THE CENTER FOR
FOOD SAFETY

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Docket No. APHIS-2010-0047
Regulatory Analysis and Development
PPD, APHIS
Station 3A-03.8
4700 River Road Unit 118
Riverdale, MD 20737-1238

RE: Comments on APHIS Docket No. APHIS-2010-0047

Comments to USDA APHIS on Draft Environmental Impact Statement for Glyphosate Tolerant Event H7-1 Sugar Beets

Center for Food Safety - Science Comments II:
Environmental consequences of increased glyphosate-based herbicide use with H7-1 sugar beets

A. Glyphosate-based herbicide use will increase dramatically under Alternatives 2 or 3, compared to Alternative 1.

Alternative 1 "would deny the petition seeking a determination of nonregulated status of H7-1 sugar beets." Alternative 2, APHIS' "preferred alternative", would result in full deregulation, and Alternative 3 would extend the partial deregulation currently in force. (Draft Environmental Impact Statement, DEIS, at iv-v)

According to APHIS, glyphosate use would be much less with conventional sugar beets grown under Alternative 1 in comparison to glyphosate use with H7-1 sugar beets under Alternatives 2 or 3. They estimate that 56,000 lbs would be used if nonregulated status is denied, compared with 2,1491,900 lbs if full deregulation is granted – a 4,450% increase (DEIS at 436, Table 4-3). This is no doubt an underestimate: glyphosate use per acre is likely to increase further as weed control with glyphosate becomes more difficult due to resistant and tolerant weeds (see CFS-Science Comments 1).

Under Alternatives 2 or 3, not only would the total amount of glyphosate increase, the window of glyphosate use during the season would widen. Instead of a single glyphosate application occurring mainly for pre-planting burndown, glyphosate-based herbicides would be applied an additional one or more times during the growing season and, thus, potentially come into contact with different animals, plants, and microorganisms at different stages in their lifecycles, resulting in more detrimental impacts to eco-systems.

B. APHIS does not carefully consider many recent studies showing adverse environmental impacts of glyphosate-based herbicides.

APHIS claims that the increased glyphosate use likely to accompany planting of H7-1 sugar beets is a benefit to the environment to the extent that glyphosate replaces other herbicides commonly used with sugar beets that are considered to be more toxic. They also argue that glyphosate use with H7-1 sugar beets will lead to environmental benefits from changes in farming practices, such as greater adoption of conservation tillage.

These claims must be backed up with careful evaluation of scientific studies on the environmental consequences of increased glyphosate-based herbicide use. In this DEIS APHIS does not in fact provide a thorough, up-to-date analysis of science related to environmental impacts of increased glyphosate-based herbicide use because they leave out or gloss over important implications of key studies.

Use of glyphosate-based herbicides is tied to the Roundup Ready system and they will be applied to all H7-1 acres, so analysis of the consequences of increased glyphosate-based herbicide use is integral to the DEIS. Increased glyphosate-based herbicide use should be evaluated independently of the herbicides it might replace. If it is found to have negative environmental consequences, these should be considered in the DEIS irrespective of risks of other herbicides. Unlike glyphosate, the kinds and amounts of other herbicides used on conventional sugar beets are not proscribed by that system and can be changed by growers in response to environmental concerns.

In fact, the risks of non-glyphosate herbicides should be added to the risks of glyphosate, not subtracted from them. APHIS bases calculations of environmental benefits on the use of glyphosate instead of a suite of other herbicides and tillage practices. These calculations assume that glyphosate will be the only weed control method used with H7-1 sugar beets. However, APHIS also claims that H7-1 farmers will use good weed resistance management methods, and part of good weed resistance management is the use of other tools in addition to glyphosate. Other tools are herbicides with different modes of action, tillage, and hoeing. The mix of weed control practices used by farmers practicing good weed resistance management for H1-7 sugar beets will greatly affect calculations of environmental consequences.

For example, in analyzing the consequences of using less glyphosate and more of other herbicides under Alternative 1, APHIS relies upon life-cycle assessment calculations to state that "[e]missions related to global warming, ozone depletion, summer smog and carcinogenicity, among others, were found to be lower in glyphosate-tolerant crop systems compared to conventional crop systems (Bennett et al., 2004)." (DEIS at 602). However, Bennett and colleagues assumed that glyphosate would only be applied twice post-emergence, and that no other weed control methods would be used (Bennett et al. 2004, Table 1). They stressed that results and possibly rankings of herbicides would change with assumptions: "Clearly, these results are dependent on the number of herbicide spray applications under each growing system, as well as the nature of the herbicides applied. Indeed, sensitivity analyses revealed that assumptions concerning the number of applications and the amount of herbicide applied to the crop were most important in affecting a number of impacts." (Bennett et al. 2004, p. 276) There were also serious data limitations with this analysis: "Guinee (2002) notes that, for any LCA [Life Cycle Analysis], 'in practice, data are frequently obsolete, incomparable or of unknown quality'." Finally, the relative life-cycle impacts of different herbicides are likely to vary with particular growing sites and seasons, rather than be fixed at all locations.

Therefore, the environmental advantages calculated for glyphosate over other herbicides and tillage may not hold in real-world situations, and calculated benefits certainly will decrease as growers incorporate weed-resistance management.

1. Glyphosate-based herbicides and the environment: APHIS does not analyze and incorporate results from the most recent research showing off-site movement of glyphosate and surfactants via soil, air, water and plant tissues that could impact wild animals and plants.

APHIS asserts that an increase in glyphosate use with Alternatives 2 and 3 will be less harmful to the environment in general than the herbicides used instead if Alternative 1 is adopted: "Alternative 1 is expected to result in usage of herbicides that could have more environmental impacts than glyphosate, which is the predominant herbicide applied under Alternatives 2 and 3. This includes impacts to animals, micro-organisms, non-target plants, human health, and environmental quality of the physical environment." (DEIS at vii).

However, APHIS does not adequately consider the extent to which glyphosate and associated surfactants will move off-site via soil and water. They stress the tight binding of glyphosate and surfactants to soil particles, on the one hand, and their rapid degradation by bacteria in soil on the other hand. They also state that conservation tillage, increased by RR crop adoption, will further reduce the movement of glyphosate off-site via soil-particle-laden water runoff. Thus, they conclude that wild animals and plants outside of sugar beet fields will have little contact with glyphosate and surfactants via soil and water.

Recent, relevant studies of actual behavior of glyphosate-based herbicides in water, air and soil, and within plant residues, need to be more carefully analyzed and incorporated into the DEIS.

