



CENTER FOR
FOOD SAFETY

Docket No. APHIS-2013-0043

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10 October 2014

Comments to USDA APHIS on the Draft Environmental Impact Statement for Determinations of Nonregulated Status for Dicamba Resistant Soybean and Cotton Varieties, Monsanto Petitions (10-188-01p and 12 -185-01p)

Center for Food Safety, Science Comments II

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These comments submitted by Center for Food Safety are one of three sets of comments from our organization. Legal comments and a second set of science comments (Science Comments I, by Bill Freese) are also being submitted separately. The filenames for these documents match the citations in the text, and are all incorporated as (e.g. Benbrook 2012). Full citations are in the reference list at the end of this text.

We have also submitted to this docket our earlier comments to EPA on new use registrations for dicamba on dicamba-resistant soybeans and cotton, and comments to APHIS on deregulation petitions and scoping for the draft EIS, as well as all the references cited in those comments:

Appendix A: CFS Science Comments to EPA on registration of dicamba for new use on MON 87701, dicamba-resistant cotton, 1/13/2018

Appendix B: CFS Science Comments to EPA on registration of dicamba for new use on MON 87708, dicamba-resistant soybean, 9/21/2012

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Appendix C: CFS Comments to USDA on petitions for deregulation of MON 87708 soybean (9/11/2012) and MON 88701 cotton (4/29/2013)

Appendix D: CFS Scoping Comments to USDA on Notice of Intent to Prepare an Environmental Impact Statement on MON 87708 soybean and MON 88701 Cotton, 7/17/2013

Appendix E: CFS Legal Comments to EPA on registration of dicamba for new use on MON 87701 cotton, 1/18/2013

Appendix F: CFS Legal Comments to EPA on registration of dicamba for new use on MON 87708 soybean, 9/21/2012

Appendix G: CFS Science Comments I to USDA on the Draft Environmental Impact Statement for 2,4-D Resistant Corn and Soybeans, 3/11/14

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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 MON 87708 dicamba-resistant soybean and MON 88701 dicamba- and glufosinate-resistant cotton (hereafter referred to as Xtend soybean and cotton). Non-target organisms affected include, but are not limited to, plants growing within and near Xtend soybean and cotton 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 adequately consider compositional changes in parts of the plants used by the diverse array of species that will interact with Xtend soybean and cotton. Nor does APHIS consider impacts to these organisms from applications and off-target movement of herbicides used as part of the Xtend system. In addition, APHIS fails to consider the differences in potentially toxic herbicide metabolites between Xtend soybean and cotton and unmodified recipient organisms that may harm non-target organisms that consume or come in contact with Xtend soybean and cotton 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 Xtend soybean and cotton (DEIS at 140, emphasis added):

The EPA is conducting an independent assessment of direct and indirect effects associated with the use of dicamba on MON 88701 cotton and MON 87708 soybean, concurrently with the development of this DEIS.

...

These direct effects of dicamba use are outside the scope of this DEIS. APHIS decisions regarding the regulated status of the petitions for these new GE varieties will be made independently of the results of the EPA assessments. 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 dicamba on these varieties. While the EPA is still evaluating Monsanto's application requesting the new uses of dicamba on MON 88701 cotton and MON 87708 soybean, APHIS sees as reasonably foreseeable that requirements similar to those imposed on Dow Agrosciences' (DAS) Enlist Duo containing glyphosate and the choline salt of 2,4-D related to weed resistance management will be imposed on Monsanto's registration of DGA formulated dicamba (M1691).

APHIS explicitly states this and other assumptions when justifying its relegation of herbicide considerations to cumulative impacts of EPA's expected registration of dicamba

herbicide combined with approval of Xtend soybean and cotton (DEIS at 143 - 144). Other assumptions by APHIS relevant to non-target organisms are:

APHIS considers that herbicide applications will conform to the EPA-registered uses for cotton and soybean.

