THE CENTER FOR
FOOD SAFETY

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Comments to USDA APHIS on Dow AgroSciences LLC; Draft Environmental Impact Statement for Determination of Nonregulated Status of Herbicide Resistant Corn and Soybeans

Center for Food Safety, Science Comments II

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These comments submitted by Center for Food Safety are one of two sets of science comments from our organization. Legal comments are also being submitted. The references cited have been uploaded as supporting materials. The filenames for these documents match the citations in the text, and are all incorporated as (e.g. Benbrook 2012). Full citations are included at the end.

These comments supplement and incorporate by reference our earlier two rounds of comments on the draft Environmental Assessments for event DAS-68416-4 (Enlist soybeans) and event DAS-40278-9 (Enlist corn), as well as all the references submitted previously. The previous CFS science comments on the draft EAs have been resubmitted as Appendix B. The previous CFS science comments will be cited in this text as "CFS Science Soy", "CFS Science Corn I", and "CFS Science Corn II".

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Overview

In its analyses of its proposed Plant Protection Act approval decision, APHIS fails to adequately consider, among other things, the effects on non-target organisms of approving Enlist corn and soybeans. Non-target organisms affected include, but are not limited to, plants growing within and near Enlist crop fields, wildlife such as migratory birds and butterflies, threatened and endangered species, and species beneficial to agriculture, such as pollinators, mycorrhizal fungi, nitrogen fixing bacteria, and predators of plant pests. APHIS does not consider impacts to these organisms from applications and off-target movement of herbicides used as part of the Enlist system. Nor does it consider the differences in potentially toxic herbicide metabolites between Enlist corn and soybeans and unmodified recipient organisms that may harm non-target organisms that consume or come in contact with Enlist corn and soybean plant parts.

APHIS excludes important analyses of herbicide impacts to non-target organisms based on invalid assumptions

In its assessments, APHIS excludes impacts of applications and off-target herbicide movement, and impacts of herbicide metabolites, saying that only EPA has authority to regulate pesticides, and that EPA's regulation will mitigate any adverse impacts to health and environment. For example, APHIS defers to EPA's regulations to mitigate harm from herbicide use on Enlist corn and soybeans:

> EPA is conducting an independent assessment of direct and indirect effects associated with the use of 2,4-D on DAS-40278-9 corn, DAS-68416-4 soybean, or DAS-44406-6 soybean concurrent with the development of this EIS. These effects are outside the scope of this EIS. APHIS decisions for the petitions for these new GE varieties will be made independent of the results of the EPA assessment. <u>One assumption of the APHIS analysis is that EPA will establish label restrictions that will ensure the safety standards for human health and the environment associated with the use of Enlist Duo™ on these three varieties will be met.</u> Therefore, APHIS' analysis in this section focuses on cumulative impacts associated with these varieties including the development of HR weeds due to herbicide application and changes in management practices resulting from their use. (EIS at 118, emphasis added)

APHIS explicitly states this and other assumptions when justifying its relegation of herbicide considerations to cumulative impacts of EPA's expected registration of Enlist Duo herbicide combined with approval of Enlist corn and soybeans (EIS at 118 - 119). Other assumptions by APHIS relevant to non-target organisms are:

> <u>APHIS assumes that herbicide applications will conform to the EPA-registered uses for corn and soybean that are summarized in Appendix 7 and 8.</u> …
>
> <u>APHIS assumes that drift from 2,4-D and other pesticide applications will be mitigated to an acceptable level by the registration requirements established by EPA</u>. (EIS at 119, underline added)

APHIS does not provide evidence in support of these assumptions, however. In fact, there is evidence to the contrary, as discussed by CFS in previous comments (CFS Science Soy at 43 – 45, 76 - 78), and below. APHIS's reliance on these assumptions is thus contrary to sound science, and renders its conclusions arbitrary, since the agency refused to analyze important impacts of its proposed action.

EPA's label restrictions have not ensured safety standards for the environment from use of herbicides on previously approved resistant crops

There is good evidence that EPA's label restrictions have not "….ensure[d] the safety standards for human health and the environment associated with the use of…" herbicides with previously approved herbicide resistant crops. For example, glyphosate applications on glyphosate-resistant corn and soybeans, presumably used according to label instructions, has essentially eradicated common milkweed from fields in the Midwest (CFS Science Soy at 79 - 80). Common milkweed in corn and soybean fields is the most important food plant for monarch butterfly larvae in North America, producing almost 80% of the butterflies that overwinter in Mexico (Pleasants and Oberhauser 2012). Monarch populations have plummeted in recent years, with the lowest overwintering population ever recorded this year (Rendón-Salinas & Tavera-Alonso 2014), continuing an alarming 20-year decline of more than 90% (Brower et al. 2011, 2012), and raising concern that the entire migration is in jeopardy. Scientists have linked this dramatic decline in monarchs in large part to loss of breeding habitat from milkweed eradication by glyphosate use on glyphosate-resistant crops (Pleasants and Oberhauser 2012).

EPA's label regulations failed to prevent this important harm to the environment, even though monarch biologists predicted the result soon after glyphosate-resistant crops were approved (e.g., Simpson 1999, Hartzler and Buhler 2000, Brower 2001). Now, in the EIS, APHIS has failed to assess impacts of approving Enlist corn and soybeans on monarchs, even after learning of harm from previous herbicide-resistant corn and soybean approval decisions, and seeing the evidence that EPA's label restrictions were not protective. APHIS must consider how approval of Enlist corn and soybeans will impact milkweeds and monarchs, including associated use of herbicides, rather than improperly deferring responsibility for assessment to EPA (discussed in more detail below).

EPA's registration has not mitigated drift from herbicides to acceptable levels

Also, in spite of EPA's regulation, off-target herbicide movement, including drift of glyphosate applied on glyphosate-resistant crops, has resulted in many incidents where non-target organisms were harmed (US-EPA 2009). Glyphosate use has increased dramatically in concert with widespread adoption of glyphosate resistant crops (CFS Science Soy at 21 - 26). Even though glyphosate is not volatile, it nevertheless has become one of the most common herbicides detected in air and rain samples as fine droplets become airborne (Chang et al. 2011, Majewski et al. 2014). Glyphosate and its metabolites are also frequently measured in runoff and surface water (Battaglin et al. 2009, Coupe et al. 2012), glyphosate-resistant soybean samples (Bøhn et al. 2013), and in urine from both rural and urban people (Curwin et al. 2007a, 2007b). In other words, glyphosate is now practically ubiquitous in the environment. In some cases, glyphosate is measured at levels that can harm non-target organisms, such as amphibians (Relyea 2011) and plants (US-EPA 2009). Much of this glyphosate is likely to have originated in labeled applications to glyphosate-resistant crops (Coupe et al. 2012, Majewski et al. 2014). Many people find this level of off-target movement, including drift, to be unacceptable (for example, growers whose crops have been injured: CFS Science Soy at 43 – 44). APHIS does not provide evidence that off-site movement of 2,4-D used with Enlist corn and soybeans will be mitigated by EPA's regulations any more effectively, and its assumption to the contrary is belied by past crop experiences and sound science. In fact, 2,4-D's volatility makes off-site movement even more prevalent and APHIS's reliance on EPA further misplaced.

Not all herbicide applications to HR crops in the past have conformed to EPA's requirements

APHIS' assumption that herbicide use on Enlist corn and soybeans will always conform to EPA-registered uses as described in Appendix 7 and 8, where APHIS describes what label it assumes EPA will require, is also unfounded, because it is contrary to experience with previously approved herbicide-resistant crops. There are well known examples of off-label applications of herbicides to resistant crops in certain circumstances where growers find benefits (CFS Science Corn II at 36: use of glufosinate on WideStrike cotton), and APHIS has not analyzed the conditions under which off-label use is likely to occur with Enlist corn and soybeans in order to assess risks. Also, herbicides are sometimes applied when environmental conditions are not as required on the label (AAPCO 2002).

APHIS arbitrarily considers herbicide impacts from 2,4-D use on Enlist corn and soybeans, but refuses to consider these impacts in other contexts

Although it claims that direct and indirect effects of 2,4-D use on Enlist corn and soybeans are outside of the scope of the EIS, APHIS nevertheless considers impacts of herbicide use with Enlist corn and soybeans when assessing socioeconomic impacts of increased weed resistance to those herbicides – a harm that only will occur if the herbicides are registered and used on Enlist crops:

> Because of the likely adverse socioeconomic impacts that would result in the event that 2,4-D resistant weeds would be selected from the expected increased 2,4-D use on Enlist™ crops, APHIS believed these impacts may be significant. Therefore APHIS concluded that, for the three Enlist™ varieties that are the subject of this EIS, it will, at its discretion, prepare an EIS to further analyze the potential for selection of 2,4-D-resistant weeds and other potential impacts that may occur from making determinations of nonregulated status for these varieties. This EIS limits its analysis of herbicide use to the cumulative impacts that occur from the selection of herbicide resistant weeds and the changes in management practices that result. (EIS, at xi)

Limiting the scope of its EIS to cumulative impacts from 2,4-D selection of resistant weeds and resulting agricultural practices when there are many other direct, indirect and cumulative impacts of herbicide use with Enlist corn and soybeans, including to non-target organisms, is arbitrary and contrary to sound science.

