THE CENTER FOR
FOOD SAFETY

Docket No. APHIS-2012-0019

Regulatory Analysis and Development, PPD, APHIS
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11 September 2012

Comments to USDA APHIS on Draft Environmental Assessment and Draft Plant Pest Risk Assessment for Dow AgroSciences Petition (09-349-01p) for Determination of Nonregulated Status of Event DAS-68416-4: 2,4-D- and glufosinate-resistant soybean

Center for Food Safety, Science Comments

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1. Notes on science comments

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

2. The impact of approval of DAS-68416-4 soybean on herbicide use

a. Summary of herbicide use

Dow's DAS-68416-4 soybean is genetically engineered for resistance to 2,4-D and glufosinate, and if deregulated would be marketed with additional resistance to glyphosate – fostering greater use of three herbicide classes. APHIS must assess DAS-68416-4 soybean as Dow intends it to be used, as a weed control system. Very little 2,4-D and glufosinate are presently used in soybean production because both herbicides cause severe damage to soybeans. DAS-68416-4 soybean eliminates this risk of crop injury. Depending upon how widely DAS-68416-4 soybeans are grown, they would increase 2,4-D use on soybeans from 3-4 million lbs. at present to 67 to 135 million lbs. per year by 2025; glufosinate use on soybeans would likewise increase from 0.5 million lbs. at present to 19 to 38 million lbs. per year. Glyphosate would also likely continue to be used at high levels, such that overall herbicide use on soybeans would increase dramatically. DAS-68416-4 soybean would also substantially alter herbicide use patterns. The consequences of these substantial changes in farming practice are addressed elsewhere in these comments. The draft EA (DEA) is undermined by numerous, fundamental errors of fact and interpretation; lacks any quantitative assessment of herbicide use aside from speculative and conclusory statements; relies unduly on Dow and other industry sources; neglects to consider quality data from APHIS' USDA sister agencies; and excludes important considerations. As a result, the DEA does not meet the "sound science" standard demanded by NEPA for environmental assessments.

b.Introduction

Dow AgroSciences seeks deregulation of DAS-68416-4 soybean, a variety of soybean genetically engineered to withstand direct application of high rates of phenoxy auxin herbicides, specifically 2,4-dichlorophenoxyacetic acid (2,4-D), as well as glufosinate. Dow will stack DAS-68416-4 soybean with glyphosate-resistance for resistance to three herbicide classes.

Herbicide-resistant (HR) crops are weed control systems involving one or more post-emergence applications of the HR crop-associated herbicide(s). Dow describes DAS-68416-4 soybean in precisely these terms, as the "Enlist Weed Control System" (DAS 2011a), with the brand name "Enlist" referring to both the HR trait and Dow's 2,4-D herbicide. Monsanto describes its HR crops in similar terms: "The utilization of Roundup agricultural herbicides plus Roundup Ready soybean, collectively referred to as the Roundup Ready soybean system..."[1] APHIS must assess both DAS-68416-4 soybean in its own right and as a component of an HR soybean system, in which 2,4-D and glufosinate would be used in different amounts and in altered patterns by virtue of the genetically engineered resistance to these herbicides in DAS-68416-4 soybean seed. The certain use of glyphosate must also be assessed. As shown below, APHIS has failed to provide an adequate assessment of the DAS-68416-4 soybean system.

According to APHIS and Dow, the purpose of the DAS-68416-4 soybean system is give farmers herbicidal options to manage difficult weeds, in particular weeds that have evolved resistance to glyphosate and ALS inhibitor herbicides (Petition at 127). This purposed use of DAS-68416-4 soybean cannot be assessed without careful consideration of the herbicidal components of the system, any more than an automobile can properly assessed without putting gasoline in the tank and subjecting it to a road test. APHIS' failure to assess Monsanto's glyphosate-resistant, Roundup Ready (RR) crop varieties as HR crop systems led to unregulated cultivation, which as discussed elsewhere generated the glyphosate-resistant weed epidemic that is now the rationale for Dow's soybean. APHIS' preferred alternative in the draft Environmental Assessment (DEA) – full deregulation – would repeat this same mistake with the DAS-68416-4 soybean system, and trigger impacts still more severe than did its RR crop predecessors.

APHIS' assessment of herbicide use in the DEA is seriously deficient, and does not meet the "sound science" standard demanded by NEPA for environmental assessments (EAs). The draft EA (DEA) is undermined by numerous, fundamental errors of fact; lacks any quantitative assessment of herbicide use; relies excessively and cursorily upon Dow's conclusions with no critical analysis of the same; and excludes important considerations.

c. DAS-68416-4 soybean will lead to sharply increased use of 2,4-D in American agriculture

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[1] From: "Petition for the Determination of Nonregulated Status for Roundup Ready2Yield Soybean MON89788," submitted to USDA by Monsanto on June 27, 2006 (revised November 3, 2006), APHIS Docket No. APHIS-2006-0195, p. 4).

APHIS concedes that its proposed full deregulation of DAS-68416-4 soybean "has the potential to result in an increase in the amount of 2,4-D that may be used and the time of year that it may be applied on soybeans" (DEA at 79), and that "glufosinate use on soybean could increase" (DEA at 80), yet provides no estimate, however rough, of the magnitude of the expected increase in use of either herbicide. Neither does APHIS offer an assessment of overall herbicide use, but rather merely speculates that: "The 2,4-D applied may likely [sic] replace other herbicides currently used on soybeans, and, as a result, the overall amount of herbicides used on soybeans may not change" (DEA at 79). Such loose and completely undocumented speculation obviously does not qualify as an assessment.

CFS undertakes to provide the missing assessment below, based on: 1) Dow's proposed label for DAS 68416-4; and 2) A projection of herbicide use with synthetic auxin-resistant soybeans by Pennsylvania State University weed scientists Dave Mortensen and colleagues (Mortensen et al 2012).

i. 2,4-D rates and EPA label restrictions

At present, 2,4-D use on soybeans is limited to pre-plant/burndown use at a maximum of 1 lb/acre/year. Dow has proposed a tripling of the label to 3 lbs/acre per year for DAS-68416-4 soybean (see Figure 47 below, from Petition at 126). The preplant/burndown label limit would remain unchanged at 1 lb/acre, while the proposed registration would add two applications of 0.5 to 1 lb/acre POST, for total permitted post-emergence use of 2 lbs/acre/season up through the R2, full-flowering stage of development.

Enlist soybeans would increase both preplant and post-emergence use of 2,4-D. Preplant usage is currently constrained by 2,4-D's (limited) residual activity – the potential for emerging seedlings to be damaged by 2,4-D applied soon before crop emergence. According to Dow (Petition at 123):

> Currently, for soybeans without the *aad-12* gene, 2,4-D can be applied only as a burndown or pre-emergence application at up to 1.0 lb ae/A (1120 g ae/ha). 2,4-D currently cannot be applied at burndown or pre-emergence to conventional soybeans any later than 7-15 days (0.5 - 1.0 lbs ae/A, or 560 - 1120 g ae/ha of ester formulations) or 15-30 days (0.5 – 1.0 lbs ae/A, or 560 - 1120 g ae/ha of amine formulations) prior to planting, due to potential for crop injury.

Thus, Enlist soybeans would eliminate the risk of crop injury from application of 2,4-D at 0-15 days pre-plant (ester formulations) or 0-30 days pre-plant (amine formulations) that obtains for currently grown soybean varieties.[2] This expanded pre-plant application window would permit growers to more fully exploit the 1 lb/acre pre-plant portion of the label. At present, average pre-plant usage of 2,4-D is 0.5 lb/acre, just half that permitted.

However, the much bigger impact would be to enable entirely new post-emergence use of 2,4-D, up to 2 lbs/acre per year. In 2011, fully 94% of U.S. soybean acreage was planted to Roundup

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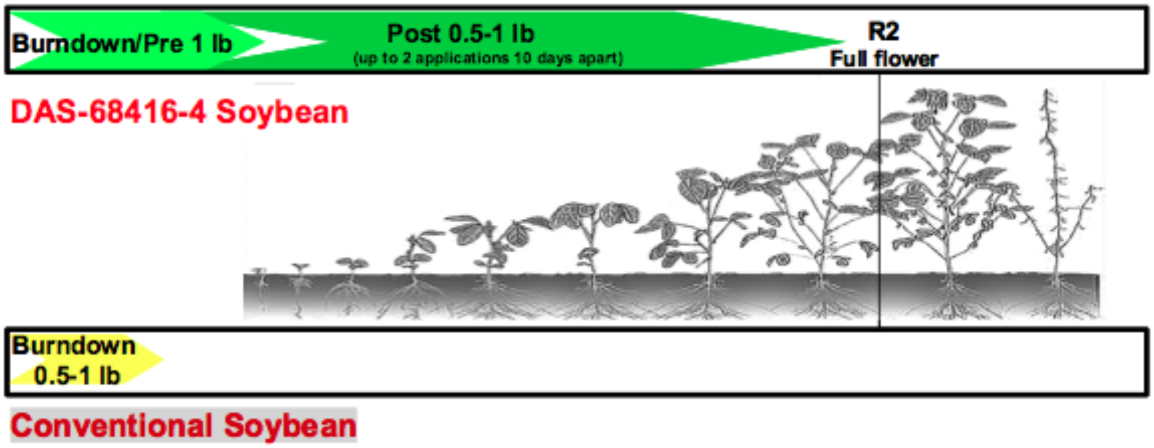
[2] CFS has no information on the residual activity of 2,4-D choline salt, but it is presumably similar.

Ready varieties. Roundup Ready soybeans have fostered a huge shift to weed control programs that rely exclusively or primarily on post-emergence use of glyphosate. Growers who are already accustomed to a POST weed control regime with RR soybeans, and who then switch to adopt Enlist soybeans, would be likely to make substantial use of the post-emergence portion of the label.

USDA NASS data show that glyphosate was applied at an annual rate of 0.63 lbs/acre on cotton, and 0.69 lbs/acre on soybeans, in 1996, at the dawn of the Roundup Ready era. All or nearly all[3] of this usage was pre-plant or pre-emergence, since POST applications would severely damage conventional cotton and soybeans. By 2007, annual glyphosate use on cotton had tripled to 1.89 lbs/acre, while by 2006 glyphosate use on soybeans had doubled to 1.36 lbs/acre (Benbrook 2009a, Table 4.1, p. 29). All or nearly all of this increased use was comprised of POST applications enabled by Roundup Ready versions of these crops, which had become dominant by those years. One can expect similarly sharp increases in post-emergence use of 2,4-D.

Figure 47. 2,4-D herbicide application timing and rates for conventional and DAS-68416-4 soybeans



From Dow Petition (p. 126)

ii. Projection by Mortensen et al. (2012)

A recent peer-reviewed publication in the journal BioScience by Penn State weed scientist David Mortensen and colleagues offers an estimate of increased herbicide use with introduction and widespread adoption of synthetic auxin-resistant soybeans, which includes both DAS-68416-4 soybean and Monsanto's dicamba-resistant soybeans (Mortensen et al. 2012).[4] Figure 4 and its caption from that publication are reproduced below; the caption provides an explanation of the assumptions used in their projection.

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[3] There was limited adoption of RR soybeans in 1996, the first year of its introduction.

[4] 2,4-D and dicamba are closely related members of the synthetic auxin class of herbicides, and are used at similar rates. Mortensen et al address these two HR soybean varieties together for purposes of the projection because they present many of the same risks.

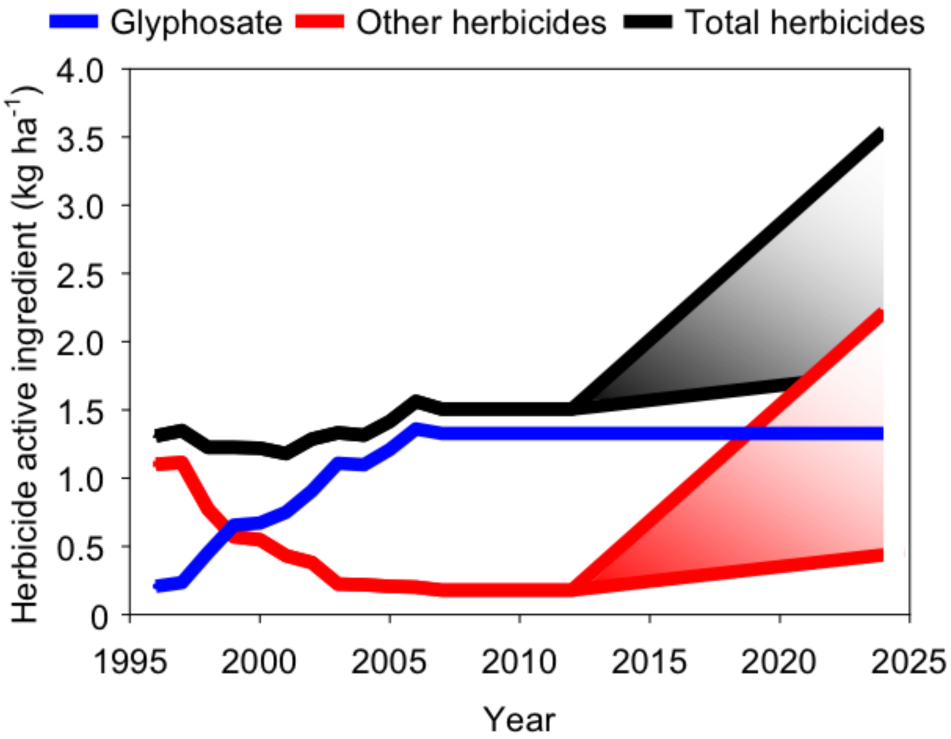

Figure 4. Total herbicide active ingredient applied to soybean in the United States. The data from 1996 to 2007 are adapted from Figure 2-1 in NRC (2010), and the projected data are based on herbicide programs described by Arnevik (2010) and Olson and Peterson (2011). To forecast herbicide rates from 2008 to 2013 we assumed that the applications of glyphosate and other herbicides will remain constant at 2007 levels until 2013, when new resistant soybean varieties are likely to become available. We estimated yearly increases in synthetic auxin herbicides (assumed to drive increases in other herbicides) by assuming that the adoption of stacked synthetic auxin–resistant cultivars mirrors the adoption of glyphosate-resistant cultivars, such that 91% of soybean hectares are resistant to synthetic auxin herbicides within 12 years. We further assumed that all soybean hectares with stacked resistance to glyphosate and synthetic auxin herbicides will receive an annual application of glyphosate and dicamba or 2,4-D. We assumed that the use rates of glyphosate will remain at current levels, and our estimates for dicamba and 2,4-D encompass lower (0.28 kilograms [kg] per hectare [ha]) and higher (2.24 kg per ha) use rates, which are in line with the rates currently used on tolerant crops (i.e., corn and wheat) and with rates being researched and promoted by Dow and Monsanto.

Mortensen et al.'s Figure 4 shows annual baseline herbicide use of roughly 1.5 kg/ha (= 1.34 lbs/acre) from 2007 to 2012. The first auxin-resistant soybean is assumed to be introduced in 2013. The rate of adoption of auxin-resistant crops is projected to mirror that of Roundup Ready crops: from 0 to 91% over 12 years. A broad range of possible auxin usage is projected on auxin-resistant soybean acres: from 0.28 kg/ha/year (0.25 lbs/acre/year) to 2.24 kg/ha/year (2 lbs/acre/year).

The low-end auxin projection of 0.28 kg/ha (0.25 lbs/acre) is based on current auxin usage on crops, such as corn and wheat, that have natural tolerance to auxin herbicides. The high-end auxin projection of 2.24 kg/ha (2 lbs./acre) is based on herbicide programs for auxin-resistant crops being promoted by Dow and Monsanto. Soybean acreage has averaged 74.1 million acres (30 million ha) from 2007 to 2011. If one assumes unchanged soybean acreage in 2025, then the projection estimates that 67.4 million acres (91% of 74.1 million) or 27.3 million ha would be planted to auxin-resistant varieties in 2025.

Based on the low-end projection, auxin use on soybeans in 2025 would be 7.6 million kg (16.9 million lbs). With the high-end projection, auxin usage on soybeans would be 61.2 million kg or 134.8 million lbs. The lower-end projection is clearly a substantial underestimate, since 0.25 lb/acre is just half the average annual rate of 2,4-D currently applied in soybean production (0.5 lb/acre),[5] and represents pre-emergence 2,4-D use.

Farmers who grow DAS-68416-4 soybean will likely make some combination of pre- and post-emergence applications of 2,4-D, weighted to POST applications. Both the average POST application rate and the average number of POST applications will gradually climb from 2013 to 2025 as weed shifts occur to more 2,4-D-tolerant species, and 2,4-D-resistant weeds evolve. This development will mirror the rising rate and number of applications of glyphosate with increasing adoption of RR crops and emergence of GR weeds (see DAS 2011f, Figures 3.5 & 3.6, pp. 28-29). By 2025, growers of DAS-68416-4 soybean would likely apply 2,4-D at annual rates of 0.5 to 1 lb/acre pre-emergence, and 1 to 1.5 lb/acre post-emergence, for total annual use of 2 lbs/acre, two-thirds of the maximum seasonal use permitted by the label.

Mortensen et al.'s estimate for aggregate adoption of 2,4-D/dicamba-resistant soybeans is based on the rate of adoption of glyphosate-resistant soybeans, or 0 to 91% of soybean acreage over 12 years. Ninety-one percent of land planted to soybeans is roughly 67 million acres. Is this a realistic adoption scenario?

Dow is targeting growers who have glyphosate-resistant and ALS-inhibitor-resistant weeds as its most likely market (DAS 2011d). It is estimated that one or more glyphosate-resistant weeds now infest 30 million acres of U.S. cropland, with acreage infested growing rapidly, mostly in soybeans and cotton (see discussion below). Several years ago, Syngenta projected that GR weeds would infest 38 million acres by 2013 (Syngenta 2009). Dow projects that nearly all soybean acres will be infested by GR weeds by 2020 (DAS 2011f, Table 11, at 78).[6] This doesn't take into account U.S. acreage infested with ALS inhibitor-resistant weeds, which CFS estimates at over 20 million acres based on reports listed in the International Survey of Herbicide-Resistant Weeds. If as Dow assumes soybean growers with resistant weeds are likely adopters of DAS 68416-4 soybean, then

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[5] DEA at 22-23, Table 7, which also shows the average rate of dicamba applied in soybean production in 2006, which is 0.25 lb/acre/year.

[6] Dow's estimate counts each acre infested with a single GR weed separately; hence acres that have two or three GR weeds are counted twice or three times. Using this system, Dow estimated that 70% of Northern and 116.2% of Southern state soybean acreage are infested with a GR weed in 2012, rising to 131.9% and 187.7%, respectively, by 2020. While difficult to interpret, Dow's projection clearly points to the great majority of soybean acres being infested with GR weeds by 2020.

Mortensen et al.'s projection of 91% adoption of auxin-resistant crops by 2025 appears to be justified.

However, even if one doubts that resistant weeds will expand so dramatically, there are additional factors at play that will likely increase adoption beyond what would be expected purely from resistant weed-infested acreage. For instance, Mortensen et al. describe several important "agronomic drivers" that would boost adoption, including "defensive use" by farmers who wish only to protect their soybeans from injury by auxin drift emanating from the fields of neighbors who grow the corresponding auxin-resistant soybeans or corn. Such "defensive" reasons for HR crop adoption have been noted for Roundup Ready corn, for instance in Arkansas (Baldwin 2010). Marketing decisions by Dow and Monsanto will also be important. Monsanto has pursued an aggressive "trait penetration" strategy with its Roundup Ready crops, which is most evident with Roundup Ready corn. By incorporating the Roundup Ready trait in the most desirable corn hybrids, and withdrawing non-RR versions of those same hybrids, farmers who have little or no use for the RR trait nevertheless purchase hybrids containing it. Some then go on to make use of the trait (for which they have paid a premium) even though they originally did not want it. Dow may also choose to pursue such a strategy, increasing farmer adoption of Enlist soybeans beyond what it would otherwise be. Having purchased soybeans with the AAD-12 enzyme, whether for defensive reasons or from lack of suitable non-2,4-D-resistant varieties, farmers are then more likely to utilize it through application of 2,4-D.

If the 67.4 million acres of auxin-resistant soybeans projected by 2025 were equally divided between 2,4-D and dicamba-resistant varieties, one would expect over 67 million additional lbs. of 2,4-D to be sprayed on soybeans (2 lbs./acre x ½ of 67.4 million acres), but it could well be 100 million lbs or more annually if Enlist soybeans prove to be more popular than Monsanto's dicamba-resistant soy, or the latter is not deregulated by USDA or commercially introduced.

In 2006, three forms of 2,4-D were applied to 10% of soybean acreage. An average of 1.0 application was made to treated acreage, at an average application rate of 0.48 lbs/acre and seasonal use of 0.50 lbs/acre (DEA, Table 7, at 22). Overall usage in the 19 Program States, representing 96% of soybean acreage, was 3.53 million lbs, for an estimated 3.67 million lbs nationally.

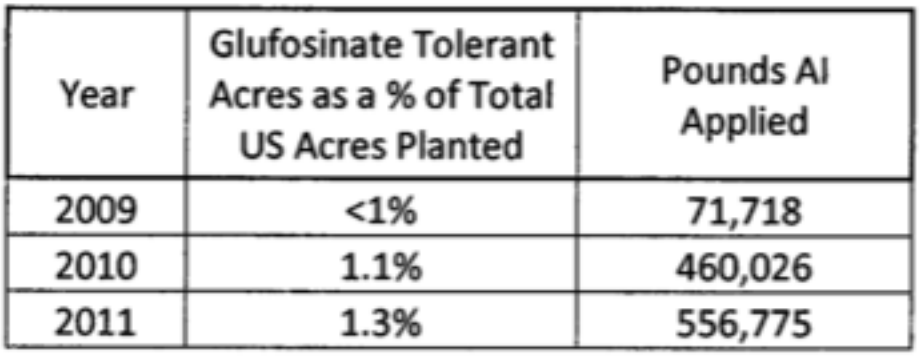
Assuming that DAS-68416-4 soybean is grown on 45-46% of soybean acres (1/2 of 91%) in 2025, 2,4-D use on soybeans would increase by 18-fold over current levels to 67 million lbs. Assuming adoption on 91% of acres, 2,4-D use would increase by over 36-fold, to 135 million lbs. The table below provides further possible scenarios. Growers are unlikely to apply the full label rate of 2,4-D (3 lbs/acre/year), but this scenario is included to indicate the maximum legally permissible use of 2,4-D on DAS-68416-4 soybean for this projection.

Proportion of Label	Amount applied (acre/year)	Amount applied (45.5% Adoption)	Amount applied (91% Adoption)	Fold increase over current use
1/3	1 lb	38.7 million lbs	67.4 million lbs	10.5 to 18.4
2/3	2 lbs	67.4 million lbs	134.8 million lbs	18.4 to 36.7
Max. Rate	3 lbs	106.1 million lbs	202.2 million lbs	28.9 to 55.1

The latest available estimate for overall 2,4-D use in American agriculture is 27 million lbs (EPA Pesticide Use 2011), with 3.7 million lbs. applied to soybeans and 23.3 million lbs. applied to other crops. Thus, introduction of DAS-68416-4 soybean has the potential to increase overall agricultural use of 2,4-D to 90.7 to 158.1 million lbs.[7] per year. This represents a 3.4 to 5.9-fold increase in agricultural use of 2,4-D under the assumptions of this projection.

d. DAS-68416-4 soybean will lead to increased use of glufosinate in American agriculture

Glufosinate has traditionally been used very little on soybeans. The latest USDA NASS report on soybean herbicide use (2006) does not list glufosinate among the 37 herbicides used nationally on soybeans (DEA, Table 7, at 22-23). EPA's latest estimate indicates annual use of glufosinate on soybeans of just 10,000 lbs per year (EPA 2007). However, Bayer CropScience introduced glufosinate-resistant, LibertyLink soybeans in 2009. According to "third party proprietary data" provided to the EPA by Dow, glufosinate use has increased sharply with rising adoption of LL soybeans.



Year	Glufosinate Tolerant Acres as a % of Total US Acres Planted	Pounds AI Applied
2009	<1%	71,718
2010	1.1%	460,026
2011	1.3%	556,775

Source: Third Party Proprietary Data

From: DAS (2011h). "Supplementary documentation in support of draft environmental assessment: Glufosinate use on soybeans," Dow AgroSciences, Nov. 16, 2011.

Based on these data provided by Dow, the table below shows that glufosinate was used on glufosinate-resistant soybeans at annual rates of 0.54 and 0.57 lbs/acre in 2010 and 2011, respectively.

Year	Glufosinate-Resistant Soy as % Total Soy Acres	Total Soybean Acres (thousands)	Glufosinate-Resistant Soybean Acres (thousands)	Pounds Glufosinate Applied (a.i.)	Pounds Glufosinate per Acre (a.i.)
2009	< 1%	77,451		71,718	
2010	1.1%	77,404	851	460,026	0.54
2011	1.3%	75,208	978	556,775	0.57

Source: DAS (2011h); USDA NASS for soybean acreage figures.

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[7] 90.7 million lbs. = 67.4 million lbs (soybeans) + 23.3 million lbs. (existing uses); 158.1 million lbs. = 134.8 million lbs. (soybeans) + 23.3 million lbs (existing uses).

Dow is not seeking any label change for use of glufosinate on DAS 68416-4 (DEA at 5), so If growers choose to make use of the crop's glufosinate resistance, they will be subject to the same label limits as growers of LibertyLink (LL) soybeans, which prescribe a seasonal maximum rate of 1.2 lbs ai/acre (DEA at 28). Growers of LL soybeans are presently utilizing nearly half of the maximum permitted by the label (0.57 of 1.2 lbs/acre/year). Based on this usage rate and the DAS-68416-4 soybean adoption scenario presented above, glufosinate use with DAS-68416-4 soybean could increase to 19.2 million lbs (assuming 45.5% adoption) or 38.4 million lbs (assuming 91% adoption) by 2025. This would represent a roughly 35 to 70-fold increase over current use of glufosinate on soybeans. The maximum label rate scenario, while perhaps unlikely, is included to indicate the maximum legally permissible use of glufosinate on DAS 68416-4 for this projection.

Usage Assumption	**Amount applied (acre/year)**	**Amount applied (45.5% Adoption)**	**Amount applied (91% Adoption)**	**Fold increase over current use**
Average current use	0.57 lb	19.2 million lbs	38.4 million lbs	34.2 to 68.6
Max. label	1.2 lbs	40.4 million lbs	80.9 million lbs	72.1 to 144.5

EPA's latest estimate of overall glufosinate use in American agriculture is 1.36 million lbs per year, with corn (900,000 lbs) and cotton (300,000 lbs) accounting for the vast majority (EPA 2007). Because this estimate is based on crop years 2001-2006, it does not account for glufosinate use on LL soybeans, discussed above, which brings total agricultural use of glufosinate to 1.92 million lbs. (1.36 + 0.56 million).[8] Thus, DAS-68416-4 soybean has the potential to increase overall agricultural use of glufosinate to 20.6 to 39.8 million lbs.[9] per year. This would represent a more than ten to twenty-fold increase in agricultural glufosinate use, based on average current (2011) use on LL soybeans and the 45.5% and 91% adoption scenarios.

e. DAS-68416-4 will sharply increase overall herbicide use on soybeans

As noted above, APHIS offers no assessment of overall herbicide use on DAS-68416-4 soybean, but rather only the weakest of undocumented speculations: "The 2,4-D applied may likely [sic] replace other herbicides currently used on soybeans, and, as a result, the overall amount of herbicides used on soybeans may not change" (DEA at 79).

In fact, overall herbicide use on soybeans will increase sharply with DAS-68416-4 soybean, as 2,4-D is highly unlikely to significantly "replace other herbicides currently used on soybeans."

Penn State weed scientists Dave Mortensen and colleagues project continuing use of glyphosate at current rates with adoption of 2,4-D and dicamba-resistant soybeans (see caption to figure above).

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[8] APHIS' Figure 6 (DEA at 29) portrays total glufosinate use of just 0.981 million lbs, less than half of current usage, because it is based on 1999-2004 data.

[9] 20.6 million lbs. = 19.2 (soybeans) + 1.4 (existing non-soybean uses); 39.8 million lbs. = 38.4 (soybeans) + 1.4 (existing non-soybean uses).

Thus, they see little or no potential for 2,4-D to displace use of glyphosate with DAS-68416-4 soybean.