For example, in discussion of glyphosate in water (DEIS at 339-342), there is no analysis of how frequently glyphosate is now found in surface waters near Roundup Ready crops after applications. APHIS relies on review articles that cite data predating adoption of herbicide resistant crops (e.g. Cerdeira and Duke 2006 review citing a Franz review from 1997 and a research article from 1993), rather than examining relevant USGS studies undertaken specifically to fill in the data gaps about how the Roundup Ready cropping system is affecting glyphosate levels (for example, Battaglin et al. 2005, 2009). The most recent USGS studies by Coupe et al. (2011) and Chang et al. (2011) are mentioned (DEIS at 333, 342, 610) but key conclusions are omitted (see below). These recent studies conclude that glyphosate and presumably surfactants are found in most surface water samples when measured after herbicide applications, showing that off-site movement is much more common in areas where Roundup Ready crops are grown than APHIS states. Also, glyphosate is found in air and rain in concentrations higher than more volatile herbicides, against APHIS' predictions based on physical properties, showing the importance of basing risk assessments on real-world data.

a. Glyphosate-based herbicides are found frequently in surface waters.

In 2002, the USGS began a monitoring program for glyphosate and its main degradation product, AMPA, in Midwestern streams (Battaglin et al. 2005), because "…the use of glyphosate is increasing rapidly, and there is limited understanding of its environmental fate". Glyphosate was detected in about a third of the streams sampled, after rain events following pre-emergence, post-emergence and harvest seasons. The highest measurement in these streams was 8.7 ug/l in a harvest-season sample.

The authors encouraged further study of glyphosate in water:

> "However, it also appears that glyphosate and AMPA are more mobile or persistent in aquatic environments than earlier research and monitoring suggested (Giesy et al., 2000). Additional monitoring for glyphosate to include summer low flow and wintertime samples could provide the information needed to determine which use, fate, and transport factors have the most influence on their environmental occurrence. Additional monitoring will be needed to determine if the increasing use of glyphosate results in increasing glyphosate and AMPA concentrations in Midwestern streams."

In follow-up studies, Battaglin and his colleagues observed vernal pools and streams near herbicide application sites in National Parks (Battaglin et al. 2009):

> "Vernal pools are sensitive environments that provide critical habitat for many species, including amphibians. These small water bodies are not always protected by pesticide label requirements for no-spray buffer zones, and the occurrence of pesticides in them is poorly documented…" (Abstract)

All vernal pools and ponds that they sampled were used by amphibians for breeding (p. 284), and samples were taken from water bodies before and after nearby glyphosate applications, with control samples from water bodies that were not near such applications. At some of the parks, "selected water samples were collected during or just after the first substantial rainfall that occurred after the use of glyphosate adjacent to the study sites." (p. 292) Overall, glyphosate was measured in 32% of samples, but 87% of the samples taken from ponds and streams near applications contained glyphosate. (p. 294-296, Table 4; Riley Spring Pond, Rock Creek at Riley Spring Pond, Lock 7 Vernal Pool, C&O Canal at Lock 7, Field-side wetland, Rands Ditch, and Browns Pond were water bodies near glyphosate application sites.)

One of the vernal pools (Riley Spring Pond) had over 300 ug/l glyphosate (and presumably proportional surfactant concentrations) after nearby applications, showing that vernal pools are vulnerable to significant contamination. The important point is that glyphosate applications near vernal pools do have the potential to result in high concentrations of herbicide components in the water.

In spite of the reported strong sorption to soil, glyphosate (and presumably surfactants) is commonly found in surface water where RR crops are grown, not just in vernal pools. The most recent USGS studies designed to measure the impacts of RR cropping systems on glyphosate levels in surface waters (Coupe et al. 2011) found glyphosate in 59% (SFIR New Providence site in Iowa), 72% (SFIR Blairsburg site in Iowa), and 100% (three sites in the Bogue Phalia basin in Mississippi) of samples taken every 1 to 2 weeks over two years from streams and subsurface drains.

Use-patterns of glyphosate-based herbicides in H7-1 fields, where it can be applied season-long, will result in frequent surface water contamination throughout the growing period. Researchers concluded that more water samples in Mississippi contained glyphosate than in Iowa because “Mississippi has nearly a continuous application of glyphosate for 9 months, whereas glyphosate applications in Iowa are limited to a few months in spring and summer.” The highest water concentrations of glyphosate occurred in samples taken after applications, during periods of high rainfall, which were not unlikely events.

They conclude: “These data suggest that glyphosate will be detected in surface water in agricultural basins where it is used”, and that concentrations will be determined by the amount of glyphosate applied relative to the size of the basin, water pathway characteristics, and weather patterns – factors that change from year to year.

b. Glyphosate-based herbicides are found in air and rain.

The fact that glyphosate is not volatile does not keep it out of the air where it comes down in rain, and this source of off-site movement was mentioned in the DEIS (p. 333), but then not considered in assessing risks to animals and plants, particularly listed species.

Even though glyphosate is not volatile, it moves off-site via air and in rainfall at about the same frequency and at higher concentrations compared to other herbicides that are more volatile because glyphosate is used so much in RR cropping systems (Chang et al. 2011). In this USGS study, glyphosate was measured in air and rain samples, weekly, during 2 growing seasons in the same areas sampled for surface water contamination by Coupe et al. (2011). They detected glyphosate in 60-100% of samples. “The detection frequency and median concentrations of glyphosate in both air and rain were not substantially different compared with other current-use herbicides, but maximum glyphosate concentrations were greater.” (p. 551) “These results are somewhat surprising insofar as the other herbicides are more volatile than glyphosate. The relatively elevated levels of glyphosate probably are due to its frequent use in these agricultural areas in conjunction with the genetically modified crops.” (p. 552)

Glyphosate gets into the air from spray drift and wind erosion of soil-bound glyphosate (p. 553), and then comes down later – sometimes after weeks - in rain, indicating that some portion was in small enough droplets to remain airborne. " At both study locations [in Mississippi and Iowa], the highest air concentrations were observed in the weeks during the application season when little or no rainfall occurred. These conditions gave a strong source of glyphosate to the air but no strong removal mechanism." (p. 553)

The amount of glyphosate taken out of the air in rain is substantial, given the large amount of glyphosate applied to RR crops, and must contribute to surface water contamination, in addition to glyphosate from other sources. "It is not known what percentage of the applied glyphosate is introduced into the air, but it was estimated that up to 0.7% of application is removed from the air in rainfall." (p. 555)

Instead of taking these new facts into account in assessing risks to amphibians and fish of the greatly increased glyphosate usage that will accompany deregulation of H7-1 sugar beets, APHIS cites the 2008 review by Borggaard and Gimsing to state that "….runoff and erosional movement of soils with sorbed glyphosate and surfactant to surface waters should be limited to storm events…" (p. 511). Even this conclusion underestimates the likelihood of off-site movement, given that "storm events" are quite common, but completely misses the implications of increased surface water contamination due to adoption of glyphosate-resistant cropping systems, as actually measured rather than hypothesized.

c. Glyphosate-based herbicides accumulate inside of crop tissues.