APHIS assumes that the impacts associated with drift from dicamba and other pesticide applications will be mitigated to an acceptable level by the registration requirements established by EPA. (DEIS at 144, 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 (Appendix A, B, D; 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, have essentially eradicated common milkweed from fields in the Midwest (Monarch ESA Petition 2014). 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 past winter (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. In fact, CFS and Center for Biological Diversity are lead petitioners, joined by Xerces Society and monarch scientist Lincoln Brower, on a petition to FWS to list monarchs as "threatened" under the Endangered Species Act, submitted August 26, 2014 (Monarch ESA Petition 2014). Scientists have linked this dramatic decline in monarchs and threat to their population 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 DEIS, APHIS has failed to assess impacts of approving Xtend soybean and cotton 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 Xtend soybean and cotton 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 (Monarch ESA Petition 2014 at 45 – 72). 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, Battaglin et al. 2014), 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). APHIS does not provide evidence that off-site movement of dicamba and other herbicides used with Xtend soybean and cotton 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, dicamba's volatility – even with reduced volatility formulations - 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 Xtend soybean and cotton will always conform to EPA-registered uses as described in Appendix 7, where APHIS describes what label it assumes EPA will require based on the proposed label for Dow's 2,4-D resistant corn and soybeans, is also unfounded, because it is contrary to experience with previously approved herbicide-resistant crops. There are well known examples of unsupported applications of herbicides to resistant crops in certain circumstances where growers find benefits (Roberson 2011: use of glufosinate on WideStrike cotton), and APHIS has not analyzed the conditions under which off-label use is likely to occur with Xtend soybean and cotton 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 some impacts from herbicide use on Xtend soybean and cotton, but refuses to consider these impacts in other contexts

APHIS claims that direct and indirect effects of dicamba and other herbicide use on Xtend soybean and cotton are outside of the scope of the DEIS:

The scope of this DEIS covers the direct and indirect impacts that would result from the cultivation and use of these varieties. EPA, in its registration process, is considering any direct and indirect impacts from the proposed new uses of dicamba on these varieties. APHIS is relying on EPA's authoritative assessments and will not duplicate the assessments prepared by EPA. However, in this DEIS, APHIS does consider (see Chapter 5) the potential cumulative impacts that could result in the event that it approves the petitions for nonregulated status of MON 87708 soybean and MON 88701 cotton and EPA registers the proposed new uses of dicamba on these crop varieties. (DEIS at viii)

Limiting the scope of its DEIS to some cumulative impacts from dicamba when there are many other direct, indirect and cumulative impacts of herbicide use with Xtend soybean and cotton, including to non-target organisms, is arbitrary and contrary to sound science.

Impacts of the APHIS approval of Xtend soybean and cotton must be assessed by APHIS under realistic scenarios, considering all reasonably foreseeable factors. Neither APHIS nor Monsanto provides any reason that a farmer would buy and plant Xtend crops unless he or she planned to use dicamba, alone or in combination with other herbicides to which the crops are resistant, on those fields, since the engineered traits confer no advantage in environments where the herbicides are absent. There will be no impacts of the approval action by APHIS if Xtend soybean and cotton are not grown at all, and yet this is the scenario APHIS assesses for all direct and indirect impacts (DEIS at 137).

In fact, APHIS fully expects dicamba alone, and a pre-mixed herbicide formulation containing dicamba and glyphosate, to be registered by EPA for use on the soybean and cotton that are being considered in this DEIS (see, for example, DEIS at 138: "The action Monsanto requested of EPA, which is approval of the use of dicamba on cotton and corn [sic; should be "cotton and soybean"], will be concurrent with a determination of nonregulated status for MON 88701 cotton and MON 87708 soybean. A reasonably foreseeable action is that EPA will approve registration of formulations of Xtendimax™ (dicamba registered with EPA as M1691). This herbicide product contains a diglycolamine (DGA) salt formulation of dicamba and would likely be offered as a premix with glyphosate as well (as Roundup Xtend™)."). Therefore, analyses of its approval action and considering "alternatives" that do not take into account the use of dicamba are inappropriately based on an obviously unrealistic scenario where no dicamba is applied (see, for example, section 4.2, DEIS at 129, where APHIS states there will be no direct or indirect effects of approval on agronomic practices, because "... growers would be able to plant MON 88701 cotton and MON 87708 soybean, but would not be able to make applications of dicamba other than