APHIS' erroneous speculation is likely based on a misplaced analogy to the effect that the Roundup Ready soybean system had in displacing use of other herbicides with glyphosate. As shown in Table 6 and Figure 3 (DEA at 20-21), soybean farmers made significant use of a large number of different herbicides in 1995, the year prior to introduction of RR soybeans. With massive adoption of RR soybeans, growers came to rely almost exclusively on glyphosate for weed control. Glyphosate displaced most other herbicides because it kills a broader range of weeds than perhaps any other herbicide on the market, one important reason it has been dubbed a "once-in-a-century" herbicide.[10] Glyphosate's broad-spectrum activity – on broadleaf and grassy weeds alike, the two major classes of weeds – made supplemental use of other herbicides unnecessary to achieve adequate weed control. In contrast, non-glyphosate herbicides must be used in combinations to achieve the same broad spectrum of weed control. Of course, sole reliance on glyphosate in RR crop systems was what triggered the glyphosate-resistant weed epidemic, as discussed below.

In contrast, 2,4-D has a much narrower spectrum of activity than glyphosate. 2,4-D is a broadleaf herbicide that provides little or no control of grassy weeds (DEA at 26). Troublesome grass weeds in soybeans that 2,4-D does not control include foxtail, crabgrass, barnyardgrass, volunteer corn, wild oat, woolly cupgrass, shattercane, fall panicum, Johnsongrass and quackgrass (Petition, Table 30, p. 122). Thus, glyphosate will continue to be used, together with 2,4-D, to control such grass weeds, which also explains why Dow intends to stack DAS 68416-4 with glyphosate-resistance and market a premix 2,4-D/glyphosate combination product for use with DAS 68416-4. These considerations also explain why Mortensen et al. (2012) assumed continued use of glyphosate at current levels with DAS-68416-4 soybean in their projection, as discussed above. In short, sharply rising use of 2,4-D to control glyphosate-resistant weeds is unlikely to displace glyphosate, which will continue to be applied at high levels to control the many troublesome grassy weeds that 2,4-D does not kill.

Neither is there an significant potential for 2,4-D use with DAS-68416-4 soybean to displace non-glyphosate herbicides, for the simple fact that so little of such herbicides are used in soybean production. Glyphosate was used on 96% of soybean acres in 2006; the next most widely used herbicide was 2,4-D, two forms of which were applied to 10% of soybean acreage.[11] No other herbicide was applied to more than 4% of soybean acreage (DEA at 22, Table 7).[12]

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[10] Duke SO & Powles SB (2008). "Glyphosate: a once-in-a-century herbicide," Pest Management Science 64: 319-325.

[11] As noted above, 2,4-D is only used pre-emergence on currently grown soybeans due to their sensitivity to the herbicide.

[12] Comparison of Table 7 to Table 6 (DEA at 20-21) reveals several sloppy errors in Table 6. The entry for "Acetic acid (2,4-D)" should be 2,4-D, 2-EHE, which is the abbreviation for 2,4-D, 2-ethylhexyl ester (EHE). See http://npic.orst.edu/factsheets/2,4-DTech.pdf. APHIS also lists glyphosate as being used on 92% of soybean acres in Table 6; while this is true of the isopropylamine salt ("isop. salt") of glyphosate, other forms of glyphosate are applied to an additional 4% of soybean acreage, for 96% total.

Simple calculations based on APHIS' Table 7 (DEA at 22) show that glyphosate comprised 90% of total herbicide use on soybeans in 2006, versus 10% for all non-glyphosate herbicides combined.[13] Furthermore, Table 7 reveals that of the five most widely used non-glyphosate, non-2,4-D herbicides by area applied (chlorimuron-ethyl, clethodim, flumioxazin, imazethapyr and pendimethalin), four are used at much lower rates than either 2,4-D or glyphosate (0.017-0.102 lbs/acre/year). Thus, even to the extent that 2,4-D and/or glufosinate might displace some or most of the non-glyphosate herbicides used on soybeans, the effect on overall herbicide use would be minimal.

APHIS cites "reduced use of soil-applied herbicides" (DEA at 92) as a potential advantage of the Enlist soybean system. APHIS is apparently unaware that: 1) Post-emergence use of glyphosate with RR soybeans has practically eliminated use of non-glyphosate (which includes soil-applied) herbicides, as discussed above; thus there is extremely little room for any "reduction"; 2) One of the leading recommendations of weed scientists to prevent and manage glyphosate-resistant weeds is ***to make greater use of soil-applied, residual herbicides*** (Peterson 2012, pp. 4-5), although for the most part HR crop farmers have not followed this advice;[14] and 3) Dow recommends use of a soil-applied herbicide in the Enlist soybean system to mitigate evolution of resistant weeds (DAS 2011d, p. 7). Thus, to the extent that growers of Enlist soybeans realize the "potential advantage" of eliminating what little soil-applied herbicides some may still presently use, they will be sharply increasing the likelihood of fostering weeds resistant to 2,4-D, glufosinate and/or glyphosate. As discussed below, however, the history of herbicide use with RR crops suggests strongly that growers of DAS-68416-4 soybean will ***not*** use soil-applied herbicides, and instead rely completely on pre-emergence and even more on post-emergence use of 2,4-D, together with POST applications of glyphosate and to a lesser extent glufosinate, which is the intended use of the "Enlist Weed Control System" for soybeans.

Based on the projection by Mortensen et al. (2012) and the foregoing discussion, widespread adoption of DAS-68416-4 soybean would lead to a massive increase in overall soybean herbicide use. Taking 2006 as the baseline, and assuming 45.5% adoption with application of 2 lbs/acre/year 2,4-D, soybean herbicide use would increase by 67.4 million lbs., from 107.5 million lbs.[15] to 174.9 million lbs, a 63% increase. We have assumed no use of glufosinate, since Dow is heavily marketing DAS-68416-4 soybean for use with 2,4-D and glyphosate. Yet the near-certain emergence of weeds resistant to both herbicides (discussed in the following section) would likely drive additional use of glufosinate, which as projected above could add another 19 to 38 million lbs. of herbicide use.

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[13] Total glyphosate use is 92,856,000 lbs. Overall herbicide use is 103,156,000 lbs. Total glyphosate use includes "sulfosate," which despite its name is another form of glyphosate: the trimethylsulfonium salt of glyphosate. See: http://biotechterms.org/sourcebook/savetermretrieve.php3?Sulfosate. These figures apply to the 72.9 million acres of soybeans grown in the 19 Program States covered in USDA NASS's survey (DEA at 23, Table 7), which represents 96% of total soybeans grown in that year (USDA NASS AgChem 2006, p. 2). To account for soybean herbicide use in states not surveyed, divide these figures by 0.96, yielding 96.725 million lbs. glyphosate and 107.454 million lbs. total herbicide use.

[14] In a survey of weed scientists by Dow, use of one or more residual herbicides was rated as the most effective of 10 practices for managing the potential development of glyphosate-resistant weeds (DAS 2011f, p. 156, Question 3, practice "f").

[15] See calculation in footnote 13 above.

In light of this analysis, APHIS' speculation that "the overall amount of herbicides used on soybeans may not change" (DEA at 79) must be rejected as completely at odds with expert scientific projections and abundant record evidence. Overall herbicide use is conservatively estimated to increase by 63% with introduction of DAS-68416-4.

f. Assessments of herbicide use as influenced by Roundup Ready crops

APHIS maintains that "[h]erbicide usage trends since the adoption of GE crops are the subject of much debate" (DEA at 22), citing Benbrook (2009a) for an increase in herbicide use with herbicide-resistant crops and several other studies that purport to show that herbicide use has either decreased or remained constant. One study APHIS cites for decreased herbicide use is Fernandez-Cornejo and Caswell (2006). Inspection of that study reveals that the authors charted herbicide use on soybeans and corn only through 2002, and on cotton through 2001, leaving out more recent data[16] that reflect sharply rising herbicide use in response to the epidemic of glyphosate-resistant weeds, which emerged in the year 2000 (see discussion below). CFS provides a chart based on gold-standard USDA National Agricultural Statistics Service data (the same data source used by Fernandez-Cornejo and Caswell) that shows, incontrovertibly, the massive increase in per acre herbicide use on soybeans and cotton since 2001, and the more modest rise in corn since 2002, as increasing adoption of RR crops drove weed resistance and greater herbicide use in response. The USDA data graphed below are also entirely inconsistent with the supposition of "relative stability in the amount of herbicide active ingredients applied to soybeans (Brookes and Barfoot (2010)" (DEA at 22). Brookes and Barfoot are pesticide industry contractors, the principals of an outfit called PG Economics, which regularly disseminates misinformation about GE crops. Benbrook (2009a) provides the only assessment cited by APHIS that comports with the USDA NASS data displayed below. Chapter 6 of Benbrook (2009a) also debunks other studies by PG Economics and other pesticide industry front groups.

APHIS' continued reliance on decade-old data and misinformation from the pesticide industry and its contractors (CFS has critiqued such studies in many past comments on APHIS GE crop assessments), as well as its continued refusal to consult the gold standard data produced by its sister agency, NASS, is entirely inconsistent with the "sound science" standard demanded of it by the National Environmental Policy Act and other federal laws. This deficiency must be redressed in the context of an Environmental Impact Statement that relies on sound science and accurate data.

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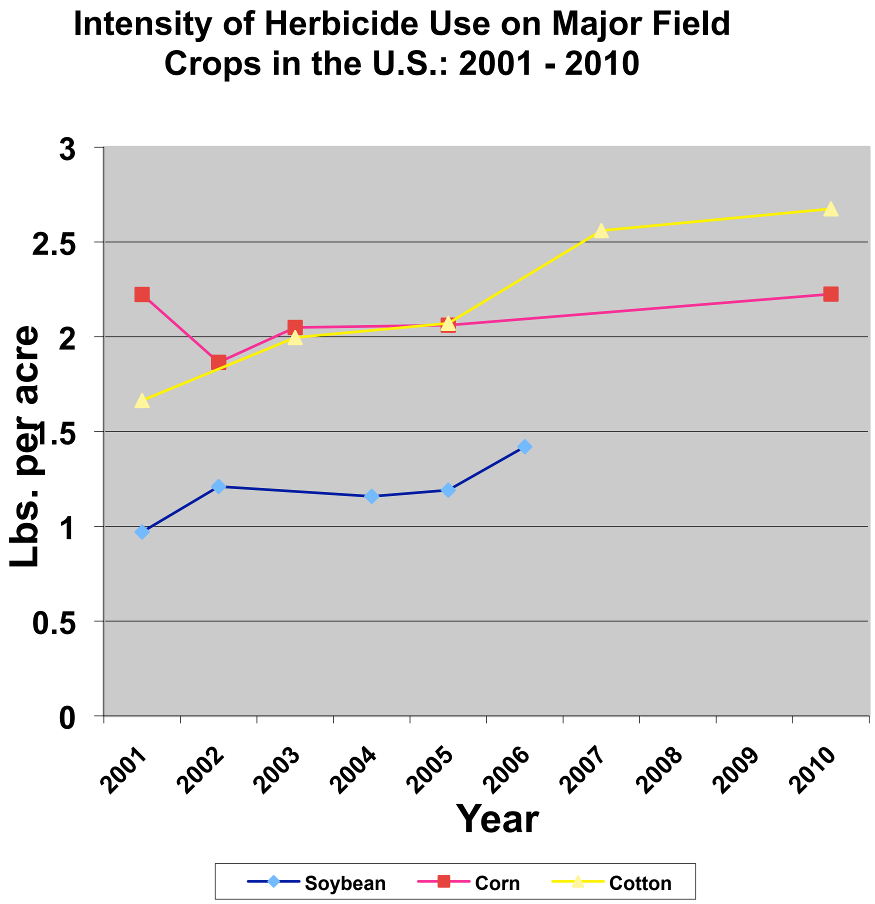
[16] See Figure 8, p. 13, reproduced below. The authors of this 2006 publication for some unexplained reason left out of their Figure 8 more recent USDA NASS data from the very same data source that they used to construct Figure 8.



Notes: Average annual per acre herbicide use on soybean, soybeans and cotton from 2002-2010. **Source:** "Agricultural Chemical Usage: Field Crops Summary," USDA National Agricultural Statistics Service, for the respective years. USDA does not collect data every year for each crop. For instance, no soybean data has been collected since 2006, and no corn data was collected from 2006 to 2009. 2010 corn and cotton data in USDA-NASS AgChem (2010). http://usda.mannlib.soybeanell.edu/MannUsda/viewDocumentInfo.do?documentID=1560

g. Conclusions: Dow's flawed herbicide use assessment has no merit and must not be relied upon by APHIS

APHIS' reliance on misinformation from industry sources for past estimates of herbicide use with Roundup Ready crop systems (discussed above) clearly prejudiced it in favor of uncritical acceptance of Dow's similar claims with respect to DAS-68416-4 soybean (DEA at 126-128). Dow compares various projected herbicide regimes to control glyphosate-resistant weeds, with and without introduction of DAS-68416-4 soybean, to estimate the impact of its Enlist Weed Control System on herbicide use. To this end, case studies of soybean herbicide use were carried out in five states, each geared to a particular glyphosate-resistant weed species (DAS 2011f, p. 123). However, it is extremely difficult to assess Dow's herbicide use projections because they are completely non-transparent.

First, Dow utilizes "market research data" provided by a "third party proprietary data source" to determine which herbicides to model in the various states (DAS 2011f at 124). Dow does not provide even the name of the firm providing the data, but does state that it ".... has purchased licenses to access the proprietary databases for marketing and business analysis purposes" (Id). Dow is a paying customer of this "third-party propriety data source." The "source" has a clear financial disincentive (a conflict of interest) to provide data that might not support Dow's preferred conclusion of reduced herbicide use. The apparent unwillingness of the source to be named as the provider of the data can only decrease our confidence in the assessment based upon them.

Second, the provenance of the various soybean herbicide regimes that Dow utilizes in its simulation of baseline herbicide use ***without DAS-68416-4 soybean*** (DAS 2011f at 93-97) is entirely unclear. Dow speaks vaguely of gathering current recommendations for herbicide regimes to control glyphosate-resistant weeds from university weed scientists (Id at 127), yet for most GR weed species only "general recommendations were found," giving Dow full play to rig a complex numbers game biased towards ***excessively high herbicide use rates*** in regimes ***without DAS-68416-4 soybean*** (Id at 128-129).

Third, Dow consistently biases its soybean herbicide use simulations ***with DAS-68416-4 soybean*** downwards by assuming only a single post-emergence application of 2,4-D at the rate of 0.71 ae lb/acre, often together with a low rate of glyphosate (0.75 ae lb/acre) (DAS 2011f at 93-97). Yet the proposed label allows two post-emergence 2,4-D applications of up to 1 lb/acre each, nearly 3 time the amount modeled by Dow (Petition at 77). In practice, growers of Enlist soybeans would often use a higher rate of 2,4-D, and/or make two rather than one application, especially as weeds gradually evolve 2,4-D tolerance or resistance. In addition, Dow does not appear to model any pre-emergence use of 2,4-D, despite the proposed label allowing an additional pre-emergence application of up to 1 lb/acre, and growers' long history of using 2,4-D pre-emergence. In short, the total seasonal use of 2,4-D in most scenarios – 0.71 lb/acre – is less than 1/4th that permitted by the proposed label (3 lbs/acre).

Fourth, the entire simulation is extremely non-transparent. For instance, Dow does not break out the application rates of the various herbicides used in most herbicide regimes, but rather aggregates them into a single combined figure, making it extremely difficult to parse out how much of which herbicide was used. This would seem to serve the purpose of clouding the biases mentioned above: excessively high rates for herbicide regimes without Enlist soybeans, and excessively low ones for programs with Enlist soybeans. The result is a presentation that cannot be given any credence as a simulation of herbicide use with or without Enlist soybeans. Even APHIS cautions that the 0.71 lb/acre 2,4-D modeled in these scenarios is well below the single application label rate (DEA at 126), though APHIS fails to note Dow's failure to model scenarios where 2 or 3 applications of 2,4-D are made.

Dow's cost estimates of herbicide regimes with DAS-68416-4 soybean have absolutely no value, and should be completely disregarded by APHIS, for two important reasons (see DAS 2011f at 130, the footnote designated by the single asterisk, for the following discussion). First, the price Dow models for 2,4-D is based on generic 2,4-D currently available in the marketplace, not its proprietary choline salt of 2,4-D that is intended for use on DAS-68416-4 soybean. Generic 2,4-D is among the cheapest herbicides available (U of Tenn 2011, p. 94), while Dow's proprietary choline salt of 2,4-D is likely to be much more expensive. Second, Dow does not include any technology fee for the "DHT Technology," the 2,4-D resistance trait. Technology fees for other GE crop seeds comprise a major portion, in some cases half or more, of the total seed price. This, too, leads to a huge "underpricing" of weed control with Enlist soybeans. Dow's only explanation for these lapses is that the "cost of specially formulated 2,4-D that will be registered for use on crops containing DHT technology," and the technology fee for the resistance trait itself, "had not been established at the time this analysis was undertaken." Dow should have either made reasonable estimates of these increased costs, or abstained from undertaking this assessment altogether, because the assessment in the present form gives a seriously deceptive and potentially huge underestimate of the cost of weed control with Enlist soybeans.

3. Herbicide-resistant weeds and approval of DAS-68416-4 soybean

a. Summary

U.S. agriculture's undue reliance on single-tactic, chemical-intensive weed control generates huge costs in the form of herbicide-resistant weeds – costs that could be avoided or greatly lessened with sustainable integrated weed management techniques that emphasize non-herbicidal tactics. Herbicide-resistant crop systems promote still more rapid evolution of resistant weeds. The history of glyphosate-resistant weed emergence must be carefully heeded, yet APHIS has provided no assessment of it. Weeds resistant to synthetic auxin herbicides, the class to which 2,4-D belongs, are already numerous. Multiple herbicide-resistant weeds are a rapidly growing threat. Some existing populations of resistant weeds already rate the designation "noxious," and they will be made still more intractable and costly if they evolve additional resistance to 2,4-D. Recent scientific studies reveal that 2,4-D-resistant crop systems will likely accomplish just this. Volunteer DAS-68416-4 soybean may become a problematic "resistant weed" in its own right, by virtue of its resistance to three herbicides, and perhaps still others with cross-pollination by other HR soybean varieties. Stewardship strategies proposed by Dow are quite similar to those of Monsanto with Roundup Ready crops, which have utterly failed to prevent resistant weed emergence. A population of one of the most problematic glyphosate-resistant weeds, common waterhemp, has recently been identified with high-level resistance to 2,4-D, and lower-level resistance to dicamba, prompting weed scientists to call for "mandatory stewardship practices" for 2,4-D and dicamba-resistant crops. Yet APHIS provides no assessment of weed resistance stewardship, nor does it consider an alternative involving mandatory weed resistance management, in the DEA. APHIS completely failed to assess the still higher risks of weed resistance that would accompany deregulation of DAS-68416-4 together with 2,4-D-resistant corn, even though APHIS has given preliminary approval of 2,4-D-resistant corn as well. Under the preferred alternative, the massive increase in use of 2,4-D accompanying DAS-68416-4 soybean and subsequent 2,4-D crops will very likely trigger rapid emergence of increasingly intractable, multiple herbicide-resistant weeds that will harm farmers, the environment, and the interests of American agriculture.

b. Weed management vs. weed eradication

Weeds can compete with crop plants for nutrients, water and sunlight, and thereby inhibit crop growth and potentially reduce yield. While less dramatic than the ravages of insect pests or disease agents, weeds nevertheless present farmers with a more consistent challenge from year to year. However, properly managed weeds need not interfere with crop growth. For instance, organically managed has been shown to yield as well as conventionally grown varieties despite several-fold higher weed densities (Ryan et al. 2010). Long-term cropping trials at the Rodale Institute reveal that average yields of organically grown soybean were equivalent to those of conventionally grown soybean, despite six times greater weed biomass in the organic system (Ryan et al. 2009). Weeds can even benefit crops – by providing ground cover that inhibits soil erosion and attendant loss of soil nutrients, habitat for beneficial organisms such as ground beetles that consume weed seeds, and organic matter that when returned to the soil increases fertility and soil tilth (Liebman 1993). These complex interrelationships between crops and weeds would seem to call for an approach characterized by careful management rather than indiscriminate eradication of weeds.

Farmers have developed many non-chemical weed management techniques, techniques that often provide multiple benefits, and which might not be utilized specifically or primarily for weed control (see generally Liebman-Davis 2009). For instance, crop rotation has been shown to significantly reduce weed densities versus monoculture situations where the same crop is grown each year (Liebman 1993). Cover crops – plants other than the main cash crop that are usually seeded in the fall and killed off in the spring – provide weed suppression benefits through exudation of allelopathic compounds into the soil that inhibit weed germination, and when terminated in the spring provide a weed-suppressive mat for the follow-on main crop. Common cover crops include cereals (rye, oats, wheat, barley), grasses (ryegrass, sudangrass), and legumes (hairy vetch and various clovers. Intercropping – seeding an additional crop amidst the main crop – suppresses weeds by acting as a living mulch that competes with and crowds out weeds, and can provide additional income as well (Liebman 1993). One common example is intercropping oats with alfalfa. Higher planting densities can result in more rapid closure of the crop "canopy," which shades out and so inhibits the growth of weeds. Fertilization practices that favor crop over weeds include injection of manure below the soil surface rather than broadcast application over the surface. Techniques that conserve weed seed predators, such as ground beetles, can reduce the "weed seed bank" and so lower weed pressure. In addition, judicious use of tillage in a manner that does not contribute to soil erosion is also a useful means to control weeds.

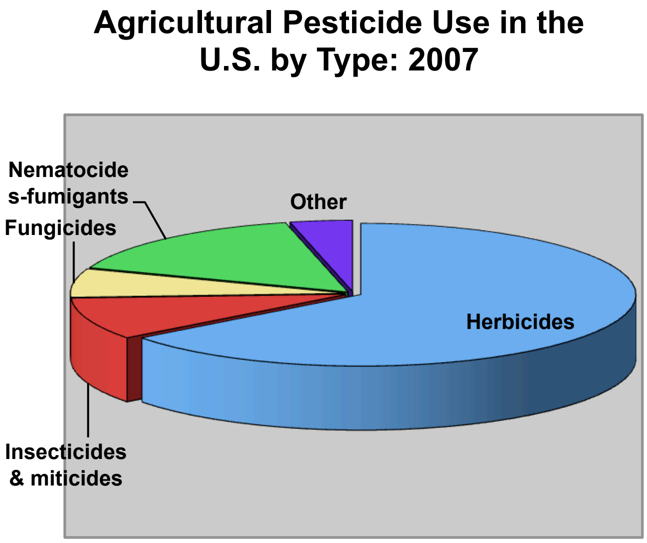
Unfortunately, with the exception of crop rotation and tillage, such techniques are little used in mainstream agriculture. This is in no way inevitable. Education and outreach by extension officers, financial incentives to adopt improved practices, and regulatory requirements are just a few of the mechanisms that could be utilized to encourage adoption of more integrated weed management systems (IWM) that prioritize non-chemical tactics (Mortensen et al. 2012). Meanwhile, the problems generated by the prevailing chemical-intensive approach to weed control are becoming ever more serious. APHIS provides no assessment of IWM systems or non-chemical tactics as an alternative to deregulation of DAS-68416-4 soybean for the stated purpose of Dow's product, to provide a means to control glyphosate-resistant weeds (DEA at 3).

c. The high costs of herbicide-only weed control

In 2007, U.S. farmers spent $4.2 billion dollars to apply 442 million lbs of herbicide, and uncounted billions more on technology fees for herbicide-resistance traits in major crops. Overall, the U.S. accounts for one-quarter of world herbicide use (EPA Pesticide Use 2011, Tables 3.1, 5.2, 5.6). Surely this intensive herbicidal onslaught should make American fields among the most weed-free in the world. But such is not the case. As farmers gradually came to rely more on herbicides as the preferred and then often the sole means to control weeds, herbicide-resistant weeds have become increasingly severe and costly.

The first major wave of herbicide-resistance came in the 1970s and 1980s as weeds evolved resistance to the heavily used triazines, such as atrazine (see Benbrook 2009a for this discussion). The next major wave of resistance comprised weeds resistant to ALS inhibiting herbicides in the 1980s and 1990s. Just five years intervened between introduction of the first ALS inhibitor herbicide in 1982 and the first resistant weed population (1987). One of the major factors persuading farmers to adopt Roundup Ready, glyphosate-resistant crops was the prevalence of weeds resistant to ALS inhibitors. Weeds have evolved resistance at least 21 "modes of action," or herbicide classes, in the world (ISHRW HR Weed Ranking 4/22/11).

According to the USDA's Agricultural Research Service, up to 25% of pest (including weed) control expenditures are spent to manage pesticide (including herbicide) resistance in the target pest (USDA ARS Action Plan 2008-13-App. II). With an estimated $7 billion spent each year on chemical-intensive weed control (USDA ARS IWMU-1), herbicide-resistant weeds thus cost U.S. growers roughly $1.7 billion (0.25 x $7 billion) annually. These expenditures to manage resistance equate to tens and perhaps over 100 million lbs of the over 400 million lbs of agricultural herbicide active ingredient applied to American crops each year (see figure below), as growers increase rates and make additional applications to kill expanding populations of resistant weeds



Herbicides comprise by far the largest category of pesticides, defined as any chemical used to kill plant, insect or disease-causing pests. In 2007, the last year for which the Environmental Protection Agency has published comprehensive data, weedkillers (herbicides) accounted for 442 million lbs of the 684 million lbs of chemical pesticides used in U.S. agriculture, nearly seven-fold more than the insecticides that many associate with the term "pesticide." Source: "Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates," U.S. Environmental Protection Agency, 2011, Table 3.4 (EPA Pesticide Use 2011 in supporting materials).

Increasing the rate and number of applications, however, rapidly leads to further resistance, followed by adding additional herbicides into the mix, beginning the resistance cycle all over again, just as overused antibiotics breed resistant bacteria. This process, dubbed the pesticide treadmill, has afflicted most major families of herbicides, and will only accelerate as U.S. agriculture becomes increasingly dependent on crops engineered for resistance to one or more members of this by far largest class of pesticides (Kilman 2010). APHIS provides no assessment of the impacts or costs to farmers of past herbicide use and the resistant weeds it has triggered, an assessment that it critical to inform a similar analysis of DAS-68416-4 soybean's impacts.

Besides costing farmers economically via herbicide-resistant weeds, a chemical-intensive pest control regime also has serious public health and environmental consequences. Various pesticides are known or suspected to elevate one's risk for cancer, neurological disorders, or endocrine and immune system dysfunction. Epidemiological studies of cancer suggest that farmers in many countries, including the U.S., have higher rates of immune system and other cancers (USDA ERS AREI 2000). Little is known about the chronic, long-term effects of exposure to low doses of many pesticides, especially in combinations. Pesticides deemed relatively safe and widely used for decades have had to be banned in light of scientific studies demonstrating harm to human health or the environment. Pesticides also pollute surface and ground water, harming amphibians, fish and other wildlife.

Herbicide-resistant weeds thus lead directly to adverse impacts on farmers, the environment and public health. Adverse impacts include the increased costs incurred by growers for additional herbicides to control them, greater farmer exposure to herbicides and consumer exposure to herbicide residues in food and water, soil erosion and greater fuel use and emissions from increased use of mechanical tillage to control resistant weeds, environmental impacts from herbicide runoff, and in some cases substantial labor costs for manual weed control. These are some of the costs of unsustainable weed control practices, the clearest manifestation of which is evolution of herbicide-resistant weeds. APHIS provides no meaningful assessment of the costs to farmers or U.S. agriculture from the reasonably foreseeable evolution of weeds resistant to 2,4-D or glufosinate.

d. Why herbicide-resistant crop systems promote rapid evolution of resistant weeds

Herbicide-resistant (HR) crop systems such as DAS-68416-4 soybean involve post-emergence application of one or more herbicides to a crop that has been bred or genetically engineered to survive application of the herbicide(s). These HR crop systems promote more rapid evolution of herbicide-resistant weeds than non-HR crop uses of the associated herbicides. This is explained by several characteristic features of these crop systems.

HR crops foster more ***frequent*** use of and ***overreliance*** on the herbicide(s) they are engineered to resist. When widely adopted, they also lead to more ***extensive*** use of HR crop-associated herbicide(s). Herbicide use on HR crops also tends to occur ***later in the season***, when weeds are larger. Each of these factors contributes to rapid evolution of resistant weeds by favoring the survival and propagation of initially rare individuals that have genetic mutations lending them resistance. Over time, as their susceptible brethren are killed off, these rare individuals become more numerous, and eventually dominate the weed population.

High frequency of use means frequent suppression of susceptible weeds, offering (at frequent intervals) a competition-free environment for any resistant individuals to thrive. Overreliance on the HR crop-associated herbicide(s) means little opportunity for resistant individuals to be killed off by alternative weed control methods, thus increasing the likelihood they will survive to propagate and dominate the local weed population. Widespread use of the HR crop system increases the number of individual weeds exposed to the associated herbicide(s), thus increasing the likelihood that there exists among them those individuals with the rare genetic predisposition that confers resistance. The delay in application fostered by HR crop systems means more weeds become larger and more difficult to kill; thus, a greater proportion of weeds survive to sexual maturity, and any resistant individuals among them are more likely to propagate resistance via cross-pollination of susceptible individuals or through deposition of resistant seeds in the seed bank; in short, a higher likelihood of resistance evolution.