Glyphosate sequestered inside of RR crop tissues degrades more slowly than glyphosate in soil, with implications for herbicide fate and risk assessment that were ignored by APHIS.

Roundup Ready crops have been genetically engineered with a form of EPSPS that is not inhibited by glyphosate, so that the whole field – crops and weeds – can be sprayed without killing the crop. Although glyphosate isn't lethal to Roundup Ready plants, some is intercepted and moves from the leaves and stems to growing parts of the Roundup Ready plant in a similar way to susceptible plants, although translocation continues for a longer time after application, and the distribution pattern may be different (Feng and Chiu 2005, Geiger et al. 2009, Hetherington et al. 1999). When glyphosate encounters the altered EPSPS, the enzyme continues to function.

Because Roundup Ready plants don't die from being sprayed, the glyphosate from herbicide applications is likely to stay in the plant longer than it would in a dying weed. In a dying weed, the plant tissues disintegrate into the soil where microorganisms degrade the glyphosate, more slowly than if the glyphosate was applied to soil directly (Doublet et al. 2009). But within many Roundup Ready crops (including sugar beets), glyphosate mainly remains as glyphosate, without being broken down (Duke 2011). Eventually, some glyphosate leaves the plant. As glyphosate moves through the plant from source to sink, some goes to roots, where it exits the plant in root hairs that are sloughed off, and also exits by exudation from roots into the soil with other nutrients and molecules (Kremer et al. 2005, Laitinen et al. 2007). Older leaves containing glyphosate are shed as the plant ages. Thus the total amount of glyphosate in the plant decreases as the Roundup Ready plant grows. However, some of the glyphosate remaining redistributes and its concentration can increase over time in reproductive structures such as seeds, or in storage tissues such as rhizomes, even as total plant levels decrease (Feng and Chiu 2005, Geiger et al. 1999, Thomas et al. 2007). Later additional herbicide applications can add new glyphosate, resulting in higher glyphosate concentrations in these reproductive and storage organs, as well (Arrequi et al. 2003). This means that in Roundup Ready crop systems glyphosate (and presumably surfactants) can be stored within the ecosystem longer, inside of Roundup Ready plant tissues.

Many wild animal species are likely to eat storage organs or flowers, fruits and seeds of Roundup Ready plants, and thus potentially will have dietary exposures to glyphosate and surfactants over a longer period of time during the year than they would if they were eating conventional crops, where their exposure to leaves and stems of treated weeds would be more transient.

Doublet and coworkers (2009) conclude that plant-sequestered glyphosate should be factored into risk assessments: "Following application, pesticides can be intercepted and absorbed by weeds and/or crops. Plants containing pesticides residues may then reach the soil during the crop cycle or after harvest. However, the fate in soil of pesticides residues in plants is unknown….Absorption of both herbicides in plant delays their subsequent soil-degradation, and particularly, glyphosate persistence in soil could increase from two to six times. The modifications of herbicide degradation in soil due to interception by plants should be considered for environmental risks assessment." [Doublet et al. 2009, p. 582, abstract]

2. Glyphosate-based herbicides and the environment: APHIS does not incorporate the most recent research on impacts of glyphosate-based herbicides on amphibians.

a. Amphibians are of special concern in agricultural landscapes.

Amphibians, officially listed as endangered or not, are in decline worldwide, and habitat loss, much of it to agriculture, is a major cause (Mann et al. 2009). Their natural habitats gone, amphibians do use land and water in and near farming operations:

> "The fact that many species have been able to persist in agricultural landscapes is testimony to the one saving grace of agriculture… – the near permanent availability of water. Extraction of groundwater and the establishment of weirs, irrigation channels and dams has, in the case of some species, inadvertently provided breeding habitat where otherwise habitat has been destroyed." (Mann et al. 2009)

Amphibians pay a price for living with farming, though:

> "…agricultural practice changes continuously. In particular, chemicals in the form of pesticides and fertilizers are being applied in greater varieties, combinations, and to a greater extent than ever before, and represent a significant suite of pollutants. Data collated on the IUCN Red List of Endangered Species website for 2008 indicate that after habitat loss, pollution is the next major threatening process to amphibian populations (Fig. 1)." (Mann et al. 2009)

The very water resources provided by agriculture can harbor pesticide pollution that negatively impacts the amphibian populations there:

> "A large proportion of the amphibian life cycle occurs in ponds, streams, and temporary pools that are often associated with agricultural areas receiving pesticides…. breeding and larval development of amphibians occurs in spring and summer and coincides with the application of pesticides and fertilizers on agricultural lands. When considering these factors in addition to the large quantities of various herbicides, insecticides and fungicides presently used in agricultural production the resulting impacts on anurans have the potential to be significant." (Mann et al. 2009)

Of animals tested so far, amphibians of many species are more sensitive to glyphosate-based herbicides than many other taxa (review: Mann et al. 2009; Dinehart et al. 2010, Relyea 2005a, 2005b, 2006, review: 2011; Relyea and Jones 2009; Bernal et al. 2009a, 2009b, 2010). Amphibians are poisoned by glyphosate-containing herbicides at larval and adult stages, so could be exposed via soil, water or contact with sprayed foliage or crop tissues, in addition to being sprayed directly. They could also ingest insects that had eaten glyphosate-treated plants, and thus be exposed to glyphosate and perhaps surfactants via food.

APHIS is clear about the fact that aquatic animals are likely to inhabit nearby water bodies, but equivocates on the question of whether terrestrial-phase amphibians are likely to be present in sugar beet fields, and thus whether they will be exposed to more glyphosate-based herbicides with adoption of H7-1 sugar beets. First they claim that amphibians do not use sugar beet fields for food or shelter (DEIS, pp. 229-230):

> "Several species of amphibians (e.g., frogs, toads, salamanders) and fish might be located in water bodies adjacent to or downstream from H7-1 sugar beet fields. Amphibians use a wide range of aquatic habitats for their breeding sites. The presence of large numbers of adult-stage amphibians in sugar beet fields is not expected because agricultural fields are not ideal habitat for amphibians due to relatively constant disturbances associated with agriculture (e.g., tilling and pesticide application). As mentioned above, some farmers apply insecticides to their agricultural fields to minimize insect damage. In so doing, farmers reduce the food source (insects) for terrestrial-phase amphibians, forcing the species to forage in other areas. Likewise, fish are not expected in agricultural fields, although they may exist in nearby surface waters

> that receive runoff from the sugar beet fields during storm events or from spray drift that enters water bodies directly from ground or aerial applications."