currently approved by EPA.... Therefore, the types of agronomic practices used to cultivate these cotton and soybean varieties, such as tillage, crop rotation, fertilization, and pesticide use, would be similar to those currently used.”).

Impacts to non-target organisms of applications of herbicides on Xtend soybean and cotton must be considered

Herbicide use on Xtend soybean and cotton may harm non-target species within and around those fields, and must be considered by APHIS in its assessments. APHIS does admit that herbicide use in agriculture impacts biodiversity (DEIS at 189), 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 Tschardt 2007, Hyvonen and Huusela-Veistola 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 Xtend 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. (DEIS at 189)

Elsewhere, APHIS does predict that dicamba use will increase dramatically with adoption of Xtend soybean and cotton. Impacts of this APHIS approval-associated increase in the specific herbicide dicamba, and the other herbicides Xtend soybean and cotton 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 dicamba 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 Xtend cotton must also be analyzed by APHIS. Although APHIS follows Monsanto in assuming glufosinate use will decrease with approval of Xtend soybean and cotton, CFS has determined that glufosinate use is likely to increase (CFS

Science Comments I submitted for this DEIS). 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 Xtend cotton 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 Xtend cotton, 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 Xtend cotton. These are significant adverse impacts that APHIS must assess and meaningfully consider in its assessments.

APHIS does not consider pests and pathogen impacts of herbicide use with Xtend soybean and cotton

CFS commented on potential pest and pathogen impacts of herbicides used with Xtend soybean and cotton to crops and non-target organisms (Appendix A at 64, Appendix B at 56 - 57), concluding that drift of herbicides can cause symptoms similar to injury from pests and pathogens, and herbicides can suppress or stimulate pests and pathogens, as well.

More specifically, 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 Xtend soybean and cotton, and it does not do so in the DEIS.

APHIS does not adequately consider risks to species beneficial to agriculture

Beneficial microorganisms

Beneficial microorganisms include species in the rhizosphere of soybean and cotton, 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).

Two classes of microorganisms that are particularly beneficial to soybean production are nitrogen-fixing bacteria and mycorrhizal fungi, as APHIS acknowledges (DEIS at 71-71). In fact, most soybean growers do not apply nitrogen fertilizers (DEIS at 80), 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).

Xtend soybean is the first broadleaved plant that will be sprayed directly with dicamba. Therefore, it is crucial that APHIS analyzes and assesses risks to rhizobium and the nitrogen fixation process in Xtend soybeans under realistic field conditions that include herbicides that Xtend soybeans have been engineered to withstand. APHIS does not analyze or assess impacts of dicamba as used on Xtend soybean in any specific way, nor does Monsanto provide any specific data or observations on nitrogen fixation in Xtend soybean with associated dicamba use.

Herbicide use on resistant crops has been shown to affect soil microbes. 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, Zobiolo et al. 2010, Bohm et al. 2009).

If approval of Xtend soybean 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 (DEIS at 183).

Xtend cotton is also glufosinate resistant, in addition to being dicamba 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.”

Beneficial fungi

Impacts of the approval of Xtend soybean and cotton interactions with beneficial fungi also are not specifically considered by APHIS. Both soybeans and cotton 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 root exudates. APHIS must assess impacts of its proposed approval of Xtend soybean and cotton 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 Xtend soybeans have been engineered to withstand.