Below, we discuss these resistant weed-promoting features of HR crop systems in more detail, with particular reference to systems involving glyphosate-resistance (Roundup Ready) and 2,4-D-resistance.

GE seeds in general, including HR seeds, are substantially more expensive than conventional seeds (Benbrook 2009b). Their higher cost is attributable to a substantial premium (often called a technology fee) for the herbicide-resistance trait. This premium constitutes a financial incentive for the grower to fully exploit the trait through frequent and often exclusive use of the associated herbicide(s), and a disincentive to incur additional costs by purchasing other, often more expensive herbicides.

> The cost of RR [Roundup Ready] alfalfa seed, including the technology fee, is generally twice or more than that of conventional alfalfa seed. Naturally, growers will want to recoup their investment as quickly as possible. Therefore, considerable economic incentive exists for the producer to rely solely on repeated glyphosate applications alone as a weed control program. (Orloff et al. 2009, p. 9).

Dow has not revealed its pricing for 2,4-D soybean seed, but it is likely to be considerably more expensive than currently available GE varieties, based on Dow's profit projections. Dow CEO Andrew Liveris estimates that its 2,4-D resistance trait "is worth two to three times more than SmartStax corn seed, developed with Monsanto, which has a net present value of $500 million…" (Kaskey 2010). GE SmartStax corn is the most expensive corn seed on the market (Tomich 2010). In order to capture this $1 to $1.5 billion in revenue, Dow would likely have to charge a substantial premium for 2,4-D- and glufosinate-resistant seeds beyond that charged for current GE seeds.

Overreliance is especially favored when the associated herbicide(s) are effective at killing a broad range of weeds, which tends to make other weed control practices less needed, at least until weed resistance emerges. Glyphosate is such a broad-spectrum herbicide; 2,4-D provides control of most broadleaf weeds. Applied together or sequentially, glyphosate and 2,4-D would initially provide broad-spectrum control of soybean weeds, making use of other weed control measures unnecessary until the inevitable evolution of 2,4-D resistance, often in populations already resistant to glyphosate and/or other herbicides. Greater use of non-chemical weed control tactics is the only way to avoid the evolution of increasingly intractable, multiple HR weeds.

Frequent use and overreliance are also fostered when the HR crop-associated herbicide(s) are inexpensive relative to other herbicides. Monsanto lowered the price of Roundup herbicide (active ingredient: glyphosate) in the late 1990s to encourage farmers to adopt Roundup Ready crop systems and rely exclusively on glyphosate for weed control (Barboza 2001),[17] and the price has fallen further since then (DAS 2011f, Figure 1.2, p. 11).[18] 2,4-D is even cheaper than glyphosate, and in fact is one of the least inexpensive herbicides on the market (U of Tenn 2011, p. 94). As suggested by Orloff et al. (2009), quoted above, overreliance on HR crop-associated herbicide(s) is particularly favored when the HR trait premium is high and the herbicide's price is low, the likely scenario with DAS-68416-4 soybean.

One of the key changes wrought by herbicide-resistant crop systems is a strong shift to "post-

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[17] Monsanto has greatly increased the price of RR seed to compensate for reduced income from sale of Roundup.

[18] This and other "DAS" references refer to submissions to APHIS by Dow AgroSciences, which may be found in the bibliography of APHIS' DEA.

emergence"[19] herbicide application, which generally occurs later in the season on larger weeds, versus early-season use on smaller weeds or prior to weed emergence that is more characteristic of conventional crops. It is important to understand that facilitation of post-emergence herbicide use as the sole or primary means of weed control is the *sine qua non* of HR crop systems, not an incidental feature. Early-season uses include soil-applied herbicides put down around time of planting; these herbicides have residual activity to kill emerging weeds for weeks after application. The Roundup Ready soybean system has practically eliminated use of soil-applied, or indeed of any herbicide other than glyphosate.

Weed scientist Paul Neve has simulated the rate at which weeds evolve resistance to glyphosate under various application regimes (Neve 2008). His results show unambiguously that the post-emergence use of glyphosate unique to glyphosate-resistant crop systems fosters resistant weeds much more readily than traditional uses ("prior to crop emergence") typical of conventional crops. This is consistent with the massive emergence of glyphosate-resistant weeds only after glyphosate-resistant crops were introduced (see below):

> Glyphosate use for weed control prior to crop emergence is associated with low risks of resistance. These low risks can be further reduced by applying glyphosate in sequence with other broad-spectrum herbicides prior to crop seeding. Post-emergence glyphosate use, associated with glyphosate-resistant crops, very significantly increases risks of resistance evolution. (Neve 2008)

One way that glyphosate-resistant crop systems promote emergence of resistant weeds is by facilitating delayed post-emergence application to larger weeds:

> Growers rapidly adopted glyphosate-resistant crops and, at least initially, did not have to rely on preventive soil-applied herbicides. Growers could wait to treat weeds until they emerged and still be certain to get control. ***Many growers waited until the weeds were large in the hope that all the weeds had emerged and only one application would be needed. Today, experts are challenging this practice from both an economic and a sustainability perspective.*** (Green et al. 2007, emphasis added)

> Following the widespread adoption of glyphosate-resistant soybean, ***there has been a subtle trend toward delaying the initial postemergence application longer than was once common***. Because glyphosate provides no residual weed control and application rates can be adjusted to match weed size, ***producers hope that delaying the initial postemergence application will allow enough additional weeds to emerge so that a second application will not be necessary.*** (Hagar 2004, emphasis added)

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[19] That is, application after the seed has sprouted or "emerged," through much of the crop's life. Post-emergence use is often not possible, or only at lower rates, with conventional crops, which would thereby be killed or injured.

University of Minnesota weed scientist Jeff Gunsolus notes that: "Larger weeds are more apt to survive a postemergence application and develop resistance." (as quoted in Pocock 2012). University of Arkansas weed scientist Ken Smith notes that application of Ignite (glufosinate) to cotton plants with dual resistance to glyphosate and glufosinate (Widestrike) in order to control large glyphosate-resistant weeds risks generating still more intractable weeds resistant to both herbicides (as quoted in Barnes 2011, emphasis added):

> Many growers who use Ignite on WideStrike varieties do so after they discover they have glyphosate-resistant weeds, according to Smith. To combat this, ***growers will make an application of Ignite on weeds that, on occasion, have grown too big to be controlled by the chemistry. This creates a dangerous scenario which could possibly encourage weeds to develop resistance to glufosinate***, the key chemistry in Ignite. ***The end-result, according to Smith, would be disastrous***.

It should be noted that Dr. Smith's concern is that weeds will evolve resistance to the same two herbicides to which the HR crop is resistant, which both undermines the utility of the crop and creates a potentially noxious HR weed that becomes extremely difficult to control. As discussed further below, this tendency for weeds to mimic the herbicide resistances in the crop is a general feature of HR crop systems, and sets up a futile and costly chemical arms race between HR crops and weeds. APHIS fails to provide any assessment of the special proclivity of HR crop systems, or DAS-68416-4 soybean in particular, to trigger evolution of resistant weeds. This is a serious deficiency, as APHIS concedes frequently that it is the emergence of glyphosate-resistant weeds that forms the rationale for DAS-68416-4 soybean.

e. Overview of glyphosate-resistant crops and weeds

A discussion of glyphosate-resistant (GR) crops and weeds is important for two reasons. First, the rapid emergence of GR weeds in RR crop systems is evidence of the resistant weed-promoting effect of HR crop systems in general, as discussed above, and provides insight into the risks of resistant weed evolution in the context of the Enlist soybean and corn systems. Second, the prevalence of glyphosate-resistant weeds is the motivating factor in Dow's introduction and farmers' potential adoption of Enlist crops.

Glyphosate-resistant crops represent by far the major HR crop system in American and world agriculture, and provide an exemplary lesson in how HR crop systems trigger HR weeds (see Benbrook 2009a for following discussion). Glyphosate was first introduced in 1974. Despite considerable use of the herbicide, for the next 22 years there were no confirmed reports of glyphosate-resistant weeds. A few small and isolated populations of resistant weeds – mainly rigid and Italian ryegrass and goosegrass – emerged in the late 1990s, attributable to intensive glyphosate use in orchards (e.g. Malaysia, Chile, California) or in wheat production (Australia).

Significant populations of glyphosate-resistant weeds have only emerged since the year 2000, four years after the first Roundup Ready (RR) crop system (RR soybeans) was introduced in 1996, followed by RR cotton & canola in 1997 and RR soybean in 1998 (Monsanto History undated).[20] According to the International Survey of Herbicide-Resistant Weeds (ISHRW), multiple populations of 23 weed species are resistant to glyphosate in one or more countries today; of these, 26 populations of ten species are also resistant to herbicides in one to three other families of chemistry in addition to glyphosate (ISHRW GR Weeds 4/22/12).[21] Based on acreage infested, GR weeds have emerged overwhelmingly in soybeans, cotton and soybean in countries, primarily the U.S., where RR crop systems predominate (see CFS RRSB 2010, which has further analysis of GR weeds).

The first glyphosate-resistant (GR) weed population confirmed in the U.S., reported in 1998, was rigid ryegrass, infesting several thousand acres in California almond orchards (ISHRW GR Weeds 4/22/12). Beginning in the year 2000 in Delaware, glyphosate-resistant horseweed rapidly emerged in Roundup Ready soybeans and cotton in the East and South. Just twelve years later, glyphosate-resistant biotypes of 13 species are now found in the U.S., and they infest millions of acres of cropland in at least 27 states (ISHRW GR Weeds 4/22/12).[22]

Based on Center for Food Safety's periodic compilation of data from the ISHRW website over the past four years, glyphosate-resistant weeds in the U.S. have evolved at an accelerated rate in recent years. As of November 2007, ISHRW recorded eight weed species resistant to glyphosate, covering up to 3,200 sites on up to 2.4 million acres. By early 2012, as many as 239,851 sites on up to 16,683,100 acres were documented to be infested by glyphosate-resistant weeds (CFS GR Weed List 2012). This astonishing proliferation of resistant weeds – an over 70-fold increase in number of sites and 7-fold increase in acreage – is portrayed in the figure at the end of this section. This chart and two additional charts portraying GR weeds by crop setting and farm production region are found in the file entitled at CFS GR Weed Charts (2012).

However, the true extent of GR weeds is much greater than even the maximum figures shown in the graph, because "...the voluntary basis of the contributions [to ISHRW] likely results in underestimation of the extent of resistance to herbicides, including glyphosate" (NRC 2010, p. 2-12). Many examples could be cited to illustrate to what extent ISHRW underestimates the extent of GR weed populations, but one will suffice. Illinois weed scientist Bryan Young recently reported 5-6 million acres of Illinois cropland infested with glyphosate-resistant waterhemp (as quoted in Lawton 2012, confirmed with Dr. Young, personal communication). Yet ISHRW lists GR waterhemp as infesting just 100 acres in Illinois (ISHRW Illinois Waterhemp). Inclusion of this

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[20] APHIS mistakenly states that RR cotton was introduced in 1993 and RR soybean in 1996 (DEA at 59, 66, 70, 72), in the latter case conflating the year APHIS received Monsanto's petition for deregulation and the year Monsanto commercially introduced RR soybean. APHIS did not deregulate RR soybean until 1997. See http://www.aphis.usda.gov/brs/aphisdocs2/96_31701p_com.pdf and APHIS (1997). It is not clear why APHIS is four years off on the year of RR cotton introduction.

[21] A population of one additional weed species (for 24 total) has evolved resistance to glyphosate since the cited 4/22/12 list was compiled, spiny amaranth in Mississippi. See http://www.weedscience.org/Case/Case.asp?ResistID=5682.

[22] Now 14 weed species, in at least 30 states. GR weeds have been documented in three additional states since this 4/22/12 list was compiled. For South Dakota and Wisconsin, see list at http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go. For Montana, see AgNews (2012). Thus, all 10 major soybean growing states how have GR weeds (DEA at 63).

single updated report in the ISHRW system would raise the GR weed infested acreage by one-third. It appears that much or all of this waterhemp is resistant to ALS inhibitors as well, with a significant portion also resistant to PPO inhibitors and/or triazine herbicides (Tranel 2010).

Dr. Ian Heap, who manages the ISHRW website cited above, confirms that: "The survey is definitely too low because researchers report the first cases and enter in the area infested. Often they don't return in subsequent years to keep updating the survey." Dr. Heap estimates that "there are about 40 million acres affected by glyphosate-resistant weeds," but notes that if one accounts for "overlapping acres" infested with more than one GR weed, "the estimate probably comes down to about 30 million actual acres" (Heap 2012). Dow has even higher estimates of GR weed-infested acreage (DAS 2011f, pp. 77-78), which Heap believes are excessive. Thus, actual acreage infested with glyphosate-resistant weeds is roughly double the 16.7 million acres reported by ISHRW and shown in the figure below. However, the figure can be assumed to accurately capture the tremendous rate of GR weed emergence.

Early on, most resistant weed populations were driven by intensive glyphosate use associated with RR soybeans and RR cotton. However, adoption of soybean with the Roundup Ready trait has increased sharply in recent years, from 20% to 72% of national soybean acres from just 2004 to 2011. The increasing reliance on glyphosate associated with the growing use of RR soybean/RR soybean rotations is likely responsible for the rapid emergence of resistant weeds in the Midwest and Northern Plain states. In general, more GR weeds are emerging on agricultural land planted to several crops that are predominantly Roundup Ready in the U.S., which since 2008 includes sugar beets. The most recent example is the emergence of GR common waterhemp on land planted to soybeans and sugar beets in North Dakota (ISHRW GR Weeds 4/22/12).

Populations of some glyphosate-resistant weeds, such as GR Palmer amaranth, GR horseweed, GR giant ragweed, and GR common waterhemp, are properly regarded as noxious weeds. The increased use of herbicides and increased use of soil-eroding tillage operations to control them cause harm to the environment and natural resources (e.g. loss of soil and increased runoff of agricultural chemicals). When not properly managed due to the difficulty of controlling them, these noxious weeds can sharply reduce yields, while successful control efforts often involve a several-fold increase in weed control costs, in either case harms to the interests of agriculture. A brief, documented overview of these harms is provided in Benbrook (2009a, Chapter 4).

APHIS provides essentially no analysis of the emergence of glyphosate-resistant weeds, a significant flaw in the DEA for several reasons. First, the rapid emergence of GR weeds in RR crop systems is evidence of the resistant weed-promoting effect of HR crop systems in general, as discussed above, and a proper analysis would have provided APHIS with important insights into the risks of resistant weed evolution in the context of the DAS-68416-4 soybean system. Second, APHIS repeatedly acknowledges that the prevalence of glyphosate-resistant weeds is the motivating factor in Dow's introduction and farmers' potential adoption of DAS-68416-4 soybean (e.g. DEA at 3). Without a proper understanding of the prevalence of GR weeds,[23] it is impossible

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[23] APHIS cites an outdated 2010 figure for glyphosate-resistant infested acreage of 6% of total land planted to corn, soybeans and cotton, which comes to just 10-11 million acres (DEA at 25). As discussed above, the true figure is at least three-fold more, or 30-40 million acres.

to gauge even roughly how widely Dow's soybean would be adopted, and the magnitude of increase in the use of herbicides, such as 2,4-D and glufosinate, entailed by the proposed deregulation, both crucial factors in assessing the herbicide-resistant weed threat posed by the DAS-68416-4 soybean system.

APHIS provides no empirical assessment of farmer use of resistant weed mitigation measures at all, but rather flaccidly relies on Dow's stewardship program (DEA at 78), which is quite similar to Monsanto's stewardship program for RR crops. APHIS knows that such stewardship has failed, otherwise GR weeds would not be such a serious problem; yet there is no assessment of the flaws of past stewardship plans or how they might be improved, which might have informed APHIS' assessment of the efficacy, if any, of Dow's stewardship recommendations. APHIS should have assessed an alternative that included mandatory weed resistance management plans for DAS-68416-4 soybean, as recently recommended by weed scientists from Nebraska and Illinois (Bernards et al. 2012), discussed below. These are all, to say the least, grave deficiencies in the DEA that demand redress in the context of an Environmental Impact Statement.

f. Synthetic auxin-resistant crops and weeds

2,4-D is the most important and widely used member of the synthetic auxin class of herbicides, which act by mimicking plant growth hormones such as indole acetic acid. (DEA at 27). Dow scientists maintain that deployment of 2,4-D-resistant crop systems such as DAS-68416-4 soybean would be unlikely to foster evolution of weeds resistant to 2,4-D for several reasons: 1) Very few weeds have thus far evolved resistance to the herbicide; 2) 2,4-D's mode of action is complex, suggesting that multiple mutations would be needed to confer resistance; and 3) 2,4-D will be used in combination or rotation with glyphosate, which would require weeds to evolve resistance to both at once, which is regarded as unlikely (Wright et al. 2010).

There are several serious flaws in these arguments, which were persuasively rebutted by Mortensen et al. (2012). First, the ISHRW website lists 43 biotypes of 29 different weed species with resistance to synthetic auxin herbicides (ISHRW SynAux Weeds 4/22/12). Of the 21 herbicide classes to which weeds have evolved resistance, synthetic auxin-resistant weeds rank fourth in terms of number of resistant biotypes (ISHRW HR Weed Ranking 4/22/12). The majority of the 29 auxin-resistant species (17) are resistant to 2,4-D.[24] This is hardly "very few 2,4-D–resistant weed species" (Wright et al. 2010).

The second argument is equally specious. In most cases, scientists have not elucidated the precise mechanisms by which weeds evolve resistance, making predictions about the likelihood of weed resistance on this basis extremely hazardous. Monsanto scientists likewise predicted very little chance of glyphosate-resistant weed evolution in the 1990s (Bradshaw et al. 1997), and for much the same reasons as put forward by Dow's scientists. These predictions were of course disastrously wrong, but they did help quell concerns about GR weed evolution as Monsanto was introducing its Roundup Ready crops. Interestingly, only one GR weed had been identified by the

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[24] Mortensen et al. (2012) report 16 species, but an additional one has arisen since publication of that paper, discussed further below.

time the first RR crop was introduced in 1996 (ISHRW GR Weeds 4/22/12), in contrast to the 17 weed species with biotypes resistant to 2,4-D today.

The experience with glyphosate-resistant weeds demonstrates that neither a narrow focus on the biochemical nuances of resistance mechanisms, nor the frequency of resistance evolution in the past, provide an accurate basis for forecasting what will happen when the herbicide in question is used in the context of an herbicide-resistant crop system. What it does demonstrate is that the characteristic ways in which HR crop systems are used in the field, as discussed above, make them far more likely to trigger evolution of resistant weeds than non-HR crop uses of those same herbicides.

Dow's third argument, that use of both 2,4-D and glyphosate on DAS-68416-4 soybean stacked with glyphosate resistance will hinder evolution of weeds resistant to either one, also lacks merit. This argument ignores the fact that the huge extent of existing GR weed populations – with many billions of individual weeds on roughly 30 million infested acres – make it near certain that some among them will have the rare genetic mutations conferring resistance to 2,4-D ***as well***. Penn State weed scientists Mortensen et al. (2012) provide the mathematical exposition (emphasis added):

> First, when a herbicide with a new mode of action is introduced into a region or cropping system in which weeds resistant to an older mode of action are already widespread and problematic, the probability of selecting for multiple target-site resistance is not the product of two independent, low-probability mutations. In fact, the value is closer to the simple probability of finding a resistance mutation to the new mode of action within a population already extensively resistant to the old mode of action. For instance, in Tennessee, an estimated 0.8–2 million ha of soybean crops are infested with glyphosate-resistant horseweed (C. canadensis) (Heap 2011). Assuming seedling densities of 100 per m^2 or 10^6 per ha (Dauer et al. 2007) and a mutation frequency for synthetic auxin resistance of 10^{-9}, ***this implies that next spring, there will be 800–2000 horseweed seedlings in the infested area that possess combined resistance to glyphosate and a synthetic auxin herbicide*** ((2×10^6 ha infested with glyphosate resistance) x (10^6 seedlings per ha) x (1 synthetic auxin–resistant seedling per 10^9 seedlings) = 2000 multiple-resistant seedlings). In this example, these seedlings would be located in the very fields where farmers would most likely want to plant the new stacked glyphosate- and synthetic auxin–resistant soybean varieties (the fields where glyphosate-resistant horseweed problems are already acute). Once glyphosate and synthetic auxin herbicides have been applied to these fields and have killed the large number of susceptible genotypes, these few resistant individuals would have a strong competitive advantage and would be able to spread and multiply rapidly in the presence of the herbicide combination.

The upshot is that 2,4-D-resistant crop systems like DAS-68416-4 soybean will very likely foster rapid evolution of weeds resistant to 2,4-D and/or glyphosate. In those cases where the GR weed populations in 2,4-D crop fields already have resistance to one or more additional modes of action, the result will be evolution of still more intractable weeds with multiple-herbicide resistance, including to 2,4-D.

g. Multiple herbicide-resistant crops and weeds

Mortensen et al. (2012) note that there are currently 108 biotypes of 38 weed species possessing simultaneous resistance to two more classes of herbicide, and that 44% of them have appeared since 2005. Since herbicide-resistant weeds began to emerge in a significant way around 1970 (triazine-resistant weeds),[25] this means that nearly half of multiple HR weed biotypes have emerged in just the past seven years of our 40-year history of significant weed resistance. This global trend is also occurring in the U.S., where acreage infested with multiple HR weeds has increased by 400% over just the three years from November 2007 to November 2010 (Freese 2010, p. 15). There are at least 12 biotypes of weeds resistant to glyphosate and one or more other herbicide families in the U.S. (11) and Canada (1) that are attributable to RR crop systems, all but one having emerged since 2005 (ISHRW GR Weeds 4/22/12).

The progressive acquisition of resistances to different herbicide classes has the insidious effect of accelerating evolution of resistance to those ever fewer herbicides that remain effective. This is well-expressed by Bernards et al. (2012) with reference to multiple-herbicide-resistant waterhemp, though it applies more generally:

> The accumulation of multiple-resistance genes within populations and even within individual plants is of particular concern. This resistance stacking limits chemical options for managing waterhemp and, where weed management depends primarily on chemical weed control, results in additional selection pressure for the evolution of resistance to the few herbicides that are still effective.

There is already evidence that the scenario of 2,4-D resistance evolving in weeds already resistant to one or more herbicide classes, as depicted by Mortensen et al. (2012), will occur with four especially problematic species of weeds: horseweed, Palmer amaranth, waterhemp and kochia.

i. Horseweed

Horseweed, or marestail, is the most prevalent GR weed. First discovered in 2000 in Delaware, GR horseweed has emerged in just over a decade to infest up to 8.4 million acres in 20 states (CFS GR Weed List 2012[26]), up from 3.3 million acres in 16 states in February 2009 (Benbrook 2009a, p. 35). It is particularly prevalent in Tennessee, Kansas and Illinois, with populations infesting up to 5 million, 2 million and 1 million acres, respectively. GR horseweed in Mississippi is also resistant to paraquat, the first time multiple resistance to these two herbicides has been documented, while Ohio has glyphosate/ALS inhibitor-resistant[27] horseweed.

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[25] A few auxin-resistant biotypes emerged in the 1950s and 1960s.

[26] Consult this chart for data in the following discussion. It should also be noted that these acreage-infested estimates are highly conservative, in view of the underreporting in the ISHRW system, as discussed above.

[27] CFS suspects that GR weeds that are also resistant to ALS inhibitor herbicides are greatly underreported by ISHRW; this is certainly the case with waterhemp (see discussion below).

Weed scientists regard GR horseweed as a "worst-case scenario" in RR cropping systems because this weed is well adapted to no-tillage planting systems popular among GR crop growers. It also produces up to 200,000 seeds per plant, and its seeds can disperse extremely long distances in the wind (Owen 2008), which may partly explain the prevalence of GR horseweed.

GR horseweed can reduce cotton yields by 40 to 70% (Laws 2006), and is also problematic in soybeans. As long ago as 2003, Arkansas weed scientist Ken Smith estimated that Arkansas growers would have to spend as much as $9 million to combat glyphosate-resistant horseweed in 2004 (AP 2003). An uncontrolled outbreak of GR horseweed in Arkansas could reduce the income of cotton and soybean farmers by nearly $500 million, based on projected loss in yield of 50% in 900,000 acres of cotton and a 25% yield loss in the over three million acres of soybeans (James 2005). Tennessee is especially hard hit, with up to 5 million acres of both cotton and soybeans infested with GR horseweed.

Because GR horseweed is often controlled with tillage, it has led to abandonment of conservation tillage practices on substantial cotton acreage in Tennessee and Arkansas, with similar trends reported in Mississippi and Missouri (Laws 2006) and perhaps other states. This in turn increases soil erosion. An NRC committee reported that increased tillage and increased herbicide use are common responses to glyphosate-resistant weeds (NRC 2010). Evolution of multiple herbicide-resistance reduces options for chemical control and so increases the chances for still more soil-eroding tillage.

The many farmers with GR and multiple-HR horseweed would be prime candidates for Dow's Enlist soybeans and corn. Yet Purdue University weed scientists have flagged horseweed as a weed with the genetic "plasticity" to readily evolve resistance to multiple herbicides:

> Multiple-resistant and cross-resistant horseweed populations have evolved to various combinations of the previous herbicide modes of action in Israel, Michigan, and Ohio (Heap 2009), providing evidence for the plasticity of this weed. (Kruger et al. (2010a).[28]

These same scientists have already founded increased tolerance to 2,4-D in some horseweed populations, demonstrating the high potential for horseweed to evolve additional resistance to 2,4-D in the context of heavy postemergence use of 2,4-D, as enabled by Enlist soybeans:

> With the announcement that Dow AgroSciences intends to insert genes which convey 2,4-D resistance into corn (Zea mays L.), soybean [Glycine max (L.) Merr.), and cotton (Gossypium hirsutum L.) (Dow AgroSciences 2007), ***the use of 2,4-D postemergence on agronomic crop areas could dramatically increase***. This increased 2,4-D use will increase selection pressure for more tolerant weed populations such as horseweed population 34, potentially leading to the evolution of creeping resistance similar to that described by Gressel (1995). (Kruger et al. 2008, emphasis added)

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[28] As noted above, horseweed has also evolved dual resistance to glyphosate and paraquat in Mississippi.

The Purdue team underscores two common practices employed by farmers, particularly in the context of (multiple) HR weed systems, that would further increase the risks of 2,4-D resistance evolution in horseweed: reducing rates of herbicides when used in combination in tank mixes; and delaying application until weeds become larger, more difficult to kill, and hence more likely to propagate resistance.

> ***The use of 2,4-D in conjunction with glyphosate is often accompanied by a reduction in the rate of 2,4-D, further increasing the likelihood that horseweed plants will survive the application***. (Kruger et al. 2008, emphasis added).

According to the same team:

> With the announcement of 2,4-D resistance traits being transformed into corn and soybean (Wright et al. 2005), it is likely that POST selection pressure with 2,4-D will increase. Additionally, if 2,4-D is used POST, ***the herbicide applications will be applied to larger plants***, creating a need for understanding the impact of horseweed size on the efficacy of 2,4-D. (Kruger et al. 2010a, emphasis added)

Follow-up research addressed this very question – "the impact of horseweed size on the efficacy of 2,4-D" – and found that larger weeds became much more difficult to control:

> ***While it is realistic to expect growers to spray horseweed plants after they start to bolt,*** the results show that timely applications to [small] horseweed rosettes are the best approach for controlling these weeds with growth regulator herbicides [2,4-D and dicamba]. ***Growers should be advised to control horseweed plants before they reach 30 cm in height because after that the plants became much more difficult to control***. (Kruger et al. 2010b, emphasis added)

As discussed above, increased survival of larger weeds means a greater likelihood of resistant individuals among them surviving to propagate resistance via cross-pollination or seed production. And as the authors acknowledge, it is "realistic" to expect late application of 2,4-D with Enlist crops, because that is precisely the point of these crop systems, as also demonstrated with the history of RR crops. Illinois agronomist E.L. Knake states that "2,4-D is ***more effective on larger weeds*** than are most other herbicides" (Knake 1996, emphasis added).

