to select for

[71] As of August 23, 2007, 352 of 970 active permits (36.3%) involved an HT trait. Some permits involve multiple traits. Information obtained from USDA's website "Field Test Release Applications in the U.S." at http://www.isb.vt.edu/cfdocs/fieldtests1.cfm. For total number of active permits, select "Date/Range" box. On the next page, select "Field Test Permits Currently in Effect" and "Full Record," then "Create Excel File." To arrive at the total number of active permits, it is necessary to delete entries with "Denied" or "Withdrawn" under the "Status" column. For total number of active permits involving HT, go to the original screen and select "Phenotype Category." On next page, select "Herbicide Tolerance (HT)" and "Field Test Permits Currently in Effect" and "Full Record," then "Create Excel File." Delete "Denied" and "Withdrawn" entries as above. Figures current as of August 23, 2007. Note that figures will change as new field test permit applications are received and as permits lapse and become inactive.

[72] Glyphosate-tolerance represents 62% of HT crop field tests, while tolerance to dicamba, or dicamba and glyphosate, represent another 11%. In 18% of HT crop field tests, the herbicide to which the crop is tolerant is considered "confidential business information," or is simply not reported, so there may well be more than eight different HT traits in development. On dicamba-tolerant crops, see also Behrens et al (2007). "Dicamba Resistance: Enlarging and Preserving Biotechnology-Based Weed Management Strategies," *Science*, 1185-1188, May 25, 2007.

the rare individual plants of a particular weed species that naturally possess genetic resistance to the herbicide. Given the chance to reproduce, the number of resistant individuals increases as their susceptible brethren are killed off, and over time the genetically resistant plants form a larger and larger percentage of the weed population. Secondly, frequent and extensive use of a particular herbicide can also cause weed shifts. Weed shifts occur when populations of a weed species with greater natural resistance to a particular herbicide gradually supplant populations of other weed species with lesser resistance. In either case, herbicide-resistant weeds are a growing and costly problem for American farmers.

Factors that promote development of herbicide-resistant weeds include:
1) **Frequency of natural resistance:** More frequent occurrence of natural resistance to the herbicide in individuals of a particular weed species, or greater natural resistance in the weed species as a whole;
2) **Selection pressure:** Frequent and extensive use of a particular herbicide, which increases the "selection pressure" for resistant weeds or resistant weed species;
3) **Overreliance:** Excessive reliance on a particular herbicide to the exclusion of other weed control methods, including other herbicides; and
4) **Delayed application:** The longer a weed is allowed to grow, the harder it is to kill, and the more likely it is to reproduce. Delaying application of an herbicide increases the potential for weeds, including resistant individuals, to survive and propagate.

Facets of the HT Crop System that Foster Rapid Development of HR Weeds

Herbicide tolerance permits the HT plant to survive application of a single herbicide that would otherwise kill the [non-biotech] plant. Herbicide tolerance thus allows "over-the-top" application of the herbicide to more easily kill nearby weeds without killing or severely injuring the plant itself. "Over-the-top" is one form of "post-emergence" herbicide application, or spraying after the crop seed has "emerged" or sprouted. The alternative herbicide regime more common with conventional, non-HT varieties is called "pre-emergence." That is, an herbicide that retains its activity for weeks is applied to the soil before the crop actually sprouts so as to suppress "weed competition" in the critical early life of the plant. Pre-emergence herbicides are also used, though to a lesser extent, with HT crops. HT crops permit greater flexibility in the timing of herbicide applications, allow for herbicide use over a greater portion of the life of the plant, and in general simplify weed management by reducing the number of different weed killers applied. The chief advantages cited for HT crops are convenience and the ability to cover more acres (i.e. reduced labor inputs per acre),[73] both of which are of particular value to larger farmers.[74]

HT crop systems facilitate more rapid development of HR weeds versus conventional crops in three of the four ways cited above. The HT crop's tolerance to a particular herbicide ensures that that herbicide will be used more frequently than it would be with a corresponding conventional crop. This is because: a) only the herbicide the HT crop is

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[73] Duffy, M. (2001). "Who Benefits from Biotechnology," presentation at the American Seed Trade Association Meeting, Chicago, Ill., Dec. 5-7, 2001.
http://www.leopold.iastate.edu/pubs/speech/files/120501-who_benefits_from_biotechnology.pdf

[74] Benbrook, C. (2005). "Rust, resistance, run down soils, and rising costs: problems facing soybean producers in Argentina," AgBioTech InfoNet, Technical Paper No. 8, Jan. 2005.
http://www.aidenvironment.org/soy/08_rust_resistance_run_down_soils.pdf

engineered to tolerate can be applied "over-the-top" without fear of damaging the crop itself, leading farmers to apply it in preference to other herbicides; and b) herbicide-tolerance greatly widens the "application window" for that herbicide, facilitating repeated applications of the herbicide through part or all of the crop's life span. The ability to apply the herbicide through part or all of the crop's life span also facilitates delayed application, which allows weeds to grow larger, increasing the chances that resistant weeds will survive to reproduce and propagate. HT crops are specifically engineered to facilitate simplified weed control that relies primarily on the single herbicide to which the crop is tolerant, leading to overreliance on that herbicide (DEIS, p. 119). The substantial price premium (technology fee) a farmer pays for HT crop seed offers a further inducement to make use of its engineered property through increased reliance on the herbicide to which the crop is tolerant. Finally, the growing trend to plant different HT crops tolerant to the same herbicide "in rotation" (e.g. corn following soy) increases selection pressure for resistant weeds over longer time spans.