Predators of crop pests

Predators of crop pests may be harmed by use of herbicides on Xtend soybean and cotton, and this was not analyzed by APHIS in the DEIS. 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 Safety 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 applications 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 arthropods, 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 and birds

Some mammals and birds are beneficial to agriculture, including cotton 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, such as bats, reduce insect pests, and may provide billions of dollars of services to US agriculture each year (Boyles et al. 2011, 2013). Some birds also control agricultural pests and provide other ecosystem services (Whelan et al. 2008).

APHIS does not analyze risks to beneficial mammals and birds from the use of dicamba and glufosinate with Xtend soybean and cotton, even though APHIS includes information from EPA about risks to mammals and birds in Appendix 8. Direct adverse effects from chronic exposure to dicamba for both listed and non-listed mammals, and from acute exposure for both listed and non-listed birds, have been identified by EPA in screening level risk assessments for the dicamba use patterns being planned for Xtend soybean and cotton (DEIS at Appendix 8, pp.12-13 appendix). EPA also identified the potential for indirect risks to mammals and birds from modification of their habitat by dicamba use with Xtend crops (DEIS at Appendix 8, p. 13). CFS has commented on risks from dicamba use to mammals, birds and other animals, as well (Appendix A, Appendix B).

Of the herbicides considered for invasive species control by the US Forest Service in the Pacific Northwest, dicamba is of special concern for mammals and birds (USDA Forest Service 2005):

Dicamba, triclopyr, and 2,4-D have the highest potential to adversely affect wildlife. Dicamba has a relatively low acute toxicity to adult animals, in terms of direct lethal doses, but adverse effects on reproduction and nervous systems occur at much lower doses. Dicamba shows a consistent pattern of increased toxicity to larger sized animals, across several species and animal types (i.e. birds and mammals). Dicamba exposures exceed the toxicity indices for five scenarios at the typical application rate, and nine scenarios at the highest application rate. (Bautista 2005, p. 22)

Based on this analysis, in their Record of Decision for the invasive plant control program the Forest Service decided not to use dicamba or 2,4-D:

I recognize the cost-effectiveness of 2,4-D and dicamba. It has been commonly and widely used on both private and public lands for the last several decades. At the Regional scale, however, no situations were found where these herbicides would be absolutely necessary. These herbicides are inherently more risky than the ten I am approving for use. Forest Service risk assessments consistently place these two herbicides in higher risk categories for human beings, large mammal and birds (see FEIS Chapter 4.4 and 4.5). (USDA Forest Service 2005 at 25)

These concerns about dicamba impacts on wild animals, including beneficial mammals, will only be amplified by the increased use of dicamba with Xtend soybean and cotton.

Glufosinate use on Xtend soybean and cotton 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 cotton and soybeans are mainly self-pollinating, pollinators necessary for other crops and wild plants are known to collect nectar from soybeans (Krupke et al. 2012), and pollen and nectar from cotton (Borem et al. 2003, Röse et al. 2006), and to use the other plant species found within and around cotton

and soybean fields for food and other habitat requirements. Thus APHIS must assess the impacts on pollinators of herbicide use with Xtend soybean and cotton, but they did not do so in the DEIS.

CFS discussed impacts on pollinators of dicamba use with Xtend soybean and cotton at length (soybean: Appendix B at 62 – 64, cotton: Appendix A at 70 – 73, and below in relation to nectar plants used by monarchs).

Glufosinate use with Xtend cotton 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 *Calpodis 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 Xtend cotton 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 cotton, it may translocate to nectar later, even if the applications occur well before flower formation. It may also be present in extrafloral nectaries.

APHIS should examine data on glufosinate levels in flowers and extrafloral nectaries of Xtend cotton 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 discussed 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.

Risks to monarch butterflies from herbicide use associated with approval of Xtend soybean 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, yet APHIS does not analyze impacts to monarchs of approving Xtend soybean and cotton in the DEIS.

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). In the winter of 2013/2014, counts indicated 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). In fact, CFS and Center for Biological Diversity are lead petitioners, joined by Xerces Society and monarch scientist Lincoln Brower, on a petition to FWS to list monarchs as “threatened” under the Endangered Species Act, submitted August 26, 2014 (Monarch ESA Petition 2014).