Impacts of the APHIS approval of Enlist corn and soybeans must be assessed by APHIS under realistic scenarios, considering all reasonably foreseeable factors. Neither APHIS nor Dow provides any reason that a farmer would buy and plant Enlist crops unless he or she planned to use 2,4-D and glyphosate on those fields, since the engineered traits confer no advantage in environments where the herbicides are absent.

In fact, APHIS fully expects Enlist Duo pre-mixed herbicide formulation containing 2,4-D and glyphosate to be registered by EPA for use on the corn and soybeans that are being considered in this EIS (see, for example, EIS at ix: "If APHIS approves the three petitions for nonregulated status for Enlist™ corn and soybean, it is reasonably foreseeable that EPA will independently approve registration of Enlist Duo™ herbicide for use on these GE plant varieties."). APHIS has stated this expectation in the EIS (at ix, and elsewhere), and has been working closely with EPA to coordinate actions related to its approval. Therefore, analyses of its approval action and considering "alternatives" that do not take into account the use of 2,4-D and glyphosate are inappropriately based on an obviously unrealistic scenario where no 2,4-D is applied (see, for example, EIS at 108, where APHIS states there will be no direct or indirect effects of approval on agronomic practices and costs of production, because "…currently growers cannot use any herbicides differently on Enlist™ corn or soybean than are available to them for other corn and soybean varieties. That is because Enlist Duo™ is not registered for use on these corn or soybean events until EPA approves the label.").

Impacts to non-target organisms of applications of herbicides on Enlist corn and soybeans must be considered

Herbicide use on Enlist corn and soybeans may harm non-target species within and around those fields (CFS Science Soy at 76 – 93, CFS Science Corn II at 12 - 25), and must be considered by APHIS in its assessments. APHIS does admit that herbicide use in agriculture impacts biodiversity (EIS at 143), as part of its cursory look at cumulative impacts. However, APHIS relies on a few industry-associated reviews instead of the large body of independent, peer-reviewed primary studies and reviews that are available on impacts of agricultural practices on biodiversity, so does not base its assessment on sound science. For example, there are many recent reviews and studies of impacts to biodiversity of organic agriculture compared with other agricultural regimes (e.g., Andersson et al. 2012, Blaauw and Isaacs 2012, Gaba et al. 2013, Gabriel and Tscharntke 2007, Hyvonen and Huuselaveistola 2008, Kennedy et al. 2013, Kremen and Miles 2012, Lynch 2012, Morandin and Winston 2005, Nicholls and Altieri 2012, Power et al. 2012, de Snoo et al. 2013, Tuck et al. 2014).

In addition, APHIS skirts the impacts of the specific herbicides that will be used on Enlist crops, saying that herbicide use cannot be predicted:

> Herbicide use in agricultural fields can impact biodiversity by decreasing weed quantities or causing a shift in weed species. This can affect insects, birds, and mammals that use these weeds. The quantity and type of herbicide use associated with conventional and GE crops depends on many variables, including cropping systems, type and abundance of weeds, production practices, and individual grower decisions. (EIS at 143)

Elsewhere, APHIS does predict that 2,4-D use will increase dramatically with adoption of Enlist corn and soybeans. Impacts of this APHIS approval-associated increase in the specific herbicide 2,4-D, and the other herbicides Enlist corn and soybeans were engineered to withstand, must be assessed, rather than waved away by claims that quantity and type of herbicides used are too variable to predict.

For example, APHIS does not assess impacts of increased use of 2,4-D combined with glyphosate on monarch butterflies, even though this important non-target species is already impacted by herbicide use with herbicide-resistant corn and soybeans (discussed below).

Impacts of glufosinate use on Enlist soybeans must also be analyzed by APHIS. Glufosinate is a potent broad-spectrum herbicide, toxic to non-target crops and wild plants at low levels via drift and runoff of water and soil (Carpenter and Boutin 2010, EPA EFED Glufosinate 2013). Therefore glufosinate use on Enlist soybeans will impact non-target crops and wild plants, including threatened and endangered plants, with consequences for biodiversity. In addition, glufosinate is directly toxic to some animals at environmentally relevant concentrations. Beneficial insects may be particularly at risk from glufosinate use on Enlsit soybeans, including predatory mites and spiders, and lepidopteran pollinators (discussed below). Mammals present in the agroecosystem may experience chronic toxicity. Pest and pathogen levels may be altered. Also, threatened and endangered animals may be put at greater risk by glufosinate use on Enlist soybeans. These are significant adverse impacts that APHIS must assess and meaningfully consider in its assessments.

In addition, APHIS fails to analyze impacts of quizalofop use on Enlist corn, even though Enlist corn is engineered to resist this herbicide via the same enzyme that confers resistance to 2,4-D.

APHIS does not consider pests and pathogen impacts of herbicide use with Enlist corn and soybeans

CFS commented on potential pest and pathogen impacts of herbicides used with Enlist soybeans to crops and non-target organisms (CFS Science Soy at 44), concluding that drift of 2,4-D can cause symptoms similar to injury from pests and pathogens, and herbicides can suppress or stimulate pests and pathogens, as well.

In addition, glufosinate has been shown to affect various plant pathogens, both after applications to resistant crops, and in culture (reviewed in Sanyal and Shrestha 2008). Some effects of glufosinate on pathogens may be beneficial for agriculture, and some may be harmful. In glufosinate-resistant rice, glufosinate has been shown to trigger transcription of pathogenesis-related genes and other defense systems that act in concert with direct suppression to protect the GE rice from blast and brown leaf spot diseases (Ahn 2008). In contrast, glufosinate may be harmful to agriculture by suppression of pathogens of weeds and pests, allowing those weeds and pests to cause more damage.

Therefore, APHIS must consider the changes in pests and pathogens of non-target plants as a result of increased herbicide use and different patterns of herbicide use resulting from approval of Enlist corn and soybeans, and it does not do so in the EIS.

APHIS does not adequately consider risks to species beneficial to agriculture

Beneficial microorganisms

Beneficial microorganisms include species in the rhizosphere of corn and soybeans, and on leaf and stem surfaces that mediate nutrient relationships, diseases, and environmental stresses. Also, soil microbes are involved with decomposition, nutrient cycling, and other functions (Cheeke et al. 2013).

APHIS describes in general terms the importance of microbes in agricultural soils. Herbicide use is one factor APHIS identifies as influencing microbial populations:

> The main factors affecting microbial population size and diversity include: (1) the plant species, cultivars, and developmental stages present, which provide specific carbon and energy inputs into the soil; (2) soil type (determined by texture, structure, organic matter, aggregate stability, pH, and nutrient content); (3) geographic location; (4) season; (5) weather; (6) agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Young and Ritz, 2000; Kowalchuk *et al.*, 2003; Garbeva *et al.*, 2004). (EIS at 37)

Even though APHIS acknowledges that herbicides can affect microbes, the impacts on soil microbes of the specific changes in herbicide use as a result of its proposed approval of Enlist corn and soybeans are not considered by APHIS.

Two classes of microorganisms that are particularly beneficial to soybean production are nitrogen-fixing bacteria and mycorrhizal fungi, as APHIS acknowledges:

> An important group of soil microorganisms associated with legumes, including soybean, are the mutualists. These include mycorrhizal fungi, nitrogen-fixing bacteria, and some free-living microbes that have co-evolved with plants that supply nutrients to and obtain food from their plant hosts (USDA-NRCS, 2004). Legumes have developed symbiotic relationships with specific nitrogen-fixing bacteria in the family *Rhizobiaceae* that induce the formation of root nodules where bacteria may carry out the reduction of atmospheric nitrogen into ammonia (NHGage, 2004). *Bradyrhizobium japonicum* is the rhizobium bacteria specifically associated with soybeans (Franzen, 1999). (EIS at 99)

In fact, most soybean growers do not apply nitrogen fertilizers (EIS at 79), since usually all nitrogen needed for plant growth is obtained from the association of soybeans with symbiotic rhizobia and nitrogen already available in the soil (Ruark 2009).