Below, we present empirical evidence supporting each of these arguments for glyphosate-tolerant crops. It is impossible to make any generalizations regarding frequency of natural resistance, which varies according to the weed species and the herbicide in question.

The Emergence of Glyphosate-Resistant Weeds with Glyphosate-Tolerant Crop Systems

Monsanto first introduced glyphosate in the U.S. in 1976,[75] and for two decades it was used primarily to control weeds in orchards. There were no reports of glyphosate-resistant weeds over these two decades. The first glyphosate-resistant weed populations in the U.S. (rigid ryegrass) were found in almond orchards. In 1998, glyphosate-resistant rigid ryegrass was reported to infest from 11 to 50 sites covering 1,001 to 10,000 acres in California.[76] Glyphosate use increased dramatically with the introduction of Monsanto's Roundup Ready soybeans in 1995, Roundup Ready cotton and canola in 1997, and Roundup Ready corn in 1998.[77] Serious concern over glyphosate-resistant weeds was reported in 2001:

> "Resistance to glyphosate (Roundup) is emerging all around the world, potentially jeopardizing the 2.5 billion dollar market for genetically modified herbicide tolerant crops"[78]

Scientists who identified the first glyphosate-resistant weed (horseweed) in Delaware in 2000 attributed their evolution to the continuous planting of Roundup Ready crops.[79] Ten prominent weed scientists confirmed this assessment in 2004:

> "It is well known that glyphosate-resistant horseweed (also known as marestail) populations have been selected in Roundup Ready soybean and

[75] Monsanto (2007). Monsanto History, last accessed 1/31/07. See http://www.monsanto.com/monsanto/layout/about_us/timeline/default.asp

[76] Weed Science Society of America, see http://www.weedscience.org/Case/Case.asp?ResistID=1034 (last visited Sept. 9, 2007).

[77] Monsanto (2007). Monsanto History, *see supra*, note 23.

[78] Farmers Weekly (2001). "Glyphosate resistance is showing a worldwide rise," *Farmers Weekly*, Nov. 23, 2001. http://www.connectotel.com/gmfood/fw231101.txt, (last visited Sept. 9, 2007).

[79] "Herbicide-resistant Weed Identified in First State," University of Delaware press release, February 22, 2001, online at http://www.rec.udel.edu/weed_sci/weedfacts/marestail_resistance.htm (last visited Sept. 9, 2007).

> cotton cropping systems. Resistance was first reported in Delaware in 2000, a mere 5 years after the introduction of Roundup Ready soybean. Since that initial report, glyphosate-resistant horseweed is now reported in 12 states and is estimated to affect 1.5 million acres in Tennessee alone."

It is estimated that glyphosate-resistant weeds now infest over 3,000 sites in 17 states.[80] Multiple populations of 8 different weed species have developed resistance since the year 2000: Palmer amaranth, common waterhemp, common ragweed, giant ragweed, horseweed, Italian ryegrass, rigid ryegrass and hairy fleabane.[81] Other weeds developing resistance to glyphosate or becoming more prevalent due to glyphosate-induced weed shifts, include velvetleaf,[82] cocklebur and lambsquarters,[83] morning glories,[84] and tropical spiderwort.[85] Johnson grass, as well as annual grasses such as goosegrass (confirmed glyphosate-resistant biotypes in Malaysia), foxtails, crowfootgrass, signal grasses, panicums, and crabgrasses, all have a history of developing resistance to multiple herbicides,[86] making development of glyphosate-resistance more likely in these species.

While glyphosate-resistant weeds are worst in the South and East, they are rapidly spreading throughout the Midwest. Missouri is now home to populations of at least three confirmed glyphosate-resistant weeds – common waterhemp, common ragweed and horseweed – and glyphosate-resistant horseweed was confirmed in Nebraska in 2006. ***A survey of farmers in the Midwest found that 24% of farmers in the northern Midwest and 29% in the south say they have glyphosate-resistant weeds.***[87] Weed experts in the Midwest are predicting further spread of glyphosate-resistant weeds in their states. For instance, Michael Owen, agronomist at Iowa State University, is concerned that with over 90% of soybeans in Iowa planted to Roundup Ready varieties, the rapid adoption of Roundup Ready corn will lead to "an increasing number of crop acres where glyphosate will follow glyphosate" in the popular corn-soybean rotation,[88] vastly increasing selection pressure for glyphosate-resistant weeds (see below). Owen's concerns about the increasing use of RR corn are borne out by the facts. Acreage planted to Roundup Ready corn is growing at an extremely rapid clip,

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80 Compiled from data on glyphosate-resistant weeds at Weed Science Society of America, at: http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go (last visited Sept. 9, 2007).

81 http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go (last visited Sept. 9, 2007).

82 Owen, M.D.K. (1997). North American Developments in Herbicide-Tolerant Crops. Proceedings of the British Crop Protection Conference, Brighton, UK, BCPC: Brighton, UK. 3:955–963.

83 Roberson, R. (2006). "Pigweed not only threat to glyphosate resistance," Southeast Farm Press, Oct. 19, 2006.

84 UGA (2004). "Morning glories creeping their way around popular herbicide, new UGA research reports," University of Georgia, August 23, 2004.

85 USDA ARS (2004). "Little-known weed causing big trouble in Southeast," USDA ARS News Service, August 24, 2004. The spread of tropical spiderwort resistant to glyphosate, particularly in Georgia, is associated with the dramatic increase in Roundup Ready cotton acreage in recent years.

86 Robinson, E. (2005). "Will weed shifts hurt glyphosate's effectiveness?" Delta Farm Press, Feb. 16, 2005.

87 Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.

