THE CENTER FOR
FOOD SAFETY

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Regulatory Analysis and Development
PPD, APHIS
Station 3A-03.8
4700 River Road Unit 118
Riverdale, MD 20737-1238

Comments to USDA APHIS on Environmental Assessment for the Determination of Nonregulated Status of Herbicide-Tolerant DAS-40278-9 Corn*, Zea mays*, Event DAS-40278-9

Center for Food Safety, Science Comments II

Overview of environmental impacts

APHIS concludes that approving DAS-40278-9 corn will have no greater impact on the environment and endangered species than the "no action" alternative (DEA p. 49). In fact, APHIS finds that using the DAS-40278-9 crop system is likely to benefit the environment to the extent that it facilitates conservation tillage: approval of DAS-40278-9 corn "…is anticipated to allow growers to maintain their conservation tillage practices, thus preserving and enhancing soil and water quality, and providing the attendant benefits to biodiversity from those improvements." (DEA p. 88, biodiversity; also see the same argument for water quality, p. 67; soil, p. 69 - 70; climate change, p. 73; animal communities, p. 74, 77; and cumulative effects on animals, plants and biodiversity, p. 89).

APHIS also bases their conclusions that environmental harms are no greater with DAS-40278-9 corn on the assumption that there will be no increase in the amount of herbicide used per acre or per season and thus no change in risks, as stated in the Petition: "By maintaining the same, or reduced, application rates and maximum seasonal use rate there should be no change in the ecological risk assessments or endangered species assessments for 2,4-D or quizalofop uses with DAS-40278-9 corn." (Petition p. 116).

These conclusions finding no difference in environmental impacts between approving and not approving DAS-40278-9 corn have weak underpinnings in science. APHIS greatly overestimates the contribution of herbicide resistant crops to adoption of no-till in corn, and inflates the environmental benefits of herbicide dependent no-till methods. Also, APHIS does not factor in the unsustainable future of conservation tillage systems that are completely dependent on substituting herbicides for tillage. Weeds develop resistance to herbicides more quickly when combined with herbicide-resistant crop systems so that herbicide-dependent conservation tillage will require more herbicides and a return to tillage as time goes by (CFS Science Comments I), negating any short-term benefits of soil retention.

At the same time, APHIS underestimates the risk of injury to non-target plants and animals due to off-site movement of herbicides because they compare impacts of using the amount of 2,4-D *allowed* on conventional corn to the amount *allowed* on DAS-40278-9 corn, rather than considering the amount that growers actually use. Nor does APHIS project the total increase of 2,4-D and quizalofop use on corn acres overall. Without a realistic estimate of the differences in herbicide use, APHIS cannot make an informed assessment of risks to the environment and endangered species.

Below, in Part 1 we first critically assess the claim that herbicide-resistant crops like DAS-40278-9 have promoted or would promote or preserve conservation tillage. As will be demonstrated, this claim is highly dubious at best, and outright deceptive at worst. Second, we assess some of the claimed benefits of conservation tillage, finding that some are justified while others are greatly exaggerated, and some negative impacts are not assessed. Finally, we comment on harms to the environment and endangered species from increased herbicide use with the DAS-40278-9 system.

In Parts 2 and 3, we analyze the potential impacts of activity of the engineered enzyme itself in DAS-40278-9 corn, including in pollen. The metabolites formed from activity of the enzyme may affect wildlife, and particularly honey bees and other corn-eating animals, including endangered species.

Respectfully submitted,
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Part 1: Conservation Tillage, Environment and Endangered Species

Herbicide-resistant corn and conservation tillage

APHIS provides no independent assessment of a possible association between DAS-40278-9 and conservation tillage. Its few statements to this effect are conclusory (see references above), and heavily reliant on DAS' assessment in supplemental information submitted for the petition (DAS 2011d, as cited in DEA). Interestingly, this assessment primarily addresses conservation tillage in connection with DAS-40278-9's predecessor HR crop system, Monsanto's glyphosate-resistant crops. As APHIS notes: "Glyphosate-tolerant crops have been identified [by DAS] as facilitating the adoption of conservation tillage practices (DAS 2011d)" (DEA p. 70). Below, we address APHIS' and DAS' assessment, upon which APHIS relies.

DAS' argument in DAS (2011d) is simple. Glyphosate-resistant crop systems have promoted adoption of conservation tillage. At the same time, they have triggered massive emergence of glyphosate-resistant (GR) weeds. Increasingly intractable GR weeds constrained some farmers to use tillage (mechanical weed control) to remove them, which is tantamount to abandonment of conservation tillage. Introduction of the DAS-40278-9 corn system and 2,4-D-resistant soybeans would allow farmers to apply 2,4-D to kill weeds instead of using tillage, thereby "preserving" the conservation tillage benefits purportedly conferred by its predecessor HR system, glyphosate-resistant crops.

Even if one accepts this story at face value, it begs a very important question: If glyphosate-resistant crops promoted conservation tillage (in the short term), but are well on the way to undermining it just a decade later, what is to stop DAS-40278-9 from triggering a repeat of this boom-bust cycle? On this point, neither APHIS nor DAS has any satisfactory answers, as we discuss further in the resistant weeds section of CFS Science Comments I.

However, DAS' argument fails on its face. There is very strong evidence demonstrating that glyphosate-resistant crop systems are not responsible for any meaningful increase in conservation tillage, particularly in corn. This in turn casts strong doubt on the supposition that DAS-40278-9 will have any meaningful impact.

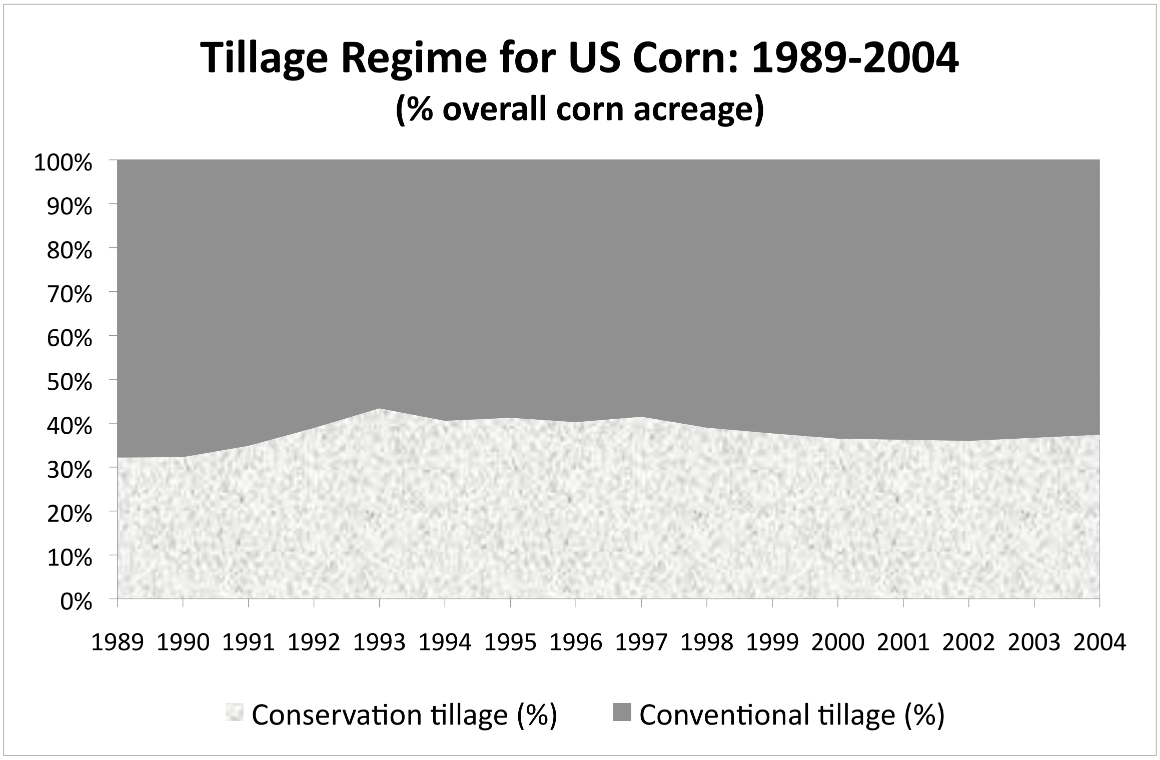
Even in the case of glyphosate-resistant (GR) soybeans, where there is in fact a correlation between adoption of GR seeds and conservation tillage practices, the causation is from conservation tillage adoption to use of GR soybeans, not the contrary. This is the conclusion of USDA ERS economists, who conducted an econometric analysis to address this very question (USDA ERS 2002, p. 29). First, they note the stagnating adoption of conservation tillage in soybeans in the years following GR soybean introduction:

> Adoption of conservation tillage for soybeans grew (at a decreasing rate) from about 25 percent of the soybean acreage in 1990 to 48 percent in 1995 (fig. 11), the 5-year period previous to the introduction of herbicide-tolerant soybeans. Growth of conservation tillage increased further in 1996, but then appears to have stagnated between 50 and 60 percent in the following years.

Their econometric analysis reached the following conclusion with respect to no-till:

> According to the econometric model results, using 1997 ARMS survey data, farmers using no-till for soybeans were found to have a higher probability of adopting herbicide-tolerant seed, but using herbicide-tolerant seed did not significantly affect no-till adoption.

The available evidence provides even less support in the case of HR corn. The chart below shows percentage of US corn cultivated using conservation tillage methods vs. conventional tillage, using USDA's definitions of the terms (USDA ERS AREI 2002, p. 23). Conservation tillage includes methods that leave 30% or more of the field's surface covered with plant residue, and includes no-till, ridge-till and mulch-till methods, while conventional tillage is defined as reduced till (15%-30% residue left) or intensive till (0-15% residue). Plant residue inhibits soil erosion.



Sources: For 1989-2000, see: USDA ERS AREI (2002) in supporting materials: "Agricultural Resources and Environmental Indicators: Soil Management and Conservation," US Dept. of Agriculture, Economic Research Service, Chapter 4.2, Table 4.2.9: "Tillage systems used on major crops, contiguous 48 states: 1989-2000." For 2002-2004, see CTIC (2002, 2004). CTIC = Conservation Tillage Information Center. Data not available for 1999, 2001, or 2003; those values were interpolated. CTIC data for 2006, 2007 and 2008 were based on far too few counties to permit extrapolation to national trends in conservation tillage on corn.

Conservation tillage (con-till) climbed rapidly from 32.2% in 1989 to its historical peak in 1993, when 43.4% of national corn acres were managed using no-till, ridge-till or mulch-till. Con-till remained above 40% through 1997, then dipped below 40% in 1998. There has been no trend since that time, with the proportion of corn-growing land under con-till fluctuating from 36-39% through 2004. We note that CTIC figures used in 2002 and 2004 were based on surveys of farmer practice in 3,092 counties. Unfortunately, CTIC collected data from far too few counties in 2006 to 2008 (67 to 375 of 3,092) counties to permit legitimate extrapolation to national trends (CTIC 2006, 2007, 2008). An agronomist with NRCS agrees that since 2004, there has not been enough data collected to make any national predictions on crop residue management (personal communication to Bill Freese, 4/6/09, see Widman 2009 in supporting materials). Thus, we exclude those data from the graph above.

Herbicide-resistant corn was introduced in 1996, when it occupied just 3% of corn acres. Adoption was slower than for other crops, reaching only 15% by 2003 and 20% by 2004 (see USDA ERS GE Adoption 2011 and graph above). If HR corn were strongly associated with promotion of conservation tillage, one would expect it to be reflected in at least the latter years of the graph above (e.g. 2003 and 2004).

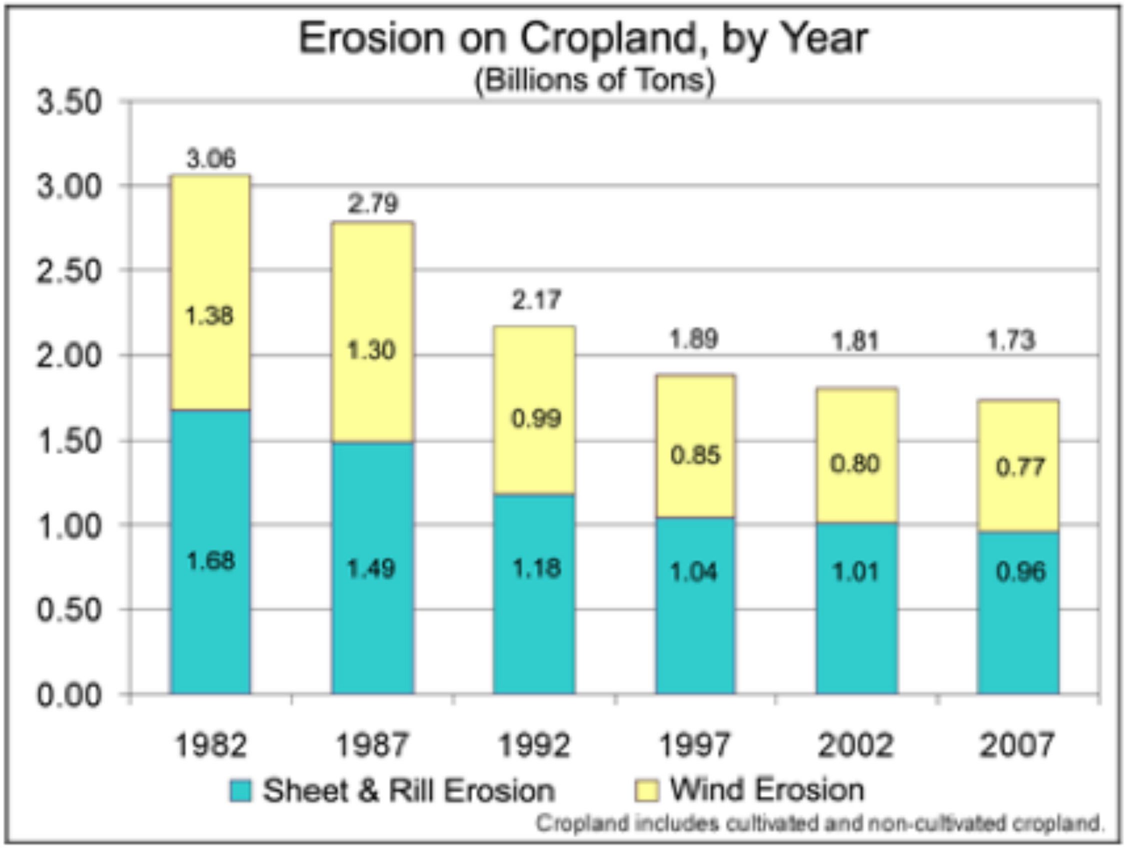
However, that is not the case. These USDA data on corn tillage regimes offer no support for APHIS' and DAS' notion that herbicide-resistant corn promotes conservation tillage, and in fact offer some grounds for disputing it.