This tendency to delay application to kill larger weeds will be greatly facilitated by the entirely new post-emergence use of 2,4-D enabled by DAS-68416-4 soybean. The proposed label permits 2 post-emergence applications of up to 1 lb/acre each, up through the time when soybeans are in full bloom (R2). And of course, delayed application will also be facilitated by the high-level 2,4-D resistance of the Enlist crops themselves, permitting many-fold higher rates without risk of crop injury,[29] higher rates which are needed for larger weeds. Thus, advising growers to spray weeds when they are small will likely not be any more effective with Enlist soybeans than were similar recommendations made for glyphosate with Roundup Ready crops.

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[29] Petition at 118, Table 28, showing little or no crop injury from application of 2 to 4 lbs/acre of 2,4-D.

Thus, the Enlist soybean system is very likely to promote rapid evolution of horseweed resistant to 2,4-D, often in combination with glyphosate-resistance. As noted above, tillage is a frequent response to glyphosate-resistant horseweed, and will be a still more frequent response to 2,4-D/glyphosate-resistant horseweed, since 2,4-D will be eliminated as an alternative control option. APHIS nowhere assesses the reasonably foreseeable impact of increased tillage to control weeds that become resistant to 2,4-D and/or glyphosate in the context of DAS-68416-4 soybean cultivation. This is a serious deficiency in the DEA that must be remedied in the context of an EIS.

CFS also notes that Dow is aware of the three studies by Kruger et al (2008, 2010a, 2010b) discussed above, as Dow has provided funding for these studies.[30] As these studies provide clear evidence for the likely emergence of 2,4-D-resistant weeds with introduction of 2,4-D-resistant soybeans and corn, Dow's failure to cite and discuss them is a clear violation of 7 CFR 340.6(c)(4), which requires petitioners to supply: "Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism…" Dow's violation is further compounded by its deceitful affirmation that: "DAS knows of no study results or other observations associated with DAS-68416-4 soybean that would be anticipated to result in adverse consequences from introduction" (Petition at 138).

ii. Waterhemp

Waterhemp is regarded as one of the worst weeds in the Corn Belt. It grows to a height of 2-3 meters, and emerges late into the growing season. Controlled trials in Illinois demonstrated that late-season waterhemp reduced corn yields in Illinois by 13-59%, while waterhemp emerging throughout the season cut yields by up to 74% (Steckel-Sprague 2004).

ISHRW lists 11 biotypes of GR waterhemp, all of which have emerged since 2005 in corn, soybeans, cotton and/or sugar beets, almost certainly all in RR crop systems (CFS GR Weed List). While ISHRW records up to 1.1 million acres infested with GR waterhemp, this is a vast underestimate. As noted above, Illinois weed scientist Bryan Young estimates a substantial 5-6 million acres infested with GR waterhemp in his state.

Waterhemp has an astounding ability to evolve resistance to herbicides. Biotypes resistant to one to four herbicide families have been identified in several Midwest and Southern states, from North Dakota to Tennessee (see CFS GR Weed List 2012 and ISHRW GR Weeds for those resistant to glyphosate). Triple herbicide-resistant waterhemp infests up to one million acres in Missouri, while populations resistant to four herbicide classes, sardonically called "QuadStack Waterhemp" (Tranel 2010), have arisen in Illinois. Tranel's investigations suggest that the 5-6 million acres of GR waterhemp in Illinois noted above are all resistant to ALS inhibitors, with some additionally resistant to PPO inhibitors and/or triazines.

Tranel states that multiple herbicide-resistant waterhemp "appears to be on the threshold of becoming an unmanageable problem in soybean," and is quite concerned that if already multiple herbicide-resistant waterhemp evolves resistance to additional herbicides, "soybean production

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[30] See "Acknowledgements" section at end of each study.

may not be practical in many Midwest fields" (Tranel et al 2010). Corn is often rotated with soybeans, and so could be similarly affected.

In early 2011, waterhemp was identified as the first weed to have evolved resistance to a relatively new class of herbicides, HPPD inhibitors, the fifth "mode of action" to which waterhemp has evolved resistance (Science Daily 2011), prompting weed scientist Aaron Hagar to comment that "we are running out of options" to control this weed. Populations of waterhemp in Iowa and Illinois are resistant to HPPD inhibitors and two other modes of action (ISHRW Waterhemp 2012).

Just months later, waterhemp resistant to its sixth mode of action, 2,4-D, was discovered, and it is potentially resistant to the popular corn herbicides atrazine and metolachlor as well, which would make it particularly difficult to manage (UNL 2011). The weed scientists who discovered this resistant weed population clearly understand the likelihood that 2,4-D resistant crop systems – "if used as the primary tool to manage weeds already resistant to other herbicides," the hallmark of these systems – will lead to still more intractable, multiple herbicide-resistant weeds:

> New technologies that confer resistance to 2,4-D and dicamba (both synthetic auxins) are being developed to provide additional herbicide options for postemergence weed control in soybean and cotton. The development of 2,4-D resistant waterhemp in this field is a reminder and a caution that these new technologies, if used as the primary tool to manage weeds already resistant to other herbicides such as glyphosate, atrazine or ALS-inhibitors, will eventually result in new herbicide resistant populations evolving. (UNL 2011)

In a peer-reviewed publication about this same waterhemp population, these scientists call for mandatory weed resistance prevention measures for DAS-68416-4 soybean and similar HR crops:

> ***The commercialization of soybean, cotton and corn resistant to 2,4-D and dicamba should be accompanied by mandatory stewardship practices*** that will minimize the selection pressure imposed on other waterhemp populations to evolve resistance to the synthetic auxin herbicides. (Bernards et al. 2012, emphasis added)

A close reading of this paper helps explain the concerns of these weed scientists. First, the 2,4-D-resistant waterhemp population is resistant to extremely high rates of 2,4-D, with some plants surviving application of 35,840 grams/hectare of 2,4-D, equivalent to 32 lbs/acre, or 32 times the maximum single 2,4-D application rate in the proposed label for 2,4-D use on DAS-68416-4 soybean. Second, this population also has lower-level resistance to dicamba. This is important because it suggests that waterhemp has the capacity to evolve simultaneous resistance to both 2,4-D and dicamba, even without application of dicamba (no dicamba use was reported on the field where this weed evolved 2,4-D resistance); and because the elimination of 2,4-D as an effective control option is compounded by the elimination or at least erosion of the efficacy of a second important control tool, dicamba. Third, as noted above, waterhemp is one of the most damaging weeds in the Corn Belt, and multiple herbicide-resistance makes it still more damaging and expensive to control.

It is interesting to note that the field where this waterhemp evolved resistance to 2,4-D and dicamba had also been regularly treated with atrazine and metolachlor: "Since 1996, atrazine, metolachlor, and 2,4-D were applied annually to control annual grasses and broadleaf weeds" (Bernards et al. 2012). This suggests the possibility of resistance to atrazine and/or metolachlor as well: "Research is underway at UNL to determine whether this waterhemp population has developed resistance to additional herbicide mechanisms-of-action" (UNL 2011).

Use of multiple herbicides is supposed to forestall evolution of resistance to any single herbicide (Petition at 132, Table 33). At least in the case of this waterhemp population, this strategy apparently did not work. Atrazine-resistant waterhemp has been reported in Nebraska and other states, and is particularly prevalent in Kansas, with up to 1 million infested acres reported.[31] Thus, it is possible that this population had previously evolved resistance to atrazine. This demonstrates the potential, discussed above with reference to glyphosate-resistant horseweed, for "resistance-stacking." More broadly, it casts doubt on the ability of multiple herbicide use on multiple herbicide-resistant crops such as DAS-68416-4 soybean to forestall the emergence of herbicide-resistant weeds. On the contrary, Bernards and colleagues' call for mandatory stewardship practices suggests that HR crops, as explained above, are particularly prone to foster rapid evolution of weed resistance.

iii. Palmer amaranth

Perhaps the most destructive and feared weed in all of U.S. agriculture is glyphosate-resistant Palmer amaranth (see Benbrook 2009a, Chapter 4). Second only to GR horseweed in prevalence, GR Palmer amaranth is estimated to infest 112,000 to over 220,000 fields covering up to 7.0 million acres in 12 states, all but one in corn, cotton and/or soybeans (CFS GR Weed List 2012). Best known for plaguing cotton and soybean growers in Southern states, this weed is rapidly emerging in Corn Belt states like Illinois and Missouri; a small population was even reported recently in Michigan (ISHRW GR Weed List 4/22/12) and also in Ohio (Ohio Farmer 2012). Palmer amaranth is feared especially because of its extremely rapid growth – several inches per day – which means it can literally outgrow a busy farmer's best attempts to control it while still small enough to be killed. It also produces a huge number of seeds, so just one mature weed can ensure continuing problems in future years by pouring hundreds of thousands of resistant weed seeds into the "weed seed bank." Left unchecked, its stem can become baseball bat breadth, and is tough enough to damage cotton pickers. Glyphosate-resistant Palmer amaranth can dramatically cut yields by a third or more, and occasionally causes abandonment of cropland too weedy to salvage. In Georgia, Arkansas and other states, farmers have resorted to hiring weeding crews to manually hoe this weed on hundreds of thousands of acres, tripling weed control costs (Haire 2010). Herbicide regimes of six to eight different chemicals, including toxic organic arsenical herbicides such as MSMA otherwise being phased out (EPA 2009, p. 3), are recommended to control it (Culpepper and Kichler 2009).

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[31] See entries for "photosystem II inhibitors," the class of herbicides to which atrazine belongs, at http://www.weedscience.org/Summary/USpeciesCountry.asp?lstWeedID=219&FmCommonName=Go.

At least three states (Mississippi, Georgia and Tennessee) have Palmer amaranth resistant to both glyphosate and ALS inhibitors; the most recent one, reported in 2011, infests over 100,000 sites covering up to 2 million acres in Tennessee (CFS GR Weed List 2012). Palmer amaranth belongs to the same genus as common waterhemp (*Amaranthus*), and to some extent can interbreed with it. Both have considerably genetic diversity. The demonstrated ability of waterhemp to evolve resistance to 2,4-D suggests that a similar potential likely exists in Palmer amaranth. Growers with GR and multiple HR Palmer amaranth would be prime candidates to adopt Enlist weed control systems, and utilize them under the proposed registrations. The likely emergence of 2,4-D resistance in Palmer amaranth, including populations already resistant to glyphosate and/or ALS inhibitors, would undermine the efficacy of existing, pre-emergence use of 2,4-D in battling this serious weed threat.

iv. Kochia

Kochia is a fourth serious weed, described further at CFS (2010). It has evolved widespread resistance to many different herbicides, and is on the ISHRW's list of the top ten most important herbicide-resistant weed species (ISHRW Worst HR Weeds). Limited populations of glyphosate-resistant kochia first emerged in Kansas in 2007, but recent reports suggest that it is now likely prevalent in the entire western third of Kansas, as well as parts of Colorado (Stahlman et al. 2011). A second population identified in Nebraska (2009) was first listed on ISHRW in December of 2011, and a third in South Dakota (2011), infesting up to 10,000 acres, was first listed in May of 2012. Kochia resistant to both glyphosate and ALS inhibitors was recently identified in Alberta, Canada (2012), while GR kochia resistant to kochia was just identified in North Dakota.[32] All of the US populations emerged in corn, soybeans and/or cotton (almost certainly RR versions), while the Canadian population emerged in cereals and "cropland" that may also include RR crops.

Stahlman et al. (2011) state that the original four populations in Kansas likely evolved glyphosate-resistance independently, but the rapid emergence across such a broad swath of the state suggests the potential for spread of the original populations, perhaps by resistant seed dispersal, as kochia "tumbleweed" can disperse seeds at considerable distances (see CFS 2010). CFS (2010) also documents that kochia is a serious weed of both alfalfa and sugarbeets, Roundup Ready versions of which have been recently introduced and are widely grown. GR kochia infesting these RR crops would seriously impair the efficacy of the RR trait; likewise, selection pressure from glyphosate use with these crop systems (especially in rotation with other RR crops, as seen particularly with RR sugar beets, which are frequently rotated with RR corn and/or RR soybeans) could rapidly lead to still more extensive emergence of GR kochia.

Four biotypes of kochia have also evolved resistance to synthetic auxin herbicides, the class to which 2,4-D belongs (ISHRW Kochia 2012). While none are listed as resistant to 2,4-D, all are resistant to dicamba, a closely-related auxin herbicide, which may indicate that kochia is a likely candidate for evolution of resistance to 2,4-D. As noted above, use of 2,4-D without dicamba appears to have selected for resistance to both herbicides in waterhemp. The rapid emergence of

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[32] See entries under Kochia at http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go.

GR biotypes in RR crop systems may induce growers to adopt Enlist corn and/or soybeans to control it; and kochia's demonstrated propensity to evolve resistance to dicamba may make it more likely to also evolve resistance to the closely related 2,4-D.

h. Potential for glufosinate resistant weeds

DAS-68416-4 soybeans are being heavily marketed for use with 2,4-D and/or glyphosate, but glufosinate could also be applied. As discussed above, glufosinate use on soybeans has been growing rapidly since the 2009 introduction of LibertyLink soybeans, but is still used on just 1.3% of soybean acres. Overall use of glufosinate in U.S. agriculture is minuscule – estimated above at just 1.92 million lbs./year – in comparison to 2,4-D and glyphosate, hence there has been much less selection pressure for weeds to evolve resistance to glufosinate. The entirely foreseeable emergence of weeds resistant to both 2,4-D and glyphosate with the introduction of DAS-68416-4 soybean would drive greater use of glufosinate in the future. However, there is already reason to question the efficacy of glufosinate in forestalling or managing resistance to 2,4-D and/or glyphosate in the event that it were to be used.

Avila-Garcia and Mallory-Smith (2011) have recently discovered Italian ryegrass resistant to both glyphosate and glufosinate in an orchard with a history of glyphosate use, but where little or no glufosinate had been used, and suspect a common, non-target site mechanism – reduced translocation – for resistance to both herbicides. ***They regard the potential for evolution of resistance to both herbicides where both glyphosate- and glufosinate crops are grown as an "alarming weed management issue."*** Growers of DAS-68416-4 soybean who have GR Italian ryegrass infestations would likely rely heavily on glufosinate, since the broadleaf herbicide 2,4-D would be ineffective on Italian ryegrass. APHIS notes the existence of this weed in a single sentence (DEA at 48), but provides absolutely no discussion of the potential implications for introduction of DAS 68416-4.

Tranel et al. (2010) find that glufosinate may soon be the only effective post-emergence herbicide option for control of already multiple-HR waterhemp in soybeans; that glufosinate is not well-suited to control this weed; and that "there is no reason to expect [waterhemp] will not evolve resistance to glufosinate if this herbicide is widely used." As noted above, waterhemp has already evolved massive resistance to glyphosate and has shown the ability to develop resistance to 2,4-D as well.

Dow touts its Enlist corn and soybean systems as the "solution" to weeds resistant to glyphosate, ALS inhibitors (DAS 2011d), as well as other modes of action, just as RR crop systems were regarded as the solution to prior resistance, particularly epidemic ALS inhibitor-resistant weed populations in soybeans. As documented above, dual resistance to glyphosate and ALS inhibitors is quite common in weeds, particularly common waterhemp and Palmer amaranth. If HR crop systems really did "solve" resistant weed problems, as Dow maintains, one would certainly not expect multiple HR weeds to expand dramatically with their use – yet that is precisely what has happened with its predecessor Roundup Ready system. As also discussed above, there are already very good scientific reasons to suspect that the major near-term consequences of widespread use of Enlist soybeans would be to foster additional resistance to 2,4-D, with extremely serious consequences for farmers, the agricultural economy, the environment and public health.

i. Stewardship

It is highly doubtful whether Dow's stewardship plan for DAS-68416-4 soybean (DAS 2011d) will be effective in forestalling weed resistance to 2,4-D. For at least 15 years, companies and weed scientists have touted voluntary stewardship guidelines and best management practices as the chief bulwark against evolution of resistant weeds in the context of HR crop systems. These programs and exhortations have demonstrably failed with Roundup Ready crops, or there would not be an epidemic of glyphosate-resistant weeds. A critical assessment of Monsanto's failed stewardship messages, practices and actions may be useful to inform an assessment of Dow's similar approach.

Monsanto insisted that weeds would not evolve glyphosate resistance to any serious extent when RR crops were first being introduced, based mostly on assumptions concerning the presumed rarity of glyphosate-resistance mutations, the lack of glyphosate-resistant weed evolution up to that time, and nuances of the herbicide's mode of action (Bradshaw et al. 1997). Many weed scientists were not convinced, and called for serious measures to forestall evolution of GR weeds (Freese 2010, question 1). Monsanto introduced its RR crops as "RR crop systems" designed for sole reliance on glyphosate for weed control. Even several years after GR weeds had emerged, Monsanto promoted "glyphosate-only" weed control programs in farm press advertisements that leading weed scientists castigated as irresponsible for promoting weed resistance (Hartzler et al. 2004). Monsanto continues to tout voluntary stewardship programs as an effective means to forestall or manage GR weeds, despite their obvious failure.

Dow scientists have similarly denied that weeds will evolve resistance to 2,4-D to any significant degree, based on the molecular nuances of 2,4-D's mode of action and the presumed rarity of 2,4-D resistant weeds up to this time (Petition at 173, Wright et al. 2010), quite similar to the fallacious arguments of Monsanto's scientists 15 years ago. When their assessment was effectively challenged (Egan et al. 2011), these same scientists fell back on the argument that stewardship recommendations would effectively prevent emergence of 2,4-D resistance (Wright et al. 2011). Yet Dow, like Monsanto before it, presents DAS-6814-6 as the "Enlist Weed Control ***System***" (DAS 2011a), suggesting to farmers a self-contained system for weed control involving sole reliance on post-emergence use of 2,4-D and glyphosate. Thus, there is little or nothing to distinguish Dow's stewardship program (DAS 2011d) from Monsanto's failed approach.

If Dow were serious about stewardship, the company would include a requirement that farmers not plant Enlist soybean in rotation with Enlist corn, which the company is also poised to introduce. This is because corn and soybeans are quite often grown in a two-year rotation (DEA at 15), and continual use of 2,4-D and/or glyphosate on both corn and soybeans, year-in and year-out, is the surest way to foster rapid evolution of 2,4-D-resistance in already glyphosate-resistant weeds.[33] Yet Dow's stewardship plan for DAS-68416-4 soybean has no such requirement, and in fact does not even mention Enlist corn (DAS 2011d). APHIS likewise provides no assessment of the resistant weed-promoting impact of DAS-68416-4 soybean grown in rotation with Enlist corn, which is also pending deregulation. This is a subject that quite obviously should have been assessed in the cumulative impacts section of the draft EA. This deficiency must be redressed in the context of an Environmental Impact Statement.

The need to consider the cumulative impacts of all 2,4-D-resistant crops together (as well as dicamba-resistant crops) is underscored by Bernards et al. (2012), whose call for "mandatory stewardship practices" encompassed "soybean, cotton and corn resistant to 2,4-D and dicamba." Furthermore, the demand for "mandatory" practices is an implicit acknowledgement of the failure of voluntary programs such as Dow's. APHIS fails to provide any critical assessment of Dow's stewardship plan.

j. Spread of weed resistance and tragedy of the commons

Weeds evolve resistance through strong selection pressure from frequent and late application as well as overreliance on particular herbicides, as fostered especially by HR crop systems. However, once resistant populations of out-crossing weeds emerge, even small ones, they can propagate resistance via cross-pollinating their susceptible counterparts (Webster & Sosnoskie 2010). It is estimated that common waterhemp pollen can travel for one-half mile in windy conditions, and so spread resistance to neighbors' fields via cross-pollination (Nordby et al. 2007). A recent study was undertaken to measure waterhemp pollen flow because "[p]ollen dispersal in annual weed species may pose a considerable threat to weed management, especially for out-crossing species, because it efficiently spreads herbicide resistance genes long distances," because the "severe infestations and frequent incidence [of waterhemp] arise from its rapid evolution of resistance to many herbicides," and because "there is high potential that resistance genes can be transferred among populations [of waterhemp] at a landscape scale through pollen migration" (Liu et al. (2012). The study found that ALS inhibitor-resistant waterhemp pollen could travel 800 meters (the greatest distance tested) to successfully pollinate susceptible waterhemp; and that waterhemp pollen can remain viable for up to 120 hours, increasing the potential for spread of resistance traits.

A second recent study made similar findings with respect to pollen flow from glyphosate-resistant to glyphosate-susceptible Palmer amaranth (Sosnoskie et al. 2012). In this study, susceptible sentinel plants were planted at distances up to 250-300 meters from GR Palmer amaranth. From 20-40% of the progeny of the sentinel plants at the furthest distances proved resistant to glyphosate, demonstrating that glyphosate resistance can be spread considerable distances by pollen flow in Palmer amaranth.

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[33] As noted above, the increasing use of RR corn by farmers already growing RR soybeans led to continual, year-in, year-out glyphosate selection pressure that is the major factor driving GR weed evolution in the Midwest.

Whether out-crossing or inbreeding, those resistant individuals with lightweight seeds can disperse at great distances. Dauer et al. (2009) found that the lightweight, airborne seeds of horseweed, the most prevalent GR weed (CFS GR Weed List 2012), can travel for tens to hundreds of kilometers in the wind, which is likely an important factor its prevalence. Hybridization among related weeds is another potential means by which resistance could be spread, for instance by weeds in the problematic *Amaranthus* genus (Gaines et al. 2012).

Thus, even farmers who employ sound practices to prevent emergence of herbicide-resistant weeds themselves can have their fields infested with resistant weeds from those of other farmers. With reference to GR weeds, Webster & Sosnoskie (2010) present this as a tragedy of the commons dilemma, in which weed susceptibility to glyphosate is the common resource being squandered. Since responsible practices by individual farmers to prevent evolution of weed resistance in their fields cannot prevent weed resistance from spreading to their fields as indicated above, there is less incentive for any farmer to even try to undertake such prevention measures.

The weed science community as a whole has only begun to grapple with the implications of the ***spread*** of resistance, particularly as it relates to the efficacy of weed resistance management recommendations based solely on individual farmers reducing selection pressure. It may not be effective or rational for farmers to commit resources to resistance management in the absence some assurance that other farmers in their area will do likewise. This suggests the need for a wholly different approach that is capable of ensuring a high degree of area-wide adoption of sound weed resistance management practices. This represents still another reason to implement mandatory stewardship practices to forestall emergence of 2,4-D-resistant weeds in the context of DAS-68416-4 soybean and similar crops. APHIS did not assess the dispersal of herbicide resistance traits via pollen or seed dispersal or its implications for stewardship practices in the draft Environmental Assessment, another deficiency demanding redress in an EIS.

k. Volunteer DAS-68416-4 soybean

Volunteer soybeans are not normally considered problematic weeds, but with the advent of RR soybeans there are some reports that glyphosate-resistance makes them more difficult to control. For instance, York et al. (2005) report that volunteer glyphosate-resistant soybean can be a problematic weed in glyphosate-resistant cotton planted the next season. They note in general that: “Volunteer crop plants are considered to be weeds because they can reduce crop yield and quality and reduce harvesting efficiency.” York and colleagues tested several herbicidal options to control GR soybean volunteers, including pyrithiobac, trifloxysulfuron, and each herbicide mixed with MSMA, an arsenic-based herbicide that EPA is in the process of phasing out due to its toxicity, though an exemption has been made for continued use in cotton to control GR Palmer amaranth (EPA 2009). They also note that paraquat can be used to control GR soybean volunteers prior to emergence of cotton. Some farmers have also reported problematic volunteer RR soybean in the following year’s corn, and sought advice from extension agents on how to deal with it (Gunsolus 2010). Recommendations include use of 2,4-D, dicamba, atrazine and/or other herbicides. In both cases, it is glyphosate-resistance that has made volunteer soybean a control problem for farmers, and necessitated the use of more toxic herbicides for control.

DAS-68416-2 soybean volunteers would possess resistance to 2,4-D, glyphosate and glufosinate, making them a more difficult challenge for farmers, reducing herbicidal control options versus volunteers that have resistance only to glyphosate, and necessitating use of more toxic herbicides or tillage to control.

Dow discusses the potential for DAS-68416-4 soybean to cross with soybeans possessing other herbicide resistance traits to produce soybean volunteers with resistance to additional herbicides (Petition at 127-28). Indeed, three different GE soybean events with resistance to dicamba (Monsanto), the HPPD inhibitor isoxaflutole (BASF), and imidazolinone herbicides (Bayer) are presently pending deregulation decisions by USDA (APHIS Pending Dereg 2012).[34] Such crossing could result in volunteer soybeans resistant to four or more classes of herbicide.

Dow notes that multiple-herbicide resistant soybean volunteers could be controlled with mechanical tillage, or use of paraquat, fluometuron or atrazine. One purpose of DAS-68416-4 soybean is supposed to be to avoid tillage to control resistant weeds, yet Enlist soybean volunteers (possibly stacked with resistance to additional herbicides) could increase use of soil-eroding tillage. Paraquat is a nerve toxin that has been linked to increased incidence of Parkinson’s disease, is the culprit in hundreds of thousands of pesticide poisoning cases each year worldwide (both accidental and suicidal), and is widely acknowledged to be among the most toxic of herbicides (Neumeister and Isenring 2011). Atrazine is an endocrine-disrupting herbicide and probable human carcinogen that has been banned in the EU due to its toxicity and the difficulty of keeping atrazine from contaminating water (Sass and Colangelo 2006). Atrazine is primarily a corn herbicide that is used little if at all on soybeans (DEA at 22-23, Table 7: atrazine is not listed), thus the introduction of DAS-68416-4 soybean could lead, via multiple HR soybean volunteers, to new use of this toxic herbicide in soybean production.

As a general matter, such “resistance stacking” speeds evolution to those herbicides that remain effective. It limits chemical options for managing weeds, and “where weed management depends primarily on chemical weed control, results in additional selection pressure for the evolution of resistance to the few herbicides that are still effective” (Bernards et al. 2012). While this statement was made with reference to HR waterhemp, it applies more generally to multiple HR weeds, including volunteer DAS-68416-4 soybeans.

Soybean is primarily a self-pollinating crop, but the potential for perhaps considerable cross-pollination is suggested by the frequency with which pollinators – bees (honeybees and wild bees), wasps and flies – visit soybean fields (Anonymous 2012, O’Neal & Gill 2012). Insect pollinators are known to effect pollination at considerable distances from the source plants, including from primarily self-pollinating crops (e.g. Pasquet et al. 2008). Even if soybean cross-pollination is relatively uncommon, it could give rise to problematic volunteer HR soybean control problems where it does occur, with the adverse consequences noted above.

This potentially serious plant pest issue presented by DAS-68416-4 soybean went completely unexamined in both the DEA and Plant Pest Risk Assessment.

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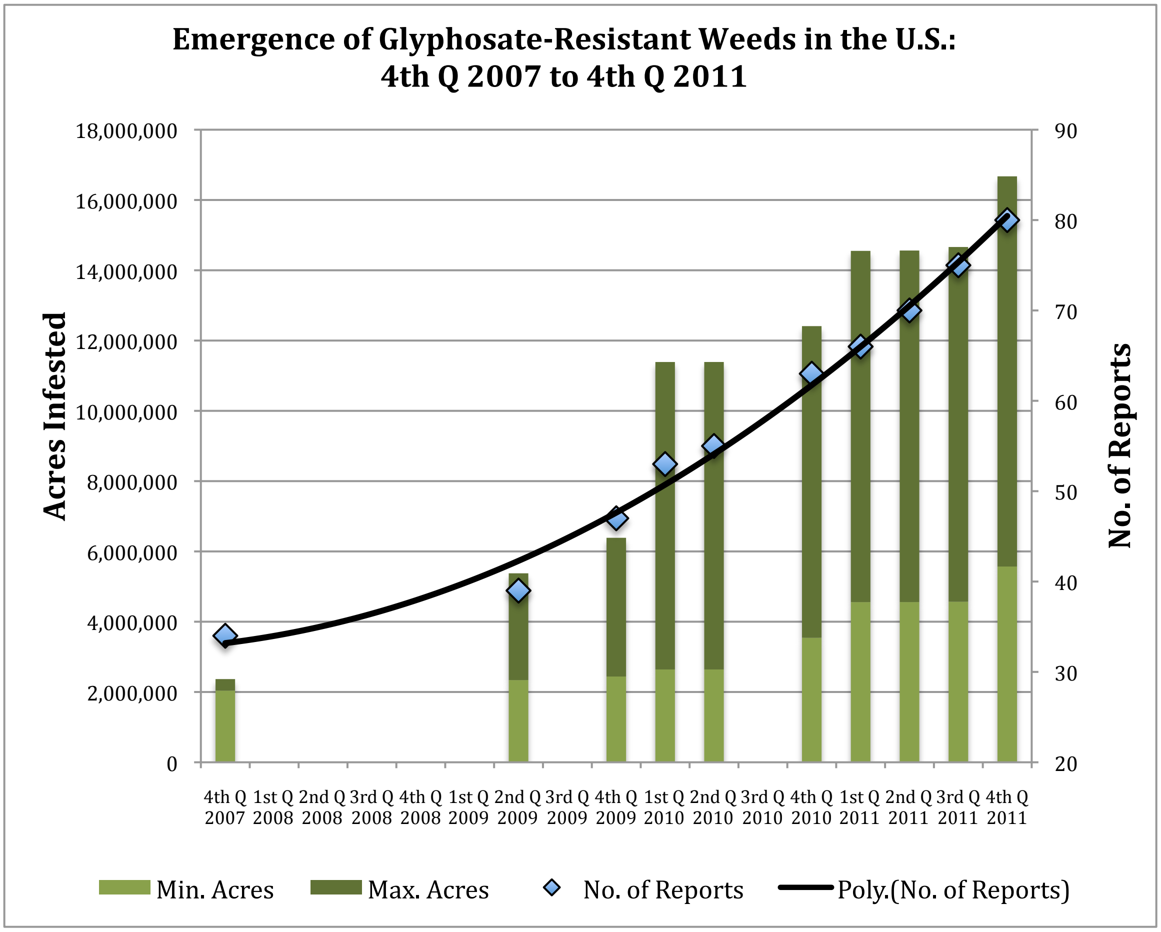
[34] See entries at http://www.aphis.usda.gov/biotechnology/not_reg.html, last visited 8/22/12.