88 Owen, M.D.K. (2005). "Update 2005 on Herbicide Resistant Weeds and Weed Population Shifts," 2005 Integrated Crop Management Conference, Iowa State University.

from just 7.8 million acres in 2002 to 24.8 million acres in 2005, to 32.7 million acres in 2006,[89] or more than a four-fold increase in just four years.

In the DEIS, APHIS inexplicably makes no reference to a single glyphosate-resistant weed population in the U.S. Instead, it cites only decade-old reports of glyphosate-resistant ryegrass populations in Australia (DEIS, p. 120). Rather than analyze up to date information on glyphosate-resistant weeds, APHIS ignores of the galloping course of glyphosate-resistant weed development on millions of acres of American cropland that has occurred in the last decade.

While weed scientists universally acknowledge that the Roundup Ready crop system has fostered rapid development of glyphosate-resistant weeds on millions of acres of cropland, APHIS turns a blind eye to these facts and instead cites studies – again, from the mid 1990s – that ***predict*** slow development of glyphosate resistant weeds (DEIS, p. 120). These predictions – in studies published in 1993 and 1997 – were made before Roundup Ready crops were (widely) planted, and thus were of necessity made in the absence of the empirical data we now have demonstrating that RR crop systems foster rapid development of glyphosate-resistant weeds. The predictions were based on the presumed rarity of natural glyphosate resistance in weed populations. According to a 2005 farm press article quoting leading weed scientists, including Stanley Culpepper of the University of Georgia:

> "The frequency of resistance to glyphosate is unknown but thought to be very low. In that regard, one might assume the potential for resistance to glyphosate is low. And, until recently, that was the prevailing opinion among weed scientists. ***The situation changed, however, with the wide-spread adoption of Roundup Ready technology.*** According to Culpepper, one must also consider the frequency of use. ***"The extensive use of glyphosate on multiple crops certainly increases the risk of resistance evolution.***" (emphasis added)[90]

In other words, no matter how rare natural resistance to glyphosate is among weed populations, that resistance has been amplified due to the tremendous selection pressure exerted by "the extensive use of glyphosate on multiple crops." In fact, the apparent rarity of natural glyphosate resistance in weeds means that greater selection pressure is required to select for (i.e. foster the development of) resistant weeds than would be the case if natural resistance were less prevalent. Thus, the ***fact*** of rapid glyphosate-resistant weed development speaks directly to the tremendous selection pressure exerted by Roundup Ready crop systems, in the form of the increased extent and frequency of glyphosate application, together with overreliance on glyphosate to the exclusion of other weed control methods.

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89 "Monsanto biotechnology trait acreage: fiscal years 1996 to 2006," updated Oct. 11, 2006, available at http://www.monsanto.com/pdf/pubs/2006/Q42006Acreage.pdf (last visited Sept. 9, 2007).

90 Yancy, C.H. (2005). "Weed scientists develop plan to combat glyphosate resistance," *Southeast Farm Press*, June 1, 2005, available at http://southeastfarmpress.com/mag/farming_weed_scientists_develop/ (last visited Sept. 9, 2007).

Striking evidence of farmers' overreliance on glyphosate to the exclusion of other weed control methods is provided by a recent survey of 400 farmers in the U.S. Midwest conducted by Syngenta. The researchers found that ***56% of soybean growers in northern states and 42% in southern states use glyphosate as their sole herbicide***. As a result, says USDA plant physiologist Stephen Duke: "the selective pressure for weeds to develop resistance has been huge."[91] The author of a study cited by USDA concurs: "the selection pressure imposed by extensive and exclusive glyphosate use will ***undoubtedly*** result in an increasing frequency of reports of glyphosate-resistant weed biotypes."[92] (emphasis added).

USDA recognizes, but fails to assess this important aspect of HT crop systems. USDA at once states that HT crop systems promote "simpler weed management strategies based on fewer herbicides," but then acknowledges that this "advantage" leads to a serious problem: "overreliance on fewer weed-management strategies will result in the evolution of resistance to the more useful herbicides or population shifts to naturally resistant weed species" (DEIS, p. 119).

USDA's failure to assess and as necessary regulate HT crop systems is no minor lapse. Agronomist Stephen Powles of the Western Australian Herbicide Resistance Initiative states: ***"Glyphosate is as important to world agriculture as penicillin is to human health."***[93] North Carolina weed scientist Alan York has glyphosate-resistant weeds "potentially the worst threat [to cotton] since the boll weevil," the devastating pest that virtually ended cotton-growing in the U.S. until an intensive spraying program eradicated it in some states in the late 1970s and early 1980s.[94] York concedes that: "Resistance is not unique with glyphosate," but goes on to state that: ***"What makes glyphosate resistance so important is our level of dependence on glyphosate***" (emphasis added).[95] Weed scientists report that there are no new herbicides with different "modes of action" on the horizon. Thus, the loss of glyphosate as an effective means of weed control poses serious problems for U.S. agriculture.[96]

Glyphosate-Tolerant Crop Systems Have Led to Increased Herbicide Use

1) Increased Use of Glyphosate

Glyphosate-tolerant crops have dramatically increased glyphosate use by all measures – number of acres treated, amount applied, as well as frequency and rate of application.

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[91] Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.
[92] Young, B.G. (2006). "Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops," *Weed Technology* 20: 301-307. Young (2006) is cited at DEIS, p. 119, but APHIS missed this finding.
[93] Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.
[94] Minor, E. (2006). "Herbicide-resistant weed worries farmers," *Associated Press*, 12/18/06. http://www.enn.com/top_stories/article/5679 (last visited Sept. 9, 2007).
[95] Yancy, C. (2005). "Weed scientists develop plan to combat glyphosate resistance," Southeast Farm Press, June 3, 2005.
[96] Roberson, R. (2006). "Pigweed not only threat to glyphosate resistance," *Southeast Farm Press*, October 19, 2006. http://southeastfarmpress.com/news/101906-herbicide-resistance/

The number of acres treated with glyphosate is reflected in RR crop adoption figures, since glyphosate is invariably applied to RR crops. As noted above, RR crops were planted on over 114 million acres in 2006.