But elsewhere in the DEIS APHIS seemed to assume that terrestrial-phase amphibians might frequent sugar beet fields, after all:

> p. 507:
> "If farmers allow the land to go fallow for a year and continue to plow the land, the amount of groundcover for terrestrial-phase adult amphibians that use the agricultural field to forage on ground-dwelling insects would be reduced."
>
> p. 512-513: "Impacts on Amphibians and Fish from Crop Management Practices. As discussed in section III.B, growing H7-1 sugar beets does not require in-crop tillage due to the use of glyphosate, thus reducing the amount of tillage compared to Alternative 1. Growers therefore can plant H7-1 sugar beets closer together compared to conventional sugar beets that require in-crop tillage. Under this conservation tillage system, there would be more extensive groundcover for terrestrial-phase amphibians to forage and disperse compared to Alternative 1, thus decreasing the probability of an individual being preyed upon and increasing the individual's chances of long-term survival and reproduction."

Without direct information about amphibian populations in and near sugar beets, APHIS should err on the side of caution and assume the presence of amphibians, as they did when assessing the impacts of denying deregulation, and then assess the potential impacts using the latest science.

b. Glyphosate-based herbicides are toxic to amphibians.

On average, most of the studies of glyphosate-based herbicide toxicity to larval amphibians found that half of them died within 4 days at between 0.8 and 3.2 mg a.e./liter of glyphosate. Formulations with different surfactants gave similar results (Relyea 2006, 2010; Bernal et al. 2009a; Dinehart et al. 2010; Williams and Semlitsch 2010). To protect most larval amphibians from harm, the concentration of glyphosate would have to be much lower, by an order of magnitude, which would be in the range of concentrations found in some vernal pools and other surface waters (Battaglin et al. 2009, Relyea 2010).

Adult or terrestrial frogs that are directly over-sprayed have LC50 4-day values of 4.5 to 22.8 kg a.e./ha (Bernal et al. 2009b), which means that spray rates must be much lower than that to protect most frogs from harm. If the amounts were lowered by an order of magnitude to provide a margin of safety, the allowed spray rates for H7-1 sugar beets, 0.86 – 1.26 kg a.e./ha (Monsanto Technology Use Guide 2011), would be in the toxic range (Relyea 2010).

Sub-lethal effect of glyphosate-based herbicides are perhaps more important than lethality, given that they are likely to occur at concentrations encountered more often in nature, and there are now several studies showing sub-lethal impacts:

> “At sub-lethal concentrations, exposure to POEA [a common surfactant in glyphosate-based herbicides] or glyphosate/ POEA formulations has been variously reported to result in delayed development (Howe et al., 2004), accelerated development (Cauble and Wagner, 2005), reduced size at metamorphosis (Howe et al., 2004; Cauble and Wagner, 2005), developmental malformations of the tail, mouth, eye and head (Lajmanovich et al., 2003; Howe et al., 2004), histological indications of intersex (Howe et al., 2004) and symptoms of oxidative stress (Costa et al., 2008).” (Mann et al. 2009)

c. Formulations used on herbicide-resistant crops are more toxic than glyphosate alone.

Results from various labs differ in the degree to which formulations versus glyphosate itself cause problems, which is not surprising. Studies using commercial formulations are difficult to compare because adjuvant mixes differ over time and between brands. Often researchers are unable to find out what components are present because of trade secret protections. Therefore, they are seldom able to include formulation-minus-active-ingredient controls in experiments.

Difficulties notwithstanding, testing formulations for environmental consequences is important. Most tests done for regulatory approval involve the active ingredient alone, or include studies on one common surfactant alone (Cox and Surgan 2006). According to Cox and Surgan (2006), testing the active ingredient is insufficient for gauging toxicity of a formulation. In some cases, increased toxicity in formulations results from interactions between the active ingredient and adjuvants, in other cases increased toxicity is primarily due to adjuvants alone. They conclude that inert “ingredients can increase the ability of pesticide formulations to affect significant toxicological end points, including developmental neurotoxicity, genotoxicity, and disruption of hormone function. They can also increase exposure by increasing dermal absorption, decreasing the efficacy of protective clothing, and increasing environmental mobility and persistence. Inert ingredients can increase the phytotoxicity of pesticide formulations as well as the toxicity to fish, amphibians, and microorganisms.”

APHIS stresses that glyphosate-based herbicide formulations with POEA are not approved for use over water (DEIS, p. 511). It is true that many glyphosate-based herbicide formulations are not approved for use over water, and probably none of the formulations labeled for use on H7-1 sugar beets are approved for use over water. However, many studies have now shown that glyphosate and formulation components get into water bodies via known and unknown routes even when being used according to directions, and that vernal pools, so important for amphibian reproduction, often escape regulation (Relyea 2006, 2010). As just discussed, Battaglin et al. (2009) showed that water bodies adjacent to glyphosate-treated fields can also be contaminated at levels toxic to amphibians, so APHIS needs to reconsider the impacts of in light of real-world practices and outcomes.

There is another issue related to glyphosate vs. glyphosate-based formulations, and that has to do with differences in how glyphosate and the surfactants act in the environment. Some surfactants, including the POEA known to be toxic to amphibians, do not break down as rapidly as glyphosate. This results in an underestimate of environmental risks:

> "… All the studies mentioned above expressed the toxicity of glyphosate/POEA formulations in terms of glyphosate concentration, which can be deceptive considering the toxicity is allegedly as a consequence of exposure to the formulation surfactants. This situation arises because surfactants in general, including POEA, are typically a mixture of closely related oligomers (e.g. Mann and Boddy, 2000) and measuring surfactants as discrete compounds is difficult. However, if the quantities of surfactant used in a formulation vary, then the toxicity data will also vary because it is dependent on the ratio of glyphosate to surfactant. Furthermore, analysis of water samples for glyphosate as a proximate measurement of the concentrations of associated surfactants is likely to underestimate the risk because the environmental persistence of surfactants may be higher than the active ingredient. Glyphosate has an aquatic half-life ranging from 2 to 14 days, whereas that of the associated POEA surfactant (Monsanto's MON 0818) in the environment has been conservatively estimated at 21–41 days (Giesy et al., 2000)". (Mann et al. 2009)

<u>d. Glyphosate-based herbicides have indirect effects on amphibians via effects on plants.</u>

There are also indirect impacts of glyphosate-based herbicide use on amphibians. For aquatic organisms, effects of glyphosate on water quality, including types and amounts of algae and microorganisms, may be as important in the long run as direct toxicity Vera et al. (2010) looked at the effects of Roundup on algae and microorganisms that attach to surfaces, called periphyton, a favorite food of tadpoles. They showed that even a single glyphosate application to water is able to change the balance of algae and other microbes. The changes may be mediated by the phosphate contribution made by glyphosate itself, as well as by the direct toxicity of glyphosate to some microorganisms, and its stimulating effects on others.