Enlist soybeans are the first broadleaved plant that will be sprayed directly with 2,4-D, and also the only genetically engineered crop that harbors symbiotic nitrogen-fixing bacteria. Therefore, it is crucial that APHIS analyzes and assesses risks to rhizobium and the nitrogen fixation process in Enlist soybeans under realistic field conditions that include herbicides that Enlist soybeans have been engineered to withstand. APHIS does not analyze or assess impacts of 2,4-D as used on Enlist soybeans in any specific way, nor does Dow provide any specific data or observations on nitrogen fixation in Enlist soybeans with or without associated 2,4-D use.

Enlist soybeans are also glufosinate resistant. Some studies have shown negative effects of glufosinate on beneficial microbes. Pampulha et al. (2007) treated soil in laboratory microcosms with the glufosinate formulation “Liberty” at different concentrations and durations, and then determined the types, numbers and functional activity of culturable microorganisms – bacteria, fungi, and actinomycetes; cellulolytic fungi, nitrite oxidizing bacteria, and dehydrogenase activity. They found a complex pattern of changes in number and activity of microbes. However, the most dramatic change in response to glufosinate was a large decrease in dehydrogenase activity over time, which they say is a good indicator of general microbial activity. They conclude that glufosinate use "may have injurious effects on soil microorganisms and their activities."

APHIS does make a general statement that "[s]everal reviews of the investigations into the impact of GE plants on microbial soil communities found that most of the studies examining distinctive microbial traits concluded that there was either minor or no detectable non-target effects…" (EIS, at 99). In fact, glyphosate use on glyphosate-resistant soybeans has been shown to impair nitrogen-fixing bacteria in some circumstances (Zablotwicz and Reddy 2007, Kremer and Means 2009, Zobiole et al. 2010, Bohm et al. 2009). And, more importantly, none of these reviews include studies of use of 2,4-D on GE, resistant soybeans, or use of 2,4-D on any GE crop.

If approval of Enlist soybeans does lead to a reduction in nitrogen fixation in soybeans, then soybean growers may need to add more nitrogen fertilizer to their fields, with increased socioeconomic costs and environmental impacts. Impacts on nitrogen fixation need to be ascertained before concluding, as APHIS does, that agronomic inputs will not be changed by a deregulation decision (EIS, p. 121).

Beneficial fungi

Impacts of the approval of Enlist corn and soybean interactions with beneficial fungi also are not specifically considered by APHIS. Both corn and soybeans benefit from being infected by mycorrhizal fungi that live in their roots. These fungi facilitate movement of nutrients from the soil, protect against pathogens, and moderate effects of drought (Harrier and Watson 2003, Cheeke et al. 2013: Chapter 7). A wide range of agronomic practices influences the numbers and kinds of mycorrhizal fungi. Studies have even shown that corn varieties genetically engineered with insect-resistant Bt traits inhibit mycorrhizae in certain conditions (Cheeke et al. 2013: Chapter 8), possibly due to changes in rood exudates. APHIS must assess impacts of its proposed approval of Enlist corn and soybeans on mycorrhizal fungi under realistic field conditions covering a range of stresses that these fungi are known to ameliorate, and that include applications of the herbicides Enlist soybeans have been engineered to withstand.

Predators of crop pests

Predators of crop pests may be harmed by use of herbicides on Enlist corn and soybeans, and this was not analyzed by APHIS in the EIS. For example, glufosinate is toxic via a metabolic pathway found in animals and microorganisms, as well as plants, and some animals are injured or killed by herbicidal doses (EPA EFED Glufosinate 2013). Arachnids such as mites and spiders are particularly sensitive to glufosinate.

Although some mite species are serious agricultural pests of many crops, including corn, the use of pesticides for their control is not generally an effective strategy. Pesticides fail because many pest mites have developed resistance; while predatory mites, spiders and other insects that are important for keeping pest mite populations low are susceptible. Therefore, Integrated Pest

Management systems are recommended, where healthy predator populations are encouraged (Peairs 2010).

Glufosinate can harm predatory mites. Experiments on the direct toxicity of various pesticides to a predator mite found in Virginia vineyards showed glufosinate to be particularly toxic, causing 100% mortality within a day (Metzger and Pfeiffer 2002). Although the dose used was greater than that for resistant corn, lower doses were not tested.

Further experiments on glufosinate and beneficial arthropods were carried out in conjunction with a risk assessment by the European Food Safety Authority (EFSA 2005), and included glufosinate applications as used on corn:

> The European Food Safey Authority (EFSA 2005) evaluated a series of extended laboratory and semi-field studies on beneficial insects including the parasitoid wasp (*Aphidius rhopalosiphi*), predatory mite (*Typhlodromus pyri*), wolf spider (*Pardosa* ssp.), green lacewing (*Chrysoperla carnea*), ground beetle (*Poecilus cupreus*), and rove beetle (*Aleochara bilineata*). "Severe" effects were observed with a potential for population recovery in one season when glufosinate was applied at rates consistent with use on glufosinate-resistant corn (two application at 0.8 kgai/ha) (EPA EFED Glufosinate 2013 at 95)

Although there was "potential for population recovery in one season", the risks to beneficial insects were considered to be high enough to warrant mitigation:

> As described in the EFSA (2005) report, the EFSA Peer Review Coordination (EPCO) expert meeting (April 2004, ecotoxicology) recommended mitigation measures for risk to nontarget arthropoods, such as a 5-m buffer zone when glufosinate is applied to corn or potatoes. (EPA EFED Glufosinate 2013 at 95).

Data from EPA also indicates that large buffers may be required to protect non-target terrestrial plants from injury (EPA EFED Glufosinate 2013 at 98), and thus reduce harm to non-target predatory mites and spiders, and other beneficial arthropods.

Beneficial mammals

Some mammals are considered beneficial to agriculture, including corn and soybeans. For example, some rodents eat weed seeds, reducing the weed seed bank (EFSA 2005), or become food for predators that control pest species. Other mammals are predators of corn and soybean pests.

APHIS does not analyze risks to beneficial mammals from the use of 2,4-D with Enlist corn and soybeans, even though APHIS includes information from EPA in Appendix 8. Both acute and chronic risks to mammals have been identified by EPA in screening level risk assessments for the 2,4-D use patterns being planned for Enlist corn and soybeans (EIS at 8-10 appendix). EPA also identified indirect risks to mammals from modification of their habitat by 2,4-D use with Enlist crops (EIS at 8-10). CFS has commented on risks from 2,4-D use to mammals and other animals, as well (CFS Science Soy at 83).

Glufosinate use on Enlist corn and soybeans is likely to exceed levels of concern for chronic risk to mammals that eat insects, and plant parts other than strictly fruits, seeds and grains (EPA EFED Glufosinate 2013 at 70), as summarized:

> The screening level assessment with preliminary refinements concludes that the use of glufosinate in accordance with registered labels results in chronic risk to mammals that exceeds the Agency's chronic risk Level of Concern (LOC). Adverse effects in mammals following chronic exposure to glufosinate in laboratory studies include reductions in growth and in offspring fitness and viability; these effects are seen across generations and in multiple species (EPA EFED Glufosinate 2013 at 5).

Chronic effects of glufosinate at the expected exposure levels in laboratory studies "include reductions in parental and offspring growth and offspring viability. These effects have been observed in multiple studies and have been shown to extend to the second generation (no subsequent generations were tested)." (EPA EFED Glufosinate 2013 at 92)

Formulated products are more acutely toxic to mammals than the active ingredient alone by an order of magnitude (EPA EFED Glufosinate 2013 at 91), and formulations may also cause chronic toxicity at lower levels.

EFSA identified a high risk to mammals from glufosinate use in glufosinate-resistant corn based on chronic toxicity, and considered it to be "critical area of concern" (EFSA 2005).

Pollinators

Pollinators are beneficial to agriculture. Even though corn is wind-pollinated, and soybeans are mainly self-pollinating, pollinators necessary for other crops and wild plants are known to collect pollen from corn and nectar from soybeans (Krupke et al. 2012), and pollinators use the other plant species found within and around corn and soybean for food and other habitat requirements. Thus APHIS must assess the impacts on pollinators of herbicide use with Enlist corn and soybeans, but they did not do so in the EIS.

CFS discussed impacts on pollinators of 2,4-D use with Enlist corn and soybeans are at length (CFS Science Corn II at 35 – 41, and below in relation to nectar plants used by monarchs.

Glufosinate use with Enlist soybeans may have direct effects on lepidopteran (butterfly and moth) pollinators when larvae eat glufosinate-containing pollen, nectar or leaves, either after direct over-spray or from drift. Laboratory experiments with the skipper butterfly *Calpodes ethlias* showed that larvae fed glufosinate-coated leaves were injured or killed by inhibition of glutamine synthase, at doses "comparable to the amount that might realistically be acquired by feeding on GLA [glufosinate]-treated crops." These studies were done with the active ingredient, not a full formulation, and so may have underestimated field toxicity (Kutlesa and Caveney 2001).