Overall glyphosate use in American agriculture has jumped 10-fold from just 1995 to present,[97] tracking the dramatic rise in RR crop acreage. The amount of glyphosate applied to cotton climbed 753% from 1992 to 2002.[98] The introduction in 2006 of Roundup Ready Flex cotton, which tolerates twice the application rate of original RR cotton and also permits glyphosate application throughout the cotton plant's growing season,[99] promises to lead to continued increases in glyphosate use on cotton. In 2006, 91.886 million lbs of glyphosate were applied to soybeans alone, an astounding 42% increase from the previous year. Glyphosate use on corn has also increased rapidly, rising more than seven-fold from 3.3 million lbs in 2002 to 23.9 million lbs. in 2005, the latest year for which USDA statistics are available.[100]

Both the number and rate of glyphosate applications have also increased. From just 2002 to 2006, annual glyphosate applications to soybeans increased by a substantial 24%, from 1.07 to 1.33 lbs/acre.[101] Glyphosate use on corn has risen even more rapidly, from 0.71 lbs/acre in 2002 to 0.96 lbs/acre in 2005, a 32% rise in just three years.[102]

2) Increased Use of Other Herbicides

Since a major feature of HT crop systems is reliance on the single herbicide to which the HT crop is tolerant, one would expect declining use of other weed control methods, including other herbicides. However, the facts do not bear out this expectation. In an exhaustive analysis of USDA data, Charles Benbrook, former head of the Board on Agriculture of the National Academy of Sciences, has demonstrated that the widespread adoption of Roundup

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97 Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.

98 Steckel, L., S. Culpepper and K. Smith (2006). "The Impact of Glyphosate-Resistant Horseweed and Pigweed on Cotton Weed Management and Costs," Power Point presentation at Cotton Incorporated's "Crop Management Seminar," Memphis, 2006.
http://www.cottoninc.com/CropManagementSeminar2006/SeminarProceedings/images/Steckle%20Larry.pdf

99 Bennett, D. (2005). "A look at Roundup Ready Flex cotton," *Delta Farm Press*, 2/24/05, http://deltafarmpress.com/news/050224-roundup-flex/.

100 USDA NASS (2007). "Agricultural Chemical Usage: 2006 Field Crops Summary," National Agricultural Statistics Service, U.S. Dept. of Agriculture, May 2007.
http://www.nass.usda.gov/Publications/Todays_Reports/reports/agcs0507.pdf; USDA NASS (2006). "Agricultural Chemical Usage: 2005 Field Crops Summary," National Agricultural Statistics Service, U.S. Dept. of Agriculture, May 2006.
http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC//2000s/2006/AgriChemUsFC-05-17-2006.pdf; USDA NASS (2003). "Agricultural Chemical Usage: 2002 Field Crops Summary," National Agricultural Statistics Service, U.S. Dept. of Agriculture, May 2003.
http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC//2000s/2003/AgriChemUsFC-05-14-2003.pdf

101 From an average 1.4 applications of 0.74 lbs. glyphosate per acre in 2002 to an average 1.7 applications of 0.80 lbs./acre in 2006. See USDA NASS reports cited in last footnote.

102 From an average 1.1 applications of 0.64 pounds per acre in 2002 to 1.3 applications of 0.73 lbs./acre in 2005. See USDA NASS reports cited above.

Ready crops has increased ***overall herbicide use*** by 138 million lbs. from 1996-2004.[103] Interestingly, Roundup Ready crops slightly reduced herbicide use from 1996-1999, before increased reliance on glyphosate as the near-exclusive weed control method spurred a dramatic rise in glyphosate-resistant weed populations, which in turn has driven accelerating use of both glyphosate and other herbicides since the year 2000.

USDA turns a blind eye to this development, noting merely that "there are methods, such as crop rotation, to minimize the development of herbicide-tolerant weeds…" (DEIS at 120). The Programmatic EIS is defective because it fails to analyze this development. USDA ignores two of the most common methods that extension agents and Monsanto recommend for minimizing resistance: abandoning no-till farming, and the use of an herbicide cocktail, including suggestions for using older herbicides with high environmental toxicity that HT crop systems were supposed to supplant. Examples of such recommendations include:

As early as 2002, Ohio State Extension experts recommended using 2,4-D plus metribuzin plus paraquat as pre-emergence chemicals to control glyphosate-resistant marestail in RR soy.[104] In the same year, Syngenta recommended growers use a number of chemicals, including AAtrex®, Bicep®, DoublePlay®, Dual® MAGNUM, Gramoxone® Max, Princep®, atrazine and metribuzin, with their Roundup Ready soy, cotton and corn crops.[105]

In August 2005, reports of resistant horseweed in California prompted Monsanto to recommend that farmers should "use other chemicals" along with Roundup on their Roundup Ready crops. In addition to adding other herbicides, University of California researchers suggested tillage to control weeds.[106] In September 2005, reports of glyphosate-resistant Palmer amaranth in Georgia cotton fields prompted Monsanto to recommend that farmers use several additional herbicides with Roundup, including Prowl (pendimethalin), metolachlor, diuron and others. The company also suggested that farmers planting any RR crops use pre-emergence residual herbicides in addition to Roundup.[107] In the same year, weed scientists in Tennessee noted that Palmer amaranth in the state survived applications of up to 44 ounces per acre of Roundup, and so recommended that farmers use additional herbicides such as Clarity, 2,4-D, Gramoxone Max or Ignite.[108]

In October 2005, reports of glyphosate-resistant weeds in Roundup Ready corn and soybeans prompted Monsanto to recommend using cultivation and additional herbicides,

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103 Benbrook, C. (2004). "Genetically Engineered Crops and Pesticide Use in the United States: The First Nine Years," AgBioTech InfoNet, Technical Paper No. 7, Oct. 2004. http://www.biotech-info.net/technicalpaper7.html.