What then explains APHIS' and DAS' view that HR corn promotes conservation tillage, as expressed for instance in the following passage (DEA p. 67): "Increases in total acres dedicated to conservation tillage have been attributed to an increased use of GE crops, including corn, reducing the need for mechanical weed control (Towery and Werblow 2010; USDA-NRCS 2006b, 2010)."?

APHIS' error is evident from its phrasing, which refers to "GE crops" in a general way rather than to herbicide-resistant corn. This is confirmed by consultation of its references. The Towery and Werblow piece cited by APHIS makes the claim that Roundup Ready ***soybeans*** have contributed to greater use of no-till, but makes no such claim for herbicide-resistant corn. Neither does USDA-NRCS (2006b, as cited in DEA) have any figures on conservation tillage in corn. Instead, Tables 2a and 2b of that work cite aggregate data for "cropland" under conservation tillage from 1990-2004. Although which crops are covered by these data are not specified, the high acreage figures (over 290 million acres of cropland) make it clear that NRCS aggregated all major field crops (e.g. corn, soybeans, cotton, wheat and others). Since corn represents only a bit over one-fourth of cropland, the, in any case, small increase in overall cropland under conservation tillage from 2002 to 2004 is likely attributable to other crops. This is confirmed by the specific data on corn presented above, showing no appreciable change.

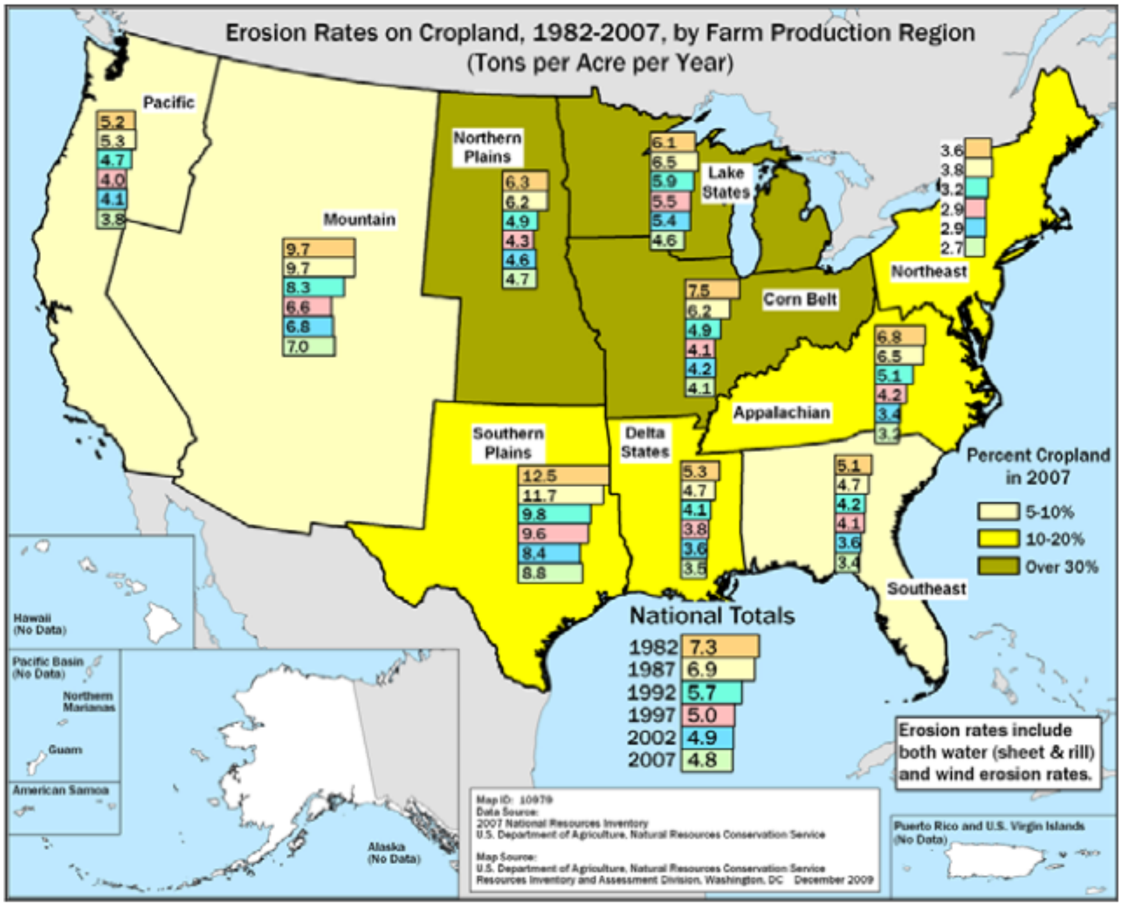
The third reference cited by APHIS, USDA-NRCS (2010), actually offers further evidence ***against*** its contention. The USDA's National Resources Conservation Service (NRCS) was formerly known as the Soil Conservation Service, signifying one of its major missions: to promote soil conservation, especially in the agricultural sector. NRCS administers Farm Conservation Plans to assist farmers in adopting sound farming practices that conserve soil, and also keeps careful track of soil erosion trends through surveys.

Below, we reproduce a chart from page 2 of NRCS' 2010 report: "2007 National Resources Inventory: Soil Erosion on Cropland" (USDA NRCS 2010, in supporting materials). The chart represents NRCS' best estimate of cropland erosion from 1987 to 2007. According to NRCS: "[E]rosion rates computed from NRI data are estimates of average annual (or expected) rates based upon long-term climate data, inherent soil and site characteristics, and cropping and management practices." Tillage regimes are the primary component of "cropping and management practices," and thus play a large role in determining soil erosion rates. It is well established that soil erosion increases with the intensity of tillage, and decreases as farmers adopt regimes that leave more plant residue on the soil (USDA ERS AREI 2002). Thus, the chart below reflects in large degree the tillage regimes used by farmers.



On a national basis, water and wind erosion on cropland declined sharply by 38% from 1982 to 1997, from 3.06 to 1.89 billion tons. In the following decade, however, soil erosion almost leveled out, declining by just 8%, from 1.89 to 1.73 billion tons. Herbicide-resistant crops were first introduced in 1996, and the area planted to them (HR soybeans, corn and cotton) increased steadily from 16.0 million acres in 1997 to 117.2 million acres in 2007 (Benbrook Supplemental 2009, Table 5). If HR crops promoted adoption of conservation tillage in any significant way, one would surely expect a much stronger decline in soil erosion over a period when their adoption increased by 100 million acres.

However, NRCS' survey offers still more compelling evidence at the regional level. The following map (from p. 3) breaks down average annual soil erosion rates, in tons per acre per year, by farm production region. For each region, rates for the six survey periods (1982, 1987, 1992, 1997, 2002 and 2007) are shown stacked from top (1982) to bottom (2007). The rates in this map are also listed in Table 36 of the report (pp. 12-16).



Erosion Rates on Cropland, 1982-2007, by Farm Production Region (Tons per Acre per Year)

Erosion rates include both water (sheet & rill) and wind erosion rates.

Map ID: 10979
Data Source:
2007 National Resources Inventory
U.S. Department of Agriculture, Natural Resources Conservation Service

Map Source:
U.S. Department of Agriculture, Natural Resources Conservation Service
Resources Inventory and Assessment Division, Washington, DC December 2009

The Corn Belt states (Iowa, Illinois, Indiana, Missouri and Ohio) and the Northern Plains states (Kansas, Nebraska, North and South Dakota) comprise two-thirds of the nation's corn and soybean acreage, and all of these states have high adoption rates of GE herbicide-resistant soybeans and corn (for corn, see USDA-ERS 2011a, cited in DEA). If APHIS' and DAS' supposition that HR crops and HR corn promotes conservation tillage were correct, one would certainly expect to see an appreciable decline in soil erosion in these two regions over the 1997 to 2007 period when HR versions of these crops were widely adopted; and that decline should be far more pronounced than the national average. However, this is not the case at all.

In the Corn Belt states, the annual erosion rate remained constant at 4.1 tons per acre, while ***erosion actually increased in the Northern Plains states*** from 4.3 to 4.7 tons per acre, in both cases bucking the national trend of modest decline. Of the eight other farm production regions, all but two (Mountain and Lake States) had declining erosion rates. Clearly, the massive adoption of HR corn and soybeans from 1997 to 2007 did not foster increased adoption of soil-conserving practices; if it had, it would have been reflected in declining rather than stable or increasing erosion rates.

The other question raised by NRCS' report is this: what explains the sharp declines in soil erosion in ALL farm production regions, including Corn Belt and Northern Plains states, in the 1982 to 1997 period before any appreciable adoption of HR crops? The answer is clear. Strong financial incentives to adopt soil-saving farming practices contained in the 1985 and 1990 Farm Bills were chiefly responsible for increased use of conservation tillage. According to Coughenour and Chamala (2000), authors of a book examining the history of conservation tillage in the U.S. and Australia:

> There is little mystery about what brought ***a sea change in farmers' tillage*** decisions as the 1990s unfolded. The compliance provisions of the 1985 Farm Bill and the 1990 amendments dramatically altered the effective policy and institutional environment. ***Farmers who wanted governmental support payments had to begin implementing their farm conservation plans (FCPs) by 1995, and their plans often included provision for conservation tillage.*** The balance of factors favoring use of no-tillage systems has also been strengthened by the progressive change in the cultural climate favoring farmers acceptance of program requirements and changes in farming practice. (p. 286, emphases added)

The NRCS concurs. In the short work referenced by APHIS above (USDA-NRCS 2006b, p. 3), they state:

> Total acres of conservation tillage systems rose steadily in the late 1980s to 37.2% of all planted acres in 1998 (Figure 2b). The implementation of Farm Bill Compliance standards containing residue management practices was largely responsible for much of this increased adoption.

"Residue management practices" refer to conservation tillage practices.

Finally, APHIS asserts without reference that: "[a]s glyphosate-resistant weed varieties have emerged, growers have returned to increased tillage as one of the weed management practices" (DEA p. 67), yet nowhere assess the ***extent*** to which farmers are actually utilizing tillage to control glyphosate-resistant weeds, much less corn farmers in particular. The only evidence that CFS has found on this point relates to cotton cultivation in the South. Cotton farmers in Tennessee, Arkansas, Missouri and Mississippi have reportedly increased their use of tillage, with declines in conservation tillage, to combat glyphosate-resistant weeds (Laws 2006). However, GR weeds have been worst in cotton and soybeans, and at least thus far less severe for corn growers. There is also evidence that increased herbicide use has been the primary response of many growers to GR weeds (see herbicide use and resistant weed sections, CFS Science Comments I). On the other hand, reasonably foreseeable weed resistance to 2,4-D resulting from introduction of the DAS-40278-9, and its potential impact on increasing herbicide use and/or tillage in response (see herbicide use and resistant weed sections, CFS Science Comments 1), is nowhere addressed in the DEA.

APHIS does not come close to providing a serious analysis to support its repeated conclusory statements with respect to conservation tillage in corn. APHIS refers to "GE crops," "herbicide-tolerant crops," "glyphosate-tolerant crops" and DAS-40278-9 interchangeably, and without distinction, as promoting conservation tillage. However, there are huge differences in management practices for corn, soybeans and cotton, which comprise the vast majority of HR crop acres. APHIS' treatment of them as an undifferentiated group just because they have a GE herbicide-resistance trait in common leads directly to the false conclusion that HR ***corn*** has promoted conservation tillage, which in turn prejudiced APHIS to accept DAS' assertions that DAS-40278-9 ***corn*** will do the same.

APHIS also frequently fails to reach its own independent assessment of the putative association of HR crops and conservation tillage (e.g. attribution to DAS re: glyphosate-resistant crops, DEA p. 66). APHIS is not even consistent it what it attributes to these other sources (attributing to the same two sources the proposition that HR/GE seeds "eliminate" (DEA p. 26) or "reduce" (DEA p. 67) mechanical weed control). APHIS' reluctance to own this assessment is perhaps understandable. As demonstrated above, neither statement is true of HR ***corn***.

APHIS also fails to assess important factors. For instance, while APHIS does provide a bare three-sentence description of the 1985 Farm Bill's requirement of soil conservation plans (DEA p. 26), there is no assessment of its impact on the adoption of conservation tillage practices, which as demonstrated above was profound. This failing is all the more striking in that APHIS cites a source that clearly states the Farm Bill was "largely responsible" for most con-till adoption (see USDA-NRCS 2006b quote above). Clearly, a proper assessment and appreciation of the important role of Farm Bill soil-conservation requirements in promoting conservation tillage practices would have altered APHIS' false, conclusory statements regarding a putative link to "GE seeds," much less HR corn or the DAS-40278-9 system.