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 (Monarch ESA Petition 2014). 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 Monarch ESA Petition 2014). 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 (Monarch Listing Petition 2014) 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 Xtend soybean on common milkweed will continue glyphosate harms in addition to new harms from Xtend-associated herbicide use

As confirmed by APHIS (DEIS at 117), Xtend soybean will be sprayed post-emergence with a pre-mix formulation of glyphosate and dicamba. In addition, other herbicide resistance traits are likely to be “stacked”, allowing use of glufosinate and other herbicides. Farmers may also apply the individual herbicides sequentially.

Xtend soybean 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, Xtend soybean is 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 dicamba at killing common milkweed

Dicamba is in the synthetic auxin class of herbicides. Synthetic auxins are generally effective on perennial broadleaf weeds because they, like glyphosate, are translocated to the root. Dicamba and 2,4-D are the auxin herbicides most frequently recommended for control of common milkweed, though neither is as consistently effective as glyphosate (Monarch ESA Petition 2014, Martin and Burnside 1984, Cramer and Burnside 1981, Bhowmik 1982).

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).

Effects of dicamba used with Xtend soybean and cotton on common milkweed

Although dicamba is 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.

Xtend soybean will greatly exacerbate the negative impacts of dicamba 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 (Monarch ESA Petition 2014)).

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 Xtend soybean approval on monarchs in its DEIS.

Herbicide drift injury from Xtend cotton 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. Various models of herbicide spray drift from ground applications suggest that from 1% (commonly) to 25% (occasionally) of the applied herbicide dose drifts beyond the field boundaries to affect wild vegetation (Holterman et al. 1997, Wang and Rautmann 2008, Boutin et al. 2014), though these models made no attempt to account for unpredictable volatilization, and models generally do not account for extreme situations.

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 Xtend soybean and cotton that are associated with use of highly active, volatile dicamba 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 Xtend soybean and cotton 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, Boutin et al. 2014, Schmitz et al. 2014a, 2014b). 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, and also come down in rain (Hill et al. 2002). Also, 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 (Boutin et al. 2004, Strandberg et al. 2012, Olszyk et al. 2013), and at different growth stages (Carpenter and Boutin 2010, Strandberg et al. 2012, Boutin et al. 2014), so that exposure can change plant population dynamics in affected areas. Dicamba and other auxin-like herbicides are particularly potent poisons for many species of plants (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 dicamba, and suffer sub-lethal injuries from drift levels at certain times in their life cycles (US-EPA 2006).

Plants – both crop and wild species – are often very sensitive to herbicide injury as flowers and pollen are forming (Olszyk et al. 2004, Strandberg et al. 2012). 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 (e.g. Strandberg et al. 2012, Schmitz et al. 2014a, 2014b).

Differential sensitivity to herbicides can lead to changes in species composition of plant communities. For example, dicamba 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, Schmitz et al. 2014b). 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 (Carvalho 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 dicamba, pose danger to nectar plants in particular

Herbicides such as dicamba 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 Xtend soybean and cotton– 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 at 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 greater 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 at 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 at 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 at 12)

Implications of this butterfly study in England are clear for use of dicamba with Xtend soybean and cotton: dicamba is an herbicide that selectively kills broadleaved plants (dicots), the main nectar source for adult butterflies, even those species whose larvae feed on grasses. Dicamba is also likely to be used more often during a season, more extensively in an area, and from year to year with Xtend soybean and cotton 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.

Several new field studies in the United States—undertaken to assess the potential effects of dicamba use with dicamba-resistant crops—support the English findings. Bohnenblust (2014) found that drift-level doses of dicamba delayed flowering of alfalfa, and both delayed and reduced flowering of common boneset (*Eupatorium perfoliatum*), a wildflower that provides resources to many insect species. In addition, common boneset flowers were less visited by all pollinators when treated with dicamba at rates simulating drift.