104 Mark Loux, and Jeff Stachler, "Is There a Marestail Problem in Your Future?" O.S.U. Extension Specialist, Weed Science, 2002.

105 Syngenta Announces Guidelines To Prevent Weed Resistance To Glyphosate Herbicides, press release, Greensboro, N.C., February 25, 2002, online at http://www.syngentacropprotection-us.com/media/article.asp?article_id=199

106 Juliana Barbassa, "Attack of the 12-foot horse weed: Herbicide-resistant strains plague California farmers." Associated Press, August 10, 2005, online at http://lists.ifas.ufl.edu/cgi-bin/wa.exe?A2=ind0508&L=sanet-mg&P=6738

107 "Investigation Confirms Case Of Glyphosate-Resistant Palmer Pigweed In Georgia." Monsanto press release, September 13, 2005

108 "Glyphosate-resistant Palmer Pigweed Found in West Tennessee." Farm Progress, staff report, September 23, 2005.

including Harness Extra, Degree Extra, Intrro, Prowl, Valor, and 2,4-D. Weed scientists also suggested using Lasso, Dual, Diuron, Gramoxone, Ignite, Suprend, Direx or MSMA, and noted that weed problems were so severe that herbicides such as Direx, Cotoran and Caporal were in short supply at retailers.[109]

In June 2006, reports of widespread populations of lambsquarters that were not controlled even with application of up to 48 oz per acre of Roundup prompted Iowa State University experts to recommend farmers use additional applications of Roundup and/or other chemicals, including Harmony GT, Ultra Blazer, and/or Phoenix herbicides.[110] Also in 2006, it was reported that farmers would rely increasingly on older herbicides such as paraquat and 2,4-D to control glyphosate-resistant weeds.[111]

In 2007, resistant weeds prompted Monsanto to recommend that farmers use tillage and apply a pre-emergence herbicide in combination with Roundup. Monsanto also noted that it would pay for an additional application of Roundup if growers still experienced weed problems after using a pre-emergence herbicide.[112] By 2007, the American Soybean Association was advocating that farmers return to multiple-herbicide weed control systems on their Roundup Ready soybeans.[113]

Finally, over-reliance on Roundup Ready crops and glyphosate has dampened research into new herbicides, meaning none are on the horizon.[114]

USDA statistics on herbicide use demonstrate that farmers are in fact using both more glyphosate (see above) as well as increased amounts of other herbicides. For instance, 2,4-D is the second most-heavily used herbicide on soybeans (after glyphosate). From 2002 to 2006, while glyphosate use on soybeans increased by an astounding 28 million lbs (44% rise), 2,4-D use on soybeans more than doubled from 1.35 to 3.53 million lbs (a 129% increase). Clearly, glyphosate is not displacing 2,4-D.[115]

Atrazine is the most heavily applied herbicide on corn, followed by acetochlor and S-metolachlor/metolachlor. At the same time that glyphosate use on corn climbed seven-fold from 2002 to 2006, atrazine use rose by nearly 22 million lbs. (60% increase), applications of acetochlor increased by over 7 million lbs (32% rise), and the amount of (S-)metolachlor

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[109] Andrew Burchett, "Glyphosate Resistant Weeds," Farm Journal, October 4, 2005.
[110] Michael Owen, "Large common lambsquarters is a problem for glyphosate." Iowa State University Extension Agronomy, June 15, 2006, online at http://www.weeds.iastate.edu/mgmt/2006/Largecommonlambsquarters.htm
[111] Roberson (2006), *see supra,* note 31.
[112] Henderson & Wenzel (2007). "War of the Weeds," Agweb.com, Feb. 16, 2007. http://www.agweb.com/Get_Article.aspx?sigcat=farmjournal&pageid=134469.
[113] Tom Sellen, "Herbicide-Resistant Weeds Force Change In Agriculture." Dow Jones, February 7, 2007, online at http://www.cattlenetwork.com/content.asp?contentid=104080
[114] Mueller, T.C., P.D. Mitchell, B.G. Young and A.S. Culpepper (2005). "Proactive versus reactive management of glyphosate-resistant or –tolerant weeds," *Weed Technology* 19:924-933; Yancy, C.H. (2005). "Weed scientists develop plan to combat glyphosate resistance," *Southeast Farm Press*, June 1, 2005. http://southeastfarmpress.com/mag/farming_weed_scientists_develop/.
[115] See USDA NASS "Agricultural Chemical Usage: Field Crop Summary" reports cited above for the appropriate year.

applied rose by nearly 9 million lbs (52% increase).[116] Clearly, glyphosate is not displacing use of the top three corn herbicides, but rather all four herbicides are being applied in substantially increased quantities. Such increased herbicide use constitutes a significant environmental impact that must be addressed in the PEIS.