The abstract provides a summary of their findings:

> "Abstract: Argentina is the second largest world producer of soybeans (after the USA) and along with the increase in planted surface and production in the country, glyphosate consumption has grown in the same way. We investigated the effects of Roundup (glyphosate formulation) on the periphyton colonization. The experiment was carried out over 42 days in ten outdoor mesocosms of different typology: ''clear'' waters with aquatic macrophytes and/or metaphyton and ''turbid'' waters with great occurrence of phytoplankton or suspended inorganic matter. The herbicide was added at 8 mg L-1 of the active ingredient (glyphosate) in five mesocosms while five were left as controls (without Roundup addition). The estimate of the dissipation rate (k) of glyphosate showed a half-life value of 4.2 days. Total phosphorus significantly increased

> in treated mesocosms due to Roundup̀ degradation what [sic] favored eutrophication process. Roundup̀ produced a clear delay in periphytic colonization in treated mesocosms and values of the periphytic mass variables (dry weight, ash-free dry weight and chlorophyll a) were always higher in control mesocosms. Despite the mortality of algae, mainly diatoms, cyanobacteria were favored in treated mesocosms. It was observed that glyphosate produced a long term shift in the typology of mesocosms, ‘‘clear’’ turning to ‘‘turbid’’, which is consistent with the regional trend in shallow lakes in the Pampa plain of Argentina. Based on our findings it is clear that agricultural practices that involve the use of herbicides such as Roundup̀ affect non-target organisms and the water quality, modifying the structure and functionality of freshwater ecosystems.” (Vera et al. 2010)

This team noticed that there were lingering effects of earlier Roundup treatments in some of the mesocosms:

> “Since the glyphosate half-life was no longer than 1 week it was assumed that no long term effect could be attained, and that after a year of recovery it would be safe to start a new experiment in the same mesocosms. However, most of the ‘‘turbid’’ mesocosms in the present experiment were those treated with glyphosate in the previous experiment and the mesocosms used as controls in the first experiment remained ‘‘clear’’ at present. Unexpectedly, we detected that a single application of glyphosate in 2005 shifted the mesocosms from a ‘‘clear’’ to a ‘‘turbid’’ state which remained until the next year. As was discussed above, the glyphosate may be adsorbed to sediments and a slow later desorption might produce a long turn effect suppressing growth of the most sensitive groups and favoring the abilities to compete of the more resistant algae. This trend in long term effect was suggested by Holtby and Baillie (1989) who reported an enhancement of periphytic production as a response to increased levels of phosphorus produced by a unique application of Roundup̀ done 1 year before their experiment, carried out in natural streams.” (Vera et al. 2010)

And they conclude: “Based on the findings obtained in our work as well as those obtained in previous researches, it is clear that agricultural practices that involve the use of herbicides such as Roundup affect non-target organisms and water quality, modifying the structure and functionality of freshwater ecosystems.” (Vera et al. 2010)

After reviewing these and other studies of glyphosate-based herbicide toxicity to amphibians, Mann and his colleagues (2009) summed up their own conclusions that “…. amphibians may be particularly susceptible to the toxic effects of these pesticides because their preferred breeding habitats are often shallow, lentic or ephemeral pools that do not necessarily constitute formal water-bodies, and which can contain higher concentrations when compared to larger water-bodies (NRA, 1996; Mann et al., 2003; Howe et al., 2004; Relyea, 2005a,b,c, 2006).” (Mann et al. 2009)

e. APHIS did not rely on these latest studies in assessing risks of increased glyphosate-based herbicide use to amphibians, including those that are threatened and endangered.

In the DEIS, APHIS examines risks to aquatic-phase amphibians using EPA studies of herbicide impacts, which are based on fish data; and terrestrial-phase amphibians impacts based mainly on the pesticide effects determination of the risks of glyphosate use to Federally Threatened California Red-legged frog from 2008 (DEIS, pp. 503 – 514). The only study on toxicity of glyphosate-based herbicides to amphibians cited specifically by APHIS in the DEIS is Relyea 2005a. The risks to amphibians, including those that are threatened and endangered, from increased glyphosate-based herbicide use associated with deregulation of H7-1 sugar beets needs to be reexamined by APHIS in light of these most recent studies specific to amphibians, discussed above, which were not considered in the DEIS or in the EPA reports upon which APHIS relies, and which show that there is more risk to amphibians from glyphosate-based herbicide use than previously thought.

3. Glyphosate-based herbicides and the environment: APHIS does not take into account key studies of negative consequences of increased glyphosate use on non-target plants.

Plants are, of course, sensitive to the herbicide glyphosate, so non-target plants are inherently at risk from inadvertent exposure, mainly from drift of spray droplets during applications.

There are no published studies of glyphosate drift exposure and effects on non-target wild plants in the fields where glyphosate use has increased due to adoption of Roundup Ready cropping systems. Therefore, APHIS should consider studies showing well-known sub-lethal effects of glyphosate on plants populations in assessing risk to non-target plants, including threatened and endangered species. Appropriate studies include those that examine simulated drift effects on non-RR crops, weeds, and wild plants. APHIS should also discuss reports of drift injury to non-RR crops as examples of real-world impacts of glyphosate use.

a. Glyphosate affects plant reproduction at drift levels.

The data regarding sub-lethal glyphosate effects on plant reproductive success are reviewed, and added to by original research, in a key peer-reviewed scientific paper, not cited in the DEIS (Blackburn and Boutin 2003).

This research specifically addresses risk to the success of wild plants from drift levels of glyphosate related to increased use of glyphosate associated with Roundup Ready crops. The author's state:

> "The use of these new crops has raised concern about an increase in reliance on glyphosate for weed control with detrimental consequences on nontarget plants and habitats due largely to the broad spectrum nature of this herbicide. The objective of this paper is twofold: (1) to review the literature on the effect of glyphosate on seed germination and early seedling growth, and (2) to present the results of a new

> experiment with several crop and noncrop species. The attempt was made to build on past findings and to add to the knowledge base in an effort to move away from studies on crop plants such as soybean and grain by focusing mainly on noncrop plant species." (Blackburn and Boutin 2003, p. 272)

Past findings are that sometimes plants that have not suffered mortality after contact with glyphosate, and in fact may not have exhibited visible symptoms of injury at all, nevertheless produce fewer seeds or seeds that have problems with germination or vigor (references cited in Blackburn and Boutin 2003). Also, plants that reproduce vegetatively from tubers or rhizomes sometimes show injury in the generation subsequent to actual glyphosate application or contact (Viator et al. 2008, Dalley and Richard 2010).