A second study explored the impact of a range of drift-level dicamba doses on the plant and arthropod communities in agricultural “edge” habitats (Egan et al. 2014). The most striking result was a significant decline in the abundance of broadleaf plants over time and with increasing dicamba dose. Impacts were observed at substantially lower levels (about one percent of the dicamba field application rate) than have been reported to affect plant communities in other studies. This study was conservative in design: dicamba alone was applied just once per year over two years. More severe impacts would be expected with longer-term use, and with the dicamba-glyphosate mix to be used with dicamba-resistant crops, which could be applied up to three times per year according to the proposed label (DEIS Appendix 8 at 8 - 9). In general, the complementary action of glyphosate and dicamba, applied in the form of Roundup Xtend to resistant crops, would kill or injure a broader range of plants more effectively, and over a broader range of plant growth stages, than either component alone.

EPA regulations do not protect nectar plants from herbicide drift injury

EPA 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, Strandberg et al. 2012, 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 Xtend soybean and cotton be approved by APHIS.

Monarchs may also be harmed by direct exposure to herbicides used with Xtend soybean and cotton

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 *Calpododes 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 Xtend cotton, and may be “stacked” into Xtend soybean.

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

APHIS makes an explicit assumption that there are no differences in composition between Xtend soybean and cotton and non-dicamba-resistant counterparts:

The APHIS PPRA did not identify any changes in MON 88701 cotton or MON 87708 soybean that would directly or indirectly affect natural or biological resources. These plants are compositionally similar to other cotton and soybean plants. The growth habits of these plants are also similar to other cotton and soybean plants. (DEIS at 143, underlining added).

However, the PPRA analysis was based on compositional comparisons that did not include dicamba residues and metabolites.

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

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 Xtend soybean and cotton, their genotypes differ from non-engineered counterparts by the addition of DNA encoding a protein with enzymatic activity that can metabolize dicamba into non-phytotoxic compounds, allowing the engineered crops to withstand otherwise lethal doses of the herbicide. This transgene is *dmo*, encoding the enzyme dicamba mono-oxygenase (DMO) that demethylates dicamba to form the non-phytotoxic 3,6-dichlorosalicylic acid (DCSA) as well as formaldehyde. For Xtend cotton, the *bar* gene has also been added, making phosphinothricin N-acetyltransferase (PAT) protein that acetylates glufosinate, inactivating it. Other genotypic changes include sequence changes as a result of insertion of the transgenes, and mutations caused by tissue culture during the engineering process. The engineered genes are embedded in the plants’ chromosomes and are passed on to all cells in the organism during development, and from one generation to the next, along with all the other soybean or cotton 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 soybean or cotton, 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 effects 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 Xtend soybean and cotton, APHIS first must describe how Xtend soybean and cotton 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 Xtend soybean and cotton 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, dicamba and some related herbicides. In its Petitions, Monsanto provides APHIS with some transgene expression data. It measured DMO protein in several plant parts and stages of development of Xtend soybean and cotton grown with different combinations of the herbicides that the introduced enzymes allow them to withstand. Monsanto also provided expression data for the PAT protein in Xtend cotton that confers resistance to glufosinate (see Monsanto Petitions for Deregulation, “Characterization of Introduced Proteins”).

APHIS uses Monsanto's description of when, where and how much of the transgenic proteins are present in Xtend soybean and cotton plants, along with analyses of protein sequence comparisons to known toxins and allergens, and *in vitro* studies of DMO protein digestion (DEIS at 111), to determine whether ingestion of the transgenic proteins themselves was likely to harm non-target animals (DEIS at 131-132, 203-204, 234).

The assumption that Monsanto's *in silico* (computer simulated) and *in vitro* studies of DMO and PAT proteins can predict toxicity of these proteins, as they exist within Xtend soybean and cotton 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 Monsanto'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.