HT Crop Systems Can Adversely Impact the Interests of Agriculture

1) Increased soil erosion from mechanical tillage to control resistant weeds

The 1990s saw a huge shift in soybean cultivation practices. From 1990-1996, farmers' use of soil-conserving conservation tillage practices increased from 25% to 60% of soybean acreage.[117] This massive shift largely pre-dated the introduction of Roundup Ready crops.[118] The following three years, from 1997 to 1999, saw a slight decline in conservation tillage acres in soybeans, though it remained near the 60% level.[119] The rise of glyphosate-resistant weeds is beginning to reverse this trend. For instance, weed scientists from California have advised farmers to use tillage with their RR soybeans to control glyphosate-resistant weeds.[120] Press reports also state that Monsanto advises farmers to use tillage with their RR soy.[121] Other experts also recommend that farmers use tillage to control glyphosate-resistant weeds.[122]

Aside from these recommendations, weed scientists have already documented increased use of tillage to control glyphosate-resistant weeds. For instance, acreage under conservation tillage in Tennessee dropped by 18% in 2004, as farmers turned back to the plow to control glyphosate-resistant horseweed; Tennessee counties with the largest cotton acreage experienced the largest decline in conservation tillage, from 80% to just 40%.[123] It is estimated that resistant horseweed has reduced the area under conservation tillage in Arkansas by 15%, with similar trends reported in Missouri and Mississippi. *Id.*

These reductions in conservation tillage due to glyphosate-resistant weeds will increase soil erosion. As glyphosate-resistant weeds continue their rapid spread, the use of tillage to control these weeds will become still more common, increasing soil erosion still more.

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116 See USDA NASS "Agricultural Chemical Usage: Field Crop Summary" reports cited above for the appropriate year.
117 Fernandez-Cornejo, J. and W.D. McBride (2002). "Adoption of Bioengineered Crops," U.S. Dept. of Agriculture, Economic Research Service, Agricultural Economic Report No. 810, May 2002. http://www.ers.usda.gov/publications/aer810/aer810.pdf. See Figure 11 on p. 29 for percentage of soybean acres grown with conservation tillage from 1990-1999.
118 The first Roundup Ready crop, RR soybeans, was introduced in 1995. In 1996, just 10% of US soy acreage was planted to RR soy.
119 Fernandez-Cornejo & McBride (2002), *see supra*, note 65.
120 Juliana Barbassa, "Attack of the 12-foot horse weed: Herbicide-resistant strains plague California farmers." Associated Press, August 10, 2005, online at http://lists.ifas.ufl.edu/cgi-bin/wa.exe?A2=ind0508&L=sanet-mg&P=6738
121 Henderson & Wenzel (2007). "War of the Weeds," Agweb.com, Feb. 16, 2007. http://www.agweb.com/Get_Article.aspx?sigcat=farmjournal&pageid=134469.
122 Andrew Burchett, "Glyphosate Resistant Weeds," Farm Journal, October 4, 2005.
123 Steckel, L., S. Culpepper and K. Smith (2006). "The Impact of Glyphosate-Resistant Horseweed and Pigweed on Cotton Weed Management and Costs," Power Point presentation at Cotton Incorporated's "Crop Management Seminar," Memphis, 2006. http://www.cottoninc.com/CropManagementSeminar2006/SeminarProceedings/images/Steckle%20Larry.pdf

2) Increased production costs from resistant weeds

As weed resistance increases, costs of weed control will also rise. APHIS must take this economic effect into account. 40 C.F.R. § 1508.8.

a) Increased expenditures on herbicides

An Arkansas weed scientist estimated that Arkansas growers would have to spend as much as $9 million to combat glyphosate-resistant horseweed in 2004.[124] The alternative is even more expensive. Left unchecked, horseweed can reduce cotton yields by 40-70%. Larry Steckel, weed scientist at the University of Tennessee, estimates that on average, glyphosate-resistant pigweed will cost cotton growers in the South an extra $40 or more per acre to control.[125] This represents a substantial burden, as cotton farmers' average expenditure on ***all*** pesticides (insecticides and herbicides) was $61 per acre in 2005.[126]

b) Other production cost increases

In 2006, Monsanto introduced a "second generation" Roundup Ready cotton known as Roundup Ready (RR) Flex. RR Flex is engineered to withstand application of roughly twice as much Roundup as first generation RR cotton, and to permit application throughout the growing season, rather than only in the early growth stages as with original RR.[127] RR Flex is a clear response to the glyphosate-resistant weed problem, which is driving growers to apply more glyphosate and to apply it more frequently. Producers who adopt RR Flex cotton in the hopes of better controlling resistant weeds will not only pay for more glyphosate, but also spend roughly 40% more for RR Flex.[128]

Since growers of RR crops are spraying Roundup more frequently to control resistant weeds, their fuel expenditures for tractor operation will increase. Those who are driven to use mechanical tillage for control of resistant weeds will likewise expend more on fuel. In particularly bad cases of glyphosate-resistant pigweed in Georgia, the necessity of hand-weeding can cost growers $92 an acre.[129]

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124 AP (2003). "Weed could cost farmers millions to fight," *Associated Press*, 6/4/03, http://www.biotech-info.net/millions_to_fight.html

125 Laws, F. (2006). "Glyphosate-resistant weeds more burden to growers' pocketbooks," *Delta Farm Press*, November 27, 2006, http://deltafarmpress.com/news/061127-glyphosate-weeds/

126 USDA ERS (2007b). Cost and return data for cotton production: 1997-2005. USDA Economic Research Service, last accessed January 12, 1997. http://www.ers.usda.gov/data/CostsandReturns/data/recent/Cott/R-USCott.xls

127 Bennett, D. (2005). "A look at Roundup Ready Flex cotton," *Delta Farm Press*, 2/24/05, http://deltafarmpress.com/news/050224-roundup-flex/.