Specific, unique properties of glyphosate explain how it can affect subsequent plant generations. Glyphosate applied to leaves and stems translocates with photosynthates to the most rapidly growing tissues and organs of plants, such as developing flowers and seeds (Feng et al. 2003, Feng and Chiu 2005). In most plant species glyphosate is not metabolized, and these plant parts not only accumulate the glyphosate but also are particularly sensitive to it (Feng et al. 2003, Chen et al. 2006). Therefore, glyphosate can cause pollen sterility (Chen et al. 2006, US Patent 4,735,649), potentially resulting in fewer seeds; or can cause seeds that form to be less viable and vigorous (Thomas et al. 2005, Walker and Oliver 2008). Different species of plants are more or less sensitive to glyphosate's sexual and vegetative reproductive effects, and the stage of development at which the plant is exposed to glyphosate influences the response, as well (Blackburn and Boutin 2003). In many cases, drift levels of glyphosate have been shown to cause these effects (Blackburn and Boutin 2003). Thus, sub-lethal doses of glyphosate can reduce the fitness of an affected plant species, reducing population levels in subsequent generations. Because other herbicides have different basic properties – for example, less efficient or no translocation to reproductive tissues, or metabolism within the plant resulting in less accumulation and persistence – the substitution of other herbicides by glyphosate is likely to have unique effects on plants.

Another factor APHIS did not consider is the impact of being able to apply glyphosate during the entire H7-1 sugar beet growing season. It is certain that wild plants will have the potential to be exposed to glyphosate during more of their growth phases, including closer to or during reproduction, making them more vulnerable to adverse reproductive outcomes. In their discussion, Blackburn and Boutin say this:

> It is difficult to predict how glyphosate exposure will change a plant community due mainly to the wide variation in maturation and growth patterns of the species present at the time of application. Noncrop species growing within crops or along field margins where they may be exposed to glyphosate through overspray or spray drift may be at different phenological stages than the crop in which or near which they are growing (Shuma et al.,1995). Some species may have mature seeds while others may have immature seeds and still others may not be reproducing yet. The future of the seeds could be affected; while in the cases where plants are not at a stage of reproduction,

> the death or declined vigour of the plant could result in no input of seeds for the next generation. Herbicide applications may reduce the production of viable seeds and thus reduce the establishment or replenishment of noncrop seed reserves in the soil (Baskin and Baskin, 1998).

Researchers at the EPA have also done studies on sub-lethal effects to plants of glyphosate applied at drift levels, concerned that risk assessments are missing important reproductive impacts (Olszyk et al. 2009, 2010). For example, using pea seed weight as a measure of reproduction, Olszyk et al. (2009) concluded:

> Significance of potential reproductive responses
> Although additional studies are needed with other crops and under field conditions, this study indicates that seed production in peas, and possibly other crops, could be reduced by low levels of herbicides found in drift scenarios. Reductions in pea seed yield were found at less than 0.01 X FAR [0.008 lb a.e./acre] for at least one experiment and growth stage for four herbicides (sulfometuron, primisulfuron, glyphosate, and glufosinate). The 0.01 rate approximates a maximum treatment approximately 30 m from the edge of a field for a ground application with a 4.5 m/s wind speed according to the AgDRIFT model [39,40]. (Olszyk et al. 2009)

b. Glyphosate affects other plant processes at drift levels.

APHIS did not weigh studies showing other types of sub-lethal effects of glyphosate. For example, here has been recent interest in non-herbicidal effects of glyphosate that may operate at drift levels, too (Duke and Dayan 2010). These include changes in biochemistry (for example, sugar increases in cane), and growth enhancement (not to be construed as an advantage of drift – not all growth increases are adaptive).

c. Species vary in sensitivity to drift levels of glyphosate.

Also, the species used in tests by EPA to determine sensitivity to glyphosate may not adequately represent the range of responses found in wild species. Dalton and Boutin (2010) studied differences in herbicide sensitivity between species under different test conditions and found:

> … the present study has shown that herbicide sensitivity is highly dependent on species and test conditions. Interactions between species, test condition, and concentration complicate extrapolation to complex ecological systems. Given that sensitivity is so heavily dependent on the particular species and test conditions used, it is unlikely that single-species tests using crop species would have much relevance for assessing the risk posed to nontarget communities.

d. Formulations affect plants differently.

As with amphibians, plants show different sensitivities depending on surfactants and other adjuvants, but formulations have not been assessed by APHIS for their impacts to non-target species (White and Boutin 2007).

e. Glyphosate drift from applications to glyphosate-resistant crops happens when other plants are likely to be most vulnerable.

Researchers at EPA used the detailed pesticide usage information, crop location maps, and developmental timetables for specific crops for Fresno County, CA, to model likelihood of injury to crops from glyphosate use (Lee et al. 2005). They found (p. 3): "Glyphosate is not as damaging to sensitive crops as 2,4-D and dicamba and other potential high risk herbicides but has greater potential to damage sensitive crops due to spray drift because it is applied throughout the year in large quantities." Confirming that glyphosate drift is particularly damaging to plants at their reproductive stages, they state (p. 32): "The likelihood of drift damage to sensitive crops depends upon when, where and how the herbicide was applied in relation to the time of increased plant sensitivity and proximity of the nontarget crop. For most crop species, the time of increased sensitivity occurs at or prior to anthesis [pollen shed]." This sensitive period means that usage patterns for H7-1 sugar beets are more likely to cause injury to non-target plants than former burndown applications (p. 15): "Postemergence application of a herbicide to a genetically-modified (GM) crop often occurs when non-GM plants are in the early reproductive growth stage and are most susceptible to damage from herbicide drift (Ghosheh et al., 1994; Hurst, 1982; Snipes et al., 1991, 1992). Consequently, more drift complaints occur in spring and summer as the use of postemergence herbicide applications increase."

Again, adoption of H7-1 sugar beets will increase the likelihood that many stages of a particular plant will be contacted by glyphosate, causing a more varied and perhaps cryptic injury pattern, often not seen until the next generation. This type of injury will be difficult to monitor and mitigate given its cryptic nature, and has been causing problems for farmers growing rice, wheat, and other non-RR crops near RR crops, from ground rigs and aerial equipment (Scott and Cartwright 2002, Scott 2005, Koger et al. 2005, Baldwin 2011, Wagner 2011). So in spite of the non-volatile nature of glyphosate and label restrictions on application rate, droplet size, wind speed, equipment set up; with ground and air applications; drift injury does happen, and needs to be taken into account in assessing risk of increased glyphosate applications longer during the season to non-target plants.

f. Sub-lethal glyphosate injuries are of particular importance for populations of threatened and endangered species.

If threatened or endangered plants are found near H7-1 sugar beet fields, farmers are supposed to take special measures as suggested by Monsanto (DEIS, Appendix F-10)). Again, however, given the cryptic nature of important sub-lethal glyphosate effects and variations in sensitivity between species under different conditions, such measures may not be adequate to protect the plants, even if the farmers do know that there are such species nearby and go to and follow the instructions on the Monsanto Pre-Serve website. In other words, the "legal precautions" represented by the EPA label use restrictions, may not be adequate given new knowledge about glyphosate effects on non-target plants.

4. Glyphosate-based herbicides and the environment: APHIS did not consider relevant research showing that changes in populations of rhizosphere microorganisms, including pathogens, that occur in Roundup Ready crop systems where glyphosate is used post-emergence, can increase risk of diseases in subsequent crops.