Nectar of glufosinate-treated Enlist soybeans may accumulate significant levels of glufosinate. Although primarily a contact herbicide, glufosinate does translocate via phloem to a limited degree, depending on the plant species (Carpenter and Boutin 2010). In experiments comparing glufosinate translocation in GE resistant canola versus a susceptible variety (Beriault et al. 1999), glufosinate translocated more readily in resistant plants. However, in both resistant and susceptible canola, glufosinate moved in the phloem to developing anthers without causing injury to tissues along the way. If glufosinate is retained in leaves of resistant soybeans, it may translocate to nectar later, even if the applications occur well before flower formation.

APHIS should examine data on glufosinate levels in flowers of Enlist soybeans after labeled applications to assess risks to beneficial pollinators.

Pollinators may also be affected by changes in habitat from glufosinate toxicity to plants. Numbers and kinds of plants can change dramatically in response to herbicide applications, with impacts that ripple through ecosystems (as discuseed in previous CFS comments, and in relation to monarchs, below). In addition, pollinators that depend on specific host plants may be affected if those plants are more sensitive to glufosinate (Pleasants and Oberhauser 2012).

Large buffers may be required to protect non-target terrestrial plants from injury (EPA EFED Glufosinate 2013 at 98), and thus reduce harm to pollinators.

APHIS also does not consider impacts of quizalofop use on corn to pollinators in the EIS.

Risks to monarch butterflies from herbicide use associated with approval of Enlist corn and soybeans are not assessed by APHIS

The recent decline of monarchs (*Danaus plexippus*) is a clear example of harm to a non-target organism from past APHIS approval of herbicide-resistant corn and soybeans, as CFS commented (CFS Science Soy at 79 -80), yet APHIS does not analyze impacts to monarchs of approving Enlist corn and soybeans in the EIS.

Monarch numbers in North America are at their lowest since records have been kept, and biologists are concerned that the monarch migration is in jeopardy (Brower et al. 2011, 2012). At their most recent peak in 1997, there were almost a billion monarch butterflies overwintering in oyamel fir trees in the central mountains of Mexico (Slayback et al. 2007). This year, counts indicate an overwintering monarch population of fewer about 33 million, by far the lowest ever measured (WWF-Mexico 2014), continuing an alarming 20-year decline of more than 90% (Brower et al. 2011, 2012).

Although there are many factors at play, scientists have shown that a critical driver of the recent steep decline in monarch butterfly numbers is loss of larval host plants in their main breeding habitat, the Midwest corn belt of the US, as CFS commented previously (CFS Science Soy at 79-80, Pleasants and Oberhauser 2012). Monarchs lay eggs exclusively on plants in the milkweed family, and the larvae that hatch from these eggs must consume milkweed leaves to complete the butterfly's lifecycle (Malcolm et al. 1993). Common milkweed has been largely eradicated from corn and soybean fields where it used to be common (Hartzler 2010, Pleasants and Oberhauser 2012), depriving monarchs of the plant they require for reproduction.

Glyphosate used with glyphosate-resistant corn and soybeans has removed common milkweed from corn and soybean fields, decimating the monarch population

Common milkweed (*Asclepias syriaca*) is a perennial plant with shoots that die back in the winter, but re-sprout from buds on spreading roots in the spring to form expanding colonies (Bhowmik 1994). Common milkweed also regrows when the plants are mowed, chopped by tillers, or treated with many kinds of herbicides that only kill aboveground plant parts, or are applied before milkweed shoots emerge in late spring (Bhowmik 1994). Thus, until recently, common milkweed has been found within and around corn and soybean fields in sufficient numbers to support a large population of monarch butterflies. In fact, in the late 1990s when monarch numbers were still high, almost half of the monarchs in Mexican winter roosts had developed on common milkweed plants in the Midwest corn belt, making this the most important habitat for maintaining the monarch population as a whole (Wassenaar and Hobson 1998).

Recently, though, the widespread adoption of genetically engineered, glyphosate-resistant corn and soybeans has triggered a precipitous decline of common milkweed, and thus of monarchs (Pleasants and Oberhauser 2012). Glyphosate, is one of the extremely few herbicides that efficiently kills milkweed (Waldecker and Wise 1985, Bhowmik 1994). Glyphosate moves throughout the plant – from sprayed leaves into roots, developing shoots and flowers – where it thwarts milkweed's reproductive strategies.

Glyphosate is particularly lethal to milkweed when used in conjunction with glyphosate-resistant corn and soybeans (patterns of glyphosate use on resistant crops are described in detail in CFS Science Soy at 6, 14 – 15, 21- 24). It is applied more frequently, at higher rates, and later in the season (during milkweed's most vulnerable flowering stage of growth) than when used with traditional crops. The increasingly common practice of growing glyphosate-resistant corn and soybeans every year means that milkweed is exposed to glyphosate every year without respite, and has no opportunity to recover. In fact, in the 15 years since glyphosate-resistant soybeans, and then corn, were approved by APHIS, common milkweed has been essentially eliminated from corn and soybean fields in the major breeding area for monarch butterflies (Hartzler 2010).

This loss of habitat for monarch butterflies, because of eradication of the only host plant that grows within corn and soybean fields in the Midwest, has been devastating. Fewer corn and soybean fields have milkweed plants, and where they do occur, the plants are more sparsely distributed. In a 1999 survey of Iowa, common milkweed was found in half of corn and soybean fields, and this milkweed occupied an aggregate area of almost 27,000 acres (Hartzler and Buhler 2000). A decade later in 2009, a second survey found that only 8% of corn and soybean fields had any milkweed plants at all, with an aggregate area of just 945 acres – a 96.5% decline (Hartzler 2010). By 2012, it is estimated that just over 1% of common milkweed remained in corn and soybean fields in Iowa compared to 1999, just a few hundred combined acres (extrapolated from Pleasants and Oberhauser 2012). It is clear that other Midwestern states have experienced similarly devastating milkweed losses, based on comparable land-use patterns and other evidence.

Rapid, large-scale changes in glyphosate use (e.g. Benbrook 2009, as cited in CFS Science Soy) are responsible for milkweed loss. Common milkweed in corn and soybean fields has been unable to survive the change in glyphosate use that accompanied approval of glyphosate-resistant corn and soybeans (Pleasants and Oberhauser 2012).

Milkweeds do still remain outside of agricultural fields in the Midwest, but there aren't enough of them to support a viable monarch population. The combined area of roadsides, Conservation Reserve Program (CRP) land, and pastures is only about 25% of corn and soybean acreage in Iowa, which is representative of the Corn Belt as a whole (Pleasants and Oberhauser 2012). In addition, monarchs produce almost four times more progeny per milkweed plant in corn and soybean fields than in non-agricultural areas (Monarch Larval Monitoring Project, as described in Pleasants and Oberhauser 2012), so agricultural milkweed is more valuable as habitat. Thus, even if non-crop lands have a higher density of milkweeds, they cannot begin to compensate for agricultural habitat lost to glyphosate use on glyphosate-resistant corn and soybeans.

Impact of APHIS approval of Enlist corn and soybeans on common milkweed will continue glyphosate harms in addition to new harms from Enlist-associated herbicide use

As confirmed by APHIS in the EIS, Enlist corn and soybeans will be sprayed post-emergence with a pre-mix formulation of glyphosate and 2,4-D. In addition, they may be sprayed glufosinate or quizalafop. Farmers may also apply the individual herbicides sequentially.

Enlist corn and soybeans will therefore not only continue to be sprayed post-emergence with glyphosate, but also with other herbicides, when common milkweed is in its most vulnerable reproductive stages (Bhowmik 1994). Even those herbicides that are weaker on perennial weeds such as milkweed (e.g. glufosinate) can be expected to cause considerable damage to aboveground plant parts. In addition, Enlist corn and soybeans are engineered to be extremely resistant to the herbicides in question, enabling application of rates higher than have ever been used before without injuring the crop. Herbicides that cause limited damage to weeds when applied at lower rates are often much more damaging at higher rates. The combination of additional active ingredients applied post-emergence, and use of higher rates, can only accelerate the demise of common milkweed in corn and soybean fields while preventing its reestablishment, especially in view of the fact that glyphosate will continue to be used at rates similar to those used at present on crops resistant to glyphosate alone.

Efficacy of 2,4-D at killing common milkweed

2,4-D is a in the synthetic auxin class of herbicides. Synthetic auxins are generally effective on perennial broadleaf weeds because the, like glyphosate, they are translocated to the root. 2,4-D and dicamba are the auxin herbicides most frequently recommended for control of common milkweed, though neither is as consistently effective as glyphosate.