Legend: This chart plots data on glyphosate-resistant weeds in the U.S. compiled from the International Survey of Herbicide-Resistant Weeds (ISHRW) as of December 31, 2011. See CFS GR Weed List (2012) for the data upon which this chart is based. The ISHRW lists reports of confirmed herbicide-resistant weeds submitted by weed scientists.[35] Each report normally contains the year of discovery, the number of sites and acreage infested by the resistant weed population, the crop or non-crop setting where the weed was found, whether or not the population is expanding, and date the report was last updated. Note that months to several years can elapse before a putative resistant weed population is confirmed as resistant and listed on the website. ISHRW reports sites and acreage infested in ranges due to the difficulty of making precise point estimates. CFS aggregated ISHRW data for all glyphosate-resistant weed reports on ten dates – 11/21/07, 2/2/09, 11/19/09, 2/25/10, 5/18/10, 11/30/10, 1/6/11, 7/5/11, 9/28/11 and 12/31/11 – corresponding to the ten bars in the graph above. The bars were assigned to the appropriate quarterly period on the x-axis. The minimum and maximum acreage values represent the aggregate lower- and upper-bound acreage infested by all glyphosate-resistant weeds listed by ISHRW on the given date. The number of reports is plotted on the secondary y-axis. ISHRW organizer Dr. Ian Heap made a point estimate of 10.4 million acres infested with GR weeds in May of 2010,[36] when the maximum acreage infested was 11.4 million acres. This suggests that the upper-bound estimates more closely approximate real world conditions. However, many reports of glyphosate-

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[35] Each report may be accessed by (and corresponds to) a link at: http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go.

[36] “WSSA supports NRC Findings on Weed Control,” Weed Science Society of America, 5/27/10. Dr. Heap is cited for the statement that 6% of total area planted to soybean, soybean and cotton in the U.S. [which is 173 million acres] is infested with GR weeds. http://www.wssa.net/WSSA/Information/WSSA%20position%20paper%20on%20herbicide%20resistance%205-27-2010.pdf.

resistant weeds in the farm press and scientific literature are never recorded by ISHRW because it is a voluntary reporting system; in other cases, old reports are not updated to reflect expanded populations. Thus, the actual acreage infested by GR weeds is likely even higher than the maximum acreage shown here.

4. Non-target crop and plant injury from herbicide drift with approval of DAS-68416-4 soybean

2,4-D is a volatile herbicide that is prone to drift beyond the field of application to damage neighboring crops and wild plants. 2,4-D vapor injures most broadleaf (i.e. non-grass) plants at extremely low levels, as low as three-billionths of a gram per liter of air (Breeze and West 1987). Particularly sensitive crops include grapes (Walker 2011), tomatoes, cotton (Bennett 2006), soybeans, sunflower, and lettuce. Two surveys of state pesticide regulators establish that 2,4-D drift is already responsible for more episodes of crop injury than any other pesticide (AAPÇO 1999, 2005).

Use of 2,4-D with DAS-68416-4 soybean would greatly increase drift injury to crops over already high levels by enabling higher rates, on much greater acreage, sprayed later in the season when neighboring crops and plants have leafed out and are thus more susceptible to drift injury (Mortensen et al. 2012). As discussed above, the magnitude of the increase in 2,4-D use with Enlist soybeans could be substantial. Overall 2,4-D use in soybean production would likely increase from 3-4 million lbs/year at present to 60-70 million lbs. The impacts on growers of many broadleaf crops could be severe.

A recent episode in California may be a harbinger of things to come. In the San Joaquin Valley, 1,000 acres of pasture were recently sprayed with 2,4-D under hot and windy conditions (Cline 2012). At least 15,000 acres of cotton and a 50-acre pomegranate orchard were damaged, with damage detected at up to 100 miles from the application site.

Although Dow claims that its 2,4-D choline is less volatile than other 2,4-D salts, it is unclear to what extent this would mitigate crop injury under field conditions. Spray drift (versus vapor drift) has more to do with weather conditions, application equipment, and the applicators' practices than with the properties of the herbicide formulation. Even if 2,4-D choline is less drift-prone, any improvement in mitigating drift that it might present will be swamped by vastly increased use. In any case, it will be difficult if not impossible to prevent the use of cheaper, highly-drift prone formulations of 2,4-D.

Vineyard operators are especially at-risk (Hebert 2004). Growers of vegetables, fruits and other smaller-acreage crops are already sparse in corn-soybean country. The introduction of Enlist soybeans could thin their ranks still further, decreasing what little crop diversity remains in the heartland. Growers of conventional and glyphosate-resistant soybeans would also be threatened by drift. There is already substantial litigation over drift-related crop injury, pitting farmer against farmer, and it would escalate dramatically with use of 2,4-D on Enlist crops under the proposed registrations (Huff 2011).

It is unclear whether such harms can be prevented or even mitigated should APHIS fully deregulate DAS-68416-4 soybean, yet there is no assessment of this serious issue in the draft EA.

APHIS must seriously assess the potential of the Enlist soybean system to increase drift-related crop injury as well as potential mitigation measures in the context of an Environmental Impact Statement.

Herbicide use in agriculture results in injury to non-target crops and wild plants, and approval of DAS-68416-4 soybean will result in increased herbicide use and changes in herbicide use patterns will increase this risk, as we have discussed in detail.

In soybeans, herbicide use, including drift, volatilization and runoff from such herbicide use, can cause poor seedling emergence, yellowing of leaves, necrotic lesions, and cupped leaves, similar to the injuries from pests or pathogens (Shumway and Scott 2012; UW Agronomy). For example, cupped leaves in soybeans, a symptom of injury from auxinic herbicides such as 2,4-D and dicamba, can also be caused by soybean mosaic virus, bean pod mottle virus and alfalfa mosaic virus (Legleiter et al. 2012).

Similarly, herbicide drift causes injuries to tomato like those from pathogen damage: "Cucumber mosaic virus and herbicide injury are almost identical. Cucumber mosaic virus causes tomato plants to yellow and become bushy and stunted. Leaves may be mottled."(Edmunds and Pottorff 2009).

As explained in detail, crop injury from herbicide use is a significant issue associated with the increased use of over-the-top applications of herbicides accompanying the cultivation of the DAS-68416-4 soybean system. In the PPRA, then, APHIS needs to consider – as a plant pest risk – the injury to non-target plants from increased herbicide use and different patterns of herbicide use as part of the DAS-68416-4 soybean system, and ways to prevent such injury.

Herbicides can also have direct effects on plant pathogens, either stimulating or suppressing the growth of particular bacteria and fungi (Duke et al. 2007; Sanyal and Shrestha 2008). Indirect effects on plant diseases are also common, and involve a variety of mechanisms: "Another potential indirect effect is alteration of plant metabolism or physiology in a way that makes it more susceptible or resistant to plant pathogens. For example, induction of higher levels of root exudate (e.g., Liu et al., 1997) or altered mineral nutrition (proposed by Neumann et al., 2006)." (Duke et al. 2007).

Herbicide dosage is important for the effects, and sometimes drift levels can stimulate the growth of pathogens, whereas full application rates suppress the same pathogens. Thus non-target plants may be at higher risk for diseases than the treated crop itself from herbicide applications: "It is not unusual for low rates of herbicides to stimulate in vitro pathogen growth (e.g., Yu et al., 1988). Hormesis (the stimulatory effect of a subtoxic level of a toxin) is common with both fungicide effects on fungi and herbicide effects on plants (Duke et al., 2006). Thus, dose rates are likely to be highly important in both direct and indirect effects of herbicides on plant disease"(Duke et al. 2007).

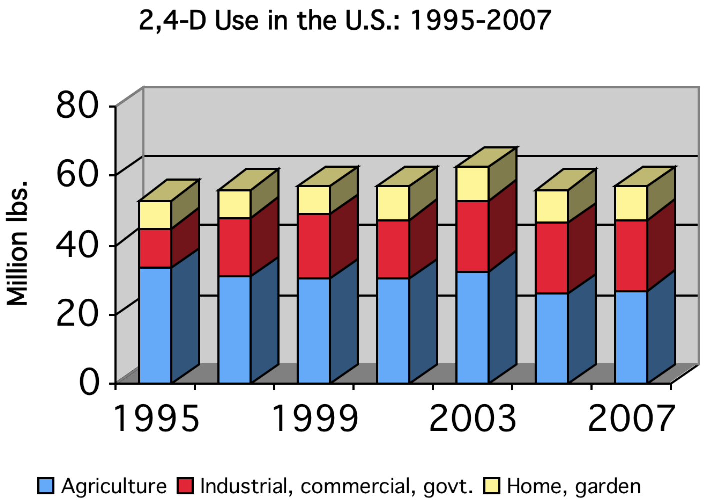
Therefore, in the PPRA, APHIS must consider the changes in pests and pathogens of non-target plants as a result of increased herbicide use and different patterns of herbicide use with the DAS-68416-4 soybean system, and they did not do so.

5. Human health and approval of DAS-68416-4 soybean

a. Health risks to farmers and the general public from exposure to 2,4-D

2,4-D is the most widely used of at least 10 compounds in the chlorophenoxy class of herbicides, which include 2,4,5-T, MCPA, dichlorprop, and mecoprop.[37] 2,4,5-T, which together with 2,4-D formed the Agent Orange defoliant used in the Vietnam War, was first subject to restrictions in 1970 on the basis of numerous studies demonstrating harm to human health that were generally linked to dioxin contaminants generated during its manufacture. However, Dow was able to extend its use for many applications through legal appeals and injunctions (Trost 1984, p. 169), until its registration was finally cancelled by EPA in 1985.

2,4-D is the main ingredient in over 1,500 pesticide products (EPA 2007). Overall use of 2,4-D in the U.S. has ranged from 53 to 63 million lbs. per year since 1995 (see chart), with the agricultural share declining from roughly 2/3 of overall use in 1995 to somewhat less than one-half by 2007, the latest year for which we have EPA data. 2,4-D has been the most heavily used pesticide in both the home/garden and the commercial-industrial-government sectors since 1995. Home/garden use of 2,4-D has increased slightly from 1995 to 2007 (from 8 to 9.5 million lbs./year), while industrial/commercial/ government use has nearly doubled over this period (11.5 to 20.5 million lbs./year). 2,4-D usage in 2007 is shown in Table 1.



Based on EPA's Pesticide Industry Sales and Usage reports from various years. Figures based on mid-points of ranges reported by EPA.

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[37] http://www.weedscience.org/summary/ChemFamilySum.asp?lstActive=&lstHRAC=24&btnSub2=Go

Use of 2,4-D in the United States (2007)		
Sector	**Amount used (min.) (lbs. a.i.)**	**Amount used (max.) (lbs. a.i)**
Home	8,000,000	11,000,000
Industry, commercial, government	19,000,000	22,000,000
Agriculture	25,000,000	29,000,000
TOTAL	**52,000,000**	**62,000,000**

Source: EPA Pesticide Use (2011). Note: EPA reports usage in ranges. a.i. = active ingredient.

The leading agricultural uses of 2,4-D in terms of pounds applied are pasture/rangeland and wheat, followed by soybeans and corn (EPA 2005, p. xi). The most recent available USDA NASS data discussed above show that 3.7 million lbs. of 2,4-D were applied to soybeans (2006). This situation would change dramatically if the Enlist soybeans are deregulated. Based on the 2,4-D usage projections discussed above, a conservative estimate places the amount of 2,4-D applied to soybeans at 67 million lbs./acre/year by 2025, an 18-fold increase over current (2006) usage. If adoption of Enlist soybeans is greater than projected, this total could easily rise to 100 million lbs. or more.

For the purposes of APHIS's cumulative environmental assessment, 2,4-D usage from the foreseeable introduction of Enlist corn must also be considered. As detailed in CFS comments on APHIS's draft EA for Enlist corn, 2,4-D use on corn would likely increase to roughly 100 million lbs. with the introduction and widespread adoption of Enlist corn, from just 3.3 million lbs. in 2010. Thus, 2,4-D use would increase to 160-170 million lbs. with the expected broad adoption of both Enlist soybeans and corn. This would represent a more than 20-fold increase over the 7 million lbs. of 2,4-D used on these two crops at present. Overall agricultural usage of 2,4-D would increase from 27 million lbs. to 180-190 million lbs.,[38] for a six to seven-fold increase in agricultural 2,4-D use. While these estimates of increased use may appear high, they are entirely consistent with the six- to seven-fold rise in agricultural use of glyphosate that has in fact occurred since the introduction of Roundup Ready crops: from 25-30 million lbs. in 1995 to 180-185 million lbs. in 2007.[39]

This vastly increased use would result in much greater exposure to 2,4-D, especially for farmers and pesticide applicators.

Impacts on Farmers and Pesticide Applicators

Farmers are in many ways healthier than the general population. They have lower mortality from heart disease; cancers of the lung, bladder, liver, colon, esophagus, rectum

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[38] Agricultural uses of 2,4-D other than corn and soybeans total 20 million lbs. (27 million for agriculture – 7 million for corn/soybeans); 160-170 + 20 = 180-190 million lbs.

[39] Glyphosate use in U.S. agriculture almost certainly exceeds 200 million lbs. today, given the sharp rise in Roundup Ready corn adoption from 2007 (52%) to 2011 (72%).

and kidney; as well as from all cancers combined. However, farmers from many countries experience higher rates of certain cancers – leukemia, non-Hodgkin's lymphoma, multiple myeloma, soft-tissue sarcoma, and cancers of the skin, lip, prostate, brain and stomach (Blair & Zahm 1995). The excess of certain cancers in farmers is striking in light of their lower mortality from most other causes. Which factors in the farming life might explain the fact that farmers are more likely to contract and die from certain cancers?

Several lines of evidence suggest that exposure to pesticides is one important factor. In broad terms, increased cancer risk coincides with pesticide use in time and space. The overall incidence of cancer in the U.S. population has risen sharply over the period of extremely rapid growth in the use of pesticides and other industrial chemicals, by 85% from 1950 to 2001 (Clapp et al 2006). Significant associations have been found between agricultural chemical use and cancer deaths in 1,497 rural U.S. counties (Steingraber 2010, p. 64).

Because direct human experimentation is unethical, the chief means to determine whether exposure to pesticides has adverse health effects is epidemiological studies. The rate or incidence of a disease in a population exposed to a particular pesticide is compared to that of a reference population of those not exposed to it. Any excess disease in the exposed population suggests that the pesticide is a risk factor that increases the likelihood of contracting the disease.

2,4-D and Non-Hodgkin's Lymphoma

Numerous epidemiological studies have reported an association between exposure to 2,4-D and non-Hodgkin's lymphoma,[40] a cancer of the white blood cells that kills 30% of those afflicted. The first studies linking 2,4-D with non-Hodgkin's lymphoma were published in Sweden thirty years ago.[41] Some of these studies also found an association with soft-tissue sarcoma, a rare and frequently fatal cancer.[42] More recently, studies published in Canada and Italy have supported these results, as have studies performed by researchers at the National Cancer Institute.[43,44,45]

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[40] *See, e.g.*, Hardell L, Eriksson M. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 85:1353-1360, 1999. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 256:1141-1147, 1986. McDuffic HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev. 10(11):1155-63, 2001; Mills PK, Yang R & Riordan D. Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988-2001. Cancer Causes and Control 16: 823-830, 2005. Selected studies included in supporting materials.

[41] Hardell L, Eriksson M, Lenner P, et al. Malignant lymphoma and exposure to chemicals especially organic solvents, chlorophenols and phenoxy acids: A case-control study. Br J Cancer 43:169-176, 1981.

[42] Hardell L, Sandstrom A. Case-control study: Soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711-717, 1979.

[43] McDuffie HH, Pahwa P, Robson D, Dosman JA, Fincham S, Spinelli JJ, McLaughlin JR. Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. J Occup Environ Med 47(8):806-16, 2005.

[44] Miligi L, Costantini AS, Veraldi A, Benvenuti A; WILL, Vineis P. Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. Ann N Y Acad Sci 1076:366-77, 2006.

Evidence from other occupational groups exposed to 2,4-D support the association found in studies of farmers. A recent study by The Dow Chemical Company of their pesticide production workers reported a 36% increase in non-Hodgkin's lymphoma in workers classified as exposed to 2,4-D, but the authors concluded the result was not statistically significant.[46]

A retrospective study of golf course superintendants (based on death certificates) revealed a proportionate mortality ratio for NHL of 237, meaning that death from NHL was 2.37 times more likely in this group than in the general population (Kross et al 1996). Many golf course superintendants have a history of pesticide application. 2,4-D is heavily used on golf courses. For instance, the authors note that 2.4-D is the most common herbicide applied to Iowa golf courses, representing about 42% of the total amount of herbicide applied.

Additional evidence of a link between 2,4-D and NHL comes from studies of canine malignant lymphoma conducted by National Cancer Institute scientists, which showed increased incidence of the canine equivalent of NHL in dogs exposed to 2,4-D-treated lawns (Hayes et al 1991, 1995).

In light of this abundant evidence, it is not surprising that scientists at the National Cancer Institute regard the link between phenoxyacetic acid herbicides[47] and NHL as "the strongest association" yet found in "epidemiological investigations focusing on pesticides" (Zahm & Blair 1995).

NHL is a disease of the lymphocytes, or white blood cells. Cancer involves uncontrolled cell growth, or proliferation. Exposure to 2,4-D increases lymphocyte replication in humans. One study of pesticide applicators found increasing lymphocyte proliferation of 11 to 14 percent greater than normal in the applicators in a manner that was directly related to 2,4-D absorbed dose (Figgs et al 2000). This finding was confirmed in a follow-up study, showing a 12-15% rise in lymphocyte proliferation, with a further indication that higher-dose exposures may cause direct damage to white blood cells, thereby increasing the risk of lymphoid cancer in humans (Holland et al 2002). These findings are consistent with, and provide mechanistic support for, the frequently-reported epidemiologic evidence linking 2,4-D exposure to non-Hodgkin's lymphoma in humans discussed above.

In 2010, approximately 65,540 people in the United States were diagnosed with non-Hodgkin's lymphoma. The incidence of this disease in the United States has increased to

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[45] Chiu BC, Blair A. Pesticides, chromosomal aberrations, and non-Hodgkin's lymphoma. J Agromedicine 14(2):250-5, 2009. Zahm SH, Blair A. Pesticides and non-Hodgkin's lymphoma. Cancer Res 1;52(19 Suppl):5485s-5488s, 1992.

[46] Burns C, Bodner K, Swaen G, Collins J, Beard K, Lee M. Cancer Incidence of 2,4-D Production Workers. Int J Environ Res Public Health 8:3579-3590, 2011.

[47] 2,4-D is by far the most widely used phenoxyacetic acid herbicide used in American agriculture.

about double the rate seen in the 1970s, even when adjusted for population size and age.[48] While this epidemic rise in NHL has slowed since 1990, the incidence rate continues to increase (see SEER chart below). As noted above, studies from around the world have shown that farmers are more likely to contract NHL than the general population, despite having a lower incidence of cancer overall, while numerous epidemiology studies have found significant associations between 2,4-D exposure and NHL. Other occupational groups exposed to 2,4-D have also been found to suffer higher rates of NHL, and animal epidemiology provides further support. In addition, several studies demonstrating increased lymphocyte proliferation with exposure to 2,4-D offer mechanistic support for this epidemiology. Based on these multiple, mutually supporting lines of evidence, it is reasonable to conclude that exposure to 2,4-D is very likely responsible for some portion of non-Hodgkin's lymphoma cases each year.

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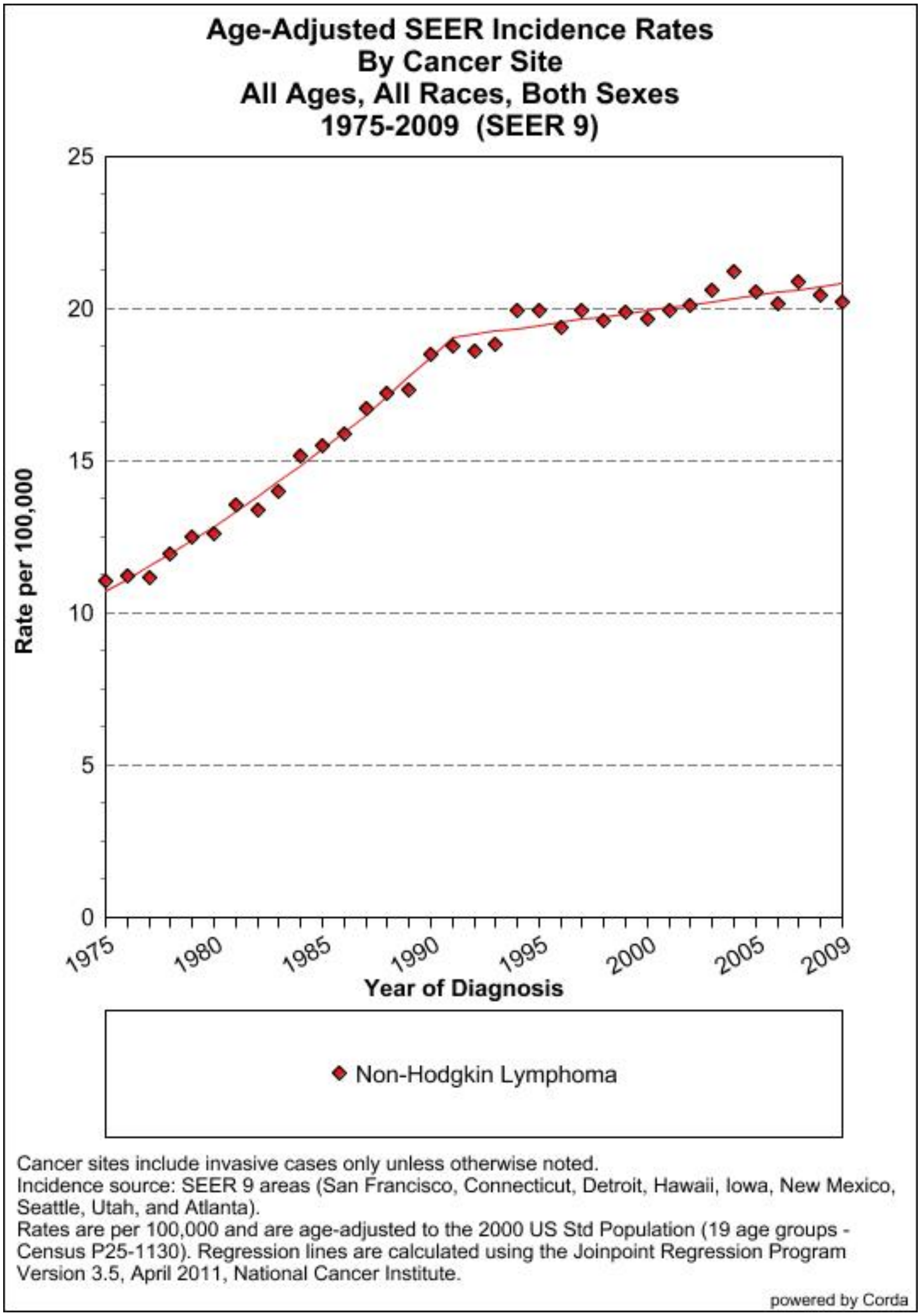
[48] Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2008, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2008/, based on November 2010 SEER data submission, posted to the SEER web site, 2011.



Age-Adjusted SEER Incidence Rates
By Cancer Site
All Ages, All Races, Both Sexes
1975-2009 (SEER 9)

Cancer sites include invasive cases only unless otherwise noted.
Incidence source: SEER 9 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta).
Rates are per 100,000 and are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1130). Regression lines are calculated using the Joinpoint Regression Program Version 3.5, April 2011, National Cancer Institute.

powered by Corda

Downloaded from http://seer.cancer.gov/, 6/21/12.

It should be noted that EPA's classification of 2,4-D as a Category D chemical ("not classifiable as to human carcinogenicity") is out of line with assessments by other authoritative bodies that take epidemiology more seriously, not to mention those by leading epidemiologists at our National Institutes of Health. For instance, the governments of Norway, Sweden and Denmark have banned 2,4-D (Boyd 2006), together with 2,4,5-T, largely on the basis of the epidemiological associations to non-Hodgkin's lymphoma and related cancers. The World Health Organization's International Agency for Research on Cancer (IARC) classifies the chlorophenoxy herbicide group, of which 2,4-D is by far the most widely used member, as "possibly carcinogenic to humans" (IARC 1987). WHO IARC finds "limited evidence in humans" for chlorophenoxy herbicides as causative agents for multiple cancers (i.e. "multiple sites") (IARC 2012). EPA incorrectly states that IARC has not assessed chlorophenoxy herbicides for carcinogenicity (EPA 2007).

The Institute of Medicine (IOM) of the National Academy of Sciences has conducted biennial reviews of the toxicity of Agent Orange compounds (2,4-D, 2,4,5-T and dioxins) for the Veteran's Administration for many years. Recognizing the difficulty in separating out the effects due to each, the IOM's approach is to assesses the toxicological evidence for what it terms "chemicals of concern," which encompass all three components of Agent Orange – 2,4-D, 2,4,5-T and the class of dioxin compounds. Like WHO's IARC, the IOM Committee seriously considers and gives substantial weight to human epidemiological evidence, in stark contrast to EPA's dismissal of the same.

In consequence, the IOM Committee's most recent review (IOM 2012) concludes as follows:

There is sufficient evidence of an association between exposure to the chemicals of interest and the following health outcomes:

* Soft-tissue sarcoma (including heart)
* Non-Hodgkin's lymphoma
* Chronic lymphocytic leukemia (CLL) (including hairy cell leukemia and other chronic B-cell leukemias)
* Hodgkin's disease
* Chloracne

Finally, EPA's assessment of the cancer risks posed by 2,4-D conflicts with the views of leading U.S. epidemiologists at the National Cancer Institute, part of the National Institutes of Health. As noted above, Dr. Aaron Blair and Dr. Sheila Hoar-Zahm regard the link between phenoxyacetic acid herbicides such as 2,4-D and NHL as "the strongest association" yet found in "epidemiological investigations focusing on pesticides" (Zahm & Blair 1995).

Parkinson's Disease

Exposure to 2,4-D (an organochlorine compound) has been associated with a significant, more than 2-fold increased risk of Parkinson's disease (odds ratio = 2.59 (1.03-6.48)) in subjects occupationally exposed to it (Tanner et al 2009). The authors of this paper note that other organochlorines have also been associated with increased risk of Parkinson's disease (PD), that excessive amounts of organochlorines have been found in the brains of people with PD, and that 2,4-D is known to affect dopaminergic neurons in experimental settings, all of which strengthen the association of 2,4-D with PD found in this study.

Spermatic Abnormalities and Birth Defects

Male pesticide applicators who applied 2,4-D exhibited lower sperm counts and more spermatic abnormalities than men who were not exposed to this herbicide.[49] In Minnesota**,** children of pesticide applicators in wheat-growing regions of Minnesota with heavy use of chlorophenoxy herbicides like 2,4-D had a disproportionately higher incidence of birth anomalies than in non-crop regions or where these herbicides were less used; moreover, this increased incidence was most pronounced among infants who were conceived in the spring, when herbicide use is greatest (Garry et al 1996). Another study that encompassed agricultural counties in Montana, North Dakota and South Dakota as well as Minnesota found significant increases in malformations of the circulatory and respiratory systems, particularly among infants conceived between April and June in wheat-growing counties where chlorophenoxy herbicides such as 2,4-D are heavily used (Schreinemacher 2003).