APHIS' assessment of these matters falls far below the standards of "sound science" and the demand to take a "hard look" required by NEPA. All conclusory statements attributing putative benefits of conservation tillage to DAS-40278-9 should be disregarded as completely unsupported.

Environmental impacts of conservation tillage

Even if DAS-40278-9 corn is managed with conservation tillage, the environmental benefits attributed to reduced tillage are not well substantiated, other than slowing soil loss.

Soil and water

APHIS says there will be less soil loss from croplands with conservation tillage, and soil and water quality will benefit from less use of pesticides, and thus fewer pesticide spills and misapplications, and fewer passes over the field with soil-compacting equipment (DEA p. 66-67, 69). However, while herbicide-facilitated no-till methods may decrease soil erosion, they do not always increase soil quality or reduce water pollution, and under some conditions actually increase agrichemical runoff, degrading water quality.

No-till and other conservation-tillage systems discourage the disturbance of the soil, which can lead to over-compaction (Fabrizzi et al. 2005, Tebrugge 1999). In the absence of soil disturbance, some studies have shown that fertilizers broadcast on the soil surface are washed off the field by rain, thus polluting waterways as well as lowering nutrient-use efficiency (Mahli et al. 1996). Pesticides also can end up at higher concentrations in runoff from fields in conservation tillage. Crop residues are left on the surface in these systems, and surface residues intercept sprayed pesticides that are then washed off during rain (Baker and Shiers 1989, Martin et al. 1978). "If this washoff water becomes a part of surface runoff, herbicide concentrations can be quite high." (Mickelson et al. 2001). Research conducted on corn herbicides confirmed these conclusions. While no-till systems had the lowest volume of runoff, the concentrations of atrazine and cyanazine in runoff water were always greater (statistically significant in most cases) in no-till systems than for the other tillage regimes (Mickelson et al. 2001).

Fertilizer and pesticides can also run off more rapidly from no-till fields into drainage ditches and then into the watershed via more extensive pores, including earthworm burrows. (Shipitalo et al. 2004, Comis 2005).

There is thus no guarantee that use of herbicide-dependent conservation tillage systems will result in overall benefits to soil and water quality, even if DAS-40278-9 corn were to increase use of conservation tillage in the short term.

Climate change

APHIS also concludes that "[t]he adoption of herbicide-tolerant crops, and the attendant increase in conservation tillage has been identified as providing climate change benefits. Conservation tillage…in addition to providing benefits to soil health, has the benefit of increasing carbon sequestration in soils." (DEA p. 73). These benefits for climate change of a purported increase in no-till corn acreage are generally unsubstantiated. Recent work by Blanco-Canqui and Lal (2008) and a careful review of the literature by USDA researchers Baker et al. (2007) cast doubt on the claim that no-till results in more carbon sequestration than tillage in most conditions. Other gases that contribute to global warming– such as nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3) – are reported to be generally higher in no-till fields, as well.

Scientists from the USDA's Agricultural Research Service and Department of Soil, Water & Climate at the University of Minnesota (Baker et al. 2007) reviewed the literature on the effects of tillage on carbon sequestration in agricultural soils and concluded that in order to accurately determine how much carbon is sequestered, it is necessary to sample the soil to a depth that the roots grow. This is because much of the carbon fixed in photosynthesis is translocated to the roots and some is exuded into the soil where it stimulates the growth of various microorganisms. The deeper roots and microorganisms may also store carbon for a longer period of time than the more shallow roots.

The vast majority of tillage-soil carbon sequestration studies have sampled no deeper than the top 30 cm (roughly 1 foot) of soil. When studies of carbon sequestration are limited to the top 30 cm of soil, more carbon is stored in no-till than tilled fields, on average. However, when the sampling includes more of the root zone (below 30 cm; corn roots can go down more than 200 cm), tilled fields have as much stored carbon as their no-till counterparts (Baker et al. 2007). In some cases, tillage results in more carbon storage. Thus, the claim that conservation tillage results in more carbon sequestration than conventional tillage seems to be a result of sampling bias.

Blanco-Canqui and Lal (2008) published a study questioning carbon sequestration in no-till fields, as well. This study covered a large geographic area, looking at farmers' fields rather than small research plots, and sampling throughout the root zone. Not only did the plowed plots store as much carbon as the no-till plots when sampled below 10 cm, three of the plowed areas sequestered more carbon.

They come to a similar conclusion about using no-till to sequester carbon as Baker and colleagues:

> This regional study shows that NT [no-till] farming impacts on SOC [soil organic carbon] and N [nitrogen] are highly variable and soil specific. In MLRAs [Major Land Resource Areas] where NT soils have greater SOC than tilled soils, the gains in SOC are limited solely to the surface soil layers (<10 cm). The net effect of NT on SOC sequestration for the whole soil profile (0-60 cm) is not significantly different from that of plow tillage…
>
> Based on the data on soil profile C distribution from previous reports and this regional study, the view that NT farming would increase SOC over PT [plow tillage] is questionable... (Blanco-Canqui and Lal 2008, p. 701)

Other greenhouse gases may also be affected by tillage systems:

- Fertilizers in no-till fields are generally more vulnerable to volatilization. Fertilizers are often applied to the surface in no-till fields (Rochette et al. 2009), which can result in up to 50% of urea being volatized as ammonia (NH_3) (Sommer et al. 2004). Some studies showed that cumulative NH_3 volatilization was three times greater in no-till than in plowed fields, attributed to the reduced ability of nitrogen to infiltrate soils in the presence of crop residues on the surface of untilled soils (Al-Kanani et al. 1992).

- Ammonia can be oxidized and transformed into the greenhouse gas N_2O.

- Once emitted, ammonia can also be rapidly converted to the aerosol ammonium (NH_{4+}) that contributes to ecosystem fertilization, acidification, and eutrophication. These processes increase methane emissions and decrease carbon sequestration through photosynthesis, thereby exacerbating climate change.

- Globally, most N_2O emissions are the result of microbial processes in soil, both aerobic nitrification and anaerobic denitrification (Smith and Conen 2004). No-till soils have demonstrated elevated levels of water-filled pore space (WFPS), determined by water content and total porosity (Mosier et al. 2006). WFPS appears to be closely related to soil microbial activity. One study demonstrated that WFPS in no-till systems to be 62% compared to 44% for plowed soils (Linn et al. 1984). Other studies have implicated no-till in greater N_2O releases, as well (Ball et al. 1999, Rice and Smith 1982, Aulakh et al. 1984).

The point of citing these studies that show exacerbation of greenhouse gas emissions and degradation of soil and water quality with no-till methods is not to discount environmental benefits of conservation tillage in specific situations, particularly when it is used with other techniques of sustainable agriculture (Davis 2010). Using sustainable methods to decrease tillage for soil conservation is indeed important. However, APHIS has relied too heavily on conservation tillage as an argument for a whole range of environmental benefits of DAS-40278-9 corn without critical analysis of the best science available.

In fact, overall environmental benefits and harms from approval of DAS-40278-9 corn are likely to have more to do with changes in herbicide use that accompany the DAS-40278-9 corn cropping system than with tillage methods that may or may not be different.

Environmental effects of increased herbicide use

Drift injury to plants and other non-target organisms

2,4-D is a particularly potent poison for many species of plants (Rasmussen 2001), especially dicotyledons (broadleaf plants) that are sensitive to very low drift levels (US-EPA 2009, Appendix F, F-28, F-30; EC25 for shoot weight of 0.037 lbs ae/acre 2,4-D 2-EHE for radish, Appendix F, F-28; EC25 for fresh weight of 0.003 lbs ae/acre 2,4-D DEA for tomato, Appendix F, F-30). Even monocots such as members of the grass and lily families can be killed by higher doses of 2,4-D, and suffer sub-lethal injuries from drift levels at certain times in their life cycles (US-EPA 2009, Appendix F, F-27, F-29; Nice et al. 2004). Hormone-mimic herbicides such as 2,4-D injure some plants at lower concentrations than other widely used herbicides. It takes almost ten times more glyphosate than 2,4-D to decrease vegetative vigor of the most sensitive plant species, for example, and over 300 times more glyphosate than 2,4-D to inhibit seedling emergence (Peterson and Hulting 2004).

Therefore, reports of injury to non-target crops from 2,4-D drift are common (see drift, CFS Science Comments I). And "… most drift complaints involved commercial applications of agricultural pesticides in rural areas and ground applications accounted for 2/3 of complaints." (Lee et al. 2005, p. 15).

Even after changes in regulations, formulations and methods of application designed to reduce drift, it is still a significant problem for specialty crop growers, partly due to the unpredictable occurrence of volatilization, movement with soil particles, and other poorly understood processes (drift section, CFS Science Comments I). For example:

> Grape vineyards, especially in regions of mixed cereal and minor crop production, have historically been exposed to auxin-type herbicides, presumably from a combination of local and regional transport. Banning dust and volatile ester formulations, restricting the timing of low volatile ester formulations, and prohibiting applications when drift is likely have all helped to minimize the damage to grapes. Unfortunately, episodic injury remains severe enough to cause economic losses to the grape industry. Our recent two years of field monitoring of 2,4-D residues supports the above assertion The movement of these highly active substances from the target site as aerosols, on/in soil wind-blown particulates, or in the gas-phase are unfortunately difficult to predict and therefore more difficult to apply consistent label language. Moreover, post-application processes are beyond the direct control or influence of pesticide applicators. Because of the high potency, mitigating injury from the use of auxin-type herbicides to sensitive crops upwind will remain difficult. (Herbert 2004).

If 2,4-D is moving off-site far enough to cause injury to crops, it is undoubtedly also causing injury to wild plants. Drift of 2,4-D is most likely to impact vegetation near the site where it is applied, so borders of fields and adjacent fencerows, wetlands, woodlands, riparian areas, and old-fields are vulnerable. These areas provide most of the biodiversity found in agricultural landscapes (e.g., Boutin and Jobin 1998). But with a volatile herbicide such as 2,4-D, injury has also occurred at locations distant from the application site, as described above with injury to grapes, putting organisms in a variety of natural areas at risk.

There have been few studies of 2,4-D drift effects on wild plants and their communities. EPA reviewed their Ecological Incident Information System (EIIS) database for “ecological incidents involving 2,4-D acid, salts and esters” through 2008 (US-EPA 2009, p. 100, and Appendix H) Reports include injury from off-site herbicide movement after applications of 2,4-D on conventional cornfields (US-EPA 2009, p. 115). Plants listed as injured from drift or runoff from agricultural areas include oak and poplar trees, but primarily concern various crops (Appendix H). There are also reports of small mammals being killed after ingesting 2,4-D used on crops, and of “kills” of aquatic organisms – fish and water snakes - after 2,4-D runoff or drift from agricultural areas (US-EPA 2009, Appendix H).

It is likely that crop injury from pesticide drift is significantly under-reported:

> When crops are damaged by off-target movement of herbicides, the affected growers may settle their differences without the intervention of government enforcement agencies or courts. However, in the absence of a damage report to a state agency or court settlement, there are no records of their occurrence, due to lack of a centralized

> herbicide incident reporting system in the United States. For incidents that are more contentious or serious, a likely sequence of events arising from herbicide damage to non-target crops may include: 1) a complaint to a state agency over damage cause[d] by an herbicide, 2) an ensuing investigation that may uncover a violation (but which may not resolve the economic loss by the farmer whose crop is affected), and 3) lawsuits that use the investigation as evidence of harm…However, the majority of lawsuits are settled out of court with the stipulation that the plaintiffs not divulge the contents of the settlement to anyone including the government. (Olszyk et al. 2004, p. 225)

When only wild plants are harmed, injury may not be noticed or reported at all. Therefore, most information about risks of herbicide exposure for wild plants and ecosystems comes from experimental studies and comparative surveys rather than from incident reports (discussed below). It is clear that non-target organisms do risk injury from 2,4-D used in agriculture, and that approval of DAS-40278-9 corn is likely to increase that risk to the extent that the crop system involves increased use of 2,4-D, and also quizalofop.

Simply, the amount of injury that non-target organisms will sustain is determined by how sensitive they are to the 2,4-D and quizalofop formulations and by the dose they receive. Therefore, it is important for APHIS to make a realistic prediction of the amount of 2,4-D and quizalofop that will be used on DAS-40278-9 corn compared to conventional varieties in order to evaluate the impacts of their alternatives.

Realistic estimates of herbicide use with DAS-40278-9 corn

APHIS admits that organisms in field edges could receive higher and more frequent doses of 2,4-D during the growing season (DEA, p. 75-76) This is because the proposed label for DAS-40278-9 allows for two applications to the growing corn plant rather than just one, and at double the application rate, than is currently allowed on conventional corn. This means that four-fold more 2,4-D can be applied to DAS-40278-9 during this critical "post-emergence" period (2 vs. 0.5 lbs/acre). In addition, an important drift-reducing measure required for application of 2,4-D to conventional corn (use of drop nozzles) has been eliminated from the proposed label for DAS-40278-9. Drop nozzles direct the spray downwards below the crop foliage where it is less likely to drift. The combination of more frequent and higher doses, applied without the use of drift-reducing drop nozzles, means that DAS-40278-9 corn presents a considerably higher risk of plant-damaging drift than 2,4-D used on conventional corn. Then APHIS passes the ball to the EPA to evaluate the consequences for the environment of these changes in allowed herbicide use. (see, for example, DEA p. 86).