The Ohio State University extension service recommends a high rate of glyphosate (2.25 lbs. a.e./acre) as the first option for control of common milkweed in non-crop or fallow field situations, but also notes that a lower rate of glyphosate (1.5 lbs ae/acre) combined with 2,4-D "can provide good control as well." Likewise for corn, a post-emergence application of glyphosate is recommended if the corn is Roundup Ready. For non-Roundup Ready corn, dicamba is the top choice – alone or combined with one of several other herbicides (Ohio State Extension, as cited in Isleib 2012).

North Dakota State University has conducted tests evaluating the efficacy of various herbicides on common milkweed (Martin and Burnside 1984, Cramer and Burnside 1981). A high rate of glyphosate (3 lbs./acre) provided the best milkweed control when evaluated the following spring. Higher than normal rates of 2,4-D (2 lbs./acre) provided lesser but still considerable levels of control, reducing milkweed stands by roughly half.

Other studies on herbicidal control of common milkweed reveal quite variable results for 2,4-D (Cramer & Burnside 1981, Bhowmik 1982). In greenhouse experiments conducted by Cramer and Burnside (1981), 2,4-D provided modest suppression of common milkweed regrowth when evaluated five weeks after application, suppression almost equal to that of glyphosate (Cramer and Burnside, Table 1). Mixtures of glyphosate and 2,4-D were one of the most effective herbicide combinations (Table 1).

Field studies designed to assess the long-term efficacy of various herbicides on common milkweed generally show that 2,4-D did not provide much control in the year or two following a single application (Bhowmik 1982). However, these experiments generally involved low rates of 2,4-D and/or application in the fall when milkweed was past its reproductive phase (post-flowering), and so presumably less susceptible to herbicidal control.

Cramer and Burnside (1981) were unable to explain the variable efficacy exhibited by 2,4-D (or that of other herbicides) in the experiments they conducted, noting merely that herbicidal control of common milkweed "is variable … and appears to be dependent on growth stage, growth rate, time of herbicide application, climatic variables, and other factors."

Effects of Enlist Duo used with Enlist corn and soybeans on common milkweed

The discussion above shows that 2,4-D suppresses common milkweed. Although not consistently as effective as glyphosate, particularly for longer-term control, its efficacy is regarded as sufficient to merit recommendations for its use on common milkweed by experienced agronomists at several universities.

Enlist corn and soybeans will greatly exacerbate the negative impacts of 2,4-D on common milkweed for several reasons: higher rates will be used; most applications will occur during milkweed's most vulnerable reproductive phase; most applications will be in combination with glyphosate; much more cropland will be sprayed; and the frequency of use will increase both within season and over years (CFS Science Soy at 78).

Combined use of two herbicides known for their efficacy in killing milkweed can only hasten its eradication from crop fields and maintain its absence, with devastating consequences for monarch butterflies. APHIS does not consider these impacts of Enlist corn and soybean approval on monarchs in its EIS.

Herbicide drift injury from Enlist corn and soybean fields to nectar plants

Although monarch larvae are selective about food plants, only thriving on milkweeds, the adult butterflies derive nutrients from a wide variety of nectar-producing flowers (Tooker et al. 2002). They depend on flowers that are in bloom in their breeding habitat during the spring and summer, and then along migration routes to winter roosts (Brower and Pyle 2004). Monarchs that are breeding during spring and summer use energy derived from nectar for flying, laying eggs, mating, and other activities. In addition, the generation that migrates in the fall converts nectar sugars into storage lipids to fuel their metabolism during winter, and perhaps also for northern migration the following spring (Brower et al. 2006).

Herbicides are toxic to plants, by definition, and their use in agricultural landscapes has resulted in changes in flowering plant populations within and around crop fields, with impacts felt throughout ecosystems. It has been shown that "[b]etween 5% (commonly) and 25% (occasionally) of the applied herbicide dose is expected to reach the vegetation in field margins and boundaries (e.g. hedgerows, woodlots, etc.) (Holterman et al., 1997; Weisser et al., 2002)." (Boutin et al. 2014).

There have been no surveys of wildflowers in agricultural landscapes before and after commercialization of previously approved herbicide-resistant crops, as important as such information is for assessing environmental impacts. However, glyphosate from use on herbicide resistant crops may have already reduced abundance and diversity of nectar plants in and around agricultural fields, from direct applications as well as spray drift (e.g. Gove et al. 2007, Blackburn and Boutin 2003). Approval of Enlist corn and soybeans that are associated with use of highly active, volatile 2,4-D with an even greater potential for causing drift injury, in addition to glyphosate, is likely to have severe impacts on nectar resources used by monarchs and other pollinators (Brower et al. 2006).

Hugely increased spray drift, volatilization and runoff from the much greater use of herbicides with Enlist corn and soybeans are likely to alter the very habitats important for biodiversity in agroecosystems, such as hedgerows, riparian areas, unmanaged field margins, and other areas where wild organisms live near fields (Freemark and Boutin 1995, Boutin and Jobin 1998, Olszyk et al. 2004). These areas harbor nectar plants for adult monarchs as well as milkweeds for larvae. Based on experiences with 2,4-D sensitive crops, for example, natural areas miles from agricultural applications of these herbicides will be at increased risk from the use of greater amounts on herbicide resistant crops, since these herbicides can volatilize under certain conditions (CFS Science Soy at and also come down in rain (Hill et al. 2002). Also, as CFS has commented, herbicides used on resistant crops are applied over a longer span of the growing season, and thus overlap a wider range of developmental stages of nearby plants, hitting them when they may be more sensitive to injury.

Plants of different species and growth stages vary in sensitivity to herbicides, putting monarchs and other pollinators at risk

Particular species of plants are more or less sensitive to specific herbicides (Olszyk et al. 2013, Boutin et al. 2004), and at different growth stages (Carpenter and Boutin 2010, Boutin et al. 2014), so that exposure can change plant population dynamics in affected areas. 2,4-D and other auxin-like herbicides such as dicamba are particularly potent poisons for many species of plants (Rasmussen 2001, US-EPA 2009), especially dicotyledons (broadleaf plants) that are sensitive to very low drift levels. Even monocots such as members of the grass and lily families can be killed by higher doses of 2,4-D or dicamba, and suffer sub-lethal injuries from drift levels at certain times in their life cycles (US-EPA 2009; Nice et al. 2004).

Plants – both crop and wild species –are often very sensitive to herbicide injury as flowers and pollen are forming (Olszyk et al. 2004). This has been clearly shown with dicamba and injury to tomato plants (Kruger *et al.* 2012) and soybeans (Griffin et al. 2013), and with glyphosate injury to rice flowers (Wagner 2011). Drift levels of dicamba have also been shown to affect asexual reproduction in potatoes (Olszyk et al. 2010), and seed production in peas (Olszyk et al. 2009), sometimes without accompanying vegetative injury. Glyphosate drift to potato plants has been responsible for causing potato shoots arising from seed potatoes in the next generation to grow abnormally or not at all (Worthington 1985), without always affecting the growth of the potato plants that were actually hit with the herbicide (Potato Council 2008). There are many other examples of differential sensitivity to particular herbicides (Boutin et al. 2014). Injury affecting flowers and vegetative propagules but not the rest of the plant can easily go undetected, nevertheless having a large impact on reproduction and thus subsequent generations.

Differential sensitivity to herbicides can lead to changes in species composition of plant communities. For example, as noted in CFS comments (CFS Science Soy at 81), 2,4-D movement away from crop fields in mid-spring may kill sensitive dicotyledonous wildflowers at seedling stages, cause male sterility in less sensitive grasses about to flower, and have little effect on younger grasses or still-dormant perennials (Olszyk et al. 2004). These impacts can cause long-term changes in the mix of plant species, favoring annual weeds and grasses over native plants and perennial forbs (broadleaved plants), for example (Boutin and Jobin 1998, Boutin et al. 2008). And if there are herbicide resistant plants in these habitats, they will of course be better able to withstand drift and may become more abundant (Watrud et al. 2011, CFS 2013a).

Pollinators are at particular risk from changes in plant populations and flowering behavior. Recently published comparisons of flowering plants in natural areas around fields that have been exposed to herbicides on a regular basis vs. near fields managed without herbicides show striking differences in abundance and kinds of plants in flower, and also in when these plants flower (Boutin et al. 2014). Hedgerows next to organic farms had more species, and many of them flowered earlier in the season and for a longer time span. These field observations confirmed greenhouse studies that showed significant delays in flowering of several species after exposure to herbicides (Boutin et al. 2014).