Independent researchers have shown that glyphosate stimulates certain plant pathogens in the rhizosphere of Roundup Ready crops, where glyphosate within or being exuded in close proximity to the microorganisms has a chance to affect them before being bound by soil (Kremer et al. 2005; Johal and Huber 2009).

Recent work by Zobiole et al. (2010b) provides the most detailed picture to date of changes in rhizosphere microorganisms in response to glyhposate application at different stages of soybean development for both first-generation Roundup Ready and second-generation Roundup Ready 2 Yield varieties. These studies show that Fusarium spp. Increased; and rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and IAA [growth hormone]-producing rhizobacteria all decreased after glyphosate was applied, relative to the same cultivars grown without glyphosate. Root and shoot biomass also decreased. They conclude that "[g]lyphosate applied to GR soybeans, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity".

Although earlier work focused on the micronutrient manganese and under what conditions its levels were reduced by glyphosate (Zobiole et al. 2010c and references therein), more recent studies show that glyphosate can also lead to lower levels of other nutrients, such as nickel (Zobiole et al. 2010a) or iron. Nitrogen fixation and other aspects of nitrogen metabolism were inhibited by glyphosate in some situations (Zobiole et al. 2010a). Photosynthesis can also be affected (Zobiole et al. 2010a). These stresses may also facilitate diseases.

These effects of glyphosate on microorganisms depend on translocation and thus are likely to be more persistent in the rhizosphere of Roundup Ready crops that continue to translocate glyphosate, as opposed to non-Roundup Ready crops and weeds that respond to glyhposate by limiting translocation (Geiger et al. 1999). Nevertheless, many of the early studies of pathogen interactions with glyphosate predate Roundup Ready technology. The experiments led to an idea that glyphosate in field situations works synergistically with fungal pathogens to kill weeds (Levesque and Rahe 1992).

Gressel (2010) has renewed interest in this phenomenon, where in Table 3, he shows that glyphosate synergizes many mycoherbicidal pathogens, perhaps by binding cations needed for plant defense mechanisms. Since this would be independent of the metabolic mode of action of glyphosate, it should occur in Roundup Ready crops just as in conventional ones.

Additionally, scientists at Purdue are currently examining the role of glyphosate-pathogen synergy in weed resistance to glyphosate (Schafer et al. 2009):

"Our findings confirm that the insensitive biotype of each weed was more sensitive to glyphosate in unsterile soil, than the sensitive biotype in sterile soil. Soil microbes play an important role in the mode of action of glyphosate. Thus, it is possible that the evolution of resistance to glyphosate may stem not only from the resistance to the herbicide itself, but also resistance to soil microbes. Further research will investigate whether or not the insensitive biotypes of common lambsquarters and giant ragweed studied here exhibit elevated levels of resistance to soil microbes."

If H7-1 sugar beets do behave similarly to Roundup Ready soybeans by changes in rhizosphere microorganism communities in response to glyphosate, then assuming the sugar beets themselves do not succumb to more severe disease symptoms, they could leave behind higher inocula of disease organisms that could infect crops in rotation. Larson et al. (2006) alluded to this possibility in their discussion: "A future direction includes understanding the impact of GR crops on rotation. Increases in Rhizoctonia root rot could increase the soil pathogen population and affect other susceptible crops in rotation with GR sugar beet, such as dry bean, soybean and corn."

Neither Larson et al. nor APHIS cited the work of Fernandez on the association between previous glyphosate use and subsequent Fusarium Head Blight (FHB) in wheat and barley. Fernandez (2009) reviewed work in this field, much of it her own, and concluded for wheat that:

> "Previous glyphosate application, nested within tillage system, was the only agronomic factor significantly associated with higher FHB levels every year of the study (Tables 3 and 4). Glyphosate's effect on the FHB index was not influenced by environmental conditions as much as for other agronomic factors whose effects on disease levels were inconsistent from year to year. Under minimum-till, application of glyphosate at least once in the previous 18 months significantly increased the mean FHB index and the proportion of fields in the high FHB index class every year."

Fernandez further observed similar associations between previous glyphosate applications to fields then planted in barley and various Fusarium diseases.

> "These studies document the positive association of glyphosate with pathogenic Fusarium spp., including F. avenaceum, F. culmorum and F. graminearum, in spikes/kernels and subcrown internodes of wheat and/or barley, and in residues of

> these crops almost a year after harvest. The exact nature of these associations was not determined. Previous research has shown that herbicides, including glyphosate, can inhibit or stimulate the growth of fungal pathogens, and can either increase or decrease disease development through direct or indirect means (Altman, 1993; Levesque and Rahe, 1992). Levesque and Rahe (1992) showed evidence that herbicides can have a direct effect on various components of the soil microflora, such as plant pathogens, antagonists, or mycorrhizae, which can potentially increase or decrease the incidence of plant disease. Pathogens able to infect weeds can also increase their inoculum potential after weeds have been sprayed with herbicides, which could subsequently affect host crops."

Clearly, more field studies need to be done to discern the effects of increased glyphosate use on specific microorganisms, and their role in diseases on H7-1 sugar beets and rotation crops, and also on drift-impacted wild plants. Such changes could linger in and near particular fields long after glyphosate use ended.

C. APHIS does not consider the effects of glyphosate use on increased weediness of and gene flow between *Beta* varieties and species.

APHIS asserts that "gene flow, hybridization, introgression and the distance that pollen, seeds or vegetative tissues move in the landscape will not change under any of the alternatives…" (DEIS, p. 454), although they then describe differences in "the likelihood of successful gene flow between any population of H7-1 sugar beets and other fields of populations of Beta spp." between the alternatives (DEIS, p. 454 – 461). Missing from the analysis is a discussion of how glyphosate use affects gene flow.

A series of important studies published recently by a group of EPA and academic scientists (Londo et al. 2010, 2011a, 2011b, Watrud et al. 2011) shows how glyphosate can both "change the gene-flow dynamics between compatible transgenic crops and weeds" and at the same time result in "an increase in the transgenic seed bank." (Londo et al. 2010). Glyphosate can also change the population dynamics over the years, favoring plants with the RR trait, with effects that last beyond the glyphosate application periods (Watrud et al. 2011).

Their experiments involved glyphosate-resistant *Brassica napus* (Canola) growing in mixed-species plant communities that included sexually compatible weedy species as well as experiments including non-compatible weedy species. Glyphosate was applied to simulate drift, and the movement and persistence of the RR transgene and population dynamics of the species were monitored over a two-year period.

Londo et al. (2010; 2011a,b) find that glyphosate spray drift does increase the "persistence of glyphosate resistance transgene in weedy plant communities due to the effect of glyphosate on plant fitness", meaning that plants containing the transgene and thus are immune to glyphosate injury, leave more offspring, not surprisingly.