However, APHIS does not consider how conventional corn growers actually use 2,4-D. Growers are *allowed* to make up to 3 applications on conventional corn in a season: 1)A 1 lb a.e./acre preplant or preemergence treatment, and then 2) either an over-the-top spray at 0.5 lb a.e./acre until the corn is 8” high, or an application made with drop nozzles after 8” but before tassel, and finally 3) they can treat pre-harvest at the dent stage with 1.5 lb a.e./acre, for a total of 3 lb a.e./acre/year. (US-EPA 2011).

The average amount *actually used* per year by corn growers that apply 2,4-D is 0.35 lb a.e/acre, just one-eighth than the amount allowed. On average, they make 1.12 applications per year instead of the three allowed (Benbrook 2012, USDA-NASS AgChem 2010).

In fact, growers apply less 2,4-D than allowed because corn can be injured by labeled rates of 2,4-D (Nice et al. 2004). Note that in the EIIS incident report cited by EPA (US-EPA 2009, Appendix H), injury to corn itself during applications is one of the most common incidents in the report. Some growers are willing to risk injury to their corn by using 2,4-D presumably because it is inexpensive and effective on some of their problem weeds, but they mitigate that risk by using 2,4-D with caution, that is, sparingly.

Because DAS-40278-9 corn is engineered to express AAD-1 protein it is resistant to much higher levels of 2,4-D than conventional corn (Petition, p. 103), at least through the V8 stage a few weeks before pollen shed, and perhaps throughout its life (tolerance testing has only been reported by DAS through the V8 stage, (see pollen gene expression, Part 3, these Comments). Without these biological constraints, growers will no longer have to balance weed control with risk of injuring their corn, and thus will be more likely to use the full rate allowed.

DAS has proposed the following schedule for spraying DAS-40278-9 corn: up to 3 applications, with 1) a preplant or preemergence application of 1 lb a.e./acre (the same as for conventional corn but with a wider window), followed by 2 & 3) up to two applications a minimum of 12 days apart before the V8 stage of development, at up to 1 lb a.e./acre each (2X the rate allowed on conventional corn), and both applications can be applied over the top of the crop (instead of requiring drop nozzles), for a season total of 3 lb a.e./acre/year (the same as for conventional corn). (DEA p. 56).

Growers will be encouraged to use the full rate per application as part of DAS' stewardship program for supposedly delaying weed resistance to 2,4-D (Blewett 2011, p. 7). Also, DAS will encourage growers to buy a premix of 2,4-D and glyphosate to apply to DAS-40278-9 corn that has been stacked with the Roundup Ready trait (US-EPA 2011), a likely combination from the start (see herbicide use, CFS Science Comments 1). With the premix, if a grower wants to apply the full rate of glyphosate per hectare to control certain weeds, they will simultaneously be obliged to apply the full rate of 2,4-D as well.

A reasonable prediction, then, for how much 2,4-D a grower is likely to use on DAS-40278-9 corn is at least one application at 1 lb a.e./acre (see also herbicide use, CFS Science Comments I). This is already about 2.5X to 3X times higher than the rate conventional growers use now per application. And if there are no biological constraints in terms of injury to their corn, it is likely that growers will use more than one application per season if weed pressure warrants it. Cost of applying more herbicide is likely to be the main constraint instead of yield loss from injury.

Timing of 2,4-D applications in the growing season

Not only will growers use a higher rate of 2,4-D, these applications are more likely to coincide with life-stages of plants that are the most sensitive to injury because the DAS-40278-9 corn itself is less sensitive to injury during spring and summer than is conventional corn. This is a general outcome of herbicide-resistant crop systems: "Increased use of herbicide-resistant technology by producers creates the possibility of off-site movement onto adjacent conventional crops. The role of total postemergence programs to control grass and broadleaf weeds has expanded with the development of herbicide-resistant crops. Because of the diversity of cropping systems in the United States, it is not uncommon for herbicide-resistant crops to be planted near susceptible conventional crops. Postemeregence application of a herbicide to a genetically-modified (GM) crop often occurs when non-GM plants are in the early reproductive growth stage and most susceptible to damage from herbicide drift….Consequently, most drift complaints occur in spring and summer as the use of postemergence herbicide applications increase." (Lee et al. 2005, p. 15) Plants – both crop and wild species –are often most sensitive to herbicide injury as pollen is forming (Olszyk et al. 2004; pollen gene expression in these Comments).

Total use of 2,4-D at landscape level

Another way that the DAS-40278-9 corn cropping system will increase 2,4-D use is by an increase in the total number of corn acres that are treated with 2,4-D, from about 10% of acres now to a likely 55% of corn acres in 2019 (Benbrook 2012, CFS Science Comments I). At a landscape level this increase will result in a larger number of individuals of a wider array of species in proximity to DAS-40278-9 corn and thus 2,4-D. Also, since corn acreage is expanding (DEA p. 53), and some of the expansion is at the expense of former Conservation Reserve Program land not recently in agricultural production (Brooke et al. 2009), more wild native species are likely to impacted by 2,4-D, including threatened and endangered species (see below).

Increased ingestion of 2,4-D residues and metabolites

Higher application rates with DAS-40278-9 corn will also leave higher levels of 2,4-D residues and metabolites in the corn tissues (see comments-metabolism, pollen), and also higher concentrations of 2,4-D in runoff. APHIS acknowledged that spraying over the top of the crop during the growing season would result in higher levels of 2,4-D in foliage, but compared the levels to those in a pre-harvest treatment of conventional corn:

> If the drop nozzle is not required, animals feeding on corn foliage during that time would receive a higher dose if they forage on the foliage during this post-emergence period. However, current labeled uses provide for a single application of 1.5 lbs. ae/acre at the pre-harvest, later stages when corn is at the hard dough or dent stage. The proposed new label does not seek such an application. Animals feeding on corn foliage

> treated pre-harvest under the current label would receive a higher dose than under the proposed new label (DEA p. 76).

Again, APHIS does not consider how 2,4-D is actually used – actual use rates on conventional corn are well below the allowed rate, and also the pre-harvest treatment is rarely used. (CFS Science Comments I) Postemergence sprays at higher rates and without drop nozzles are highly likely for DAS-40278-9 corn, so APHIS should reconsider potential risks to animal communities from eating DAS-40278-9 corn foliage or drinking runoff, including the unique metabolites as well as the parent 2,4-D residues (see metabolism, these Comments).

Impacts to biodiversity

According to APHIS, to the extent that adoption of DAS-40278-9 corn allows growers to use conservation tillage and also to reduce the use of soil-applied herbicides, this "…reduction in herbicide use and increase in conservation tillage both benefit animals, plants, and biodiversity in and around the cornfields." (DEA p. 89)

Again, these claimed benefits are baseless. First, herbicide use will increase, not decrease. There will be a large increase in use of the herbicides that DAS-40278-9 corn has been engineered to withstand, 2,4-D and quizalofop, as already discussed, with attendant harms (below). But also DAS will encourage farmers to use a mix of the very soil-applied herbicides APHIS says will be reduced: "Dow AgroSciences will be recommending the use of soil residual herbicides as a part of the Enlist Weed Control System to provide early season weed control for crop yield protection and weed resistance management by providing additional modes of action." (Scherder et al. 2012). And other herbicide-resistance traits will be stacked with DAS-40278-9, with pre-mixed herbicide combinations will sold for the specific stacks, adding additional herbicides at their full rates (CFS Science Comments I). Any benefit to specific organisms of less tillage within the fields is likely to be offset by these increases in herbicide use.

Biodiversity in cornfields

An example of harm to biodiversity in cornfields from an herbicide-resistant crop system is the recent decline in milkweed populations in Midwestern fields with probable impacts on monarch butterflies, as described in a series of studies (Hartzler and Buhler 2000, Hartzler 2010, Brower et al. 2011, Pleasants and Oberhauser 2012). The basic conclusions are well stated in the abstract of the most recent publication by Pleasants and Oberhauser (2012):

> Abstract. 1. The size of the Mexican overwintering population of monarch butterflies has decreased over the last decade. Approximately half of these butterflies come from the U.S. Midwest where larvae feed on common milkweed. There has been a large decline in milkweed in agricultural fields in the Midwest over the last decade. This loss is coincident with the increased use of glyphosate herbicide in conjunction with increased

> planting of genetically modified (GM) glyphosate-tolerant corn (maize) and soybeans (soya).
>
> 2. We investigate whether the decline in the size of the overwintering population can be attributed to a decline in monarch production owing to a loss of milkweeds in agricultural fields in the Midwest. We estimate Midwest annual monarch production using data on the number of monarch eggs per milkweed plant for milkweeds in different habitats, the density of milkweeds in different habitats, and the area occupied by those habitats on the landscape.
>
> 3. We estimate that there has been a 58% decline in milkweeds on the Midwest landscape and an 81% decline in monarch production in the Midwest from 1999 to 2010. Monarch production in the Midwest each year was positively correlated with the size of the subsequent overwintering population in Mexico. Taken together, these results strongly suggest that a loss of agricultural milkweeds is a major contributor to the decline in the monarch population.
>
> 4. The smaller monarch population size that has become the norm will make the species more vulnerable to other conservation threats.

Here we are, 16 years after the introduction of Roundup Ready soybeans, and major impacts of their widespread adoption are just now surfacing, with only a handful of researchers doing this kind of "post-market" ecological research.

The DAS-40278-9 corn cropping system will result in higher rates and more applications per season of 2,4-D, also a systemic herbicide, and likely to be used in addition to full rates of glyphosate. It is also reasonably foreseeable that in the future engineered soybeans will be treated with both 2,4-D and glyphosate, in rotation with DAS-40278-9 corn (CFS Science Comments I). Weed biodiversity, such as small populations of milkweed, within these fields won't have a chance. Tolerant and resistant weeds will come to dominate, simplifying the number of plant species in the fields, and this by definition is a decrease in biodiversity. Also, with specialist herbivores, such as the monarch butterfly that rely completely on particular plant species, other kinds of plants will not substitute for their requirements.

Besides the direct toxicity of the increased herbicides used on DAS-40278-9 corn to plant population diversity within cornfields and ramifications for animals from changes in plant diversity, there will also be an increase in herbicide exposure from residues and their metabolites in DAS-40278-9 corn tissues (see metabolism, these Comments). A wide variety of animals feed on corn leaves, flower parts, and seeds, including many beneficial organisms such as honey bees (see pollen, these Comments).

Also, some animals may be over-sprayed during applications of herbicides, and others may brush against newly sprayed foliage, receiving higher herbicide doses in DAS-40278-9 corn with possible toxic impacts (US-EPA 2009, Freemark and Boutin 1995).

Instead of delving into the ecological literature, APHIS accepted an observational study by DAS as evidence that insects feeding on corn would not be affected by DAS-40278-9 (DEA p. 75).

DAS made "ecological observations" during field trials during 2 years, with personnel noting the presence of insects in DAS-40278-9 corn vs. conventional controls. Insect damage was also noted in another set of field tests. They determined that corn-eating insects inflicted similar damage levels, and that similar kinds of insects were seen in the corn, including beneficial insects such as lacewings and lady bugs (Petition, Table 14, p. 72; DEA p. 75), and concluded that "[i]nsects, particularly insects which feed on corn, were not impacted by ingesting corn in which the aad-1 gene was incorporated." (DEA p. 75) These observations of field trials should not in any way be construed as a study of impacts of the DAS-40278-9 crop system on insects specifically or animals generally. Valid ecological studies would require hypothesis testing in a more systematic way.

APHIS needs to assess potential impacts to animals in fields of DAS-40278-9 corn in light of the foreseeable increase in exposure to herbicides and their metabolites based on realistic use scenarios and a wide range of relevant independent scientific studies in order to compare alternatives.

Biodiversity around cornfields

APHIS provides background information on the importance of management of field edges for biodiversity in maintaining beneficial insects, birds, and other wildlife in the agroecosystem (DEA p. 36 -37). They also recognize the contribution of "drainage ditches, hedgerows, riparian areas, and adjacent woodlots" as vital habitat for a range of wild plants and animals (DEA p. 37).

At the same time, APHIS acknowledges the negative impacts of pesticide exposure to wildlife and habitats: "Reduced pesticide use has a direct positive effect on wildlife by reducing the direct exposure of birds, mammals, and fish to pesticides. Indirect benefits include less alteration of suitable wildlife habitat and an available food supply of insects for insectivores…"(DEA p. 36), and, "Minimizing pesticide exposure of ditches, aquatic habitats, border areas, strip-crop areas, and non-crop habitats may help protect fish and wildlife resources…" (DEA p. 37).

However, APHIS does not take into account the impacts that increased herbicide use in DAS-40278-9 crop systems would have on those nearby habitats because they do not develop a realistic analysis of changes in herbicide use, and defer to EPA on pesticide use anyway (DEA p. 77, 88). Thus they are unable to properly compare their proposed alternatives.

Increased drift and runoff from use of 2,4-D and quizalofop with the DAS-40278-9 crop system is likely to alter the very habitats that APHIS has identified as being important for biodiversity (Freemark and Boutin 1995, Boutin and Jobin 1998, Olszyk et al. 2004). Particular species of plants are more or less sensitive to these herbicides, and at different times of the year, so that a specific drift event is likely to change the population dynamics in affected areas. For example, 2,4-D drift 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 may result in long-term changes in the mix of plant species, favoring annual weeds over native plants, for example (Boutin and Jobin 1998, Boutin et al. 2008). And if there are 2,4-D resistant plants in these habitats, they will of course be better able to withstand drift and may become more abundant (Watrud et al. 2011).

These herbicide-induced changes in plant populations can then indirectly impact "microbial communities, occurrence of plant pathogens, or diminished insect populations. Both direct and indirect effects could lead to numerous negative impacts on ecosystem services including wildlife habitat, nutrient cycling, control of soil erosion, recreation, timber or pulp production, livestock grazing, control of noxious plant species and aesthetics…." (Olszyk et al. 2004).