Such changes in which plants flower, and when, could affect monarchs as they breed and migrate, disrupting coordination between the butterflies and needed resources:

> …. organic farming promoted not only plant diversity but also plant flowering capacity whereas conventional farming inhibited flower production of the fewer plants found in adjacent hedgerows and resulted in a shift in flowering. This in turn may cause disharmony with pollinator activities as pollinators can be very sensitive to flowering events (Santandreu and Lloret, 1999). Effects on timing of flowering can have consequences on pollinating insects as they may be less able to survive in non-crop habitats during periods when crop plants are unavailable for pollination (Carvalheiro et al., 2010). Alternatively, delays in flowering time may expose flowers to unfavourable weather conditions (e.g. frost or drought). Herbicide effects appear to constitute yet another stressor affecting plant – insect interactions, adding to other stressors including

> land-use modifications at the landscape scale (Kremmen et al., 2007) that are increasingly impacting agro-ecosystems. (Boutin et al. 2014)

Herbicides selective for broadleaved plants, such as 2,4-D, pose danger to nectar plants in particular

Herbicides such as 2,4-D that selectively kill dicots may be particularly injurious to butterflies, often considered an indicator of ecosystem health. If these herbicides are applied frequently and over a broad area – as will happen with herbicide use on Enlist corn and soybeans– negative impacts on butterflies are likely to be increased. A study by Longley and Sotherton (1997) of pesticide effects on butterflies in agricultural areas of England makes this point:

> The frequency and number of pesticide applications, the spatial scale of treatment and the degree of field boundary contamination during each spray occasion will determine the extent of damage to butterfly habitats and populations, and the rate at which populations will return to their original densities. (Longley and Sotherton 1997).

Researchers implemented experimental mitigation measures to determine whether changes in pesticide use would result in more butterflies in the landscape. One of these measure involved limiting the use of "persistent broadleaf herbicides" near field edges, and instead using herbicides that were more specifically targeted against grasses:

> The outer section of a tractor-mounted spray boom (approximately 6 m) is switched off when spraying the outer edge of a crop, avoiding the use of certain chemicals (persistent broadleaf herbicides and all insecticides other than those used for controlling the spread of Barley Yellow Dwarf Virus). Whilst the rest of the field is sprayed with the usual compliment of pesticides, more selective chemicals (e.g. graminicides rather than broad-spectrum herbicides) are sprayed on the edges (Boatman and Sotherton, 1988). (Longley and Sotherton 1997, p. 8).

They found that there were indeed more butterflies after taking these measures, and also that there were more dicots, the main source of nectar, as well as more biodiversity in general:

> In addition, as a result of selective herbicide use, Conservation Headlands are rich in broadleaved plants, thereby increasing the availability of nectar resources for butterfly species. (Longley and Sotherton 1997, p. 8)
>
> The unsprayed headlands have also been shown to benefit the survival of rare weeds (Schumacher, 1987; Wilson, 1994), small mammals (Tew, 1988), beneficial invertebrates (Chiverton and Sotherton, 1991; Cowgill et al., 1993) and gamebird chicks (Rands, 1985; Rands, 1986). However, to be of long-term value for butterfly conservation, unsprayed headlands need to be maintained over consecutive years to allow the survival of those species which are univoltine and have poor powers of dispersal. (Longley and Sotherton 1997, p. 9)

In conclusion, these researchers emphasize the need for research on impacts of pesticide use over time:

> In addition to short-term studies, covering single cropping seasons, information is also needed on the effects of different spray and cropping regimes over several seasons on butterfly communities in exposed areas. Only then will it be possible to make reliable predictions and recommendations for butterfly conservation on arable farmland. (Longley and Sotherton 1997, p. 12)

Implications of this butterfly study in England are clear for use of 2,4-D with Enlist corn and soybeans: 2,4-D is an herbicide that selectively kills broadleaved plants (dicots), the main nectar source for adult butterflies, even those species whose larvae feed on grasses. 2,4-D is also likely to be used more often during a season, more extensively in an area, and from year to year with Enlist corn and soybeans than it is currently used in agriculture. This is exactly the opposite use pattern than that recommended for mitigation of pesticide impacts on butterflies, that were also shown to be protective of biodiversity in general.

A new experimental study designed to test impacts of dicamba drift, an auxin-class herbicide and thus relevant to 2,4-D, on plant and arthropod communities in agricultural "edge" habitats highlights the importance of long- term studies of herbicide impacts over a range of environments (Egan et al. 2014). These researchers applied a range of doses of dicamba, meant to simulate different levels of drift, to field margins and to plots within old fields to determine whether plant and arthropod communities changed in response. In each habitat, they sprayed dicamba one time each year for two consecutive years, and performed plant censuses throughout the growing seasons, both before and after dicamba applications. In addition to monitoring the kinds and numbers of plants, number of flowers produced by each species was also recorded. For field margins, they also did a census of arthropods at different times during the growing season. Egan and colleagues found that low drift levels of dicamba did in fact affect plant and arthropod communities, but in complex ways, depending on plant successional status of the community to begin with, and environmental conditions such as water stress when herbicides were applied. However, impacts were seen at about 1% of the field application rate – a lower level than other studies have reported, and within the range expected to occur frequently from herbicide applications associated with herbicide-resistant crops. They advise:

> In light of this variation across sites and environments, it is not possible to derive general predictions about how plants and arthropods will respond to non-target dicamba exposure. Further research is needed to better understand the species, communities, and habitat types that are most sensitive to dicamba drift and the environmental conditions during exposure that can moderate susceptibility. In the absence of predictive understanding, a precautionary emphasis on limiting non-target herbicide exposures is well-warranted. (Egan et al. 2014)

Similar cautions apply to 2,4-D use with Enlist corn and soybeans. By far the best way to limit herbicide exposure of important nectaring habitat for monarchs is to restrict post-emergence use of such herbicides.

EPA regulations do not protect nectar plants from herbicide drift injury

IEPA guidelines for protecting non-target plants from drift injury are based on toxicity tests that include too few species, tested at only a few points in their vegetative development, and therefore underestimate the range of sensitivities in communities of wild species throughout their lifecycles (Pfleeger et al. 2012, White and Boutin 2007, Olszyk et al. 2013, Boutin et al. 2014). These deficiencies in assessment of herbicide impacts will put the monarch's nectaring habitat at further risk should Enlist corn and soybeans be approved by APHIS.

Monarchs may also be harmed by direct exposure to herbicides used with Enlist corn and soybeans

Herbicides may directly harm exposed insects, such as monarchs. Some herbicides have been shown to leave residues that cause lepidopteran larvae to stop feeding on herbicide- exposed plants, and also some herbicides directly inhibit enzymes within the exposed insects (as discussed in Russell and Shultz 2009, and in Bohnenblust et al. 2013).

For example, glufosinate may have direct effects on lepidopteran pollinators when larvae eat glufosinate-containing pollen, nectar or leaves, either after direct over-spray or from drift. Laboratory experiments with the skipper butterfly *Calpodes ethlias* showed that larvae fed glufosinate-coated leaves were injured or killed by inhibition of glutamine synthase, at doses "comparable to the amount that might realistically be acquired by feeding on GLA [glufosinate]-treated crops." These studies were done with the active ingredient, not a full formulation, and so may have underestimated field toxicity (Kutlesa and Caveney 2001). Glufosinate is one of the herbicides that will be used with Enlist soybeans.

Toxicity of metabolites that result from activity of novel enzymes must be assessed for non-target organisms

When commenting on the EAs for Enlist corn and soybeans (CFS Science Soy at 84 – 94, CFS Enlist Corn II Comments at 29 - 34), CFS alerted APHIS to the need to consider potentially toxic metabolites of 2,4-D as part of its assessments, but APHIS has not done so. In fact, APHIS makes an explicit assumption that there are no differences in composition between Enlist corn and soybeans and non-2,4-D-resistant counterparts:

> The APHIS PPRA did not identify any changes in DAS-40278-9 corn, DAS-68416-4 soybean, or DAS-44406-6 soybean that would directly or indirectly affect natural or biological resources. These plants are compositionally similar to other corn and soybean plants. (EIS at 119).

However, the PPRA analysis was based on compositional comparisons made in the absence of 2,4-D.

CFS reiterates that APHIS, in making a decision to approve Enlist corn and soybeans, must go beyond a description of the genotypes resulting from genetic engineering of corn and soybeans to be 2,4-D resistant, to describe and assess the PPA impacts of significant changes in the phenotypes of Enlist corn and soybeans, in environments that they are likely to be grown. Instead, APHIS has limited its assessment of important aspects of phenotypes of Enlist corn and soybeans to environments that these crops will rarely encounter – environments that are absent applications of 2,4-D.