128 Jones, M.A. (2006). "Cotton Cultivar Evaluation & Performance in the Southeast," presentation at the 2006 Cotton Inc. Crop Management Seminar, by Michael A. Jones, Ph.D, Cotton Specialist, Clemson University, 2006. http://www.cottoninc.com/CropManagementSeminar2006/SeminarProceedings/images/3_1340_%20Michael%20A.%20Jones.pdf, slide 34.

129 Laws (2006), op. cit.

c) <u>Potential for decreased yield and other losses</u>

In Georgia, where glyphosate-resistant Palmer amaranth has been confirmed in 48 fields of Roundup Ready cotton, the resistant weed took over some fields, and the cotton had to be cut down, rather than harvested, according to University of Georgia weed scientist Stanley Culpepper.[130] Palmer amaranth can damage cotton pickers, the machines that pluck cotton from the cotton bolls.

Arkansas extension agent Mike Hamilton estimates that an uncontrolled outbreak of glyphosate-resistant horseweed in his state has the potential to cost Arkansas cotton and soybean producers nearly $500 million in losses, based on projected loss in yield of 50% in 900,000 acres of Arkansas cotton and a 25% yield loss in the over 3 million acres of Arkansas soybeans.[131]

3) Glyphosate Use Linked to Plant Disease, Mineral Deficiencies and Reduced Yield

As documented above, overall glyphosate use in the U.S. has increased ten-fold since 1995,[132] due largely to the widespread introduction of Roundup Ready soybeans and cotton, and more recently the growing adoption of Roundup Ready corn.[133] RR versions of these crops are increasingly grown in rotation, meaning that each year, more prime cropland is sprayed more frequently with glyphosate, with increasing rates applied in many areas to control resistant weeds. While glyphosate is generally regarded as less toxic than many weed killers, a growing body of research suggests that continual use of this chemical may make RR plants more susceptible to disease and prone to mineral deficiencies than conventional crops, as well as reducing their yields. In addition, recent studies suggest that Roundup is much more toxic to amphibians than previously thought.

When Roundup is sprayed on RR crops, much of the herbicide ends up on the surface of the soil, where it is degraded by microorganisms. However, some is absorbed by the plant and distributed throughout its tissues. Small amounts of glyphosate "leak" from the roots of RR plants and spread throughout the surrounding soil.[134] This root zone is home to diverse soil organisms, such as bacteria and fungi, that play critical roles in plant health and disease; and it is also where the roots absorb essential nutrients from the soil, often with the help of microorganisms.

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130 Minor, E. (2006). "Herbicide-resistant weed worries farmers," *Associated Press*, 12/18/06. available at http://www.enn.com/top_stories/article/5679 (last visited Sept. 9, 2007).
131 James, L. (2005). "Resistant weeds could be costly," Delta Farm Press, July 21, 2005.
132 Service, R.F. (2007). "A growing threat down on the farm," *Science*, May 25, 2007, pp. 1114-1117.
133 "Monsanto biotechnology trait acreage: fiscal years 1996 to 2006," updated Oct. 11, 2006. http://www.monsanto.com/pdf/pubs/2006/Q42006Acreage.pdf (last visited Sept. 9, 2007).
134 Motavalli, P.P. et al. (2004). "Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations," *J. Environ. Qual.* 33:816-824; Kremer, R.J. et al. (2005). "Glyphosate affects soybean root exudation and rhizosphere microorganisms," *International J. Analytical Environ. Chem.* 85:1165-1174; Neumann, G. et al. (2006). "Relevance of glyphosate transfer to non-target plants via the rhizosphere," *Journal of Plant Diseases and Protection* 20:963-969.

The presence of glyphosate in the root zone of RR crops can have several effects. First, it promotes the growth of certain plant disease organisms that reside in the soil, such as *Fusarium* fungi.[135] Even non-RR crops planted in fields previously treated with glyphosate are more likely to be damaged by fungal diseases such as Fusarium head blight, as has been demonstrated with wheat in Canada.[136] This research suggests that glyphosate has long-term effects that persist even after its use has been discontinued. Second, glyphosate can alter the community of soil microorganisms, interfering with the plant's absorption of important nutrients. For instance, glyphosate's toxicity to nitrogen-fixing bacteria in the soil can depress the absorption of nitrogen by RR soybeans under certain conditions, such as water deficiency, and thereby reduce yield.[137] Some scientists believe that this and other nutrient-robbing effects may account for the roughly 6% lower yields of RR versus conventional soybeans.[138]

Other research shows that Roundup Ready crops themselves are less efficient at taking up essential minerals such as manganese through their roots,[139] and that glyphosate inside plant tissues can make such minerals unavailable to the plant.[140] The resultant mineral deficiencies have been implicated in various problems, from increased disease susceptibility to inhibition of photosynthesis.

CONCLUSION

For the foregoing reasons, we request that the petition for nonregulated status for GAT soybeans be denied, and that APHIS prepare an EIS adequately addressing all the significant environmental as well as potential human and animal health impacts of this action, before any decision is made on the DuPont's deregulation petition.