Infants and Young Children

It is well-known that infants and young children are more susceptible to adverse effects of toxins, including pesticides, than adults. 2,4-D is often tracked into homes, where it may persist for months on carpets, leading to potentially significant exposures of infants and toddlers who crawl on the floor and consume 2,4-D through hand-to-mouth contact.[50] Farm children as a group, as well as children of homeowners who use 2,4-D-containing herbicides on their lawns, are at greater risk of exposure to and adverse effects from 2,4-D. As noted above, 2,4-D is the leading herbicide used in the home/garden sector.

Liver Toxicity

Leonard et al (1997) documented a case of acute hepatitis in a golfer who played on a golf course treated with 2,4-D, and who engaged in the common golfer practice of licking his golf balls. After exhaustive analysis of medical tests (e.g. liver enzyme assays) and ruling out other potential causes, the researchers concluded that there was "little doubt that our patient's hepatitis was due to ingestion of 2,4-D from his golf ball." A case of chronic hepatitis tending to cirrhosis was diagnosed in an avid golfer who had for many years frequently licked his golf balls (Johnston et al 1998). Though not confirmed to be due to 2,4-D exposure, the authors found no other cause that might explain the patient's condition.

Epidemiology vs. Animal Studies and Human Exposure Estimates

Much of the evidence for 2,4-D's toxicity presented above is based on epidemiological studies. The medical community relies heavily on epidemiology to assess the environmental causes of disease (e.g. exposure to pesticides) because human experimentation is unethical. In contrast,

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[49] Lerda D, Rizzi R. Study of reproductive function in persons occupationally exposed to 2,4-D. Mutation Research 262: 47-50, 1991.

[50] Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. Environ Health Perspect. 2001 Nov;109(11):1185-91; Marcia G. Nishioka et al., Measuring Lawn Transport of Lawn-Applied Herbicide Acids from Turf to Home: Correlation of Dislodgeable 2,4-D Turf Residues with Carpet Dust and Carpet Surface Residues, 30 ENVTL. SCI. & TECH. 3313 (1996).

the EPA relies much more heavily on extrapolation from animal experiments to assessments of human exposure based on ideal world assumptions. Canadian physicians reviewing the toxicology of 2,4-D expressed the frustration that many medical professionals feel regarding the EPA's approach:

> "…two separate bodies of evidence are considered by the regulators (animal toxicity, exposure estimates) and the medical community (epidemiology). It may not be a surprise that they reach divergent conclusions regarding the advisability of using 2,4-D on lawns where children play."[51]

The strength of using animal studies to assess pesticide risks is that they permit strict control of the conditions of exposure, including calibrated doses to facilitate identification of dose-response relationships; exclusion of confounding environmental factors, such as exposure to other compounds; and sacrifice of animals for histological and other investigations to ascertain mechanisms of action and tissue damage. However, animal studies as they are conducted by industry for EPA have many weaknesses which far outweigh their strengths.

Animal studies generally utilize only the active ingredient of a complex pesticide formulation, and therefore cannot gauge the impacts of the active ingredient under conditions of actual use, which include potential independent, additive or synergistic effects of inert ingredients in the formulation.[52] Likewise, EPA for the most part does not require any animal testing for mixtures of pesticides, or otherwise account for exposure to multiple pesticides. It is also difficult to relate any adverse impacts found in animal studies to humans due to interspecies differences in susceptibility, which may be greater or lesser in human beings. Inferences from animal studies to human beings require a multitude of often highly dubious assumptions. First, safety factors intended to account for potentially greater human susceptibility to a particular effect found in animals are often applied in the complete absence of any mechanistic understanding of that effect, and hence may be insufficiently protective in some cases. Second, such inferences rely on highly theoretical human exposure estimates that are often based on ideal-world assumptions. In particular, EPA often prescribes measures (e.g. personal protection equipment, reentry intervals) intended to reduce farmer or applicator exposure to a pesticide, and bases exposure estimates on perfect compliance with such prescriptions, despite abundant evidence that they are often not observed (Jacobs & Clapp 2008). Finally, animal studies are most commonly conducted by the pesticide's manufacturer or by a third party contracted by the manufacturer, introducing conflict of interest concerns.

The strength of epidemiology is that it bypasses these weaknesses of the animal experiment-exposure assessment model to deliver evidence on real world outcomes. For instance, any adverse impacts of a pesticide's inert ingredients are automatically accounted for, as are real-world exposures from imperfect observance or neglect of exposure mitigation measures.

EPA's Treatment of Epidemiological Studies Involving 2,4-D

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[51] Sears, M et al (2006). "Pesticide assessment: protecting public health on the home turf," Paediatr Child Health 11(4): 229-234.

[52] This is not an inherent liability of animal experiments, but the great majority of animal trials involving pesticides are in fact carried out with the active ingredient alone.

While EPA does not completely ignore epidemiology, its treatment of the evidence reveals either extreme bias against, or fundamental misconceptions about the nature of, the science. In EPA's 2005 reregistration of 2,4-D, the Agency found that none of the recent epidemiological studies "definitively linked" human cancer cases to 2,4-D (EPA 2005, p. 20). Similarly, in a recent review of epidemiological studies on 2,4-D and cancer (EPA 2004), EPA health statistician Jerome Blondell quotes from a review article on 2,4-D epidemiology: "Overall, the available evidence from epidemiological studies is not adequate to conclude that any form of cancer is causally associated with 2,4-D exposure." As EPA acknowledges, the authors of this deeply flawed review were funded by 2,4-D manufacturers (Garabrant & Philbert 2002).[53] Surprisingly, Dr. Blondell offers no critical comment on this statement. He is apparently unaware that epidemiology, by its very nature, cannot definitively prove causation.[54] Using precisely the same rationale, tobacco industry apologists long denied the epidemiology implicating tobacco smoking as a prime cause of lung cancer based on ignorance of the specific cellular mechanisms involved. While it may be safely assumed that 2,4-D puts far fewer lives at risk than smoking, this fact offers little solace to the farmer who contracts a deadly cancer from use of an EPA-approved pesticide he/she naturally assumes is safe.

In practice, EPA's treatment of epidemiological studies appears to be somewhat more nuanced, but underlying it is the demand for the "definitive link" of mechanistic causation that epidemiology cannot by its very nature deliver. Dr. Blondell repeatedly dismisses epidemiology studies on 2,4-D and cancer on the grounds that exposure to other pesticides cannot be ruled out as potential contributing factors (EPA 2004). It is important to understand that dismissal on this basis effectively excludes ALL epidemiology studies as irrelevant, since one cannot expect to find any farming or other pesticide-exposed population that is not exposed to one or more pesticides in addition to the one of interest (e.g. 2,4-D). This approach is equivalent to demanding that an epidemiological study be what it cannot be, either ethically or in practical terms: a perfectly controlled experiment involving intentional dosing of a human population with a single pesticide.

This review also suggests that EPA does not assess pesticides with common mechanisms of action as a group for the purposes of epidemiology. For instance, EPA (2004) dismisses a Canadian study (McDuffie et al 2001) that found a significant association between exposure to 2,4-D and NHL (adjusted odds ratio = 1.32, 95% confidence interval 1.01 to 1.73) primarily on the grounds that the subjects were also exposed to another, much lesser used, phenoxy herbicide (mecoprop) and the closely related auxin dicamba. The authors apparently utilized common statistical tools used in epidemiology to isolate the effects of 2,4-D, but not to the satisfaction of EPA. Dr. Blondell also discounts the study on the illegitimate grounds that stronger associations were found to other risk factors. It is relevant to note here that 2,4-D is

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[53] See EPA (2004) for full reference. Garabrant & Philbert, environmental health scientists at the University of Michigan, apparently have no familiarity with modern industrial agriculture. In their zeal to exonerate 2,4-D, they make the following wildly inaccurate statement: "Moreover, herbicides are not used on a substantial proportion of farms." On the contrary, herbicides are ***far*** more widely and heavily used than any other class of pesticides. Scientists who have such ignorance of basic facts about herbicide use in modern agriculture are simply not qualified to conduct an epidemiological review of studies on exposure to an herbicide.

[54] "Epidemiological studies can never prove causation; that is, it cannot prove that a specific risk factor actually causes the disease being studied." http://pmep.cce.cornell.edu/profiles/extoxnet/TIB/epidemiology.html.

far more widely used than any other phenoxy herbicide. Assuming that phenoxy herbicides have a similar mechanism of action in disease causation, any effects attributed to phenoxy herbicides where more than one is used would in most cases be attributable primarily to 2,4-D. For instance, EPA figures show that the second most heavily used phenoxy in agriculture is MCPA, just 2-4 million lbs. of which were used in contrast to 27 million lbs. of 2,4-D (2007). The magnitude of exposure to 2,4-D would of course increase dramatically under the proposed registrations.

A deeper criticism of the unrealistic demand that epidemiological studies deliver definitive evidence of a causative link between exposure to a single pesticide and a disease outcome is our scientific understanding that disease causation is often multifactorial (see generally Steingraber 2010). What EPA views as "confounding variables" may in some cases be "contributing factors." The removal of a pesticide implicated as a risk factor for a disease will in many cases mitigate or eliminate the adverse health outcomes associated with exposure to it in combination with other pesticides or risk factors.

If taken seriously, the multifactorial nature of much disease would lead naturally to a very different pesticide assessment regime in which "confounders" were viewed as potential contributing factors rather than grounds for dismissal. Similarly, it is very likely that the multiple factors involved in disease causation helps to explain the inconsistent results in many epidemiological studies, including those involving 2,4-D exposure.

In light of the foregoing discussion, APHIS's statement that "EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment" (DEA at 114, 115, 123) is demonstrably false. As noted above, more scientifically based regulators in other countries have banned 2,4-D based on medical epidemiology studies that EPA ignores. EPA's assessment of 2,4-D's carcinogenicity potential is out of step with assessments by leading scientists at the U.S. National Institutes of Health and by other authoritative bodies. The continuing contamination of 2,4-D with dioxins casts further doubt on EPA's assessment of 2,4-D.

Dioxin Contaminants in 2,4-D

EPA generally appears to believe that any adverse health outcomes associated with 2,4-D by medical science are attributable to dioxin contaminants, and that dioxin levels in 2,4-D have been reduced sufficiently over the past several decades to eliminate such adverse effects.

However, it is not at all clear that dioxins associated with 2,4-D are low enough to be of no concern. First, EPA acknowledges that 2,4-D is the nation's seventh largest source of dioxins, based on industry tests conducted in the early 1990s (EPA 2005, p. 83). This ranking of 2,4-D would likely be considerably higher if EPA were to include dioxins emitted in the 2,4-D production process. While CFS does not have data on dioxin emissions associated with 2,4-D production, the EPA's toxics release inventory does show that two Dow plants (Freeport Facility and Louisiana Operations) rank 3rd and 9th in dioxin emissions (EPA TRI 2010). 2,4-D use would increase dramatically with introduction and adoption of Enlist soybeans and corn.[55] The release of dioxins into the environment would be greatly increased by the increased volume of dioxin-contaminated 2,4-D applied to agricultural fields, and through corresponding increases in any dioxins released from manufacturing plants due to expanded production.

Industry-conducted tests ordered by the EPA in a 1987 data call-in show that 2,4-D from early 1990s is still contaminated with significant levels of dioxins, on average 0.70 ug/kg TEQ, equivalent to 700 ppt TEQ (EPA Dioxins in 2,4-D). Based on assurances from the pesticide industry's 2,4-D Task Force, EPA believes that the 2,4-D production process has been improved to sharply reduce dioxin contamination of 2,4-D since that time (EPA 2005, pp. 83-84). EPA stated that further dioxin testing on more recent batches of 2,4-D would be required to confirm industry claims of reduced dioxin contamination (EPA 2005, p. 84), an implicit admission that EPA regards those levels as unacceptable. But it is unclear whether such tests have been formally requested by EPA or submitted to the Agency; and the data, if any, have not been made publicly available. CFS is also unaware of any dioxin testing on 2,4-D choline in particular.

At least two studies measuring dioxin contamination of 2,4-D formulations manufactured since the early 1990s refute industry assurances of reduced dioxins upon which EPA relies. USDA Agricultural Research Service scientist Janice Huwe led a team that conducted dioxin testing of several 2,4-D formulations collected by University of Minnesota from 1993 to 1998 (Huwe et al 2003). These researchers found dioxin levels of up to 2,627 ppt [parts per trillion], which as they note exceeds the highest dioxin level of 850 ppt found in 2,4-D formulations of the 1980s tested by Schecter et al (1987). The average dioxin TEQ level of five 2,4-D formulations tested by Huwe et al (2003) was 954 ppt (see Table 1), similar to the average value of 700 ppt reported by EPA based on industry tests in the early 1990s.

Interestingly, Huwe et al conducted their study in part to investigate whether dioxins in 2,4-D and other pesticide formulations might be responsible for an observed correlation between pesticide exposure and lowered testosterone levels in pesticide applicators of the Red River Valley of Minnesota, as well as an increased ratio of female to male offspring in the offspring of these same applicators. Their results confirm the continuing presence of toxic dioxins in 2,4-D and "support the need for more investigation into possible human health effects" (Huwe et al 2003). These researchers also developed a new CALUX bioassay which they believe provides a more accurate measure of dioxin-like toxicity than the traditional HRGC-MS assay. This is worrying, as CALUX tests suggest generally greater dioxin-like toxicity in 2,4-D than standard HRGC-MS tests. APHIS fails to discuss or cite this USDA ARS-led study in its draft EA.

Australian researchers recently tested two 2,4-D formulations manufactured in 2005 and 2006, and found dioxin levels comparable to those found in 2,4-D made 10-20 years ago (Holt et al 2010). These findings cast further serious doubt on industry claims of reduced levels of

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[55] Dow has announced construction of a new 2,4-D manufacturing facility designed to meet increased demand from Enlist corn and soybeans at its Freeport, Texas plant. See http://www.dow.com/news/multimedia/media_kits/2012_04_19a/pdfs/Marketing-Brochure-CORRECTED-FINAL.PDF.

dioxin contaminants in 2,4-D.

USDA should conduct or commission independent dioxin testing of 2,4-D formulations in the context of an Environmental Impact Statement. As noted above, there are 1,500 formulations containing 2,4-D as the main ingredient, and they are produced by a multitude of different manufacturers. Such independent tests must be undertaken on a broad cross-section of 2,4-D formulations produced by these manufacturers. This is needed because various manufacturers utilize different production processes, and it is well known that the level of dioxin contamination can vary dramatically under different production conditions.[56] Until credible, independent dioxin testing is conducted on a broad range of off-the-shelf 2,4-D formulations (rather than batches of 2,4-D provided for testing purposes by 2,4-D manufacturers[57]), the actual dioxin content and toxicity of 2,4-D will remain uncertain.

Another source of dioxin contamination is the incineration of 2,4-D containers, which are often burned without the recommended triple-rinsing to remove 2,4-D residues. Tests were recently conducted by EPA scientists on standard 2.5 gallon HDPE jugs in which pesticides are commonly sold (Gullett et al 2012). It was determined that about 10 ml of 2,4-D residue remained in unrinsed containers. Incineration of unrinsed containers resulted in many-fold higher levels of PCDD/PCDF formation (both on a TEQ and even more on weight basis) than either clean or triple-rinsed 2,4-D HPDE jugs. Gullett et al note that EPA estimates that 218 million plastic nonrefillable (single use) containers for liquid pesticides are in use (2005). USDA should assess the increased dioxin emissions and exposure associated with incineration of unrinsed 2,4-D containers that would result from the vastly increased use of 2,4-D with Enlist soybeans and corn.

Dioxin Contaminants in 2,4-D in Light of EPA's Ongoing Review of Dioxin Toxicity

In February 2012, EPA issued Part 1 of its long-awaited assessment of dioxin toxicity, that pertaining to non-cancer risks (EPA 2012). EPA established a low chronic oral reference dose for dioxins of 7.0×10^{-10} mg/kg-day for non-cancer risks. The chronic oral reference dose is defined as: "An estimate of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime."[58] The assessment of cancer risks posed by dioxins is still underway.

It is essential that USDA and EPA conduct an assessment of the greatly increased exposure to dioxins that would be triggered by Enlist soybeans and corn in light of EPA's ongoing review of dioxin toxicity, both cancer and non-cancer risks. As indicated above, the assessment should include collection of reliable data on dioxin contaminants in a broad range of 2,4-D

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[56] For instance, in 1963 Dow switched to a different production process for 2,4-D and 2,4,5-T that involved higher temperatures and more dioxin contamination in order to meet increased demand for these compounds in U.S. agriculture and Vietnam (i.e. for Agent Orange) (Trost 1984, p. 79). The introduction of Enlist soybeans and corn would similarly lead to greatly increased demand for 2,4-D, and thus the incentive to utilize more "efficient" production processes that result in 2,4-D with greater levels of dioxin contaminants.

[57] The latter approach could lead to provision of 2,4-D batches that are specially manufactured to have low dioxin levels that do not reflect dioxin levels in 2,4-D produced commercially.

[58] http://www.epa.gov/iris/gloss8_arch.htm.

formulations, and associated exposure; assessment of dioxin emissions from manufacturing facilities that produce 2,4-D; and dioxin emissions from incineration of 2,4-D containers with 2,4-D residues. The assessment should take particular account of increased exposure of farmers and pesticide applicators to dioxins in 2,4-D (e.g. from mixing and otherwise handling 2,4-D concentrate) and associated with 2,4-D (from incineration of containers with 2,4-D residues).

2,4-D and the Proposed Registrations in Light of the Upcoming Registration Review of 2,4-D

EPA is scheduled to begin a registration review of 2,4-D late this year or early next year. The registration review will give EPA the opportunity to examine 2,4-D in light of the latest science on its human toxicity, and to collect and evaluate data to assess the dioxin-related concerns in light of the Agency's ongoing review of dioxin toxicity. The registration review also will also involve a detailed assessment of use patterns, without which human health and other risks cannot be adequately evaluated.

The introduction of Enlist soybeans and corn will lead to vastly increased use of 2,4-D, which will mean that farmers and the general public will be subjected to correspondingly greater exposure to this toxic pesticide. USDA should postpone any decision on DAS-68416-4 or Enlist corn until EPA has the opportunity to complete its registration review of 2,4-D, which will include an assessment of the human health implications of increased use of and exposure to 2,4-D triggered by adoption of these crops.

b. Health risks from exposure to glufosinate

APHIS also needs to assess the health impacts of glufosinate based on the increased use of glufosinate with DAS-68416-4 soybean. Instead, APHIS says that "…glufosinate use on soybean could increase in comparison to use under the No Action Alternative…glufosinate use may likely replace other herbicides currently being used on soybean; thus, the overall amount of herbicides being applied to soybeans may not change." (DEA at 80) We project a 35-fold increase over current use of glufosinate on soybeans, and a 10-fold increase in glufosinate use in agriculture as a whole if DAS-68416-4 soybean is approved, as discussed our herbicide use section of these comments. This means that more people are likely to be exposed to glufosinate, more often.

Exposure of mixers, loaders and applicators to glufosinate is of particular concern. In 2005, the European Food Safety Authority (EFSA) reviewed glufosinate ammonium and found that it's use in agriculture poses a risk to various animals, including humans. Operators using glufosinate on genetically engineered corn were at risk of unsafe exposures in spite of taking precautions, such as wearing protective clothing (EFSA 2005, p. 20).

Studies in laboratory animals showed that glufosinate caused premature deliveries, abortions and dead fetuses in rabbits, and pre-implantation losses in rats (EFSA, p. 13 – 14). These analyses led to precautionary language on the Material Safety Data Sheet for glufosinate ammonium, warning users that it is a "[s]uspected human reproductive toxicant", and that "[i]t may cause damage to organs through prolonged or repeated exposures". It also is tagged as causing a "[p]ossible risk of harm to the unborn child." (Glufosinate EU MSDS 2010).

In fact, glufosinate is one of 22 pesticides that has been identified by the EU as a reproductive, carcinogenic or mutagenic chemical and thus will not have its registration renewed in 2017.

APHIS says that "[g]lufosinate-ammonium is classified as not likely to be a human carcinogen and has no mutagenicity concern…", but fails to mention these international concerns over reproductive toxicity (DEA at 59).

Glufosinate use is currently being reviewed for health and safety by the US EPA (DEA at 104). Given the dramatic increases in use that will be brought about if DAS-68416-4 soybean is approved, APHIS should explore these impacts in an Environmental Impact Statement, including information from the EFSA and US EPA reviews.

6. Transgenic contamination of conventional and organic soybean varieties by DAS-68416-4 soybean

Approval of DAS-68416-4 soybean is likely to result in contamination of other soybean varieties with its HR transgenes. This is likely to occur via cross-pollination and seed mixing.

Although soybean is considered to be primarily a self-pollinating crop, in fact some cross-pollination by insects does occur. Honeybees housed near soybean fields use the flowers for honey, pollinating as they go (Erickson 1975, Chiari et al. 2005, Krupke and Hunt 2012, Krupke et al. 2012). Recent studies show that an array of wild pollinators also visits soybean fields (Anonymous 2012, O'Neal and Gill 2012). Presumably these insect pollinators carry soybean pollen long distances, accomplishing a low level of cross-pollination within and between fields (e.g. Pasquet et al. 2008). The extent of cross-pollination will be site-specific, depending a lot on the types and numbers of pollinators in a given location (e.g. Taki et al. 2011), making generalizations from particular studies difficult.

Transgenic contamination resulting from seed mixing can occur in different ways (Mallory-Smith and Zapiola 2008, Mellon and Rissler 2004). Retailed seeds purchased by farmers can be contaminated with the transgene, resulting in some fraction of the harvested commodity containing the trait. After harvest, bulk seeds from different sources are routinely transported, mixed and stored together, and can result in comingling of different varieties. Human error can result in mislabeling, failure to follow best practices, and so on (Marvier and VanAcker 2005).

For example, foundation seeds for non-engineered soybeans have been contaminated with transgenes:

> In 2002, the head of North Dakota State University's Foundation Seedstocks Program acknowledged that the program's foundation seed for non-engineered natto

> soybeans—the basic stock from which seeds are grown to sell to farmers—contained sequences from engineered soybeans. [Pates, M. 2002. Seed contamination raises control issues, posted November 12, 2002. On the Grand Forks Herald website at http://www.grandforks.com, accessed on January 7, 2003. The article identified Monsanto's Roundup Ready soybeans as the source of contamination.] (Natto soybeans are grown for premium food-grade products.) Three other foundation soybean seed programs—in Virginia, Missouri, and Michigan—have also recently reported genetic engineering contamination problems. [The Non-GMO Source. 2003. Concerns increase over GMO contamination of foundation seed. Volume 3, Number 6, pp. 1-2, June.] (Mellon and Rissler 2004, p. 10, internal citations included.)

In 2002, Union of Concerned Scientists did a study of transgenic contamination in a sample of popular non-engineered varieties of soybean seeds from major seed companies available for planting that year in Iowa and Illinois. They found that at least half of the soybean varieties tested contained transgenes at levels of less than 0.05 % to more than 1.0 %. These low levels of contamination nevertheless translate into large numbers of transgenic seeds in the non-engineered varieties. For example, if the soybean seed supply is contaminated at the 0.1% level, over 4 billion seeds would be transgenic (Mellon and Rissler 2004, Table 2-7, p. 29).

In another report, "A Growing Concern: Protecting the Food Supply in an Era of Pharmaceutical and Industrial Crops," UCS enlisted the assistance of several academic experts in agricultural sciences to determine whether genetically engineered pharmaceutical-producing crops could be kept out of food. This report demonstrates how difficult it is, even for pharmaceutical crops that would be grown on small acreage and under stringent confinement, to avoid contaminating food. The authors of this report examined confinement methods, such as field separation, cleaning of farm equipment, segregation of seed, and others, and found that it would still be difficult to ensure the absence of contamination. Only by taking heroic measures, such as completely geographically isolating pharmaceutical from food crops, would contamination be unlikely. UCS concluded that even though it may be theoretically possible to prevent contamination, it would not be economically feasible.

Another route of contamination that is unpredictable, but likely over time, is human error. Two academic ecologists address this in a peer-reviewed paper (Marvier and Van Acker 2005), and conclude that contamination by genetically engineered crops due to human error or other means has occurred numerous times, and is likely to continue to occur. This paper documents many instances where genetically engineered crops are known to have contaminated non-engineered crops or food. Thus, biological contamination through human error and human behavior, such as composting, exchanging seeds, or mislabeling seeds, must be addressed in an EIS.

The likelihood of contamination of soybean varieties by transgenes shown by the USC studies and past contamination incidents has important implications for the impacts of approval of DAS-68416-4 soybean:

> Both commercial and legal considerations make the presence of transgenically derived sequences in agricultural products problematic. Many transgenic varieties of crops in

> use in the United States have not been approved in other countries and their presence in imports is unlawful. In addition, many customers for U.S. exports —particularly those looking to purchase organic food or non-organic specialty products—are exhibiting a strong preference for non-genetically engineered grains and oilseeds free of some or all transgenic varieties (Mellon and Rissler 2004, p. 7).

These impacts are discussed more fully in CFS' legal comments.

7. Environmental impacts of DAS-68416-4 soybean approval

a. Overview of environmental impacts

APHIS concludes that approving of DAS-68416-4 soybean will have no greater impact on the environment and endangered species than the "no action" alternative (DEA at 71). In fact, APHIS finds that using the DAS-68416-4 soybean crop system is likely to benefit the environment to the extent that it facilitates conservation tillage (e.g. DEA at 87,89-90, 92, 114).

APHIS also assumes that there will be no greater risk from herbicide use (DEA at 71).

These conclusions finding no difference in environmental impacts between approving and not approving of DAS-68416-4 soybean have weak underpinnings in science. APHIS 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, as already discussed, 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. 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, we critically assess the claim that herbicide-resistant crops have promoted or would promote or preserve conservation tillage. 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. Then we comment on harms to the environment and endangered species from increased herbicide use with the approval of DAS-68416-4 soybean.

Finally, we analyze the potential impacts of activity of the engineered enzyme in DAS-68416-4 soybean, including in pollen and nectar. The metabolites formed from activity of the enzyme may affect wildlife, and particularly honey bees and soybean-eating animals, including endangered species.

b. Herbicide resistant crops not responsible for increased conservation tillage

APHIS links farmers' use of herbicide-resistant crops and cultivation practices that minimize soil erosion, known as conservation tillage; and then attributes to HR crops all manner of purported benefits commonly associated with conservation tillage: reduced soil erosion, declining CO2 emissions from soil, reduced fuel use on farm, as well as improved air and water quality (e.g. DEA at 16, 39, 43, 54). The great majority of herbicide-resistant crops have thus far been glyphosate-resistant, Roundup Ready varieties of soybeans, corn and cotton. APHIS presumes that DAS-68416-4 soybean will have the same benefits as those claimed for Roundup Ready crops.

APHIS' argument, closely following Dow's in DAS (2011f), 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 have prompted some farmers to use tillage (mechanical weed control) to remove them, which is tantamount to abandonment of conservation tillage. Introduction of the DAS-68416-4 soybean system would allow farmers to apply 2,4-D to kill weeds instead of using tillage, thereby "preservin 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-resistan crops promoted conservation tillage (in the short term), but are undermining it just a decade later, what is to stop DAS-68416-4 soybean from triggering a repeat of this boom-bust cycle? On this point, neither APHIS nor Dow has any satisfactory answers, and as discussed in the resistant weed section of these comments above, it is quite clear that 2,4-D resistant crops will foster rapid evolution of 2,4-D-resistant weeds and increased tillage to control them.

However, the argument presented by APHIS and Dow fails on its face, as we demonstrate below. T following discussion may be summarized as follows.

First, there is considerable doubt concerning whether HR soybean cultivation is even correlated wi conservation tillage practices.

Second, to the extent there is such a correlation, the causation is from prior adoption of conservati tillage to subsequent adoption of HR soybeans. In contrast, the adoption of HR crops does not predispose to greater use of conservation tillage.

Third, steeply declining soil erosion rates in the 1980s and the first half of the 1990s leveled out in the following decade of Roundup Ready crop adoption. These data are irreconcilable with the proposition that HR crops drive greater use of soil-saving cultivation regimes.

Fourth, soil-saving federal farm policies, not HR crops, were primarily responsible for increased us of conservation tillage and reduced soil erosion in American agriculture.

Fifth, HR crops in fact promote ***greater*** use of soil-eroding tillage to remove herbicide-resistant weeds, which the use of these crop systems fosters.