According to 7 CFR 340.6(c), required data and information must include, among other things:

> (3) A detailed description of the **differences in genotype** between the regulated article and the nonmodified recipient organism…
> (4) A detailed **description of the phenotype** of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, **expression of the gene product, new enzymes, or changes to plant metabolism**, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, **agricultural or cultivation practices, effects of the regulated article on nontarget organisms**, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

The genotype of an organism consists of its entire set of genes that contain "instructions" for making RNA and proteins that ultimately determines that organism's characteristics. For Enlist corn and soybeans, their genotypes differ from non-engineered counterparts by the addition of DNA encoding a protein with enzymatic activity that can metabolize 2,4-D into non-phytotoxic compounds, allowing the engineered crops to withstand otherwise lethal doses of the herbicide. This transgene is *aad-1* in Enlist corn and *aad-12* in Enlist soybeans, encoding the enzyme AAD, aryloxyalkanoate dioxygenase. Other genotypic changes include sequence changes as a result of insertion of the transgene, and mutations caused by tissue culture during the engineering process. The engineered gene is embedded in the plants' chromosomes and is passed on to all cells in the organism during development, and from one generation to the next, along with all the other corn or soybean genes.

The phenotype of an organism is "[t]he physical appearance or biochemical characteristics of an organism as a result of the interaction of its genotype and the environment" (Biology Online Dictionary 2014). For corn and soybeans, the phenotype includes size and shape, growth rate, response to environmental conditions such as day length or drought, pest and pathogen susceptibility, and other characteristics that can be observed. Phenotype also includes biochemical characteristics that are not visible to the naked eye, but can be measured with various devices, such as levels of proteins, carbohydrates, lipids, and metabolites that result from enzyme activity.

An example of the importance of metabolism as a phenotypic characterization comes from medicine. Genes for metabolizing specific drugs vary within human populations, so that the same dose of a drug may affect individuals differently, from being ineffective to causing a toxic overdose (Zanger and Schwab, 2013; Johansson and Ingelman-Sundberg, 2010). In some cases, how a person will respond can be predicted by examining the genotype, because particular enzymes encoded by specific gene variants have been shown to speed up or slow down metabolism of that drug. However, the most reliable way to tell is to measure the phenotype directly. Physicians measure the metabolites of specific pharmaceuticals in patients after exposing them to the drug to determine the person's metabolic phenotype – how quickly they are able to down the drug – in order to personalize doses of medications to prevent overdoses and to optimize efficacy (Gumus *et al.,* 2011).

Plants with identical genotypes are likely to have different characteristics – different phenotypes – when grown in different environments. Genes have to become active in directing synthesis of RNA and proteins in order to have any effect on the characteristics of the organism; they must be "expressed" (see Alberts et al. 2009 for review of gene expression). Genes that are not expressed do not contribute to the phenotype of the organism. Many genes are only expressed in certain tissues and organs during development. The environment also influences how genes are expressed, and what effect the proteins made from the genes will have (Richards et al. 2012). For example, some genes are only turned on in the presence of external triggers, such as light or presence of a specific chemical. Some proteins produced from gene activation only function in certain conditions, as well, needing particular levels of nutrients, range of temperatures, or presence of substrates to carry out their roles.

In order to determine impacts of Enlist corn and soybeans, APHIS first must describe how Enlist corn and soybeans differ in phenotypic characteristics as a result of the specific genetic engineering events. The first step in doing so is to determine expression patterns of the transgenes, by finding out where, when, and how much of the gene products are made in the Enlist corn and soybean plants in environments in which they are likely to be grown. In this case, the engineered gene products are enzymes that break down, or metabolize, 2,4-D and some related herbicides. In it's Petitions, Dow provides APHIS with some transgene expression data. They measured AAD-1 and AAD-12 protein in a few plant parts and stages of development of Enlist corn and soybeans grown with different combinations of the herbicides that the introduced enzymes allow them to withstand (see DAS Petitions, "Characterization of Introduced Proteins").

APHIS uses Dow's description of when, where and how much of the transgenic protein is present in Enlist corn and soybean plants, along with analyses of protein sequence comparisons to known toxins and allergens, and *in vitro* studies of AAD-1 and AAD-12 protein digestion (EIS at 111), to determine whether ingestion of the transgenic proteins themselves was likely to harm non-target animals. For example, for Enlist soybeans:

> DAS evaluated the potential allergenicity and toxicity of the AAD-12 protein following the weight-of-evidence approach (DAS, 2010a). The AAD-12 protein does not share any meaningful amino acid similarities with known allergens. The AAD-12 protein is degraded rapidly and completely in simulated gastric fluids, and the protein is not present in a glycosylated state (DAS, 2010a). The protein does not share any amino acid sequence similarities with known toxins (DAS, 2010a). The results presented by DAS suggest that the AAD-1 protein is unlikely to be a toxin in animal diets. Based on a review of this information and **the assumption that these studies serve as surrogates for direct testing**, APHIS has found no evidence that the presence of the *aad-12* gene or the expression of the AAD-12 protein would have any impact on animals, including animals beneficial to agriculture (USDA-APHIS, 2012a). (EIS at 111 – 112)

The assumption that Dow's *in silico* (computer simulated) and *in vitro* studies of AAD-1 and AAD-12 proteins can predict toxicity of these proteins, as they exist within Enlist corn and soybean plants, is unfounded. Proteins made in plants can have different properties than counterpart proteins in bacteria that were used in the simulated digestion studies, and computer analyses of coding sequences do not always identify toxins and allergens accurately (Freese and Schubert 2004). But the biggest problem with APHIS' assumption is that Dow's analyses are based on toxicity to mammals and, by extension, to humans; whereas the non-target organisms that could be impacted by approval span the taxonomic spectrum, from beneficial soil annelids (i.e. earthworms) to insect pollinators and endangered birds. Human and mammalian parameters of toxicity are simply not applicable over this range of organisms.

CFS stressed this point in our comments about analysis of harms to pollinators (CFS Science Corn II at 35 - 41, CFS Science Soy at 93 – 94). Composition of pollen, nectar and guttation liquid was not determined to assess differences resulting from the Enlist events, for example. The inadequacy for pollinators of toxicity assessments based on mammals was also stressed in a recent EPA white paper on pollinator risk assessments (EPA SAP 2012). Nor were impacts on honey bees studied by Dow in its field trials. Therefore, there are no relevant data for making an assessment of impacts of approval to honey bees or other pollinators.

In addition, APHIS must continue on in its analyses, past the characteristics of the novel proteins themselves, to determine how the functioning of the AAD enzymes changes the phenotypic characteristics of corn and soybean plants, and whether the changes could harm non-target species. As with the levels of AAD proteins, these phenotypic differences in metabolism should be described and assessed in the presence of the herbicides that will be used with Enlist corn and soybeans.

Dow's whole purpose in engineering corn and soybeans with these particular transgenes is to have the genes expressed throughout the plants at high enough levels that the resulting proteins will be *active* in converting 2,4-D to non-phytotoxic metabolites. The rate and extent of conversion of 2,4-D to metabolites, and thus the level of 2,4-D and metabolites, is the most relevant phenotypic difference to consider after looking at the properties of the novel protein itself, and this is not considered by APHIS in their assessments.

As CFS has noted (CFS Science Corn II at 29 – 34), CFS Science Soy at 84 – 92), Dow's studies of metabolites in Enlist corn and soybeans after applications of 2,4-D show that the activity of the AAD-1 and AAD-12 enzymes metabolizes 2,4-D into 2,4-DCP, that then is changed by other enzymes in the plant into conjugated forms of DCP (mainly DCP with specific sugars attached). In non-engineered corn and soybeans, little 2,4-DCP is produced after 2,4-D applications, nor are conjugated forms found at appreciable levels. 2,4-DCP has been shown to be toxic to some organisms, and conjugated forms have been shown to release 2,4-DCP during digestion, raising the specter that conjugated forms could be a delayed-release poison. Dow did not perform studies to test toxicity of these metabolites to non-target organisms, other than simply observing that insects were found in fields of Enlist corn and soybeans at levels comparable to non-engineered corn and soybeans (DAS Petition). These observations do not constitute an appropriate study of toxicity, nor do they address the range of organisms of interest. No observations of any kind were made of pollinators, beneficial soil organisms, or predators of crop pests, for example. Nevertheless, APHIS accepts these observations as evidence that no harm to animals of ingesting Enlist corn and soybeans will occur (e.g., 44406-6 soybean PPRA, at 10: "Field observations of DAS-444Ø6-6 (DAS and MS Tech 2011, section 7) revealed no negative effects on non-target organisms, suggesting that the production of the ADD-12, PAT and EPSPS proteins in the plant tissues are not toxic to organisms.").