Respectfully submitted,

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135 Kremer et al (2005), op. cit.
136 Fernandez, M.R., F. Selles, D. Gehl, R. M. DePauw and R.P. Zentner (2005). "Crop production factors associated with Fusarium Head Blight in spring wheat in Eastern Saskatchewan," *Crop Science* 45:1908-1916. http://crop.scijournals.org/cgi/content/abstract/45/5/1908.
137 King, A.C., L.C. Purcell and E.D. Vories (2001). "Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications," *Agronomy Journal* 93:179-186.
138 Benbrook, C. (2001). "Troubled Times Amid Commercial Success for Roundup Ready Soybeans: Glyphosate Efficacy is Slipping and Unstable Transgene Expression Erodes Plant Defenses and Yields," AgBioTech InfoNet Technical Paper No. 4, May 2001. http://www.biotech-info.net/troubledtimes.html.
139 Gordon, B. (2006). "Manganese nutrition of glyphosate-resistant and conventional soybeans," in: Great Plains Soil Fertility Conference Proceedings, Denver, CO, March 7-8, p. 224-226.
140 Bernards, M.L. et al (2005). "Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation," *Weed Science* 53: 787-794.

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Appendix I
The GAT Enzyme Has No History of Safe Use in Food or Feed

APHIS states: "The GAT protein sequence, which is derived from the bacterium *Bacillus licheniformis*, has been considered safe for food and feed in the U.S., Canada, and Europe (EU Commission, 2000; FDA, 2001)" (EA, p. 17). Contrary to APHIS, the GAT protein has no history of safe use in food or feed. The sources APHIS cites do not and cannot substantiate its assurance of safety.

The "FDA, 2001" citation (EA, p. 28) lists enzyme preparations that have either been approved by FDA or designated by manufacturers as being "generally recognized as safe," or GRAS.[141] The single listing that involves *B. licheniformis* relates to use of a conventional (i.e. non-genetically engineered) strain of the bacterium to produce a "mixed carbohydrase and protease enzyme product … for use in hydrolyzing proteins and carbohydrates in the preparation of alcoholic beverages, candy, nutritive sweeteners and protein hydrolysates." (FDA 2001).[142] GAT is neither a carbohydrase nor a protease, but rather an acetyltransferase. It is not known to hydrolyze proteins or carbohydrates. It has nothing to do with production of alcoholic beverages, candy, nutritive sweeteners or protein hydrolysates.

The "EU Commission, 2000" citation (EA, p. 28) likewise has nothing to do with GAT. Instead, it involves the use of *B. licheniformis* transformed with a cycloglycosyltransferase gene derived from *Thermoanaerobacter* to produce beta-cyclodextrin, "a carrier and stabilizer of food flavors, food colors and some vitamins." GAT is not a cycloglycosyltransferase enzyme, and is not used to produce beta-cyclodextrin.

A layman might perhaps think that while the APHIS citations do not directly relate to GAT, they at least refer to enzymes produced by *B. licheniformis*, and so provide some indication that GAT will not be harmful. But this naïve view has absolutely no basis in science. *B. licheniformis* has over 4,200 genes (Rey et al 2004), meaning that it generates an equal or larger number of distinct proteins, many of them enzymes. The enzyme preparations covered by APHIS's FDA and EU citations above include only a small subset of those proteins. Furthemore, GAT belongs to ***a completely different category of enzymes with entirely different function*** than those covered by FDA (2001) and EU (2000).[143]

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[141] The FDA formerly reviewed new ingredients in food as "food additives," which involved rigorous review by agency officials and FDA's approval of them as safe or denial of them as unsafe. Since the 1990s, FDA has allowed manufacturers to introduce new ingredients in food based on their own determinations of safety. Under this GRAS system, which is based on a proposed rule issued in 1997 that the Agency has not finalized, FDA does not affirm the safety of new food ingredients, but merely reviews company-supplied data and issues a letter stating that the manufacturer is responsible for the new ingredient's safety.

[142] See listing for 184.1027, accessible as indicated on FDA's website in the Code of Federal Regulations.

[143] The Enzyme Commission, maintained by the International Union of Biochemistry and Molecular Biology, catalogs enzymes by function into one of more than 550 subclasses falling into six major categories. In 1992, the Enzyme Commission had catalogued 3196 different enzymes. We find no Enzyme Commission listing for glyphosate acetyltransferase, presumably because it is too new or has not been adequately characterized. Of the six major categories, the carbohydrases and proteases covered by FDA (2001) belong to Category 3: Hydrolases. The cycloglycosyltransferase covered by EU (2000) belongs to Category 2 (class 2.4.1.19: cyclomaltodextrin glucanotransferases) (EU Commission 2000, p. 2). In contrast, GAT would belong to a completely different group of enzymes, Category 4: Transferases.

Equally important, GAT is not produced at detectable levels by the vast majority of *B. licheniformis* strains, but rather appears to be present at low levels in only a few strains.[144] The relative rarity of this enzyme in only a few strains of *B. licheniformis* is additional reason to discount prior regulatory reviews involving very different enzymes produced by more common strains of the bacterium that likely produce no GAT at all. Finally, the GAT4601 enzyme engineered into GAT soybeans has been substantially modified by DuPont-Pioneer scientists, so even if the reviews noted above HAD covered native GAT enzyme produced by a few rare strains of *B. licheniformis* [they do not], such reviews would be of little use in assessing the safety of this substantially modified variant, which is a completely novel molecule unknown to nature.

APHIS's error here is roughly equivalent to that of an automobile safety engineer who concludes that a car's electrical system is perfectly safe based on his/her vetting of the safety of the automobile's tires. We note that this is not the first time APHIS has exhibited a disturbing lack of basic scientific competence in assessments of transgenic crops. More than ever, APHIS urgently needs to follow the recommendations of a National Academy of Sciences committee and consult independent experts, as we have also repeatedly urged. Otherwise, its assessments will continue to have little or no meaning beyond a bare re-iteration of claims made by the applicant.

144 DuPont-Pioneer screened a large number of B. licheniformis strains to identify those few that exhibited (weak) resistance to glyphosate (Castle et al 2004).