There are studies of species composition in field margins (Kleijn and Snoeijing 1997) and hedgerows that border conventional fields compared with fields managed organically without herbicides (Boutin et al. 2008) showing differences in plant populations that indicate just these sorts of species shifts from herbicide exposure. Also, "[i]n controlled experiments with plant communities, Pfleeger and Zobel (1995) demonstrated that variable species responses to herbicide exposure [including 2,4-D] may alter the competitive interactions within a community. Such shifts in a community could result in changes in frequency and production and even extinction of desired species…" (Olszyk et al. 2004).

Recent experiments have shown that drift levels of the broad-spectrum herbicide glyphosate alter population structures of plants that include some herbicide-resistant individuals, favoring an increase in those with the glyphosate-resistance trait. Differences in the populations persist years after the last "drift" incident, affecting the kinds of beneficial soil fungi present and growth of subsequently planted species, for example (Watrud et al. 2011).

Animals depend on plant biodiversity for most of their needs, so it would be surprising if herbicide induced changes in plant populations had no effects on animal biodiversity around cornfields. Freemark and Boutin (1995) reviewed the literature on how herbicide use has affected wildlife, and found that, as expected, biodiversity has been affected in areas adjacent to sprayed crop fields, including types and abundance of small mammals and birds. An example of how drift levels of 2,4-D may impact animals has to do with the ability of 2,4-D to cause sterility in grasses that are in early stages of reproduction, and "…reproduction is critical for the ability of non-crop native plants to pass along their traits. Furthermore, many wildlife species depend upon seed production of non-crop plants for their food source." (Olszyk et al. 2004). Many insects depend on abundant pollen, as well (Lundgren 2009).

Based on experiences with 2,4-D sensitive crops, natural areas miles from agricultural applications of 2,4-D may also be at increased risk from the use of greater amounts of the herbicide in corn, since it can volatilize under certain conditions. (see drift in Science Comments 1, Herbert 2004).

It is clear, then, that increased use herbicides with the DAS-40278-9 crop system are likely to have negative impacts on biodiversity around cornfields, perhaps at some distance, and thus APHIS should prepare and Environmental Impact Statement that assesses these impacts.

Threatened and endangered species

APHIS independently determined that threatened and endangered species would be unaffected by approval of DAS-40278-9 corn:

> After reviewing the possible effects of allowing the environmental release of DAS-40278-9 corn, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. As a result, a detailed exposure analysis for individual species is not necessary. APHIS also considered the potential effect of a determination of nonregulated status of DAS-40278-9 corn on designated critical habitat or habitat proposed for designation, and could identify no differences from effects that would occur from the production of other corn varieties. (DEA p. 50, 118).

However, they did not take into account the use – or the increased use – of 2,4-D and quizalofop, instead deferring to the EPA (DEA 120 – 122); nor did they consider the possible toxicity of the metabolites that are present in DAS-40278-9 corn exposed to herbicide substrates of the AAD-1 protein (see metabolism and pollen gene expression, Parts 2 & 3, these Comments).

Increased herbicide use and listed species

All of the harms from increased use of herbicides on DAS-40278-9 crop systems to plants, animals, and other organisms, and to their habitats, discussed above, apply to species that are at risk of extinction. Endangered species near fields planted to DAS-40278-9 will be at increased risk from exposure to herbicides via drift of particles and vapor, runoff, accidental over-spraying, and recently sprayed plant parts and soil. Their habitats will be at higher risk of being altered from changes in plant populations with attendant impacts.

However, the stakes of herbicide exposure are higher, especially for plants: "Determination of herbicide effects to threatened and endangered plant species in native plant communities is especially critical. In the US, the federal government has listed over 500 plant species as threatened and endangered and the Nature Conservancy considers 5,000 of the 16,000 native species to be at risk. Almost 50% of these species are annuals that are dependent on seed production or the seed bank for survival, thus any reproductive effects of herbicides could affect their survival." (Olszyk et al. 2004).

Not only will more endangered species be exposed to the 2,4-D and quizalofop used with DAS-40278-9 as it is grown on existing corn acreage, but also because corn acres are expanding. More endangered species are likely to find themselves near DAS-40278-9 crop systems, because at least some of the expansion is occurring by conversion of natural areas and Conservation Reserve Program land not recently farmed. These areas are more likely to harbor wild native organisms than land intensively farmed every year for decades (Brooke et al. 2009). APHIS assumed that because corn acreage was expanding with or without approval that there impacts from the expansion would be the same with or without DAS-40278-9, not taking into account the increase in herbicide use (DEA p. 115).

The use of 2,4-D on more corn and at higher rates should have been a red flag for APHIS to consult with FWS about endangered species because of the recent Pesticide Effects Determination by EPA (US-EPA 2009) and Biological Opinion from the National Marine Fisheries Service (NMFS 2011), both finding adverse impacts to several specific endangered species. APHIS notes these findings, but does not dwell on their significance for approval of DAS-40278-9 corn, again deferring to the EPA and their potential mitigation measures (DEA p. 120). However, the detailed information in these reports leads to the inescapable conclusion that almost all threatened and endangered species would be similarly impacted by 2,4-D use at rates like those proposed for DAS-40278-9 crop systems.

Specifically, the EPA evaluated the risks of 2,4-D use to the threatened California red-legged frog (CRLF) and Alameda whipsnake (AW) and their critical habitats. This frog lives in both coastal and interior mountain ranges, using both water bodies and riparian and upland sites; and eats wide variety of plant and animal foods during its aquatic and terrestrial phases, including insects, other amphibians and an occasional small mammal. The Alameda whipsnake is found in scrub and chaparral, as well as riparian areas, grasslands and savannas; also has a varied diet that includes insects, amphibians, other reptiles, small mammals and birds (US-EPA 2009, p. 55).

Just about all of these habitats and prey types are potentially impacted by use of 2,4-D at agricultural rates, either directly or indirectly (US-EPA, summary of effects p. 11 – 25). Looking at specific use of 2,4-D applied with ground equipment on field corn or popcorn, for example, the “level of concern” is exceeded for direct effects on the terrestrial habitat of CRLF and with aerial applications for AW. “Level of concern” is exceeded for indirect effects on prey, including terrestrial invertebrates and plants, frogs, small mammals (CRLF and AW), and also for birds (AW). Small mammals were also likely to be directly impacted, based on incident reports. After going through the whole assessment process, EPA concluded that use of 2,4-D in a variety of scenarios, including on corn, was “likely to adversely affect” both the CRLF and AW via indirect effects on prey, and was likely to modify critical habitat (US-EPA 2009, p. 175 – 179). They initiated a formal consultation with FWS based on these conclusions.

Many threatened and endangered animals share the basic food and habitat requirement of CRLF and AW, including other amphibians and reptiles, but also mammals and birds. This leads to the reasonable expectation that EPA would find that use of 2,4-D on DAS-40278-9 crop systems would similarly be “likely to adversely affect” prey and habitats of threatened and endangered animals found near these cornfields.

The only EPA consultation over 2,4-D impacts on threatened and endangered species that has proceeded to the "biological opinion" stage is for Pacific salmonid fishes (NMFS 2011). These are fish species that spawn in the floodplains of the Pacific coast, and then go to sea for a few years before returning up rivers and creeks to their original spawning ground to begin again. Here the NMFS concluded that agricultural uses of 2,4-D were "likely to adversely modify" critical habitat because of injury to plants. They expressed concern about toxicity to plants from agricultural applications near riparian zones in the floodplains, for example (NMFS 2011, p, 540 – 543). Riparian vegetation "provides shade, bank stabilization, sediment, chemical and nutrient filtering, and provides a niche for the terrestrial invertebrates that are also salmon prey items…We believe the a.i. [2,4-D] will have a detrimental effect on riparian vegetation…" (NMFS 2011, p. 627 – 628).

Again, many threatened and endangered aquatic species will have similar habitat requirements for water quality and prey, including some that are in habitats near corn cultivation and thus could be impacted by the increased use of 2,4-D on DAS-40278-9 crop systems.

EPA has not yet gone through a "pesticide effects determination" for any listed species and quizalofop, although they are in the process of reviewing the registration of this herbicide and will address endangered species risks then. It is reasonable to assume that quizalofop will also have negative impacts on vegetation and thus habitats and prey of listed species.

Because of these determinations regarding 2,4-D and CRLF, AW and Pacific salmonids, combined with scientific studies on impacts of herbicides on biodiversity, APHIS should initiate consultations with FWS and NMFS concerning the approval of the DAS-40278-9 crop system.

Ingestion of DAS-40278-9 corn by listed species

Finally, APHIS did not take into account the potential toxicity of DAS-40278-9 corn to listed species that might eat corn leaves, roots, stems, or flower parts. APHIS said corn is not a host to any of the listed species they determined might be in the corn growing areas. Without seeing the list of species that APHIS considered, it is not possible to assess their assertion that corn is not a "host", but being a "host" is perhaps not the same as being a "food item". Migrating birds, for example, eat parts of the corn plant. Bees consume the pollen, and it can comprise more than half of the pollen collected (see pollen gene expression, Part 3, these Comments), and presumably other insects also utilize corn pollen, leaves and so on. Corn detritus washes into wetlands. And so on.

If any listed species do consume corn – and FWS should be involved in determining that - then APHIS must consider the differences in composition between DAS-40278-9 corn and its conventional counterparts. They reiterate that "[t]he results presented by DAS show that the incorporation of the aad-1 gene and the attendant expression of the AAD-1 protein in DAS-40278-9 corn does not result in any biologically-meaningful differences between DAS-40278-9 corn and the non-transgenic hybrid." (DEA p. 117). Again, this ignores the body of scientific information to the contrary, showing that when DAS-40278-9 corn encounters 2,4-D, metabolites form that are similar to known toxins. This should trigger a consultation with FWS (DEA, D-2, decision tree).

References cited in Part 1

Al-Kanani, T., MacKenzie, A.F. (1992). Effect of tillage practices and hay straw on ammonia volatilization from nitrogen fertilizer solution. Can. J. Soil Sci. 72:145-157.

Aulakh, M.S., Rennie, D.A., Paul, E.A. (1984). Gaseous N losses from soils under zero-till as compared to conventional-till management systems. J. Environ. Qual. 13:130-136.

Baker, J.L., Shiers, L.E. (1989). Effects of herbicide formulation and application method on washoff from corn residue. Trans. ASAE. 32: 830-833.

Baker, J.M., T.E. Ochsner, R.T. Venterea and T.J. Griffis (2007). Tillage and soil carbon sequestration – What do we really know? Agriculture, Ecosystems and Environment 118: 1-5.

Ball, B.C., Scott, A., Parker, J.P. (1999). Field N2O, CO2, and CH4 fluxes in relation to tillage, compaction and soil quality in Scotland. Soil Tillage Res. 53:29-39.

Benbrook, C (2012). "2,4-D Use on Corn: Historical Trends and Likely Upper End Reliance in 2019 With and Without Herbicide-Tolerant (HT) 2,4-D Corn," from presentation entitled: "The Good, the Bad, and the Ugly: Impacts of GE Crops in the United States," presented at the conference Pesticides: Domestic and International Perspectives from Science, Law, and Governance, National Academy of Sciences Beckman Center, Irvine, California, April 12, 2012.

Benbrook, C (2009a). "Impacts of Genetically Engineered Crops on Pesticide Use: The First Thirteen Years," The Organic Center, November 2009.

Blanco-Canqui, H. and R. Lal (2008). No-tillage and soil-profile carbon sequestration: an on-farm assessment. Soil Sci. Soc. Am. J. 72: 693-701.

Blewett, T.C., 2011. Supplemental Information for Petition for Determination of Non-regulated Status for Herbicide Tolerant DAS-40278-9 Corn:
Stewardship of Herbicide Tolerant Trait Technology for DAS-40278-9 Corn, Dow AgroSciences (in the APHIS docket)

Brooke, R., G. Fogel, A. Glaser, E. Griffin and K. Johnson, 2009. Corn ethanol and wildlife: how increases in corn plantings are affecting habitat and wildlife in the Prairie Pothole Region. A University of Michigan study published by the National Wildlife Federation, http://www.nwf.org/News-and-Magazines/Media-Center/Reports/Archive/2010/Corn-Ethanol-And-Wildlife.aspx, full report at http://www.nwf.org/News-and-Magazines/Media-Center/Reports/Archive/2010/~/media/PDFs/Wildlife/01-13-10-Corn-Ethanol-Wildlife.ashx

Boutin, C. A. Baril, and P.A. Martin, 2008. Plant diversity in crop fields and woody hedgerows of organic and conventional farms in contrasting landscapes. Agriculture Ecosystems and Environment 123: 185 – 193.

Brower, L.P., O.R. Taylor, E.H. Williams, D.A. Slayback, R.R. Zubieta and M.I. Ramírez, 2011. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity 2011; doi: 10.1111/j.1752-4598.2011.00142.x

Comis, D. (2005). Smoking out worms. Agricultural Research/September 2005: 10-11.

Coughenour, DM & S. Chamala (2000). Conservation Tillage and Cropping Innovation: Constructing the New Culture of Agriculture. Iowa State University Press, Ames, Iowa, 2000.

Davis, A.S., 2010. Cover-crop roller-crimper contributes to weed management in no-till soybean. Weed Science 58 (3): 300 – 309.

Fabrizzi, K.P., Garcia, F.O., Costa J.L., Picone, L.I., (2005). Soil water dynamics, physical properties and corn and wheat responses to minimum and no-tillage Systems in the southern Pampas of Argentina. *Soil Tillage Res*. 81:57-69.