Finally, we show that some purported benefits APHIS attributes to conservation tillage are dispute in the scientific community, while in other cases this form of tillage appears to have adverse impac This analysis also invalidates much of Dow's presentation regarding "economic and agronomic impacts" of DAS-68416-4 soybean (DAS 2011f).

i. Correlation in question

APHIS and some sources it cites posit a ***correlation*** between adoption of RR soybeans and greater use of conservation tillage practices. However, much of the data upon which this purported correlation is based come from suspect sources, such as the American Soybean Association, a lobby group that represents Monsanto and other large agrichemical-seed firms. In a widely cited assessment of the environmental impacts of glyphosate-resistant crops, Cerdeira and Duke (2006) note that:

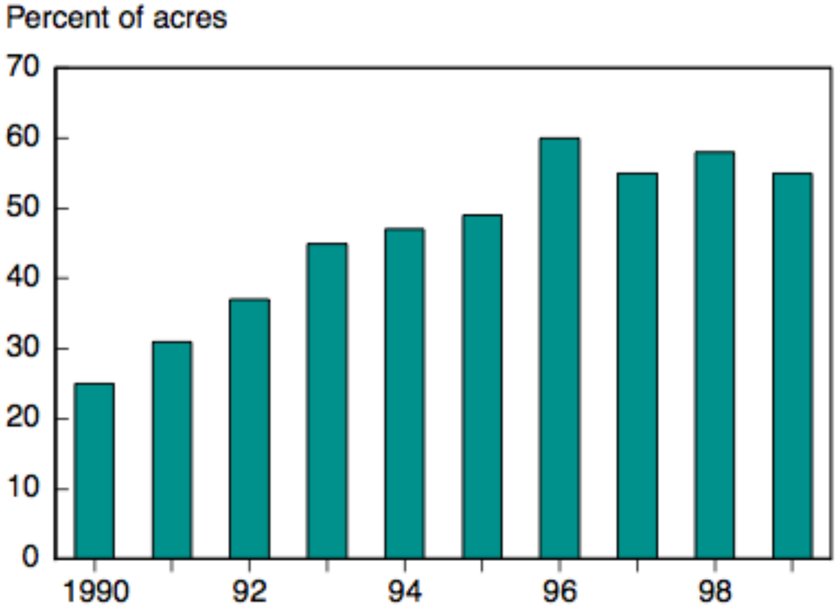
> Considering the relatively high level of potential environmental improvement that can be gained by reducing tillage, there is a remarkable paucity of refereed publications on the influence of GRCs [glyphosate-resistant crops] on tillage practices and associated environmental effects. (p. 1638).

Despite this caution, the authors proceed to base their discussion of purported reductions in soil lo and compaction from GR crop systems almost entirely on a survey conducted by the American Soybean Association (ASA) in 2001, a survey that was not subject to peer review or published in an "refereed publication." These ASA survey results are extremely difficult to reconcile with an assessment conducted the following year by USDA agricultural economists Jorge Fernandez-Cornej and William McBride. These authors have shown that adoption of conservation tillage in soybean production rose sharply in the years ***prior to*** introduction of RR soybeans, then stagnated in at leas the first four years of their cultivation (1996-1999).

> 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 (Fernandez-Cornejo & McBride 2002, p. 29).

Figure 11

Use of conservation tillage - soybeans



Source: Fernandez-Cornejo (2000) based on USDA data (USDA, 1997a updated from ARMS).

From: Fernandez-Cornejo & McBride (2002), p. 29.

Roundup Ready soybeans were introduced in 1996, and were adopted extremely rapidly. They comprised 7.4%, 17%, 44.2% and 55.8% of total soybean acreage in the years from 1996 to 1999, respectively. This represents 4.75, 11.90, 31.84 and 41.14 million acres of Roundup Ready soybeans in the corresponding years (see table below). Yet Figure 11 above shows clearly that soybean growers overall practiced conservation tillage to a considerably greater extent in 1996, when under 5 million acres were Roundup Ready, than in 1999, when RR soybean acreage had increased over eight-fold, to 41.14 million acres, to comprise over half of all soybeans grown. These data, at the very least, cast great doubt on a purported correlation between RR soybeans and use of conservation tillage.

Yet in the same publication, Fernandez-Cornejo & McBride (2002) present an "estimate," based on the same USDA Agricultural Resources Management Survey (ARMS) that served as the source of Figure 11 above, to the effect that 60% of Roundup Ready soybean acres were under conservation tillage, versus just 40% of conventional soybean acres.

> A larger portion of the acreage planted with herbicide-tolerant soybeans was under conservation tillage than was acreage growing conventional soybeans. According to estimates based on USDA's ARMS data, about 60 percent of the area planted with herbicide-tolerant soybeans was under conservation tillage in 1997 (fig. 12). In comparison, only about 40 percent of the acres planted with

> conventional soybeans were under conservation tillage the same year.(Fernandez-Cornejo and McBride 2002, p. 29)

These estimates are irreconcilable with the data portrayed in Figure 11, as shown in the table below.

	1996	**1997**	**1998**	**1999**
Total soybean acres (thousands)	64195	70005	72025	73730
Percent HR soybeans	7.4%	17.0%	44.2%	55.8%
HR soybean acres	4750	11901	31835	41141
Conventional soybean acres	59445	58104	40190	32589
Percent of total soybeans under conservation tillage (from Fernandez-Cornejo & McBride 2002, Figure 11)		54%		
Acres of soybeans under conservation tillage		37803		
Scenario assuming that HR soy cultivation correlates with conservation tillage (1997)				
60% HR soy under con-till		7141		
40% conv'l soy under con-till		23242		
Predicted soy acres under con-till		30382		
Scenario's deviation from reality				
Deficit in con-till acres		7421		
Amount by which actual con-till soy acres exceeds prediction (in percent)		24%		
Scenario assuming equal (54%) adoption of con-till on HR and conventional soybean acres		37803		

The estimate (scenario) according to which 60% of RR and just 40% of conventional soybeans were under conservation tillage in 1997 yields just 30.4 million acres of conservation-tilled (con-till) soybeans, when the true figure is 37.8 million acres, or 24% more. Clearly, the estimate linking RR soybeans to con-till soybeans is in error. Since the great majority (83%) of soybeans in 1997 were conventionally tilled, the most likely explanation for the deficit is that conventional soybean growers used conservation tillage (con-till) to a greater extent than presumed in the estimate. The scenario assuming equal adoption of con-till by the two groups gives the expected, and correct, result. The latter scenario of equal adoption of con-till is supported by the following finding.

ii. Conservation tillage leads to HR seeds, not vice versa

After noting the “correlation” between RR soybeans and conservation tillage that is critiqued above, Ferndandez-Cornejo and McBride conducted an econometric analysis to determine causation, which reached the following conclusion with respect to no-till, one form of conservation tillage:

> 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. This result seems to suggest that farmers already using no-till found herbicide-tolerant seeds to be an effective weed control mechanism that could be easily incorporated into their weed management program. On the other hand, the commercialization of herbicide-tolerant soybeans did not seem to have encouraged adoption of no-till, at least [in] the year of the survey, 1997. (p. 29)

Thus, even if one were to posit a correlation, the causation flows from prior adoption of conservation tillage to subsequent adoption of HR soybeans, not the reverse. In short, HR soybeans do not increase adoption of conservation tillage.

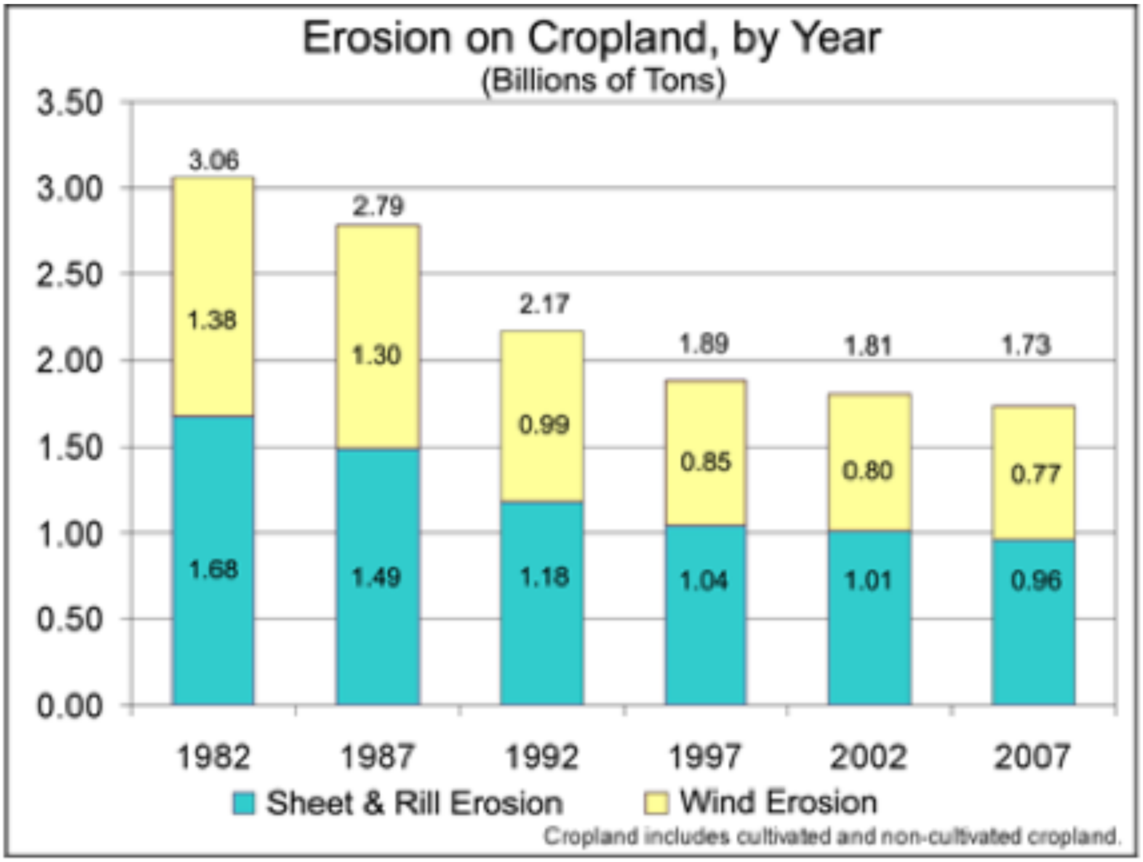
It is unclear why these trained agricultural economists did not detect this serious and obvious discrepancy in the data they presented, but it is indisputable that they did. And as indicated by APHIS’ citation of this study (DEA at 16), it has had considerable influence (together with the 2001 ASA survey noted above) in fostering the erroneous notion that RR soybeans are responsible for increased use of conservation tillage, despite the proviso regarding causation.

It is also quite striking that Fernandez-Cornejo and McBride (2002) offer absolutely no explanation for the rapid rise in conservation-tilled soybeans in the 5 years leading up to introduction of RR soybeans (1990-1995, see Figure 11). We offer an explanation of this below.

iii. Reductions in soil erosion come to an end during the decade of herbicide-resistant crop adoption

Conservation tillage is widely credited with bringing about large reductions in soil erosion rates (D at 35). Thus, if HR soybeans, corn and cotton did in fact promote greater use of conservation tillag one would clearly expect to see sharply falling soil erosion rates over the period of their widesprea adoption, in the areas where these crops are widely grown. However, gold-standard data from USDA’s soil conservation experts, the Natural Resources Conservation Service (NRCS), show that th is not the case.

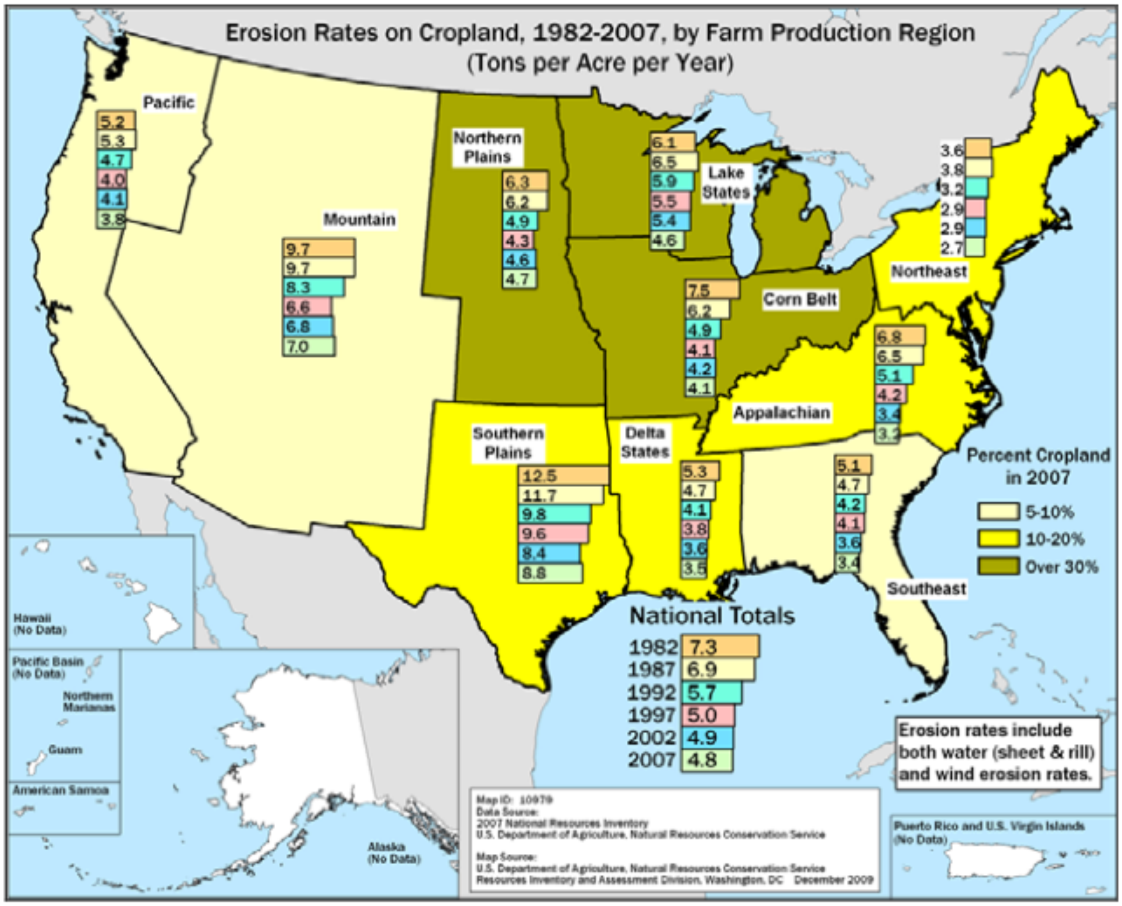
Below, we reproduce a chart from page 2 of NRCS’s 2010 report: “2007 National Resources Inventory: Soil Erosion on Cropland” (USDA NRCS 2010, in supporting materials). The chart represents NRCS’ best estimate of cropland erosion from 1987 to 2007. According to NRCS: “[E]rosion rates computed from NRI data are estimates of average annual (or expected) rates base 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," ar 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 plan residue on the soil (USDA ERS AREI 2002). Thus, the chart below reflects in large degree the tillage regimes used by farmers.



From: NRCS (2010), p. 2.

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 steadil from 16.0 million acres in 1997 to 117.2 million acres in 2007 (Benbrook Supplemental 2009, Tabl 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 m (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 list in Table 36 of the report (pp. 12-16).



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 cor (see USDA-ERS 2011a, cited in DEA). If APHIS' and Dow's supposition that HR crops and HR soybea in particular promote 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 thar 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 from 1997 tc 2007, while ***erosion actually increased in the Northern Plains states*** over this same period, 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 thar stable or increasing erosion rates.

iv. Federal farm policy triggered sharp declines in soil erosion prior to HR crop adoption

The other question raised by NRCS' report is this: what explains the sharp declines in soil erosion i 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 decline in soil erosion over this period is also consistent with increased use of conservation tillage in soybeans from 1990 to 1995, as displayed in Figure 11 above. 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 increase 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)

v. Weakening enforcement of farm conservation plans brings soil erosion reductions to an end

Soon after the 1995 deadline for implementation of farm conservation plans had passed, however, enforcement of these plans dramatically weakened, and further progress in preventing soil erosion was stymied. As explained by the Environmental Working Group:

> In 1997, after a decade of historic progress cutting soil erosion and polluted runoff from farmers' fields, America's soil, streams, lakes and rivers were improving.
>
> That historic achievement was driven by a 1985 federal law that required farmers to put conservation practices in place on their most vulnerable cropland in return for the billions of dollars of income and insurance subsidies they were getting from taxpayers. The "Highly Erodible Land Conservation" provisions of the 1985 Food Security Act required farmers to fully implement an approved soil conservation plan by 1995 on cropland that was determined to be "highly erodible." USDA's Economic Research Service (ERS) completed a comprehensive evaluation of those so-called conservation compliance provisions in 2004. ERS concluded that conservation compliance reduced soil erosion on highly erodible cropland by 331 million tons a year — a 40 percent reduction between 1982 and 1997 (USDA ERS 2004).
>
> ***Unfortunately, those gains were short-lived. Enforcement of conservation requirements weakened and in 1996 went off the rails altogether when Congress made an abortive push to phase out farm subsidies — and with them the conservation requirements.*** The phase-out of farm subsidies turned

> out to be a mirage, and Congress immediately returned to its old habits — plowing billions into farmers' hands through ad hoc disaster payments and bringing all the farm subsidies back with a vengeance in the 2002 farm bill.
>
> ***The only thing that turned out to be real was the phase-out of enforcement of conservation requirements. The result has been a decade of lost progress and mounting problems.*** (EWG 2011, p. 28, emphases added).

In short, sharp reductions in soil erosion from the mid-1980s to the mid-1990s were driven by federal farm policy that made subsidies to farmers contingent on implementation of soil conservation plans. Dramatic weakening of USDA enforcement of those plans in the mid-1990s explains the leveling off of soil erosion rates from 1997 to 2007. HR crops, adopted during this same decade, had essentially no influence on farmers' use of conservation tillage practices.

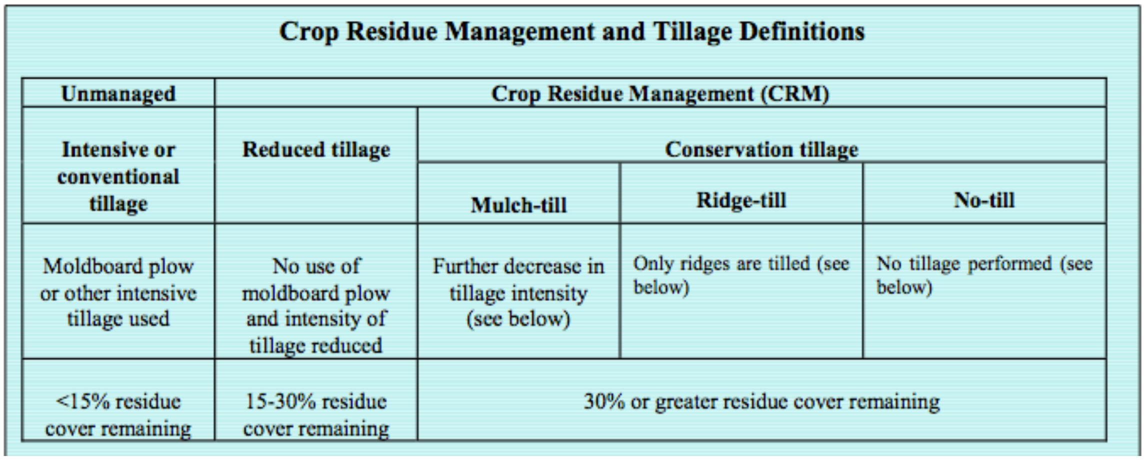
USDA's Natural Resources Conservation Service also credits federal farm policy as being "largely responsible" for increased use of soil-conserving cultivation practices. In a short work referenced by APHIS (DEA at 35), NRCS experts state:

> 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 (USDA-NRCS 2006a, p. 3).

"Residue management practices" refer to conservation tillage practices.

Surprisingly, we found no reference at all to farm conservation plans in APHIS' draft EA, despite their overriding importance in promoting conservation tillage practices that reduced soil erosion enormously in American agriculture. It is hard to avoid the conclusion that APHIS ignored this factor in order to better falsely attribute reduced soil erosion to HR crops, one of many examples of unacceptable bias in the draft EA.

APHIS does not even appear to understand what conservation tillage actually is. APHIS states that: "According to USDA Agricultural Resource Management Survey data (USDA-ERS, 2006), ***conservation tillage ranging from no-till to reduced till*** conserving 15-30% of residues was utilized on 88% of soybean acres in 2006" (DEA at 16, emphasis added). However, "reduced till" is not a form of "conservation tillage," as APHIS states. APHIS mis-cites its source, the USDA-ERS 2006 report, which clearly states: "Crop residue management systems include ***reduced till OR conservation tillage practices such as no-till, ridge-till and mulch-till***, as well as the use of cover crops and other conservation practices that provide sufficient residue cover to mitigate wind and water erosion (p. 2, emphasis added). The fact that reduced till is NOT a form of conservation tillage is further demonstrated by the following table, also from a USDA publication.



Crop Residue Management and Tillage Definitions

Unmanaged	Crop Residue Management (CRM)			
Intensive or conventional tillage	Reduced tillage	Conservation tillage		
		Mulch-till	Ridge-till	No-till
Moldboard plow or other intensive tillage used	No use of moldboard plow and intensity of tillage reduced	Further decrease in tillage intensity (see below)	Only ridges are tilled (see below)	No tillage performed (see below)
<15% residue cover remaining	15-30% residue cover remaining	30% or greater residue cover remaining		

From: USDA ERS AREI (2002), p. 23.

Conservation tillage is officially defined as cultivation practices that leave 30% or more of the soil surface covered with crop residues (USDA ERS AREI 2002, p. 23). Since reduced tillage leaves only 15-30% of the soil surface covered by crop residues, it is not a form of conservation tillage.

APHIS also cites two industry-funded studies that purport to show an association between Roundup Ready crops and conservation tillage (see DEA at 16 for following discussion). Fawcett and Towery (2002) is a publication of the Conservation Technology Information Center, whose Board of Directors includes officers from Monsanto, Syngenta, Bayer CropScience and the CropLife Foundation (a pesticide industry lobby group).[59] This report cites data from Monsanto (Table 2) and the unreliable 2001 American Soybean Association survey mentioned above (Table 3). The only objective data that is cited (from USDA's Natural Resources Conservation Service) is outdated: Figure 2 depicts sharply reduced soil erosion from cropland from 1982 to 1997, largely before the 1996 introduction of the first RR crop, Roundup Ready soybeans.

Givens et al. (2009) is a phone survey of farmers that also purports to show a correlation between increased conservation tillage and Roundup Ready crop cultivation (DEA at 16). However, several aspects of this survey raise questions as to its objectivity. First, the study does not give methodological details, referring readers to a previous survey (Shaw et al. 2009). That survey reveals that the growers who were interviewed in Givens et al. (2009) were selected from a list provided by Monsanto, raising the possibility of selection bias on the part of Monsanto. Second, the study itself was funded by Monsanto, raising similar concerns of bias.[60] Third, the one result that APHIS cites from Givens et al. (2009) referred to growers of all glyphosate-resistant crops, not glyphosate-resistant soybeans in particular, and left out an important piece of information: "…25% of farmers that had been using conventional tillage switched to no-till and 31% switched to reduced-till after adopting glyphosate-tolerant GE crops" (DEA at 16, from Givens et al., Table 2). APHIS failed to note that: 1) The largest group of growers, the remaining 44%, continued to use intensive tillage; and 2) Reduced-till is not a form of conservation tillage, as explained above. Thus, only 25% of farmers switched to a form of conservation tillage (i.e. no till), while three-fourths of the surveyed farmers continued to use forms of conventional tillage, either intensive (44%) or reduced till (31%).

Table 3 of Givens et al. (2009) shows that most of the farmers who switched tillage regimes after adopting RR crops were RR cotton growers, and even these growers switched primarily to "reduced till" rather than no-till. Furthermore, among adopters of RR soybeans who had been previously used conventional tillage, and who grew them in rotation with a non-RR crop, only 17% transitioned to no-till, versus 39% to reduced till and 44% who continued to practice conventional tillage (Table 3). Finally, this survey, which is based on numbers of growers rather

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[59] http://www.ctic.org/CTIC%20HOME/ABOUT%20CTIC/Board%20of%20Directors/, last visited 8/24/12.

[60] Monsanto and other agrichemical-seed companies provide substantial research funding to the weed science community, a problematic relationship that may bias the findings and conclusions reached in such studies.

than acreage, tells us nothing about acres of cropland under the various tillage regimes, either before or after adoption of Roundup Ready crops.

In short, none of the additional references cited by APHIS offer any credible support for its argument. All of the unbiased, credible evidence points indisputably to federal farm policy as the chief motivating factor for massive adoption of conservation tillage, and attendant sharp reductions in soil erosion. The fact that the decline in soil erosion essentially stopped when enforcement of farm conservation plans lapsed in the mid-1990s further demonstrates the importance of federal farm policy. The further fact that farmer adoption of over 100 million acres of herbicide-resistant crops from 1997 to 2007 coincided with ***stagnant or increasing*** soil erosion where HR soybeans and corn are most grown, entirely refutes APHIS' and Dow's false depiction of this matter.

c. Environmental impacts of conservation tillage

Even if DAS-68416-4 soybean is managed with conservation tillage, the environmental benefits attributed to reduced tillage are not well substantiated, other than slowing soil loss.

i. Soil and water

Although 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, 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-68416-4 soybean were to increase use of conservation tillage in the short term.

ii.Climate change

APHIS says that the "continued use of conservation tillage associated with GE crops may reduce GHG emissions as a result of increased carbon sequestration in soils, decreased fuel consumption, and the reduction of nitrogen soil amendments…" if DAS-68416-4 soybean is approved (DEA t 89). These benefits for climate change of a purported preservation of no-till sobyean 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 relies on conservation tillage as an argument for a whole range of environmental benefits of DAS-68416-4 soybean without critical analysis of the best science available.

In fact, overall environmental benefits and harms from approval of DAS-68416-4 soybean are likely to have more to do with changes in herbicide use that accompany the DAS-68416-4 soybean cropping system than with tillage methods that may or may not be different.

8. Environmental effects of increased herbicide use with approval of DAS-68416-4 soybean

a. Drift injury to plants and other non-target organisms

Glufosinate is a broad-spectrum contact herbicide, so non-target plants are at risk of injury from drift (Reddy et al. 2011). An EFSA report on glufosinate use in various agricultural scenarios identified drift injury to non-target plants as a critical area of concern that "requires risk mitigation measures such as a 5 m buffer zone." (EFSA 2005, p. 42)

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. 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).

Therefore, reports of injury to non-target crops from 2,4-D drift are common, even from registered existing uses of 2,4-D, 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, the reality remains that drift from 2,4-D applications 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, as discussed above in relation to crop injury. 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. (Hebert 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-68416-4 soybean is likely to increase that risk to the extent that the crop system involves increased use of 2,4-D.

Glufosinate is a broad-spectrum contact herbicide, so non-target plants are at risk of injury from drift (Reddy et al. 2011). An EFSA report on glufosinate use in various agricultural scenarios identified drift injury to non-target plants as a critical area of concern that "requires risk mitigation measures such as a 5 m buffer zone." (EFSA 2005, p. 42). As glufosinate use increases with approval of DAS-68416-4 soybean, more injury to non-target plants can be expected.

b. Herbicide use patterns with DAS-68416-4 soybean result in greater risk to non-target species

Simply, the amount of injury that non-target organisms will sustain is determined by how sensitive they are to the 2,4-D and glufosinate 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 glufosinate that will be used on DAS-68416-4 soybean compared to conventional varieties in order to evaluate the impacts of their alternatives, as we have done in the herbicide use section above. Because DAS-68416-4 soybean has been engineered to withstand 2,4-D and glufosinate, thus removing biological constraints, these herbicides can be used throughout the growing season and at higher rates than in non- DAS-68416-4 soybean. We predict a sharp increase in amount of 2,4-D and glufosinate used per acre.

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

Herbicide applications are more likely to coincide with life-stages of plants that are the most sensitive to injury because the DAS-68416-4 soybean itself is less sensitive to injury during spring and summer than is non- DAS-68416-4 soybean. 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).

ii. Total use of 2,4-D at landscape level

Another way that the DAS-68416-4 soybean cropping system will increase 2,4-D and glufosinate use is by increasing the total number of soybean acres that are treated with these herbicides. Within a given year, many more soybeans acres will be sprayed, as we show in our comments on herbicide use. Also, since corn and soybeans are often rotated on the same acreage within a region, and Dow is seeking approval of 2,4-D-resistant corn, it is likely that both 2,4-D-resistant corn and soybeans will be grown in proximity, greatly increasing the total acres exposed to 2,4-D in a given year. And because of the corn-soybean rotation, the likelihood that 2,4-D will be used on the same acreage year after year is greater as well.