Often, plants use specific chemicals to defend themselves against insects, and many foods that are harmless or beneficial to humans and other mammals are nonetheless toxic to particular insect species. These chemical relationships are complex. For example, broccoli and other Brassica vegetables make a variety of glucosinolates that are generally beneficial to humans, but are extremely toxic to some insect species while other insects species have evolved to utilize the same molecules for their own defense (Björkman et al. 2011).

Pollinators ingest different plant parts than humans and livestock. Composition of pollen, nectar and guttation liquid was not determined to assess differences resulting from the Xtend 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 Monsanto 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 DMO and PAT enzymes changes the phenotypic characteristics of Xtend soybean and cotton plants, and whether the changes could harm non-target species. As with the levels of DMO and PAT proteins, these phenotypic differences in metabolism should be described and assessed in the presence of the herbicides that will be used with Xtend soybean and cotton.

Monsanto'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 dicamba and glufosinate to non-phytotoxic metabolites. The rate and extent of conversion of herbicides to metabolites, and thus the level of herbicides and metabolites, is the most relevant phenotypic difference to consider after looking at the properties of the novel proteins themselves, and this is not considered by APHIS in their assessments.

Residue and metabolite data for herbicides used with Xtend soybean and cotton have been generated by Monsanto as part of its dicamba new use registration package to EPA (Monsanto Petitions for Deregulation for Xtend soybean at 551, Xtend cotton at 36), but are not available for public scrutiny. CFS has been able to find some information, though, as described in previous comments (Appendix A at 65 – 70, for both Xtend soybean and cotton).

Monsanto's studies of metabolites in Xtend soybean after applications of dicamba show that the activity of the DMO enzyme metabolizes dicamba "mainly to a glucose conjugate of 3,6-dichloro-2-hydroxybenzoic acid (DCSA), with smaller amounts of conjugates of 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA) and another glucose conjugate of DCSA. The conjugates are very complex molecules which are not readily synthesized to produce analytical reference standards" (Moran and Foster 2010 at 30). It appears, then, that the major metabolites of dicamba present in Xtend soybean are not found in the metabolism studies of non-dicamba-resistant soybean, namely glucose conjugates of DCSA and DCGA. These conjugates are present in other crop plants, but at very low levels, rather than being the major products, as here. And, although the toxicity of DCSA and DCGA has been studied, apparently the toxicity of the conjugates has not been studied.

Besides these qualitative differences, the relative levels of parent dicamba to its metabolites is "flipped" in Xtend soybean, with dicamba being low and metabolites high, instead of the other way around.

Assuming that Xtend cotton behaves similarly to Xtend soybean, we expect that glycosides of DCSA will be the main metabolites of dicamba that result from activity of the engineered enzyme in Xtend cotton. Studies of conjugated metabolites of 2,4-D, such as dichlorophenol (DCP) glycosides, show that the DCP aglycone can be released during mammalian digestion with possible impacts on health (Laurent et al. 2000, 2006; Pascal-Lorber et al. 2003, 2008, 2012). Free DCSA may also be released from conjugates during digestion. These conjugates in Xtend cotton thus need to be measured and tested for toxicity.

Another concern is whether the formaldehyde produced in the breakdown of dicamba by the engineered DMO enzyme in Xtend soybean and cotton when dicamba is applied results in formaldehyde levels over and above those that naturally occur in these crops, and that may be injurious to animals that eat the plant parts, since there can be health effects from ingestion of formaldehyde (ATSDR 2008, Fig. 1.2). Formaldehyde levels in dicamba-treated Xtend soybean and cotton tissues should be tested after applications to see if they fall below or above safe limits.

Monsanto did not describe studies to test toxicity of these metabolites to non-target organisms, other than simply observing that insects were found in fields of Xtend soybean and cotton at levels comparable to non-engineered corn and soybeans (Xtend Soybean PPRA at 19 - 20). 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 Xtend soybean and cotton will occur (e.g., DEIS at 204).