Freemark, K. and C. Boutin, 1995. Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes: A review with special reference to North America. Agriculture, Ecosystems and Environment 52: 67 – 91.

Gove, B., S.A. Power, G.P. Buckley and J. Ghazoul, 2007. Effects of herbicide spray drift and fertilizer overspray on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys. Journal of Applied Ecology 44(2): 374 – 384.

Hartzler, R.G., 2010. Reduction in common milkweed (*Asclepias syri*aca) occurrence in Iowa cropland from 1999 to 2009. Crop Protection 29: 1542 – 1544.

Hebert VR, 2004. Regional off-target movement of auxin-type herbicides. Proceedings of the International Conference on Pesticide Application for Drift Management, Kona, Hawaii. 178-183. October 27, 2004. http://pep.wsu.edu/drift04/pdf/proceedings/pg178-183_Hebert.pdf

Kleijn, D. and G.I.J. Snoeijing, 1997. Field boundary vegetation and the effects of agrochemical drift: botanical change caused by low levels of herbicide and fertilizer. The Journal of Applied Ecology 34(6): 1412 – 1425.

Laws, F. (2006). “Glyphosate-resistant weeds more burden to growers’ pocketbooks,” Delta Farm Press, Nov. 27, 2006.
http://deltafarmpress.com/news/061127-glyphosate-weeds/

Lee, H.E., C.A. Burdick and D. M. Olszyk, 2005. GIS-based risk assessment of pesticide drift case study: Fresno County, California. US EPA/600/R-05/029, March 2005. http://www.epa.gov/wed/pages/publications/authored/EPA600R-05029PesticideDriftLee.pdf

Linn, D.M., Doran, J.W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J. 48:1267-1272.

Malhi, S.S., M. Nyborg and E.D. Solberg, (1996). Influence of source, method of placement and simulated rainfall on the recovery of N-labeled fertilizers under zero-tillage. Canadian Journal of Soil Science 76: 93-100.

Martin, D.C., Baker, J.L., Erbach, D.C., Johnson, H.P., (1978). Washoff of herbicides applied to corn residue. Trans. ASAE. 21: 1164-1168.

Mickelson, S.K., Boyd, P., Baker, J.L., and S.I. Ahmed, (2001). Tillage and herbicide incorporation effects on residue cover, runoff, erosion, and herbicide loss. Soil Tillage Research 60: 55-66.

Mosier, A.R., Halvorson, A.D., Reule, C.A., Xuejun, J.L. (2006). Net global warming potential and greenhouse gas intensity in irrigated cropping systems in northeastern Colorado. J. Environ. Qual. 35:1584-1598.

NMFS (2011). "Biological Opinion: Endangered Species Act Section 7 Consultation with EPA on Registration of 2,4-D, Triclopyr BEE, Diuron, Linuron, Captan and Chlorothalonil," National Marine Fisheries Services, June 30, 2011. http://www.nmfs.noaa.gov/pr/pdfs/consultations/pesticide_opinion4.pdf

NRC (2010). "The Impact of Genetically Engineered Crops on Farm Sustainability in the United States," National Research Council, National Academy of Sciences, 2010, p. 75. http://www.nap.edu/openbook.php?record_id=12804&page=75

NRCS (2010). "2007 National Resources Inventory: Soil Erosion on Cropland," USDA NRCS, April 2010. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_012269.pdf

Olszyk, D.M., C.A. Burdick, T.G. Pfleeger, E.H. Lee and L.S. Watrud, 2004. Assessing the risks to non-target terrestrial plants from herbicides. Journal of Agricultural Meteorology 60(4): 221 – 242.

Peterson, R.K.D. and A.G. Hulting, 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. Weed Science 52(5): 834 – 844.

Pleasants, J.M. and K.S. Oberhauser, 2012. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity doi: 10.1111/j.1752-4598.2012.00196.x

Rasmussen, N., 2001. Plant hormones in war and peace: science, industry, and government in the development of herbicides in 1940s America. Isis 92(2): 291 – 316.

Rice, C.W. , Smith, M.S. (1982). Denitrification in no-till and plowed soils. Soil Sci. Soc. Am. J. 46:1168-1173.

Rochette, P., Angers A.A., Chantigny M.H., MacDonald J.D., Bissonnette N., Bertrand N. (2009). Ammonia volatilization following surface application of urea to tilled and no-till soils: A laboratory comparison. Soil Tillage Res. 103)2): 310-315.

Scherder, E.F., S.C. Ditmarsen, C.A. Gallup, P.L. Prafike, and M.W. Melichar, 2012. Enlist corn tolerance and weed control with pre followed by post herbicide programs (Abstract). 2012 Weed Science Society of America Annual Meeting, Waikoloa, HI, February 6 – 9. http://wssaabstracts.com/public/9/abstract-256.html

Shipitalo et al (2004). "Interaction of earthworm burrows and cracks in a clayey, subsurface-drained, soil," Applied Soil Ecology 26: 209-217.

Smith, K.A., and Conen, F. (2004). Impacts of land management on fluxes of trace greenhouse gases. Soil Use and Management. 20: 255-263.

Sommer S.G., Schjoerring, J.K., Denmead, O.T., (2004). Ammonia emission from mineral fertilizers and fertilized crops. Adv. Agron. 82: 557-622.

Tebrugge, F. and R.A. During, (1999). Reducing tillage intensity: a review of results from a long-term study in Germany. Soil Tillage Research 53:15-28.

USDA ERS (2002). Adoption of Bioengineered Crops. AER 810, Economic Research Service, USDA, May 2002.

USDA NASS AgChem (2006). "Agricultural Chemical Usage: 2006 Field Crop Summary," USDA National Agricultural Statistics Service.

USDA-NRCS. (2006b). Conservation Resource Brief: Soil Erosion, Number 0602 Retrieved November 9, 2010, from http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_023234.pdf (as cited in DEA)

US-EPA, 2009. Risks of 2,4-D Use to the Federally Threatened California Red-legged Frog (*Rana aurora draytonii*) and Alameda Whipsnake (*Masticophis lateralis euryxanthus*), Pesticide Effects Determination, Environmental Fate and Effects Division Office of Pesticide Programs Washington, D.C. 20460, February 20, 2009 http://www.epa.gov/espp/litstatus/effects/redleg-frog/2-4-d/analysis.pdf; Appendix H, EIIS Incident Data As of December 15, 2008 http://www.epa.gov/espp/litstatus/effects/redleg-frog/

US-EPA, 2011. EPA Registration # 62719-640, GF-2727 Herbicide, Decision Number 442889.

Watrud, L.S., G. King, J.P. Londo, R. Colasanti, B.M. Smith, R.S. Waschmann and E.H. Lee, 2011. Changes in constructed *Brassica* communities treated with glyphosate drift. Ecological Applications 21(2): 525 – 538.

Science Comments II
Part 2: Metabolism

Impacts of the activity of AAD-1 enzyme in DAS-40278-9 corn

According to APHIS, an important part of determining the "plant pest characteristics" of DAS-40278-9 corn is careful analysis of "expression of the gene product" and "new enzymes or changes to plant metabolism", and "the possibility of effects of the regulated article on non-target organisms". (PPRA, p. 7)

APHIS concurred with DAS' Petition that there is "no material difference in compositional and nutritional quality of DAS-40278-9 corn compared to other commercially available corn apart from the presence of the AAD-1 protein." (PPRA, p. 8). APHIS thus determined that there would be no impact to non-target organisms, since the composition of the corn is the same as conventional corn except for the novel AAD-1 protein that is non-toxic. (PPRA, p. 12; EA, p. 75, 86)

However, APHIS' analysis is incomplete because it does not take into account differences in composition of DAS-40278-9 corn that result from the *activity* of the novel AAD-1 protein. In fact, the AAD-1 protein is an enzyme that acts on substrates likely to be encountered by DAS-40278-9 corn to produce metabolites missing or at much lower levels in non-engineered corn (Fueng et al. 1978, Hamburg et al. 2001, Laurent et al. 2000), and some of these metabolites are suspected of being toxic to animals via ingestion (Pascal-Lorber et al. 2012, Edwards and Hutson 1986).

Specifically, the AAD-1 protein in DAS-40278-9 corn is an enzyme that breaks down several herbicides into their corresponding phenols. The herbicide substrates include some that are highly likely to be present in the environment of this corn, either because the herbicides are applied directly to the corn, or contact the corn via drift. DAS claims that "…AAD-1 is able to degrade the R-enantiomers (herbicidally active isomers) of chiral phenoxy auxins (e.g., dichlorprop and mecoprop) in addition to the achiral phenoxy auxins (e.g., 2,4-D, MCPA, 4-chrophonoxyacetic acid. See Table 1. Multiple mixes of different phenoxy auxin combinations have been used globally to address specific weed spectra and environmental conditions in various regions. Use of the AAD-1 gene in plants would afford protection to a much wider spectrum of phenoxy auxin herbicides, thereby increasing the flexibility and spectra of weeds that can be controlled, protecting from drift or other off-site phenoxy herbicide injury for the full breadth of commercially available phenoxy auxins." (DAS patent 2009, p. 4 – 6) The enzyme also degrades "…a host of commercial and non-commercial graminicidal compounds of the general class aryloxyphenoxypropionates (AOPPs). See Table 2." (DAS patent 2009, p. 6) Although the AAD-1 enzyme degrades all these herbicides, DAS has only applied for registration at this time of 2,4-D in the phenoxy auxin group, and Quizalofop in the AOPP group for direct use over the top of DAS-40278-9 corn. Other herbicide substrates may be present from drift or off-label use, though.

To assess risk to non-target organisms, then, APHIS needs to know if this new enzyme AAD-1 changes *metabolism* in DAS-40278-9 corn such that the plant has a new composition, and thus has the potential to harm non-target species.

This is not a new concern. The issue of toxins resulting from engineered 2,4-D resistance was raised in 1992 by Rebecca Goldburg, then at Environmental Defense Fund:

> Both the degradation products and accumulation of herbicides in tolerant plants need to be considered before plants can be accepted as safe.
>
> First, 2,4-D resistance can be achieved by transforming plants with a gene coding the enzyme that catalyzes the first step in the bacterial 2,4-D degradative pathway. Degradation of 2,4-D results in the formation of 2,4-dichlorophenol (2,4-DCP)", a toxic substance. (Goldburg 1992, p. 650; internal citations removed)

Since then, there has been a series of peer-reviewed, independent studies on whether 2,4-DCP or other potentially toxic metabolites do accumulate differently in 2,4-D resistant crops than conventional ones, and whether these metabolites could pose a risk to animals ingesting the plants (Laurent et al. 2000, Laurent et al. 2006, Pascal-Lorber et al. 2012). APHIS did not cite or discuss any of these relevant studies in the PPRA or in the EA.

Cotton has been engineered with an enzyme similar to AAD-1 - tfdA – that also breaks down 2,4-D into 2,4-DCP, and the metabolism of 2,4-D was followed in this cotton compared to non-engineered wild-type cotton (Laurent et al. 2000, Laurent et al. 2006). Both engineered and wild-type cotton converted 2,4-D to 2,4-DCP, but in engineered cotton "2,4-D was entirely transformed into DCP, whereas in wild[-type] cotton almost no DCP appeared." (Laurent et al. 2006) This is the first important fact, then: DCP is a major metabolite in 2,4-D resistant cotton, but not in cotton that lacks the engineered enzyme.

Also, there was a new metabolite in the engineered cotton treated with 2,4-D: DCP-glucosyl sulfate. This did not appear in wild-type cotton treated with 2,4-D but did when wild-type cotton was fed DCP directly. These researchers showed that DCP metabolism was similar in transgenic cotton plants whether it came from metabolism of 2,4-D by the novel enzyme, or from DCP supplied in the nutrient solution. They deduced that they could study the fate of DCP in different non-transformed species by just adding DCP to leaves via their petioles, and that the metabolites that form will predict what would have happened had the plants been engineered to convert 2,4-D to DCP. This allowed them to study the types of metabolites that would form if plants other than cotton were given enzymes similar to AAD-1 and tfdA, without injuring the experimental subjects with 2,4-D. They examined DCP metabolism in tomato, sugar beet, potato, and rapeseed (Laurent et al. 2006); wheat and soybean (Pascal-Lorber et al. 2003); and radish, lettuce and spinach (Pascal-Lorber et al. 2008).