At a landscape level this change in 2,4-D use pattern will result in a larger number of individuals of a wider array of species in proximity to DAS-68416-4 soybean and thus 2,4-D and glufosinate, with attendant impacts.

c. Increased ingestion of 2,4-D and glufoinate residues and metabolites

Higher application rates later in the season with DAS-68416-4 soybean will also leave higher levels of 2,4-D and glufosinate residues and metabolites in the DAS-68416-4 soybean tissues, discussed more fully below. There will also be higher concentrations of 2,4-D and glufosinate in runoff.

APHIS needs to reconsider potential risks to animal communities from eating DAS-68416-4 soybean tissues or drinking runoff, including the unique metabolites as well as the parent 2,4-D residues (see metabolism, these Comments).

d. Impacts to biodiversity

According to APHIS, "[u]nder the Preferred Alternative, potential impacts to biodiversity from runoff, spray drift, and volatilization of herbicides are not expected to be substantially different from those associated with the No Action Alternative." (DEA at 100). However, APHIS did not fully consider the impacts of the substantial changes in herbicide use amounts and patterns that are part of the DAS-68416-4 soybean system.

i. Biodiversity in soybean fields

An example of harm to biodiversity in corn fields 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, 16 years after the introduction of Roundup Ready soybeans, major impacts of their widespread adoption are just now surfacing, with only a handful of researchers doing this kind of "post-market" ecological research. APHIS needs to consider these kinds of harms, and how to prevent them, before rather than after approval of DAS-68416-4 soybean.

The DAS-68416-4 soybean 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 DAS-68416-4 soybean will be treated with 2,4-D and glyphosate, in rotation with DAS-40278-9 corn similarly treated, as we have commented. Weed biodiversity, such as small populations of milkweed, within these fields won't have a chance to survive. 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-68416-4 soybean to plant population diversity within soybean fields 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-68416-4 soybean tissues (see metabolism, these Comments). A wide variety of animals feed on soybean leaves, flower parts, and seeds, including many beneficial organisms such as honey bees (see metabolism, 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-68416-4 soybean with possible toxic impacts (US-EPA 2009, Freemark and Boutin 1995).

APHIS needs to assess potential impacts to animals in fields of DAS-68416-4 soybean 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.

ii. Biodiversity around soybean fields

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 "woodlots, fencerows, hedgerows, and wetlands" as vital habitat for a range of wild plants and animals (DEA at 53 -54). At the same time, APHIS acknowledges the negative impacts of pesticide exposure to wildlife and habitats (DEA at 54 – 55).

However, APHIS does not take into account the impacts that increased herbicide use in DAS-68416-4 soybean 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 at 56). Thus they are unable to properly compare their proposed alternatives.

Increased drift and runoff from use of 2,4-D and glufosinate with the DAS-68416-4 soybean is likely to alter the very habitats that APHIS has identified as being important for biodiversity in agroecosytems (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, as we have discussed earlier in these comments.

It is clear, then, that increased use herbicides with the DAS-68416-4 soybean are likely to have negative impacts on biodiversity around soybean fields, perhaps at some distance, and thus APHIS should prepare and Environmental Impact Statement that assesses these impacts.

e. Threatened and endangered species

APHIS needs to take into account the use – or the increased use – of 2,4-D and glufosinate, instead deferring to the EPA (DEA 135 – 136). They also need to consider the possible toxicity of the metabolites that are present in DAS-68416-4 soybean exposed to herbicide substrates of the AAD-12 protein (see metabolism, these comments).

i. Increased herbicide use and listed species

All of the harms from increased use of herbicides on DAS-68416-4 soybean 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-68416-4 soybean 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).

The use of 2,4-D on more soybeans 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-68416-4 soybean, again deferring to the EPA and their potential mitigation measures (DEA at 138). 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-68416-4 soybean.

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-68416-4 soybean would similarly be “likely to adversely affect” prey and habitats of threatened and endangered animals found near these fields.

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-68416-4 soybean.

EPA has not yet gone through a "pesticide effects determination" for any listed species and glufosinate, although they are in the process of reviewing the registration of this herbicide where they are addressing endangered species (DEA at 139). Based on ecological studies in Europe, we expect non-target plants – listed and non-listed – to be at risk from drift and runoff, with possible consequences for listed animals. Some animals may also be at direct risk from glufosinate exposure, as well (EFSA, 2005).

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-68416-4 soybean.

ii. Ingestion of DAS-68416-4 soybean by listed species

Finally, APHIS did not take into account the potential toxicity of DAS-68416-4 soybean to listed species that might eat leaves, roots, stems, or flower parts. Migrating birds, for example, eat parts of the soybean plant. Bees consume the pollen and nectar, and presumably other insects do as well. Soybean detritus washes into wetlands.

If any listed species do consume soybean APHIS must consider the differences in composition between DAS-68416-4 soybean and its conventional counterparts. APHIS reiterates that "there is no difference in the composition and nutritional quality of DAS-68416-4 soybean compared with conventional soybean varieties, apart from the presence of the AAD-12 and PAT proteins. The results presented by DAS show that the incorporation of the aad-12 and pat genes and the accompanying expression of the AAD-12 and PAT proteins in DAS-68416-4 soybean does not result in any biologically-meaningful differences between DAS-68416-4 soybean and nontransgenic hybrids." (DEA at 133). Again, this ignores the body of scientific information to the contrary, showing that when DAS-68416-4 soybean encounters 2,4-D, metabolites form that are similar to known toxins. This should trigger a consultation with FWS (DEA at 238, decision tree).

9. Impacts of the activity of AAD-12 enzyme in DAS-68416-4 soybean

In the Plant Pest Risk Assessment (PPRA) for DAS-68416-4 soybean, APHIS says that the "introduced genetic material does not result in the production of novel proteins, enzymes, or metabolites in the plant that are known to have toxic properties", and that they have "not identified any other potential mechanisms for deleterious effects on non-target organisms." (PPRA at 7). However, APHIS did not take into account independent research and Dow studies showing that potentially toxic metabolites do occur as a result of the engineered trait. USDA should carefully consider the impacts of the accumulation of novel molecules with similarity to known toxins in DAS-68416-4 soybean.

There are differences in the composition of DAS-68416-4 soybeans compared to non-2,4-D resistant varieties that result from the *activity* of the novel AAD-12 protein. The AAD-12 protein is an enzyme that acts on 2,4-D and other substrates likely to be encountered by DAS-68416-4 soybeans to produce metabolites missing or at much lower levels in non-engineered soybeans (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-12 protein in DAS-68416-4 soybean 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 crop, either because the herbicides are applied directly to the soybeans, or contact the soybeans via drift.

For the similar 2,4-D-resistant corn, Dow claims that "AAD-1 [a related enzyme] 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, emphasis added).

The AAD-12 enzyme has somewhat different activity, but also degrades 2,4-D and related phenoxy auxin herbicides into their corresponding phenols, and also would afford protection from drift of various herbicides: "…AAD-12 has significantly greater in vitro activity on 2,4-D (an achiral substrate) than AAD- 1 (Table 1) (25). Testing a series of pyridyloxyacetate compounds as substrates, we found that AAD-12 was capable of degrading the synthetic auxin herbicides triclopyr and fluroxypyr at rates of 4% and 16%, respectively, of 2,4-D (at 1 mM substrate). AADs have not previously been shown to work on substrates containing a pyridine ring. This activity gives AAD-12 potential utility for providing resistance to a wider repertoire of synthetic auxins beyond 2,4-D and thus enables expanded broadleaf weed control." (Wright et al. 2010)

Although the AAD-12 enzyme degrades multiple auxin herbicides, Dow has only applied for registration at this time of 2,4-D choline in the phenoxy auxin group for direct use over the top of DAS-68416-4 soybeans. Other herbicide substrates may be present from drift or off-label use, or may be approved for direct use in the future, though.

To assess risk to non-target organisms of approving DAS-68 416-4 soybean APHIS needs to know if the AAD-12 enzyme alters *metabolism* in DAS-68 416-4 soybean such that the plants have a new composition after 2,4-D is used, and thus have 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 Dr. 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 from the breakdown of 2,4-D 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.

a. Independent research shows that new, potentially toxic metabolites are formed in 2,4-D resistant plants

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: 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 its use on 2,4-D resistant crops with 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 [i.e., resistant] 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).

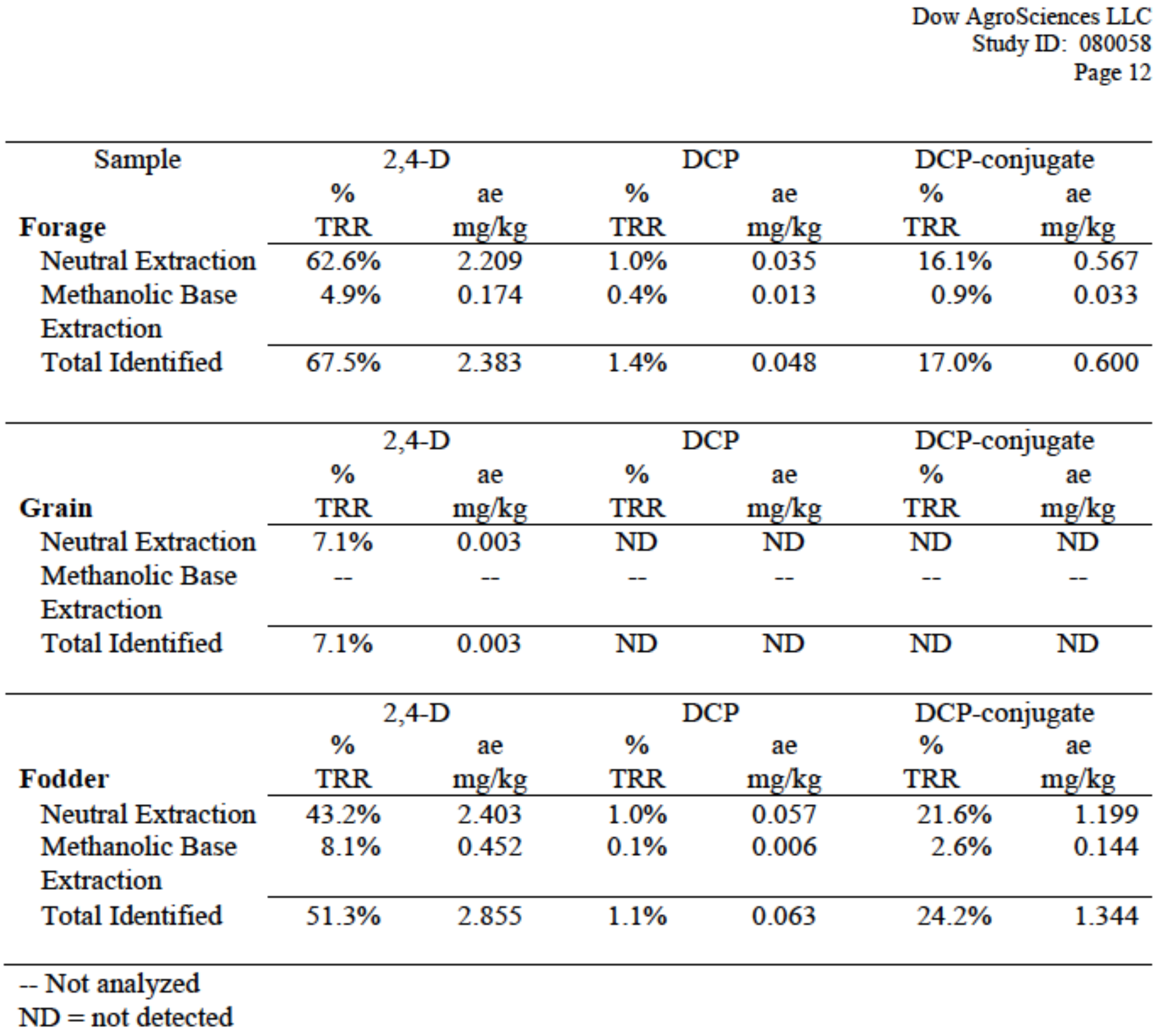
Given these results, as part of its PPRA and EA risk assessments to determine whether to approve DAS-68416-4 soybeans, APHIS must consider whether DCP and its conjugates are present in soluble fractions of DAS-68416-4 soybeans after AAD-12 enzyme acts upon 2,4-D in order to fully assess the impacts to non-target organisms and on human health.

b. Dow's studies show similar potentially toxic metabolites in DAS-40278-9 corn and DAS-68416-4 soybean

Some information about 2,4-D metabolism in 2,4-D-resistant DAS-40278-9 corn, including levels of DCP and DCP-conjugates from the breakdown of 2,4-D, is present in Dow's studies (Ma and Adlelfinskaya 2010, Rotondaro and Balcer 2010, Stagg et al. 2010, Culligan 2010; DAS Petition2011, p. 18). Such studies are clearly relevant to an assessment of non-target impacts of DAS-68416-4 soybeans as well, assuming that soybeans have similar 2,4-D and DCP metabolism to corn.

These complete studies of residues and metabolites in DAS-40278-9 corn are available on the Australia New Zealand Food Standards website (see citation for Culligan 2010, for example),but only abstracts of the analogous soybean studies are available there. We therefore base our comments on the full corn results and what was presented in the soybean abstracts.

For corn, Dow's "nature of residue" studies (Ma & Aldelfinskaya 2010, Rotondaro and Balcer 2010) that use 2,4-D formulations on DAS-40278-9 corn plants (and a different transformation event with the same enzyme) do result in measurable herbicide residues in the corn plant, as shown in the chart below (Rotondaro and Balcer 2010, p. 12). DCP levels are low in all samples, but DCP-conjugates make up a significant portion of the metabolites in forage and fodder, as expected from the independent studies discussed above. Note that neither DCP nor DCP conjugates are detected in grain. And although Dow claims that these DCP conjugates from the breakdown of 2,4-D 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). In other words, there is no conventional corn control included in the experiment to provide a baseline for novel metabolites.



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Study ID: 080058
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Sample	2,4-D		DCP		DCP-conjugate	
	%	ae	%	ae	%	ae
Forage	TRR	mg/kg	TRR	mg/kg	TRR	mg/kg
Neutral Extraction	62.6%	2.209	1.0%	0.035	16.1%	0.567
Methanolic Base Extraction	4.9%	0.174	0.4%	0.013	0.9%	0.033
Total Identified	67.5%	2.383	1.4%	0.048	17.0%	0.600

	2,4-D		DCP		DCP-conjugate	
	%	ae	%	ae	%	ae
Grain	TRR	mg/kg	TRR	mg/kg	TRR	mg/kg
Neutral Extraction	7.1%	0.003	ND	ND	ND	ND
Methanolic Base Extraction	--	--	--	--	--	--
Total Identified	7.1%	0.003	ND	ND	ND	ND

	2,4-D		DCP		DCP-conjugate	
	%	ae	%	ae	%	ae
Fodder	TRR	mg/kg	TRR	mg/kg	TRR	mg/kg
Neutral Extraction	43.2%	2.403	1.0%	0.057	21.6%	1.199
Methanolic Base Extraction	8.1%	0.452	0.1%	0.006	2.6%	0.144
Total Identified	51.3%	2.855	1.1%	0.063	24.2%	1.344

-- Not analyzed
ND = not detected

Based on this deficiency in the corn studies, APHIS should require that Dow submit a study with a conventional soybean control (with and without 2,4-D) in their residue and metabolism reports for DAS-68416-4 soybeans. It would be surprising if conventional soybeans 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 unlikely and novel metabolites.

Back to corn, although DCP conjugates were identified as major metabolites in Dow's "nature of residue" studies using radioactive tracers, this information was not incorporated into the "magnitude of residue" studies looking at total residue and metabolite levels and used to determine whether DAS-40278-9 corn would meet tolerance requirements for residues in forage and fodder (Culligan 2010). Conjugates were simply not measured in the "magnitude of residue" studies. However, the DCP levels in forage were actually higher in the "magnitude of residue" studies than the levels of 2,4-D, in contrast to the results from the "nature of residue"studies (Culligan 2010, p. 113). Even without considering the DCP conjugates, if the DCP levels Dow reports of DAS-4078-9 corn were added to the total residue of 2,4-D in forage and fodder, tolerance levels would be exceeded (Stagg et al. 2010, Culligan 2010, p. 113).

For example, the tolerance level of 2,4-D in forage is 6 µg 2,4-D per g of forage (Stagg et al. 2010, p. 29). Dow measured 3.124 µg/g 2,4-D in forage after labeled 2,4-D applications, and 3.899 µg/g of 2,4-DCP (Highest Average Field Trial measurements, Culligan 2010, p.113). If DCP were included in the current tolerances, there would be 7.029 µg/g of residues. We do not know the level of DCP conjugates in these forage samples, but we expect them to be much higher than DCP itself given the activity of the AAD-1 enzyme, so if conjugates were included in the tolerances, 2,4-D use on DAS-40278-9 corn would require a major change in tolerances.

With this DAS-40278-9 corn example as background, which residues and metabolites are reported by DAS-68416-4 soybean? First, DAS-68416-4 soybean has higher levels of 2,4-D residues and metabolites than does DAS-40278-9 corn; and second, unlike DAS-40278-9 corn, there are measurable levels of DCP and its conjugates in the soybean seed itself, in addition to the vegetative tissues (DAS FSANZ 2010)

For example, as shown in the abstract of the Nature of Residue Study, below, DAS-68416-4 soybean has 7.3 mg/kg DCP conjugates in forage, whereas DAS-40278-9 corn has 0.6 mg/kg DCP conjugates in forage. DAS-68416-4 soybean grain, unlike DAS-40278-9 corn, has detectable DCP levels, and 0.021 mg/kg DCP conjugates. DCP conjugate levels are 55X higher than DCP in these seeds, and 17X higher than DCP in forage:

> Study 090051 - A Nature of the Residue Study with [14C]-2,4-D DMA Applied to AAD-12 Soybeans
>
> ABSTRACT ONLY
>
> ……..The major radioactive component in forage and hay was 2,4-D comprising 85.8% and 59.4% of the TRR (43.117 mg a.e./kg and 21.978 mg a.e./kg), respectively. In forage and hay a DCP-acetyl-glucose conjugate comprised 12.4% and 13.4% of the TRR (6.239 mg a.e./kg and 4.969 mg a.e./kg), respectively. Most of the remaining radioactive residues in forage and hay were thought to be DCP or 2,4-D conjugates based upon liberation of DCP or 2,4-D during acid or base hydrolysis. In the mature seed, a small portion of the radioactive residue was identified as parent (1.4% of the TRR, 0.005 mg a.e./kg), only 0.1% of the TRR or <0.001 mg a.e./kg occurred as free DCP and 5.5% of the TRR or 0.021 mg a.e./kg was found as two glucose conjugates of DCP. The remaining extractable radioactivity in grain (15.6% of the TRR or 0.058 mg a.e./kg) was very polar and was composed of up to 35 separate peaks of radioactivity, the largest of which comprised 5.1% of the TRR or 0.019 mg a.e./kg. The 10.2% of the TRR or 0.038 mg a.e./kg of the seed residue extracted in the oil fraction could not be converted by acid or base hydrolysis into 2,4-D or DCP and was considered to be incorporated into natural plant lipids. Approximately 70% of the TRR in grain was non-extractable, but substantial portions (30 to 50% of the TRR) could be released by acid or base hydrolysis. Most of the radioactivity released was very polar and multi-component and was thought to be associated with natural plant constituents. Residue levels are summarized below.

Sample	2,4-D		DCP		DCP-conjugates	
	% TRR	a.e. mg/kg	% TRR	a.e. mg/kg	% TRR	a.e. mg/kg
Forage	73.5%	36.932	4.5%	2.282	14.5%	7.318
Hay	59.4%	21.978	1.4%	0.518	23.4%	8.542
Grain	1.1%	0.004	0.1%	<0.001	5.5%	0.021

In spite of DCP conjugates being so much higher than DCP, and the fact that DCP conjugates have been shown to release DCP during digestion (Pascal-Lorber et al. 2012), only 2,4-D residues and the DCP metabolite are reported in "Magnitude of Residue" studies, as was the case for corn. If we assume that DCP conjugate levels would be 55X higher than DCP levels in DAS-68416-4 soybean seeds, then we expect 2.58 mg/kg of DCP conjugates in addition to 0.047 mg/kg of DCP after 2,4-D applications (highest average field trial results), for a combined residue level of 2.63 mg/kg soybean seed:

> Study 090053 - Magnitude of the Residue of 2,4-D in/on Herbicide Tolerant Soybeans Containing the Aryloxyalkanoate Dioxygenase-12 (AAD-12) Gene
>
> ABSTRACT ONLY
> ………The current residue definition for 2,4-D does not include 2,4-DCP, but is based on only 2,4-D determined as the acid. However, 2,4-DCP was included as an analyte of interest because the herbicidal tolerance of AAD-12 soybeans to 2,4-D relies on increased plant metabolism through a pathway involving 2,4-DCP.
>
> Residue data for 2,4-D and 2,4-DCP in Soybean Seed are summarized in the following table:

Analyte/ Commodity	Target Application Rate[a] lb ae/A (g ae/ha)	PHI[b] (days)	n[c]	Residue Levels (ug/g)					
				Min.	Max.	Median[d]	Mean[d]	Std. Dev. [d]	HAFT[e]
2,4-D									
Seed	3 x 1.0 (~1120)	51-103	48	ND [f]	ND	ND	ND	NA[g]	ND
2,4-DCP									
Seed	3 x 1.0 (~1120)	51-103	48	ND	0.054	0.015	0.017	0.015	0.047

[a] 2,4-D was applied in three applications with the first being applied preemergence, the second targeted at 12 days prior to the R2 growth stage, and the third application targeted t the R2 growth stage.

[b] PHI = Pre-Harvest Interval; days between last application of, 2,4-D and collection of field sample.

[c] Two independently composited samples were collected from each treated plot at 24 trial sites = 48 samples.

[d] For statistical purposes, ND has been given the value of zero.

[e] HAFT = Highest Average Field Trial (based on average of the replicate samples from the treated plot)

[f] ND = Not detected; less than the LOD (<0.003 μg/g)

[g] NA = Not applicable

i. Risks for humans

The current tolerance level for 2,4-D residues on soybean seed is 0.02 ppm (DEA at 57), or 0.02 mg 2,4-D/kg of seeds. Therefore, if DCP was included in the definition of "2,4-D residue", as it was until recently (Stagg et al. 2010), it is likely that tolerances for DAS-68416-4 soybean would need to be raised to accommodate the residues from the new post-emergence 2,4-D applications. These applications result in highest average field trial values for DCP of 0.047 mg/kg. Adding in DCP conjugates would potentially result in much higher residue levels than allowed: 2.63 mg/kg DCP + DCP conjugates is 100-fold higher than the current tolerance level.

Humans eat soybean seeds in a variety of products. APHIS needs to consider the impacts to human health of exposure to DCP and DCP conjugates that are a result of the activity of the engineered AAD-12 enzyme in DAS-68416-4 soybean.

ii. Risks for wild animals

However, particularly for wild animals, the Dow nature and magnitude of residue studies are not adequate for determining the impacts of 2,4-D use on DAS-68416-4 soybean. Dow 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 fields.

Assuming that the soybean studies were designed similarly to corn studies, Dow applied the last 2,4-D treatment at the R-2 stage, as per label, and then waited a period of time before taking their first samples of forage and hay at the customary times. Wild animals would not necessarily wait after an application before ingesting soybean 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.

In particular, the types and levels of DCP and DCP conjugates in DAS-68416-4 soybean forage and hay after 2,4-D applications need to be compared with independent research on 2,4-D and DCP metabolism in conventional soybeans (Pascal-Lorber et al. 2003), and any differences explained. APHIS should consider levels of all expected toxic residues and metabolites; and assess impacts to non-target organisms of the novel, potentially toxic constituents expected to result when 2,4-D with DAS-68416-4 soybeans under a variety of anticipated application scenarios.

10. Impacts of AAD-12 enzyme activity in DAS-68416-4 soybeans on pollinators, including honey bees

To properly consider any unreasonable adverse effects of DAS-68416-4 soybeans, APHIS must consider how approval of DAS-68416-4 soybeans may have an unreasonable adverse effect on pollinators, which are organisms beneficial to agriculture. At a minimum, APHIS must request and examine Dow's own studies on translocation of 2,4-D into floral tissues and subsequent compositional changes due to metabolism of such 2,4-D in the pollen and nectar of engineered plants.

Dow did not measure levels of the engineered gene product AAD-1 in pollen and nectar of 68416-4 soybeans, sprayed with 2,4-D, or not (Petition at 75). Nor did they report levels of herbicide residues and metabolites in floral parts.

There is precedence for looking at gene expression in pollen as part of environmental risk assessments. Levels of gene expression in pollen have been of great interest for Bt crops, for example. 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.

Potential harmful effects of AAD-12 enzyme activity in soybeans on honeybees and the honey produced from the collection of pollen and nectar is of particular concern. Soybeans are visited by honey bees that are located near soybean fields, and honey bees gather both nectar and pollen (Chiari et al. 2005, see references cited in introduction). Surprisingly, very recent research in Iowa has shown that many species of wild pollinators also frequent soybean fields (Anonymous 2011, O'Neal and Dill 2012), as discussed in our comments on transgenic contamination.

Many honey bees do live near soybean fields during pollination season. 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 and soybeans] dominate the landscape."

If DAS-68416-4 soybeans encountered 2,4-D during flower 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 and nectar would be expected. 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 and the nectar producing cells of DAS-68416-4 soybean may be protected from the toxic effects of 2,4-D because of the expression of AAD-12, and so may accumulate more 2,4-D and other herbicide substrates than conventional soybeans: if the pollen and nectar-producing cells remains viable, they will be a stronger sinks 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 et al. 2002). Also, the 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-the 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-68416-4 soybeans (and 2,4-D resistant corn) into possible toxins (Grogan and Hunt 1979).

Taken together, the fact that honey bees are likely to collect pollen and nectar of DAS-68416-4 soybean after it has been sprayed with 2,4-D (and other herbicides that are substrates of the engineered AAD-12 protein), and that resulting residues and metabolites are likely to be different from those in conventional soybeans, we request that the EPA consider the impacts to honey bees and other pollinators, as in the guidelines for analyzing impacts of transgene products on bees set out by Malone and Pham-Delègue, for example (2001, p. 299 – 300).

10. Conclusion

As these comments make clear, APHIS' deregulation of DAS-68416-4 would have numerous adverse impacts on human health, the environment, farmers, and the agricultural economy as a whole. APHIS' draft Environmental Assessment and Plant Pest Risk Assessment do not begin to provide a serious assessment of these impacts. APHIS fails to provide any assessment of the enormous increase in 2,4-D and glufosinate use that agronomic experts regard as inevitable with use of these soybeans. Nor does APHIS consider the greatly altered use pattern of these herbicides in the context of the "Enlist Weed Control System." This altered herbicide usage pattern and increased use will trigger rapid emergence of extremely damaging weeds resistant to 2,4-D and multiple herbicides, as well as substantially increased damage to both non-target crops and wild plants via herbicide drift.

APHIS fails to provide any assessment of adverse impacts to human, particularly farmer, health that will ensue from dramatically increased exposure to 2,4-D and glufosinate.

APHIS does not provide any meaningful assessment of environmental impacts from the DAS-68416-4 soybean system. Yet as discussed above, multiple adverse effects are likely, including to threatened and endangered species, both plants and animals. Under the intended conditions of use, 2,4-D resistant soy will generate a toxic metabolite that will likely negatively impact non-target organisms, particularly honeybees and other pollinators.

APHIS' assessment shows a cavalier disregard for fundamental tenets of sound science. APHIS uncritically adopts the petitioner's assessment in its discussion of practically every issue addressed in the DEA, providing no critical analysis. Data sources and other references are selected without regard to quality. APHIS frequently relies on outdated information. Industry-sponsored misinformation, often in the form of white papers subject to no peer review, are frequently cited. Dow has intentionally omitted information known to it that points clearly to adverse consequences from introduction of DAS-68416-4, in particular studies it sponsored indicating the high likelihood of herbicide resistant weed evolution. APHIS also failed to provide any meaningful assessment of the cumulative impacts of Enlist soybeans in the context of the reasonably foreseeable introduction of other herbicide-resistant crop systems, particularly 2,4-D-resistant corn.

The many and serious flaws in the draft Environmental Assessment cannot be redressed by APHIS's usual practice of provide a "response to comments" section in a final EA. Rather, APHIS must begin anew with a comprehensive EIS that rigorously explores the full range of studies and information presented in these comments. Anything less would represent a clear abdication of APHIS' statutory duties under the Plant Protection Act, the National Environmental Policy Act, and the Endangered Species Act.

CFS would be happy to discuss the issues raised in these comments with APHIS staff in the interests of a full, rigorous and scientifically credible assessment of DAS-68416-4.

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