Therefore, to summarize, APHIS does not describe or consider important aspects of the known and potential differences in phenotypes of Xtend soybean and cotton that could harm non-target organisms, relative to the unmodified recipient organisms, in the

environmental conditions that Xtend soybean and cotton are likely to encounter. APHIS only considers toxicity of the protein products of the dmo and bar 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 Xtend crops, where dicamba, and sometimes glufosinate, will be present. In the likely and foreseeable presence of dicamba, potentially toxic metabolites accumulate in the Xtend soybean and cotton 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 Xtend soybean and cotton to assess risks to threatened and endangered species, and ignores risks from herbicide applications

Risks to listed species known to eat soybean and cotton plants are not considered adequately

Again, APHIS relies on Monsanto's presentation of "food and feed safety" of the DMO and PAT proteins, and general nutritional profiles including antinutrients, to conclude that exposure to and consumption of Xtend soybean and cotton would have no effect on threatened or endangered animal species, or those proposed for listing (Xtend cotton: DEIS at 203-204; Xtend soybean: DEIS at 207-208). 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 are likely to eat leaves, roots, nectar or pollen that was not studied for differences in nutrient composition. Birds are likely to eat insects that fed on Xtend cotton or soybean leaves, and the insects were not studied to see if they differ nutritionally. In addition, APHIS did not consider risks from potentially toxic metabolites in relevant Xtend soybean- and cotton-derived materials used by endangered species that result from activity of the introduced enzymes in the presence of dicamba or glufosinate (DEIS at 199: phenotypic effects only include resistance to the herbicides, and not presence of metabolites).

There are listed animals that forage for food in cotton and soybean fields. APHIS discussed listed birds that might be found in cotton and soybean fields (DEIS at 229, 233), discounting any significant impacts based on a study showing that at least some of these birds don't consume soybean seeds. However, soybean stems and leaves are eaten by a variety of wildlife, and some varieties are even planted for that purpose (Bennett 2001). Some listed mammals were also identified by APHIS as being found in soybean fields on occasion (DEIS at 233). More studies on habits of listed species in cotton and soybean fields are needed in order to assess risks from Xtend cotton and soybean deregulation.

APHIS does not analyze risks to listed species from exposure to herbicides used with Xtend soybean and cotton

In assessing potential effects of Xtend soybean and cotton on threatened and endangered plants, and on critical habitat that is composed of particular vegetation, APHIS does not

consider impacts of herbicide use with Xtend soybean and cotton at all. However, in Appendix 8, APHIS provides information from EPA Environmental Fate and Effects Division showing that both non-listed and listed terrestrial dicot plants are at potential risk from direct effects of drift and runoff of dicamba use on Xtend soybean and cotton, as are listed vascular aquatic plants (DEIS Appendix 8 at 12 – 13). Some non-listed and listed animals are also at risk from direct effects of exposure to dicamba, and for all of these “...there is a potential for indirect effects to taxa that might rely on plants, birds, aquatic animals, and/or mammals for some stage of their life-cycle.” (DEIS Appendix 8 at 12 – 13)

Listed aquatic animals are at particular risk from use of dicamba with Xtend soybean and cotton because dicamba is a frequent contaminant of waterways (Appendix A at 54 – 55), and there are several listed aquatic animals in soybean-growing regions (CBD 2014).

Xtend cotton is genetically engineered for resistance to glufosinate in addition to dicamba, and use of glufosinate and other herbicides likely to be “stacked” with Xtend soybean and cotton must be analyzed for harm to listed species.

APHIS cannot rely on EPA to analyze the foreseeable impacts of use of glufosinate and other herbicides on Xtend soybean and cotton, but must itself analyze impacts of these herbicides to listed species, as for use of dicamba with Xtend soybean and cotton.

Given this preview from EPA’s assessments in Appendix 8, it is clear that some listed species will be at risk from the approval action by APHIS of Xtend soybean and cotton, and that APHIS cannot improperly delegate responsibility for these potential harms of its action.

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