Combining results of these studies, it is clear that when 2,4-D is metabolized to 2,4-DCP by the engineered enzyme, this DCP is rapidly converted into conjugated forms – sugars and other molecules are added onto the DCP, depending on the plant species. These conjugated forms of DCP are stable in the plants, and might be converted back to free DCP during the digestive process in animals, posing a possible health risk:

> Offsetting the agronomic benefits of this [engineering for resistance] 2,4-D tolerant crops could increase the food safety risk, even though free DCP has not been directly found in

> plants since it is rapidly metabolised to glucoside conjugates. However, after ingestion by humans or animals, these latter could be hydrolysed by intestinal microflora in the intestinal lumen, thus liberating the toxic aglycone in the gastrointestinal tract, with subsequent absorption by intestinal mucosa. Moreover, DCP could be converted into a more toxic compound in animals. The use of 2,4-D for transgenic tolerant crops would increase the risk of health effects from exposure to this herbicide as compared with 2,4-D treatments of wild[-type] crops." (Laurent et al. 2006, p. 563; internal citations omitted)

In the latest work from the Laurent lab, the ability of animals to absorb conjugates of DCP from plants in their diet was tested directly (Pascal-Lorber et al. 2012). They first supplied radioactive DCP to radish plants via their roots. Various extracted fractions of the radishes were then fed to rats to see what happened to the DCP and DCP conjugates afterwards. They conclude:

> This study clearly demonstrates that the soluble fraction of DCP residues present in plants is bioavailable in mammals, whereas bound residues are not absorbed**. Plant DCP conjugates are likely to contribute substantially to the exposure of the general population to DCP.** This observation may be probably expanded to several categories of chemicals, including pesticides. Accordingly, our data suggest that extractable residues should then be taken into account in occurrence data regarding residues of pesticide treatment in plants. ...
>
> In conclusion, the present work focused on the comparison of oral administration to rats of DCP with plant residues, DCP- (acetyl)glucose, soluble, total, and bound residues from radish plants. DCP was rapidly absorbed and eliminated in urine equally in the form of sulfate and glucuronide conjugates. A new metabolite was also detected and characterized as a dehydrated glucuronide conjugate of DCP. DCP-(acetyl)glucose exhibited a urinary metabolic profile similar to that of DCP. Plant conjugates of DCP were absorbed in the digestive tract of rats, and the major part was eliminated in urine subsequent to biotransformation in sulfate and glucuronide conjugates. **Living organisms are thus exposed to DCP through the food chain.** The vegetal matrix seemed to influence the metabolic profiles derived from soluble and total residues as urinary elimination proceeded predominantly through glucuronidation. In addition, a plant matrix effect was also evidenced because the behavior of total residues was different (less absorption occurred) from that expected from soluble and bound residues. Bound residues seemed to be unabsorbed under our conditions and consequently should be not bioavailable and of limited toxicological concern. (Pascal-Lorber et al. 2012, p. 1734 - 1735; emphases added).

Given these results, it is important for APHIS to consider whether DCP and its conjugates are present in soluble fractions of DAS-40278-9 corn after the AAD-1 enzyme acts upon 2,4-D in order to fully assess the “plant pest risks” to non-target organisms.

DAS has in fact done studies of 2,4-D metabolism in DAS-40278-9 corn, including levels of DCP and DCP-conjugates (Ma and Adlelfinskaya 2010, Rotondaro and Balcer 2010, Stagg et al. 2010, Culligan 2010; DAS Petition 2011, p. 18). Such studies are clearly relevant to an assessment of non-target impacts of DAS-40278-9 corn.

APHIS alludes to these studies of herbicide residues and metabolites when discussing food safety for humans, referring to "... a comprehensive safety assessment of food derived from DAS-40278-9 corn" done by ANZFS (Australia/New Zealand Food Standards) (DEA, p. 97, 105). APHIS includes the following quote from the ANZFS report:

> The major residues generated on corn line DAS-40278-9 as a result of spraying with 2,4-D and Quizalofop-P-ethyl are not novel. The residues are the same as those found on conventional crops sprayed with 2,4-D or Quizalofop-P-ethyl. Residue data, derived from supervised trials, indicate that the residue levels for both herbicides are below the limit of quantitation. In the absence of any measurable exposure to either parent herbicide or their metabolites, the risk to public health and safety is likely to be negligible. (ANZFS 2011 as cited in DEA, p. 97)

However, humans eat mainly corn grain and derivatives, whereas other animals may consume leaves, stems, roots, pollen and other flower parts. These plant parts are likely to have higher herbicide residues and metabolites. Also, since studies by Laurent and his team have shown that novel metabolites are to be expected, APHIS should examine the studies upon which ANZFS has based their conclusion that residues are the same.

The DAS studies of residues and metabolites in DAS-40278-9 corn are available on the ANZFS website (http://www.foodstandards.gov.au/foodstandards/applications/applicationa1042food4758.cfm; click on the "Application" zip file). The key studies (Ma & Aldelfinskaya 2010, Rotondaro and Balcer 2010, Stagg et al. 2010, Culligan 2010) show that DAS-40278-9 corn plants (and a different transformation event with the same enzyme) do have measurable herbicide residues, that DCP levels are low, but that DCP-conjugates make up a significant portion of the metabolites in forage and fodder (e.g., Rotondaro and Balcer 2010, p. 12), as expected from the independent studies discussed above. And although DAS claims that these DCP conjugates are no different from those in conventional corn, there are no data or cited references to support their assertion (e.g., Rotondaro and Balcer 2010, p. 31-32). DAS did not include a conventional corn control in their residue and metabolism studies. In fact, it would be surprising if conventional corn made much DCP at all after application of 2,4-D (Feung et al. 1978, Hamburg et al. 2001), and therefore DCP conjugates would be an unlikely metabolite. Note that DAS did not cite or discuss any of these independent studies in their reports.

Although DCP conjugates were identified as major metabolites in DAS' nature of residue studies, this information was not incorporated into the magnitude of residue studies used to determine whether DAS-40278-9 corn would meet tolerance requirements for residues in forage and fodder. By leaving out the conjugates, it appeared as if the DCP "disappeared" rather than being stored in a form that could release DCP again later. (Culligan 2010, e.g.,p. 17). If the DCP conjugates that DAS measured in DAS-40278-9 corn were added to the total residue of 2,4-D in forage and fodder, assuming the same toxicity as free DCP and 2,4-D, it is possible that the tolerance levels set for forage and fodder would be exceeded (Stagg et al. 2010).

However, particularly for wild animals, the DAS nature and magnitude of residue studies are not adequate for determining the impacts of DAS-40278-9 corn. DAS designed their studies to simulate field conditions assuming application rates and timings according to the label, and product use by livestock and humans, not by the insects, birds, reptiles, amphibians, and other animals that visit cornfields.

DAS applied the last 2,4-D treatment at the V-8 stage, as per label, and then waited about a month before taking their first samples of forage at the customary time. Wild animals would not necessarily wait a month after an application before ingesting corn tissues. Residues and metabolites of 2,4-D are likely to be higher, and present in more soluble and thus digestible form closer to the time of application, so the food safety studies underestimate exposure for wildlife.

It is also possible that DAS-40278-9 corn could be sprayed later than the V-8 stage if farmers decide to do an off-label salvage treatment of uncontrolled weeds, as sometimes occurs (herbicide use, CFS Science Comments I); or DAS-40278-9 corn could be exposed to drift from a variety of herbicide substrates of the engineered enzyme, as suggested by DAS (DAS patent 2009, p. 6). The nature and magnitude of residue studies did not take into account any scenario other than “on label” applications, but APHIS needs to consider these reasonable possibilities since they are likely to result in higher levels of 2,4-D residues and DCP conjugates.

Clearly, APHIS should prepare an Environmental Impact Statement to assess the characteristics of DAS-40278-9 corn conferred by the activity of the novel enzyme AAD-1 and potential impacts.

References cited in Part 2

Culligan, J.F., 2010. Magnitude of the residue of 2,4-D and quizalofop-p-ethyl in/on herbicide tolerant field corn containing the aryloxyalkanoate dioxygenase-1 (ADD-1) gene. Dow AGroSciences Study Number ARA-09-15-10, Study ID Number 090052. http://www.foodstandards.gov.au/foodstandards/applications/applicationa1042food4758.cfm; click on the “Application” zip file.

DAS Patent 2009. US Patent Application Publication, US 2009/0093366 A1, Apr. 9, 2009. Novel Herbicide Resistance Genes, Dow AgroSciences LLC, inventors: Wright, T.R., J.M. Lira, D.J. Merlo and N.L. Arnold.

Edwards, V.T. and D.H. Hutson, 1986. The disposition of plant xenobiotic conjugates in animals. In: Xenobiotic Conjugation Chemistry; Paulson, G. et al; ACS Symposium Series, American Chemical Society: Washington DC.

Feung, C.S., S.L. Loerch, R.H. Hamilton and R.O. Mumma, 1978. Comparative metabolic fate of 2,4-dichlorophenoxyacetic acid in plants and plant tissue culture. Journal of Agricultural and Food Chemistry 26(5): 1064 – 1067.

Goldburg, R.J., 1992. Environmental concerns with the development of herbicide-tolerant plants. Weed Technology 6(3): 647 – 652.

Hamburg, A., V. Puvanesarajah, T.J. Burnett, D.E. Barnekow, N.D. Prekumar and G.A. Smith, 2001. Comparative degradation of [^{14}C]-2,4-dichlorophenoxyacetic acid in wheat and potato after foliar application and in wheat, radish, lettuce, and apple after soil application. Journal of Agricultural and Food Chemistry 49: 146 – 155.

Laurent, F., L. Debrauwer, E. Rathahao and R. Scalla, 2000. 2,4-Dichorophenoxyacetic acid metabolism in transgenic tolerant cotton (*Gossypium hirsutum*). Journal of Agricultural and Food Chemistry 48: 5307 – 5311.

Laurent, F., L. Debrauwer and S. Pascal-Lorber, 2006. Metabolism of [^{14}C]-2,4-dichlorophenol in edible plants. Pest Management Science 62: 558 – 564.

Ma, M. and Y.A. Adelfinskaya, 2010. A nature of the residue study with [14C]-2,4-D DMA applied to AAD-1 Corn (Event 278). Dow AGroSciences Study ID Number 090058. http://www.foodstandards.gov.au/foodstandards/applications/applicationa1042food4758.cfm; click on the "Application" zip file.

Pascal-Lorber, S., E. Rathahao, J.-P. Cravedi and F. Laurent, 2003. Uptake and metabolic fate of [^{14}C]-2,4-dichlorophenol in wheat (*Triticum aestivum*) and soybean (*Glycine max*). Journal of Agricultural and Food Chemistry 51: 4712 – 4718.

Pascal-Lorber, S., S. Despoux, E. Rathahao, C. Canlet, L. Debrauwer and F. Laurent, 2008. Metabolic fate of [^{14}C] cholorphenols in radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), and spinach (*Spinacia oleracea*). Journal of Agricultural and Food Chemistry 56: 8461 – 8469.

Pascal-Lorber, S., S. Despoux, E.L. Jamin, C. Canlet, J.-P. Cravedi and F. Laurent, 2012. Metabolic fate of 2,4-dichlorophenol and related plant residues in rats. Journal of Agricultural and Food Chemistry 60: 1728 – 1736.

Rotondaro, S.L. and J.L. Balcer, 2010. A nature of the residue study with [14C]-2,4-D Applied to AAD-1 corn, 2008. Dow AGroSciences Study ID Number 080058. http://www.foodstandards.gov.au/foodstandards/applications/applicationa1042food4758.cfm; click on the "Application" zip file.

Stagg, N.J., C.B. Cleveland, D.L. Eisenbrandt, T.C. Blewett, S.W. Rosser, B.B. Gollapudi, E.W. Carney and R.G. Ellis-Hutchings, 2010. 2,4-Dichlorophenol: relevance for 2,4-D treated corn containing the DAS AAD-1 trait. Dow AGroSciences Study ID Number 101759. http://www.foodstandards.gov.au/foodstandards/applications/applicationa1042food4758.cfm; click on the "Application" zip file.

Impacts of high expression of AAD-1 in DAS-40278-9 corn pollen

In determining whether DAS-40278-9 corn has unique characteristics that would make it more of a "plant pest risk" than conventional corn, APHIS said they considered "expression of the gene product", and "new enzymes or changes to plant metabolism", based on data presented by DAS in their Petition (PPRA, p. 7).

DAS measured levels of the engineered gene product AAD-1 in different parts of DAS-40278-9 corn, sprayed with 2,4-D and/or quizalofop, or not (Petition, p. 60, Table 8). In general, average AAD-1 protein levels were between ~3 and 14 nanograms per milligram of tissue dry weight in leaves, roots, and whole plants. However, pollen had much higher levels, with averages between 108 and 127 ng/mg dw. Thus, gene expression in pollen was between 8- and 42-fold higher than in other plant parts.

Rather than investigate whether this high level of expression in pollen might be of significance to the characteristics of DAS-40278-9 corn in various scenarios, DAS simply stated that the level of AAD-1 protein in pollen was "…in a similar range as the transgenic herbicide tolerance protein in another commercial trait", which was the enzyme conferring glyphosate resistance to corn in event MON 88017, without further elaboration (Petition, p. 59).

This total lack of commentary by DAS and APHIS regarding high expression levels in pollen is curious, given the importance of good expression of the transgene in pollen for overall performance of some glyphosate resistant crops. In Roundup Ready cotton, low transgene expression in pollen and other male reproductive cell types limits the window when glyphosate applications can be made and also the maximum rate that can be used, and is seen as a problem for effective season-long weed control (Chen et al. 2006; Yasuor et al. 2006, 2007). Companies touted the fact that a second-generation glyphosate resistance trait in cotton has better expression of the EPSPS enzyme in pollen, and thus can withstand higher rates of glyphosate during more of the growing season (Chen et al. 2006, May et al. 2004, Monsanto 2005).

Levels of gene expression in pollen have also been of great interest for Bt crops. Corn pollen expressing cry proteins is toxic to some insects should they happen to ingest the pollen in sufficient quantities (Malone and Pham-Delègue 2001). Expression levels in pollen of different corn Bt events have been compared, and there is a wide range of cry protein levels and specificities, and thus potential for harm to butterflies, for example (e.g., Mattila et al. 2005). The potential risk of exposure to Bt via pollen has been investigated for the endangered Karner Blue butterfly (Peterson et al. 2006), identifying counties where butterfly sites are located near cornfields and thus need further study.

These precedents should have prompted a discussion of whether the high AAD-1 levels in pollen of DAS-40278-9 corn are correlated with good reproductive tolerance to 2,4-D and quizalofop, and if so, what consequences this might have for how farmers use herbicides on the crop and subsequent impacts.

Also, assuming that the AAD-1 protein is metabolically active in pollen, it is important to know whether pollen has the same composition in DAS-40278-9 corn as in conventional corn, particularly when challenged with 2,4-D, quizalofop, or other herbicides that are substrates of the enzyme. Specifically, does this pollen contain residues and metabolites not found in conventional pollen that might make it toxic to any of the wide array of organisms that feed on corn pollen (Lundgren 2009, Section II: Pollinivory, Ch.6, Ch. 8), such as honey bees, for example?