The surprise in their work has to do with the finding that glyphosate spray drift changes the flowering behavior of *Brassica* spp. that do not have the transgene and are thus sensitive to the herbicide. These plants suffer temporary male sterility from drift levels of glyphosate, and thus with little pollen of their own, are more likely to be pollinated by the unaffected glyphosate-resistant plants in the population. This means that the glyphosate-resistance trait will spread much more quickly if glyphosate is present.

Londo et al. (2011a) summarize their results:

> This is the first evidence that suggests that glyphosate exposure may alter reproductive function and contribute to transgene flow potential associated with outcrossing in *Brassica* . The implication of a temporary change in self-fertility of *B. napus* is an increased window for interplant outcrossing and higher rates of gene flow to feral or volunteer *B. napus* from transgenic plants. ...In addition, transient male sterility resulting from glyphosate drift exposure could contribute to increased crop–crop gene flow and adventitious crop contamination under certain conditions. For example, in locations where nontransgenic conventionally bred and managed or organically grown canola plants exposed to glyphosate drift were within pollinator distances of feral transgenic glyphosate resistant plants. Further studies are underway to quantify the length and severity of glyphosate-drift-induced affects on reproduction and male-sterility in *B. napus*.
>
> Transient effects on self-reproduction and unidirectional gene flow may not be limited to canola and other *Brassica* species. Several weedy species such as *Amaranthus* sp., *Conyza canadensis* , *Ambrosia artemisiifolia* and *Lolium multiflorum* (Nandula et al. , 2005; Culpepper et al. , 2006) have evolved natural resistance to glyphosate applications. If exposure to sublethal glyphosate applications causes similar changes in reproduction in these other weedy species, the spread of glyphosate resistance through weedy populations would be anticipated to increase. Even in areas not subject to full concentrations of glyphosate, plants with resistance alleles would have higher pollen competitive ability, potentially increasing hybridization rates with neighboring, temporarily male-sterile plants. Spread of resistance in these populations would not be caused solely by extinction of sensitive genotypes but also by demographic swamping resulting from glyphosate drift. Additional field based assessments are needed to determine the extent and concentration of glyphosate drift from crop and roadside applications in relation to the spread of glyphosate resistant weeds.

Unlike APHIS, these researchers describe scenarios where plants are likely to encounter drift levels of glyphosate, both within agriculture – on the edges of fields where glyphosate is being applied, and outside of agriculture – along roadsides, ditches, and other wild areas where glyphosate is used for general vegetation control. In these situations, selection pressure will result in increased fitness of plants that have acquired the glyphosate-resistance trait, amplifying the impacts of rare crosses that pass on transgenes. Over time, plant populations of mixed species come to be dominated by those that have glyphosate resistance (Watrud et al. 2011).

Results of these studies on *Brassica* spp. have obvious significance for the "weediness" of *Beta* species that have crossed with H7-1 sugar beets. APHIS describes the possible impacts of movement of the glyphosate resistance trait into weedy or feral beets in the PPRA:

> Escape of the engineered trait into weed beet populations is possible if grown in close proximity to weedy or feral beets. APHIS concludes that the potential of the glyphosate tolerance trait moving from H7-1 to other sexually compatible *Beta* species in the United States is low, because of lack of ability to hybridize or proximity to sexually compatible plants. If the trait did move into wild species, these could not be controlled with glyphosate in H7-1 sugar beets. As glyphosate is not used for post-emergent control of wild beets in other *Beta* crops and other herbicides are effective to control wild beets in non-Beta crops, the potential for glyphosate tolerant weed beets to cause problems in H7-1 sugar beet fields or other crops that are resistant to glyphosate would be limited. Even if these plants become tolerant to glyphosate, there are other registered herbicides that can be used to kill them and other methods of control can still be used (OECD 2001). (PPRA 2011, p. 8)

Even if "the potential of the glyphosate tolerance trait moving from H7-1 to other sexually compatible *Beta* species in the United States is low", under glyphosate selection the trait will be selected and become common. Then these populations, unlikely to be detected and thus left "uncontrolled", will increase the likelihood of gene flow in the future.

In fact, *Brassica* populations with herbicide resistance traits have spread widely in unmanaged areas, without being controlled or monitored for over a decade. They are interbreeding, and even "stacking" different traits in combinations not found in agriculture (Schafer et al. 2011). Similarly, populations of wild cotton in Mexico have incorporated herbicide-resistance and other transgenes, also without being monitored or controlled for 15 years. (Wegier et al. 2011).

Watrud et al. (2011) discuss this possibility for *Beta* species:

> As cultivation of transgenic glyphosate-resistant sugar beet (*Beta vulgaris* L), which is a chenopod, increases in the United States, establishment of feral glyphosate-resistant sugar beet in disturbed habitats can be anticipated (Arnaud et al. 2003, Fenart et al. 2007) and perhaps should be monitored for potential unintended ecological effects.

And they conclude that glyphosate drift exposure increased the weediness of plants that have the transgene:

> Transgene escape is typically considered an environmental issue when transgenic plants survive, compete, persist, and transfer genes. We observed an interacting process between transgenic glyphosate herbicide resistance and glyphosate drift that allowed

> greater persistence of *Brassica* in a weedy community in which it is not otherwise competitive. We conclude that glyphosate drift selection is sufficient to increase the competitive ability of transgenic *Brassica* in non-agronomic environments and should be considered in management plans for monitoring and mitigating unintended ecological consequences of dispersal and establishment of glyphosate-resistant transgenic plants in disturbed habitats.

APHIS states that a certain amount of gene flow is an "unavoidable impact" in sugar beet production:

> A low level of gene flow between sugar beets and other fertile Beta species is unavoidable in sugar beet seed production practices. However, with proper mitigation measures in place, unwanted gene flow can be reduced to negligible levels. (DEIS, p. 632)

With the large increase in glyphosate use during a wider window of application throughout the growing season, drift levels are more likely to impact wild and feral *Beta* spp., in addition to non-agricultural applications so common in vegetation management. In such an environment where glyphosate selection is operating, unwanted gene flow will be favored, and consequences for future weed control and transgenic contamination of non-RR *Beta* crops need to be considered in the DEIS, and the conclusions about weediness in the PPRA should be reconsidered.

Conclusion: APHIS should choose Alternative 1: deny the petition seeking a determination of nonregulated status of H7-1 sugar beets.

APHIS has not used the latest scientific studies to carefully assess the environmental consequences of the greatly increased glyphosate-based herbicide use that will occur if Alternatives 2 or 3 are adopted. Therefore the DEIS is inadequate to fully assess the potential effects of H7-1 sugar beet deregulation on amphibians, plants, pathogenic microorganisms, and other species, including T&E species. APHIS also failed to consider evidence that greater glyphosate use will increase the likelihood of transgenic contamination and subsequent weediness of *Beta* species that inherit the glyphosate-resistance trait.

Sincerely,

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