Therefore, to summarize, APHIS does not describe or consider important aspects of the known and potential differences in phenotypes of Enlist corn and soybeans that could harm non-target organisms, relative to the unmodified recipient organisms, in the environmental conditions that Enlist corn and soybeans are likely to encounter. APHIS only considers toxicity of the protein products of the AAD-1 and AAD-12 transgenes (the earliest phenotypic character), rather than following through to consider how these new enzymes would change plant metabolism in such a way that the plants' phenotypes would differ in the most likely environment for Enlist crops, where 2,4-D will be present. In the likely and foreseeable presence of 2,4-D, potentially toxic metabolites accumulate in the Enlist corn and soybeans but not in the recipient organisms. APHIS does not consider impacts of these potential toxins as part of the approval process or other assessments.

APHIS uses inappropriate and inadequate studies of nutritional value and toxicity of Enlist corn and soybeans to assess risks to threatened and endangered species, and ignores risks from herbicide applications

Risks to listed species known to eat corn and soybeans are not considered

Again, APHIS relies on Dow's presentation of "food and feed safety" of the AAD-1 and AAD-12 proteins to conclude that exposure and consumption of Enlist corn and soybeans would have no effect on threatened or endangered animal species, or those proposed for listing (Enlist corn: EIS, at 153 – 154; Enlist soybeans: EIS, at 156 – 156). As discussed above, nutritional requirements and toxicity differ between species, so that extrapolation from mammalian requirements is not valid for assessing risk to other animal taxa. For example, insects may eat nectar or pollen that was not studied for differences in nutrient composition. Birds may eat insects that fed on corn or soybean leaves, and the insects were not studied to see if they differ nutritionally. In addition, APHIS did not look at risks from potentially toxic metabolites in relevant Enlist corn- or soybean-derived materials used by endangered species that result from activity of the introduced enzymes in the presence of 2,4-D.

APHIS claims that no listed animal species use corn and soybean plants as "hosts", without defining what is mean by host (for example, EIS at 154: "APHIS considered the possibility that DAS-40278-9 corn could serve a host plant for a threatened or endangered species. A review of the species list reveals that there are none that would use corn as a host plant."). There may or may not be listed species that use corn and soybean plants as their main food source to complete segments of their lifecycles, but there are certainly listed animals that forage for food in corn and soybean fields.

APHIS did mention listed birds that might be found in soybean fields (EIS at 153), discounting any significant impacts based on a study showing that at least some of these birds don't consume soybeans:

> Few if any TES are likely to use soybean fields because they do not provide suitable habitat. Only whooping crane (*Grus americana*), sandhill crane (*Grus canadensis pulla*), piping plover (*Charadrius melodus*), interior least tern (*Sterna antillarum*), and Sprague's pipit (*Anthus spragueii;* a candidate species) occasionally feed in farmed sites (USFWS, 2011a). These bird species may visit soybean fields during migratory periods, but would not be present during normal farming operations (Krapu *et al.*, 2004; USFWS, 2011a). In a study of soybean consumption by wildlife in Nebraska, results indicated that soybeans do not provide the high energy food source needed by cranes and waterfowl (Krapu *et al.*, 2004). (EIS p. 156)

Some listed mammals were also identified by APHIS as being found in soybean fields on occasion:

> The Delmarva fox squirrel (*Sciurus niger cinereus*), which inhabits mature forests of mixed hardwoods and pines, may be found adjacent to agricultural areas of the Delmarva Peninsula (USFWS, 2011b). … The Louisiana black bear (*Ursus americanus luteolus*), occurring in Louisiana, Mississippi, and Texas (Johnsen *et al.*, 2005), may occasionally forage on soybean; however, other crops such as corn, sugarcane, and winter wheat are preferred by the species (MSU, No Date). (EIS at 156)

APHIS fails to also consider listed species that might be found in Elist cornfields, even though in discussing soybeans APHIS admits that the Louisianna black bear prefers corn to soybeans (EIS at 156). Also, in the Nebraska study of birds in agricultural fields, cited by APHIS for lack of soybean consumption, corn is an important food source (Krapu et al. 2004).

Certainly, corn plants and seeds are eaten by at least one endangered migratory bird, the whooping crane (*Grus americana*), both as they forage naturally and in their "chow" when chicks are raised by conservation groups. Soybeans are also added to their chow. For example, the International Crane Foundation (ICF) answers questions about what cranes, including whooping cranes, eat, noting that they cranes eat enough newly sprouted corn and seeds to make them a nuisance in some fields (ICF 2014):

> **Feeding**
>
> **Q: What do the cranes at ICF eat?**
> **A:** At ICF, cranes eat "crane chow", a special blend of soy, alfalfa, fish, and corn meal, with a special vitamin supplement. All species get the same diet, although protein content changes with the season and the bird's age. Breeding females also get calcium chips in spring to help with eggshell formation, and all the cranes get shelled corn in winter, to provide extra carbohydrates.

> **Q: Do cranes cause crop damage?**
> **A:** Yes, on occasion they will. In Wisconsin, cranes may cause crop damage in corn and potato fields, where the birds may feed on newly sprouted corn plants or maturing potato tubers. Members of the ICF Field Ecology Department are involved in a long-term study of crop depredation in a study area located near Briggsville, Wisconsin. ICF researchers are working with local farmers to develop a substance to put on corn kernels that will taste bad to cranes, with the hope that this will deter them from feeding in treated fields. Farmers throughout the world are faced with this challenge, and solutions developed in Wisconsin may be useful for farmers in other countries.

Whooping cranes are not numerous enough yet to cause much damage to fields, relative to other crane species, but they are found in mixed flocks with Sandhill cranes and exhibit the same feeding behavior.

The experts at Operation Migration, who guide whooping cranes in the Eastern population with ultralight aircraft on their first migration, note that the birds forage in soybean fields as well, so may be exposed to Enlist soybean residues even if they aren't eating soybean seeds (Operation Migration 2013):

> **Karen Anne** April 1, 2013 1:35 pm
> What do the whoopers eat when there's snow on the ground?
> **Heather Ray** [from Operation Migration] April 1, 2013 4:18 pm They can and do still find a variety of foods – seeds, fruit/berries, and they travel to corn and soybean fields to consume waste grain.

The fact that whooping cranes eat young corn plants means that the birds may be present in fields shortly after over-the-top herbicide applications are made to Enlist corn. The 2,4-D residues and metabolites in newly-sprayed seedling corn have not been reported by Dow in its residue and metabolite studies, nor have Enlist corn seedlings been examined for other compositional differences, so APHIS cannot claim that food and feed studies show lack of risk to listed species.

APHIS does not analyze risks to listed species from exposure to herbicides used with Enlist corn and soybeans

In assessing potential effects of Enlist corn and soybeans on endangered plants, and on critical habitat that is composed of particular vegetation, APHIS does not consider impacts of herbicide use with Enlist corn and soybeans at all (EIS at 153, 156). However, in Appendix 8, APHIS provides information from EPA Environmental Fate and Effects Division showing that non-listed plants are at potential risk from direct effects of drift and runoff of 2,4-D choline use on Enlist corn and soybeans (EIS Appendix at 8-10). Some non-listed animals are also at risk from direct effects of exposure to 2,4-D choline, and "…all non-listed taxa [are identified] as potentially at indirect risks from the proposed uses of 2,4-D choline salt because of potential dependencies (e.g., food, shelter, habitat) on species that are directly affected." (EIS Appendix at 8-10)

Listed species identified as being at potential risk from 2,4-D choline applications to Enlist corn and soybeans are also being assessed by EPA (EIS Appendix at 8-10).

Enlist corn and soybeans are genetically engineered for resistance to herbicides in addition to glyphosate and 2,4-D, and use of these other herbicides with Enlist corn and soybeans must be analyzed for harm to listed species:

- Enlist corn is resistant to quizalofop in addition to 2,4-D, and APHIS provides information on EPA's screening level ecological risk assessment for listed and non-listed species for the proposed label for quizalofop in Appendix 8 (EIS Appendix at 8-18). There are possible direct effects to various animals and plants, and also the potential for habitat modifications for all listed taxa.

- Enlist soybeans are also resistant to glufosinate, and APHIS expects glufosinate to be used as it is on other glufosinate resistant soybean events (Liberty Link soybeans) (EIS Appendix at 8-20). CFS discusses potential risks to various taxa of glufosinate as it will be used with Enlist soybeans in relation to beneficial organisms, above.

APHIS cannot rely on EPA to analyze the foreseeable impacts of use of quizalofop and glufosinate on Enlist corn and soybeans, but must itself analyze impacts of these herbicides to listed species, as for use of 2,4-D with Enlist corn and soybeans.

Given this preview from EPA, it is clear that some listed species will be at risk from the approval action by APHIS of Enlist corn and soybeans, and that APHIS cannot improperly delegate responsibility for these potential harms of its action.

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