Consequences of reproductive tolerance

Surprisingly, DAS does not mention in the Petition or in supplementary materials whether or not DAS-40278-9 corn can be sprayed after the V8 stage of development without injury. Although V8 is the latest stage that DAS proposes for 2,4-D applications, this is well before tassel maturation and pollen shed, and is also early enough in the season that there could be weed problems after that in some conditions. If later applications of 2,4-D are safe for DAS-40278-9 corn, and weed pressure occurs, surely some growers will figure this out and will take the risk of doing an application that is not warranted by DAS or allowed in the label.

Applications later in the season may result in higher 2,4-D residues and metabolites in forage and fodder, grain, and also in pollen itself. Drift from later applications may impact different crops and wild species than earlier applications. (Olszyk et al. 2004)

DAS is undoubtedly aware that growers will use the technology in ways that are most useful to them (Anderson 2005). In 2005, DAS introduced a Bt cotton event called “WideStrike”, often stacked with a Roundup Ready trait in popular PhytoGen varieties. At this time glyphosate-resistant weeds were becoming a problem in cotton, and “[m]ostly by accident, and often serendipitously, some cotton growers found they could take a shortcut and use Widestrike-containing Phytogen varieties and spray them with glufosinate” (Roberson 2011). DAS had used a glufosinate-resistance gene as a selectable marker in the engineering process, but had not intended it to be used agronomically, so had not advertised this property. Glufosinate is now used, sometimes in addition to glyphosate, to control glyphosate-resistant Palmer amaranth and giant ragweed (Robinson 2010). Growers are willing to risk some injury to their cotton, and the loss of warranty from DAS, to manage these intractable weeds. Both DAS and Bayer, the maker of the herbicide, strongly condemn this practice, but farmers still do it because it is useful. It is also not technically illegal, apparently (“It’s controversial. It breaks rules, but not laws.” Golden 2010), since glufosinate is approved by the EPA for use in cotton at these rates (Roberson 2011).

With such high levels of AAD-1 protein in pollen, it is reasonable to assume that there will be good reproductive tolerance to 2,4-D and quizalofop, although it is not certain without results of tolerance tests. For example, Thomas et al. (2004) reported high levels of EPSPS in pollen of the Roundup Ready corn event NK603, but pollen of that corn was still negatively affected when glyphosate was applied after the V4 stage. APHIS should ask for results of reproductive tolerance tests, and discuss the implications for the "plant pest risk" characteristics of DAS-40278-9 corn that allow it to be sprayed at or closer to pollen shed.

Implications of high AAD-1 levels in pollen for bees

APHIS agreed with DAS that expression of AAD-1 would not harm beneficial non-target organisms such as honey bees or earthworms in part because the "…inserted genetic material is not secreted, is not toxic, and does not produce any substance that is secreted or that would be considered toxic." (PPRA, p. 12) However, they did not consider the impacts of the *activity* of AAD-1, particularly in pollen where it is so highly expressed. Nor did DAS include a compositional analysis of pollen of DAS-40278-9 corn with and without 2,4D and/or quizalofop in their Petition (p. 77). Therefore, APHIS' assertion that "DAS-40278-9 is agronomically and compositionally similar to other corn varieties and will not adversely affect other organisms compared to other corn varieties" (PPRA, p. 12) is based on incomplete information.

Corn produces a prodigious amount of large, protein-rich pollen (Lundgren 2009, p. 85; Roulston et al. 2000), and honeybees near cornfields collect this pollen to feed their developing brood. Recent research from USDA scientists at Purdue University in Indiana found that "[m]aize pollen was frequently collected by foraging honey bees while it was available: maize pollen comprised over 50% of the pollen collected by bees, by volume, in 10 of 20 samples." (Krupke et al. 2012, p. 2). A synthesis of 114 data sets in Switzerland showed corn to be the most common pollen source for honey bees there, too, reporting corn in the top five most common pollen sources for over half of the studies, followed by clover and dandelions (Keller et al. 2005).

And many honey bees do live near cornfields during pollination. According to Krupke and Hunt (2012) "…[m]ost commercial pollinator honey bees in the US spend May through October in the Upper Midwest where these crops [e.g., corn] dominate the landscape."

Since corn pollen can make up the bulk of pollen collected by a substantial population of honey bees, it is even more important to know whether pollen of DAS-40278-9 corn accumulates 2,4-D or other herbicide residues and metabolites differently than does conventional corn. However, information on 2,4-D levels in corn pollen was not found in the scientific literature.

If corn encountered 2,4-D during pollen development from direct application or drift, or if 2,4-D or metabolites were redistributed in the plant - from earlier applications - during growth, presence in pollen would be expected. Note that tassels begin to form just two weeks after corn emergence (Thomas et al. 2004, p. 732, citing Kiesselbach 1992). Other phloem-translocated herbicides, such as glyphosate, do accumulate in pollen along with nutrients, because developing anthers and pollen are strong "sinks". There is evidence that 2,4-D also travels to anthers because it causes male-sterility (Hsu and Kleier 1990), similar to the action of glyphosate (Yasuor et al. 2006). Other systemic pesticides are common contaminants of corn pollen (Mullin et al. 2010, Burlew 2010).

Pollen of DAS-40278-9 corn may be protected from the toxic effects of 2,4-D because of the high expression of AAD-1, and so it may accumulate more 2,4-D and other herbicide substrates than conventional corn: if the pollen remains viable, it will be a stronger sink for a longer period of time (Geiger and Bestman 1990, Chen et al. 2006). Also, the profile of metabolites is likely to be different, with more DCP-conjugates, able to release free DCP during use by developing bees.

There have been a few studies showing toxicity of 2,4-D itself to bees. According to Burlew (2010), "…Papaefthimiou et al. (2002) found cell death in the isolated atria of the honey bee heart (*Apis mellifera macedonica*) after exposure to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Only 1 µM (micro mol) of 2,4-D was required to reduce the force and frequency of heart contractions by 70% in 20 minutes. The honey bee is much more sensitive to this chemical than other insects tested, including the beetle *Tenebrio molitor*, which required more than 1000 µM of 2,4-D to produce the same result." (Papaefthimiou C., V. Pavlidou, A. Gregorc , and G.Theophilidis. 2002. The action of 2,4-dichlorophenoxyacetic acid on the isolated heart of insect and amphibian. Environmental Toxicology and Pharmacology 11: 127–140; as cited in Burlew 2010, p. 49.) Also, EPA describes a study feeding 2,4-D-formulation added to sugar water to young bees, and uses the LD50 values from that study in their risk assessments for terrestrial invertebrates (US-EPA 2009, p. 112). EPA also cites a study showing that earthworms are more sensitive to 2,4-DCP than to 2,4-D acid (US-EPA 2009, p. 112). However, because 2,4-DCP is not normally a major degradation product of 2,4-D, EPA discounts the importance of DCP for toxicity (US-EPA 2009, p. 100, citing Wahl and Ulm, 1983). They do not consider DCP conjugates since these also will be at very low levels in conventional plant tissues.

Finally, the AAD-1 enzyme itself may retain its activity in honey, even during digestion by immature bees, able to degrade herbicides brought in from sources other than DAS-40278-9 corn into possible toxins (Grogan and Hunt 1979).

Taken together, the fact that honey bees are likely to collect pollen of DAS-40278-9 corn after it has been sprayed with 2,4-D and other herbicides that are substrates of the engineered AAD-1 protein, and that resulting residues and metabolites are likely to be different from those in conventional corn, APHIS should consider the impacts to honey bees in an Environmental Impact Statement, following guidelines for analyzing impacts of transgene products on bees set out by Malone and Pham-Delègue, for example (2001, p. 299 – 300). Impacts to other animals that are likely to use corn pollen should also be examined in light of the activity of these high levels of AAD-1 in DAS-40278-9 corn pollen.

References cited in Part 3

Anderson, J.L., 2005. War on weeds: Iowa farmers and growth-regulator herbicides. Technology and Culture 46(4): 719 – 744.

Burlew, D.A., 2010. The effects of pesticide-contaminated pollen on larval development of the honey bee, *Apis mellifera*. Master's Thesis, The Evergreen State College, June 2010. http://archives.evergreen.edu/masterstheses/Accession86-10MES/burlew_daMES2010.pdf

Chen, Y.-C. S., C. Hubmeier, M. Tran, A. Martens, R.E. Cerny, R.D. Sammons and C. CaJacob, 2006. Expression of CP4 EPSPS in microspores and tapetum cells of cotton (*Gossypium hirsutum*) is critical for male reproductive development in response to late-stage glyphosate applications. Plant Biotechnology Journal 4: 477-487.

Geiger, D.R. and H.D. Bestman, 1990. Self-limitation of herbicide mobility by phytotxic action. Weed Science 38(3): 324 – 329.

Golden, G., 2010. Growers swing at pigweed with Ignite on WideStrike. Southern Farmer, January 2010, p. 7. http://magissues.farmprogress.com/STF/SF01Jan10/stf007.pdf

Grogan, D.E. and J.H. Hunt, 1979. Pollen proteases: their potential role in insect digestions. Insect Biochemistry 9: 309 – 313.

Hsu, F.C. and D.A. Kleier, 1990. Phloem mobility of xeonbiotics. III. Sensitivity of unified model to plant parameters and application to patented chemical hybridizing agents. Weed Science 38(3): 315 – 323.

Lundgren, J.G., 2009. Relationships of Natural Enemies and Non-Prey Foods. Progress in Biological Control, Vol. 7. (ebook) Springer.com: US Government, eISBN 978-1-4040-9235. Section II Pollinivory; Chapter 6: The Pollen Feeders; Chapter 8: Pollen Nutrition and Defense.

Keller, I., P. Fluri and A. Imdorf, 2005. Pollen nutrition and colony development in honey bees: part I. Bee World 86(1): 3 – 10.

Krupke, C.H. and G.J. Hunt, 2012. Protecting honey bees during corn and soybean planting season. Handout, Webinar presentation, April 9, 2012. https://gomeet.itap.purdue.edu/p32228058/

Krupke, C.H., G.J. Hunt, B.D. Eitzer, G. Andino and K. Given, 2012. Multiple routes of pesticide exposure for hone bees living near agricultural fields. PLoS ONE 7(1): e29268. doi:10.1371/journal.pone.0029268

Malone, L.A. and M.-H. Pham-Delègue, 2001. Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). Apidologie 32: 287 – 304.

Mattila, H.R., M.K. Sears and J.J. Duan, 2005. Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay. Entomologia Experimentalis et Applicata 116: 31 – 41.

May, O.L., A.S. Culpepper, R.E. Cerny, C.B. Coots, C.B. Corkern, J.T. Cothren, K.A. Croon, K.L. Ferreira, J.L. Hart, R.M. Hayes, S.A. Huber, A.B. Martens, W.B. McCloskey, M.E. Oppenhuizen, M.G. Patterson, D.B. Reynolds, Z.W. Shappley, J. Subramani, T.K. Witten, A.C. York and B.G. Mullinix, Jr., 2004. Transgenic cotton with improved resistance to glyphosate herbicide. Crop Science 44: 234-240.

Monsanto, 2005. Roundup Ready Flex Cotton: Technical Bulletin

Mullin, C.A., M. Frazier, J.L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp and J.S. Pettis, 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

Peterson, R.K.D., S.J. Meyer, A.T Wolf, J.D. Wolt and P.M. Davis, 2006. Genetically engineered plants, endangered species, and risk: a temporal and spatial exposure assessment for Karner blue butterfly larvae and Bt maize pollen. Risk Analysis 26(3): 845 – 858.

Roberson, R., 2011. Ignite on Widestrike cotton risky. Southeast Farm Press, 10/28/2011. http://southeastfarmpress.com/print/cotton/ignite-widestrike-cotton-risky

Robinson, E., 2010. Resistant giant ragweed. Delta Farm Press, 5/28/2010. http://deltafarmpress.com/management/resistant-giant-ragweed

Roulston, T.H. and J.H. Cane, 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution 222: 187 – 209.

Roulston, T.H., J.H. Cane and S.L. Buchmann, 2000. What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? Ecological Monographs 70(4): 617 – 643.

Thomas, W.E., W.A. Pline-Srnic, J.F. Thomas, K.L. Edmisten, R. Wells and J.W. Wilcut, 2004. Glyphosate negatively affects pollen viability but not pollination and seed set in glyphosate-resistant corn. Weed Science 52: 725 – 734.

US-EPA, 2009. Risks of 2,4-D Use to the Federally Threatened California Red-legged Frog (*Rana aurora draytonii*) and Alameda Whipsnake (*Masticophis lateralis euryxanthus*), Pesticide Effects Determination, Environmental Fate and Effects Division Office of Pesticide Programs Washington, D.C. 20460, February 20, 2009. http://www.epa.gov/espp/litstatus/effects/redleg-frog/2-4-d/analysis.pdf;
Appendices at http://www.epa.gov/espp/litstatus/effects/redleg-frog/

Yasuor, H., M. Abu-Abied, E. Belausov, A. Madmony, E. Sadot, J. Riov and B. Rubin, 2006. Glyphosate-induced anther indehiscence in cotton is partially temperature dependent and involves cytoskeleton and secondary wall modifications and auxin accumulation. Plant Physiology 141: 1306-1315.

Yasuor, H., J. Riov and B. Rubin, 2007. Glyphosate-induced male sterility in glyphosate-resistant cotton (*Gossypium hirsutum* L.) is associated with inhibition of anther dehiscence and reduced pollen viability. Crop Protection 26: 363 – 369.